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Residential bacteria and fungi identified by high-throughput sequencing and childhood respiratory health☆

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ABSTRACT:

The objective of this study was to examine and compare environmental microbiota from dust and children's respiratory health outcomes at ages seven and twelve.

At age seven, in-home visits were conducted for children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS). Floor dust was collected and analyzed for bacterial (16 S rRNA gene) and fungal (internal transcribed spacer region) microbiota. Respiratory outcomes, including physician-diagnosed asthma, wheeze, rhinitis, and aeroallergen sensitivity were assessed by physical examination and caregiver-report at ages seven and twelve. The associations between dust microbiota and respiratory outcomes were evaluated using Permanova, DESeq, and weighted quantile sum (WQS) regression models. Four types of WQS regression models were run to identify mixtures of fungi or bacteria that were associated with the absence or presence of health outcomes.

For alpha or beta diversity of fungi and bacteria, no significant associations were found with respiratory health outcomes. DESeq identified specific bacterial and fungal indicator taxa that were higher or lower with the presence of different health outcomes. Most individual indicator fungal species were lower with asthma and wheeze and higher with aeroallergen positivity and rhinitis, whereas bacterial data was less consistent. WQS regression models demonstrated that a combination of species might influence health outcomes. Several heavily weighted species had a strong influence on the models, and therefore, created a microbial community that was associated with the absence or presence of asthma, wheeze, rhinitis, and aeroallergen+. Weights for specific species within WQS regression models supported indicator taxa findings.

Health outcomes might be more influenced by the composition of a complex mixture of bacterial and fungal species in the indoor environment than by the absence or presence of individual species. This study demonstrates that WQS is a useful tool in evaluating mixtures in relation to potential health effects.

De-identified data upon request and approval by the PI and IRB.

1. Introduction/background

Asthma affects nearly 8% (25 million) of the US population and 300 million individuals worldwide (CDC, 2018; GINA, 2020). In the US, asthma continues to be the most common chronic disease with a prevalence in children of 7.5% (CDC, 2018). Asthma is a major burden on the healthcare system, a disruption to the families, and results in significant loss of productivity in the workplace and school (Hsu et al., 2016; Sullivan et al., 2017). Symptoms of asthma, which differ over time in intensity, occurrence, and frequency, include wheeze, shortness of breath, cough, and chest tightness (GINA, 2020). The development of allergic disease and early allergic sensitization influence the severity of childhood asthma (Del Giacco et al., 2017). In addition, most individuals with asthma also have rhinitis, although not all patients with rhinitis have asthma (Bousquet et al., 2003; Togias, 2003). Multiple genetic, epigenetic, and environmental factors play a role in the development of

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allergic disease and asthma, including exposure to tobacco smoke, outdoor air pollution, house dust mites, and mold (Beasley et al., 2015; Caillaud et al., 2018; Subbarao et al., 2009). Frequently, these environmental exposures may also trigger or worsen current asthma symptoms (Cowan and Guilbert, 2012; GINA, 2020). In contrast, other environmental factors, including endotoxin and farm animals, have been inversely associated with the development of childhood asthma (Beasley et al., 2015; Douwes et al., 2007).

Because Americans spend an estimated 87% of their time indoors (Klepeis et al., 2001), exposures occurring indoors are particularly relevant to health outcomes. Microbial exposure from the indoor environment is influenced by inhabitants' behaviors, including but not limited to pet ownership and/or living with mold or visible dampness (Murrison et al., 2019). Several studies have found low bacterial and fungal diversity in house dust to be associated with increased risk of asthma development (Dannemiller et al., 2014b; Edwards et al., 2012; Ege et al., 2011, 2012). A variety of studies have explored associations between indoor microbes or microbial components and health, including studies that found extracellular polysaccharides from *Penicillium* spp. and *Aspergillus* spp. were inversely related to asthma and wheeze (Ege et al., 2007). Other studies have found yeast exposures or their components were possibly protective against allergy and asthma in children at risk for these outcomes (Behbod et al., 2015). High (1–3)-β-D-glucan concentrations were associated with a reduced likelihood of recurrent wheezing and showed similar trends with allergen sensitization (Iossifova et al., 2007). It remains unclear if the dose, diversity, or exposure to specific microorganisms, individually or jointly, account for these conflicting microbial effects (Von Mutius and Vercelli, 2010).

High-throughput sequencing combined with quantitative polymerase chain reaction (qPCR) offers an opportunity to quantitatively study the relative abundance of microbiota in the residential environment in relation to asthma, and to explore the full microbial community in a way not possible with culture-based analysis, or even qPCR assays alone (Dannemiller et al., 2014b). Previously, we investigated the influence of environmental factors, including mold and moisture damage, on the fungal and bacterial microbiota in dust samples (Cox et al., 2021).

In this analysis, we explored relationships between the environmental microbiota and health effects. Identification of potential causal agents for adverse health effects in the indoor environment is challenging, due to the variability of microbial components as well as the lack of standardized exposure assessment methods (Tischer and Heinrich, 2013). It is unclear whether the presence of individual fungi leads to specific disease mechanisms or how fungal and bacterial effects may interact (Bush, 2020). Although some studies have explored either fungal or bacterial exposures indoors and their effects on health (Edwards et al., 2012; Reponen et al., 2012; Sharpe et al., 2015; Vesper et al., 2006), few have evaluated both fungal and bacterial microbiota exposure in late childhood with health outcomes (Adams et al., 2021; Dannemiller et al., 2016; Fu et al., 2020b). Therefore, important questions remain regarding the associations of late-childhood exposures to environmental microbiota with various health outcomes.

The objective of this cross-sectional and prospective study was to use amplicon-based sequencing of the fungal and bacterial microbiota to study their association with allergic disease and respiratory health, including asthma, wheeze, and rhinitis. Because the variability in the symptoms of pediatric asthma under the age of six makes diagnosis challenging (Trivedi and Denton, 2019) and the lack of information available regarding late-childhood exposures and health outcomes, this study explored indoor microbiota exposure in late-childhood (age seven) and the association with health outcomes at ages seven and 12. In order to accomplish this, untargeted statistical analyses, to identify microbial species associated with the health outcomes, and hypothesis-based analyses were utilized. Our hypothesis-based analyses investigated whether particular fungal species with varying moisture requirements for growth showed associations with the health outcomes.

2. Methods

2.1. In-home visits

Floor dust samples were collected and archived from in-home visits at age seven for children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS), a birth cohort study (Brunst et al., 2015; LeMasters et al., 2006; Ryan et al., 2005). Trained study staff also evaluated the primary residence and measured the total areas of moisture damage and visible mold in the home (Cox et al., 2020). Eligibility for enrollment in the CCAAPS cohort required residing at birth either $<$400 m or $>$1500 from a major road and at least one biological parent with atopy, defined as having allergic symptoms and a positive reaction on a skin prick test to at least 1 of 15 common aeroallergens (Ryan et al., 2005).

2.2. Health outcomes

Children enrolled in CCAAPS completed clinic visits at ages one, two, three, four, seven and twelve, including a physical examination, skin prick testing (age 7), radioallergosorbent (RAST) blood testing for IgE antibodies (age 12), and parent-completed questionnaires regarding their child's respiratory health (asthma, rhinitis, and wheeze) in the previous 12 months. Respiratory and allergic disease outcomes ascertained at the age seven and twelve visits are included in this analysis as described below.

2.2.1. Age 7

Aeroallergen positivity (aeroallergen+) at age seven was defined as having a positive skin prick test (SPT+) to any aeroallergen in a panel of 11 aeroallergens: white oak, elm, cat, dog, house dust mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), fescue and timothy grasses, 2 mold allergens (*Alternaria alternata* and *Penicillium* species mix), short ragweed, and German cockroach (ALK-Abelló, Round Rock, Tex) (LeMasters et al., 2006). The panel of 11 was selected for this study to allow for comparisons between ages seven and 12.

Children were defined as asthmatic at age seven based on the presence of asthma-related symptoms and airway reversibility or bronchial hyperreactivity as previously described (Brunst et al., 2015; Reponen et al., 2011). Briefly, children were defined as asthmatic if they fulfilled two criteria: (1) caregiver report of asthma symptoms (in the previous 12 months, any report of tight chest or throat, difficulty breathing or wheezing after exercise, wheezing and/or whistling in the chest) and (2) demonstration of airway reversibility (defined as $\geq$12% increase in forced expiratory volume in 1 s after bronchodilation) or a positive methacholine challenge test result (defined as a $\geq$20% decrease in baseline forced expiratory volume in 1 s at a cumulative inhaled methacholine concentration of $\leq$4 mg/mL) (Cox et al., 2020). Rhinitis at age 7 was defined as the child having sneezing or a runny, blocked, or itchy nose when he/she did not have a cold or flu in the past 12 months (Codispoti et al., 2010). Wheeze at age 7 was defined as caregiver-report of the child wheezing at least 2 times in the past 12 months (Brunst et al., 2015).

2.2.2. Age 12

Allergic sensitivity at age 12 was determined by RAST testing to a panel of 11 aeroallergens (listed above) and defined as any aeroallergen-specific IgE greater than 0.35 kU/l. Asthma at age 12 was defined as either (1) using asthma medications in the last 12 months; (2) having respiratory symptoms at least one to four times a month (difficulty breathing, shortness of breath, tight or clogged chest, chest pain or tightness) or (3) wheezing or whistling in the chest in the past 12 months one or more times. Rhinitis and wheeze symptoms at age 12 were defined by the presence of sneezing, runny, or blocked/itchy nose, and child wheezing two or more times in the past 12 months, respectively.

2.3. Characterizing the microbiota through sequencing of fungi and bacteria and universal qPCR

DNA was extracted from the archived floor dust samples using the MOBIO PowerLyzer® PowerSoil® DNA isolation kit according to the manufacturer's instructions (MOBIO, Carlsbad, California) modified by adding glass beads of different sizes (Yamamoto et al., 2012). Ultra-high-throughput microbial community analysis (Illumina MiSeq) was performed by the Research and Testing Laboratory in Lubbock, TX. The internal transcribed spacer (ITS) region of fungal ribosomal DNA was amplified by PCR with ITS1F/ITS2aR primers. For bacteria, extracted DNA was amplified with 16 S ribosomal DNA (rDNA) 515 F and 806 R primers that target the V4 region.

Total DNA concentration was measured using qPCR with universal fungal primers 5.8F1/5.8R1 that target the ITS region (Haugland, 2002) and universal bacterial primers that target the 16 S rRNA gene (Nadkarni et al., 2002) (Step One Plus, Applied Biosystems, Life Technologies, Carlsbad, CA). The assay and cycling conditions have been previously described (Cox et al., 2018; Cox, 2021).

The DADA2 Pipeline in R Statistical Analysis Software was used to remove chimeras and develop the amplicon sequence variant (ASV) table and assign taxonomy using Silva version 132 and UNITE database release date 18.11.2018 (Version 8.0) (Callahan, 2018; Callahan et al., 2016; Nilsson et al., 2019; UNITE, 2019). Analysis of the sequencing reads, including abundance and diversity analyses, was conducted at the University of Cincinnati. Relative abundance in each taxa level was based on ASVs identified. All ASVs identified to the species level were utilized for species-specific analyses, and any species with multiple ASVs were combined. Downstream analyses were conducted using the R package, phyloseq v1.28 (McMurdie and Holmes, 2013). A more detailed explanation of methods is located in the supplement.

2.4. Data analysis

All tests used p-values adjusted for multiple testing using the Benjamini-Hochberg procedure, with a p-value less than 0.05 considered significant (Benjamini and Hochberg, 1995). We examined five aspects of the microbiota data for relationships with ages 7 and 12 health outcomes: alpha diversity, beta diversity, indicator taxa, fungi with known moisture requirements, and weighted quantile sum regression.

2.4.1. Alpha diversity

Alpha diversity measures calculated for each sample, based on rarefied data, included Shannon's diversity, Simpson's Index, the number of observed ASVs (Observed), and Faith's phylogenetic diversity (PD). Univariate regression analysis was used to determine associations between fungal and bacterial alpha diversity measures (Shannon, Simpson, Observed, and PD) and ages 7 and 12 health outcomes.

2.4.2. Beta diversity

Beta diversity analysis was performed with relative abundance data. Multivariate analysis of variance using distance matrices was utilized with PERMANOVA (Adonis) and Bray Curtis to assess differences in beta diversity of the relative abundance of fungal and bacterial species associated with the presence of specific health outcomes.

2.4.3. Indicator taxa

Differential abundance testing with DESeq2 (version 1.28) (Love et al., 2016) was used to determine which fungal and bacterial species were associated with the absence vs. presence of a health outcome. We accounted for between-sample differences in the total fungal and bacterial biomass by multiplying the relative abundance values by the qPCR (total fungal or bacterial DNA) values for each sample to calculate absolute abundance (Dannemiller et al., 2014a; Jian et al., 2018). We used a modified DESeq as previously described (Cox et al., 2021). Species were sorted by requiring their presence in 20% or more of samples with an adjusted p-value <0.05. The species identified during this process were also compared to species from a previous study in which we explored indicator taxa and their association with no and high mold (defined as ≥ 0.19 m^2) or high moisture damage (defined as ≥ 0.29 m^2) (Cox et al., 2021).

2.4.4. Fungi with known moisture requirements

Fungal species were categorized into hydrophilic ($a_w \geq 0.90$), mesophilic ($0.80 \leq a_w < 0.90$), and xerophilic ($a_w < 0.80$) based on species-specific information previously compiled from the literature on minimum available moisture required for growth (Adams et al., 2020). Univariate regression analysis was used to study associations between the sum of the absolute concentrations of fungal species within each of the three water requirement categories (hydrophilic, mesophilic, and xerophilic) and health outcomes. PERMANOVA (Adonis) with Bray Curtis was performed to assess beta diversity differences of the relative abundance of fungal species within each of the three water requirement groups, and whether differences were associated with health outcomes.

2.4.5. Confounders

A directed acyclic graph (DAG) was developed for the microbiota exposure and health models to identify potential confounding variables requiring multivariate adjustment (Fig. 1) (Textor et al., 2016). A DAG, for causal paths of interest (e.g., microbiota → asthma), identifies potential biasing paths. Based on the hypothesized connections in the DAG, race (Black or non-Black), pets, neighborhood socioeconomic status, cockroaches, dust mites, and rodents were identified as variables to be included in our health models. All identified variables were adjusted for in the weighted quantile sum logistic regression models. The neighborhood socioeconomic status was determined by a deprivation index which utilizes principal components of six different 2015 American Community Survey measures. Rescaling and normalizing forces the index to range from 0 to 1, with a higher index indicating increased community deprivation (Brokamp, 2019). Race and the presence of pets, cockroaches, and rodents (mice and rats) in the home were reported by caregivers at age 7. House dust was tested for dust mite allergen (Reponen et al., 2012).

2.4.6. Weighted quantile sum index and logistic regression

Evaluating associations between fungal or bacterial species and common health outcomes is difficult due to high dimensionality and potentially high correlations between variables. In addition, traditional methods such as linear or logistic regression may lead to inaccurate inference because of collinearity and variance inflation. When the correlations between the variables of interest are high, alternative approaches such as penalized regression methods may also have several limitations. Weighted quantile sum (WQS), a statistical model for multivariate regression, attempts to overcome these issues with mixtures of many correlated variables by constructing an index weighted by the importance of particular species. The effect of this index on an outcome is then estimated using traditional methods.

The constructed WQS fungal or bacterial index was used in logistic regression models to test the association of each index with each health outcome. Initially, species were filtered by requiring their presence in 20% or more of samples. Because WQS provides a unidirectional evaluation of mixture effects, we examined each index separately as to whether it was influential for the absence or presence of each health outcome. A WQS index was developed and applied separately for the absence and presence of each health outcome. In each model, individual species were assigned a weight corresponding to their contribution to the overall index effect. Carrico et al. (2015) implemented a cutoff, τ, which was utilized to discriminate which species had a significant weight greater than zero. The generation of τ was the inverse of the number of species in the analysis. Four logistic regression models and associated weights of species were developed for each health outcome:

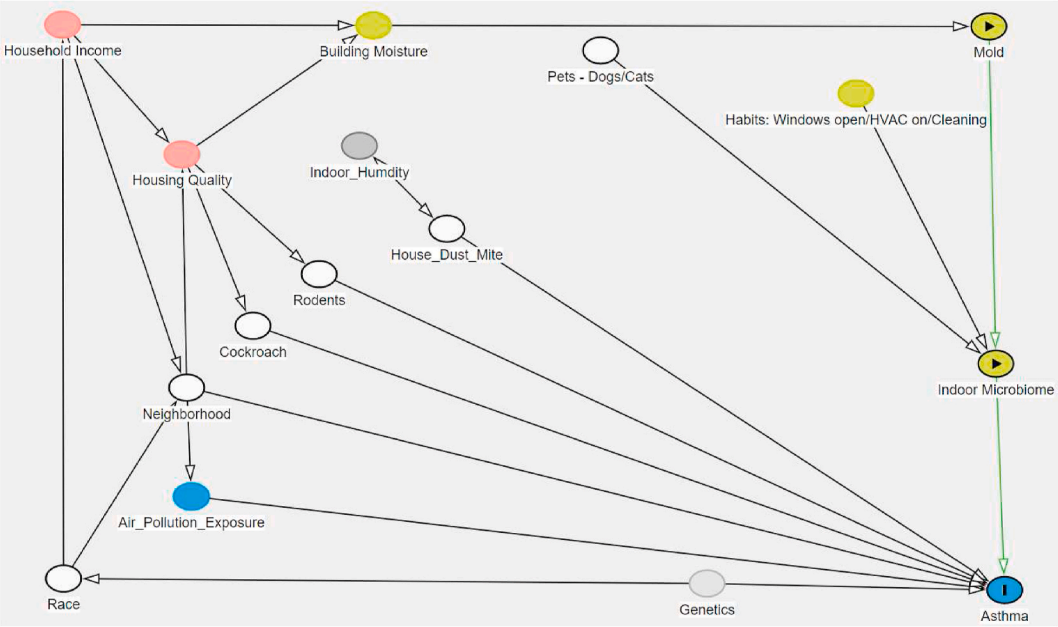

Fig. 1. Directed acyclic graph for microbiota exposure. Each circle with a dark outline represents a potential confounding variable, other than the outcome (asthma) and exposure (microbiota). A green arrow connecting two circles is a causal path of interest for the analysis (e.g., microbiota → asthma). A black arrow means no bias on that path, and a red arrow (not present) would mean potential bias. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bacterial species associated with the presence or the absence of the health outcomes and fungal species associated with the presence or the absence of the health outcomes. The effect of each WQS index on outcomes was estimated while adjusting for confounders identified by DAGs.

3. Results

A summary of health outcomes assessed at ages 7 and 12 (asthma, wheeze, rhinitis, and aeroallergen+) among the participants is presented in Table 1 and Fig. 2. Due to missing health outcomes, the number of subjects included in analyses varied by health outcomes. Of the 170 participants who had dust samples collected and a health assessment at age 7, 29 (17%) had wheeze, 77 (46%) were aeroallergen+, and 76 (45%) reported rhinitis symptoms in the previous 12 months. Thirty-one participants (19%) assessed at age 7 had asthma, and individuals who had asthma symptoms but were not able to complete either the bronchial hyperresponsiveness pulmonary function test or methacholine challenge (n = 4) were excluded from age 7 asthma analysis. Of the 170 age 7 participants, 112 individuals participated in a follow-up study and had their health assessed at age 12, which included 42 (38%) with asthma, 22 (20%) with wheeze, 59 (53%) with aeroallergen+ and 68 (61%) with rhinitis.

3.1. Microbiota data

For fungi, an average of 7354 (range 186–26,276) sequences per sample was identified, including a total of 3855 different amplicon sequence variants (ASVs). For bacteria, an average of 28,234 (range 6033–53,036) sequences per sample was identified, including a total of 11,150 ASVs. Rarefaction curves for alpha diversity fungal analyses are presented in the supplement (Figure S1). For fungal samples, 1000 reads was selected as the threshold for rarefaction, as this maintained 1942 taxa and excluded only 6 samples. For bacterial analyses, the minimum read count was 6033, which was used as the number of reads to rarefy the data for alpha diversity analyses. Species identified in the indicator taxa analysis, weighted quantile sum regression models, and fungi with known moisture requirements were verified on the NCBI database, and species closely related to the target sequences (>98% identity match and >98% query cover) have been identified previously (Cox et al., 2021). A total of 759 fungal and 798 bacterial species were identified, and from those, 59 fungal and 80 bacterial species were identified in at least 20% of the samples and subjected to DESeq and WQS regression analyses.

Fungal and bacterial taxa with the highest average relative abundance with and without each health outcome are shown in Tables S1 and S2, respectively. For fungi, Ascomycota was the most abundant phylum with an average relative abundance of 85.6%. Similarly, the most abundant class was Dothideomycetes with an average relative abundance of 59.7%. The respective taxa in order, family, and genus levels were Pleosporates (52.1%), Didymellaceae (30.5%), and *Epicoccum* (19.2%). *Cyberlindnera jadinii* (20.0%) and *Epicoccum nigrum* (25.7%) were the most abundant fungal species.

For bacteria, Proteobacteria was the phylum with the highest average relative abundance of 30.0%. The respective taxa in class, order, family, and genus levels were Actinobacteria (22.8%), Corynebacteriales (13.5%), Corynebacteriaceae (13.4%), and *Corynebacterium* (13.8%). *Acinetobacter lwoffii* (7.4%) and *Rothia mucilaginosa* (4.2%) were the most abundant bacterial species.

3.2. Alpha and beta diversity

Univariate regressions between alpha diversity measures of the fungal and bacterial microbiota (Shannon, Simpson, Observed, Phylogenetic diversity) and health outcomes did not show any significant associations using adjusted p-values (Table S3). Prior to adjusting p-values for multiple comparisons, fungal Simpson alpha diversity measure was significantly associated with asthma at age 12 (odds ratio [OR] = 0.005, p = 0.03). After adjustment, however, the association lost significance (p = 0.24). The differences in fungal and bacterial beta diversity measures and ages 7 and 12 health outcomes were calculated with PERMANOVA. R^2 and adjusted p-values, shown in Table S4, demonstrate that beta diversity was not associated with the absence or presence of each health outcome.

Table 1
Race, gender, parental asthma, presence of pets and income and the prevalence of asthma, wheeze aeroallergen positivity (+) and rhinitis at ages 7 and 12.

	Asthma			Wheeze			Aeroallergen+			Rhinitis		
	N	n	%	N	n	%	N	n	%	N	n	%
Age 7												
Overall	166	31	19	170	29	17	168	77	46	169	76	45
African American	47	13	28	48	9	19	48	24	50	48	21	44
Not African American	119	18	15	122	20	16	120	53	44	121	55	45
Male	95	19	20	95	19	20	94	48	51	94	46	49
Female	71	12	17	75	10	13	74	29	39	75	30	40
Parental asthma	64	17	27	66	16	24	65	33	51	66	29	44
No parental asthma	102	14	14	104	13	13	103	44	43	103	47	46
Pets	79	12	15	82	13	16	80	34	43	82	36	44
No Pets	87	19	22	88	16	18	88	43	49	87	40	46
Income												
Up to 29,999	38	15	39	38	14	37	38	18	47	38	13	34
30 k to 69,999	47	6	13	47	8	17	47	19	40	47	21	45
70 k and above	79	9	11	83	6	7.2	81	38	47	82	41	50
Age 12												
Overall	112	42	38	112	22	20	112	59	53	112	68	61
African American	34	17	50	34	9	26	34	19	56	34	20	59
Not African American	78	25	32	78	13	17	78	40	51	78	48	62
Male	60	21	35	60	13	22	60	36	60	60	40	67
Female	52	21	40	52	9	17	52	23	44	52	28	54
Parental asthma	42	20	48	42	10	24	42	23	55	42	26	62
No parental asthma	70	22	31	70	12	17	70	36	51	70	42	60
Pets	51	17	33	51	10	20	51	26	51	51	28	55
No Pets	61	25	41	61	12	20	61	33	54	61	40	66
Income												
Up to 29,999	30	15	50	30	9	30	30	14	47	30	15	50
30 k to 69,999	31	10	33	31	5	16	31	17	55	31	22	71
70 k and above	50	16	32	50	8	16	50	27	54	50	30	60

N - Total number of subjects with and without the health outcome-.
n - number of subjects with the variable and positive for the health outcome-.
% - percentage of n/N-.

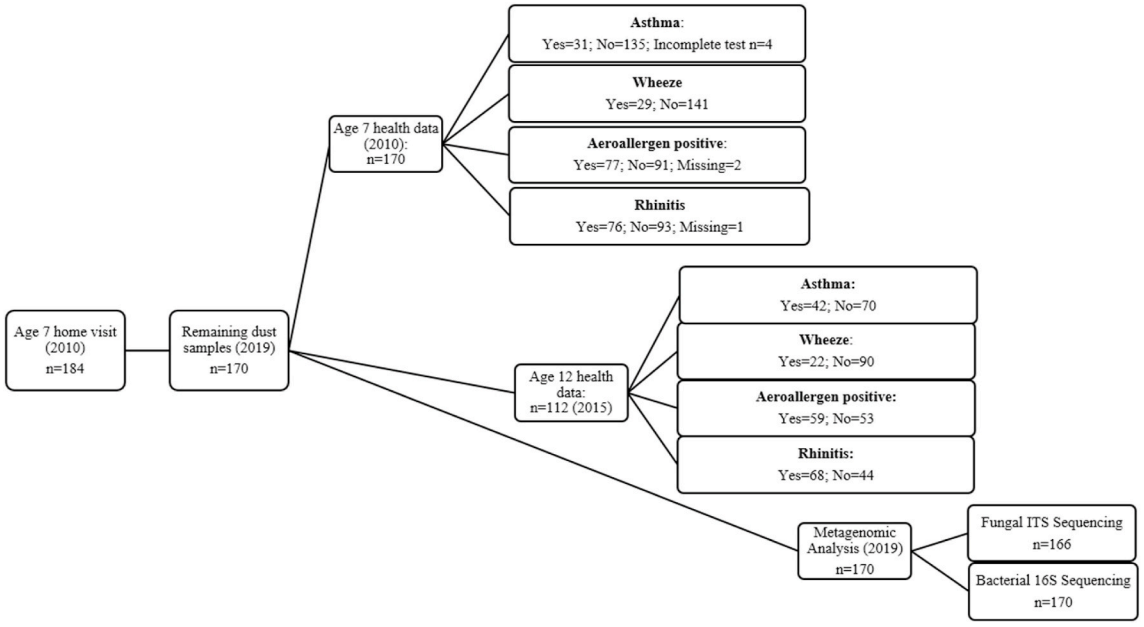

Fig. 2. Flowchart of the number of participants with available data and health outcomes. Wheezing two or more times in the previous 12 months.

3.3. Fungal species with known water requirements

The univariate regression between the sum of absolute concentrations of fungal species with similar water requirements (xerophiles, mesophiles, and hydrophiles) and the health outcomes showed no significant associations (Table S5). Additionally, PERMANOVA did not show any significant differences between the beta diversity measures of fungal species grouped by water requirements and the absence vs. presence of ages 7 and 12 health outcomes (Table S5).

3.4. Indicator taxa

3.4.1. Fungus

Fifty-nine fungal species were identified in at least 20% of the samples and were subject to DESeq analysis. Table 2 contains a list of fungal species that were significantly different, based on differential abundance, between the absence vs. presence of health outcomes for ages 7 and 12. The results reported below are species with log 2-fold change greater than 2 or less than −2 as identified by DESeq analyses. The abundance of a species was significantly higher with the presence of a health outcome (positive DESeq value) or significantly lower (negative DESeq value) with the presence of a health outcome compared to the absence of that outcome.

Overall, DESeq identified 20 fungal species were lower with the presence of asthma and wheeze, and also identified 13 fungal species were higher with the presence of rhinitis and aeroallergen+. With the presence of asthma, 10 species were lower at age 7, and 14 species were lower at age 12. Of the ages 7 and 12 species that were lower with the presence of asthma, seven species overlapped: *Arthrocatena tenebrio, Coniosporium apollinis, Curvularia americana, Devriesia strelitziicola, Gibberella intricans, Toxicocladosporium irritans*, and *Vishniacozyma victoriae*. With the presence of age 7 wheeze, 10 species were lower and one was higher, and with the presence of age 12 wheeze, 11 species were lower and one was higher. Of the ages 7 and 12 species that were lower with the presence of wheeze, five overlapped, including *Aspergillus cibarius, Coniosporium apollinis, Exophiala xenobiotica, Gibberella intricans* and *Toxicocladosporium irritans*. With the presence of aeroallergen+, 3 species were lower for age 7 and one species was lower for age 12. *Toxicocladosporium irritans* was lower with the presence of with aeroallergen+ at both ages. In addition, 8 species were higher with the presence of aeroallergen+ at age 12. With the presence of age 7 rhinitis, one species was higher (*Coniosporium apollinis*) and one was lower (*Toxicocladosporium irritans*). With the presence of age 12 rhinitis, 10 species were higher, and two of those species also were higher at age 7.

In summary, the species that were lower with the presence of any health outcome included: *Arthrocatena tenebrio, Aspergillus cibarius, Bipolaris maydis, Curvularia lunata, Devriesia strelitziicola, Mycosphaerella tassiana, Nigrospora oryzae, Papiliotrema aurea, Phaeosphaeria podocarpi, Saitozyma flava, Vishniacozyma carnescens* and *Vishniacozyma victoriae*. The species that were higher with the presence of any health outcome included: *Aspergillus sydowii*, *Candida tropicalis*, *Curvibasidium cygneicollum, Diutina catenulata, Fusarium acutatum* and *Naganishia albida*. Species that were higher or lower, depending on the health outcome, included *Candida parapsilosis, Coniosporium apollinis, Curvularia americana, Exophiala xenobiotica, Filobasidium oeirense, Gibberella intricans, Rhodotorula mucilaginosa, Rhodotorula taiwanensis* and *Toxicocladosporium irritans*.

3.4.2. Bacteria

Table 3 contains a list of bacterial species that were significantly different, based on differential abundance, between the absence vs. presence of health outcomes for ages 7 and 12. Overall, DESeq identified

Table 2
Fungal species that were significantly associated in DESeq with the health outcomes of asthma, wheeze, aeroallergen positivity (+) and rhinitis at ages 7 and 12.

	Indicator Species	Asthma		Wheeze		Aeroallergen+		Rhinitis	
		Age 7	Age 12	Age 7	Age 12	Age 7	Age 12	Age 7	Age 12
		(Log 2-Fold Change)							
Lower with presence of health outcomes	*Arthrocatena tenebrio*	**−2.69**	**−2.04**	**−3.28**	–	–	–	–	–
	Aspergillus cibarius	–	**−2.76**	**−2.15**	**−2.46**	–	–	–	–
	Bipolaris maydis	–	**−2.45**	–	–	–	–	–	–
	Curvularia lunata	–	**−2.16**	–	**−2.73**	–	–	–	–
	Devriesia strelitziicola	**−2.22**	**−2.55**	**−3.71**	–	–	–	–	–
	Mycosphaerella tassiana	–	**−2.46**	–	−2.03	–	–	–	–
	Nigrospora oryzae	–	**−2.45**	–	**−2.70**	–	–	–	–
	Papiliotrema aurea	**−2.03**	–	–	–	–	–	–	–
	Phaeosphaeria podocarpi	–	−2.45	–	–	–	–	–	–
	Saitozyma flava	–	**−2.07**	–	–	–	–	–	–
	Vishniacozyma carnescens	–	–	–	**−2.11**	–	–	–	–
	Vishniacozyma victoriae	**−2.20**	**−2.60**	–	**−2.34**	–	–	–	–
Lower And Higher with presence of health outcomes	*Candida parapsilosis*	–	–	**−2.98**	–	–	–	–	**3.39**
	Coniosporium apollinis	**−3.31**	**−3.89**	**−4.35**	**−3.41**	−2.57	–	**2.16**	2.57
	Curvularia americana	−2.56	−3.10	–	−2.62	–	2.71	–	2.78
	Exophiala xenobiotica	**−2.61**	–	**−2.38**	**−2.31**	–	**2.12**	–	–
	Filobasidium oeirense	–	–	**−2.91**	–	–	–	–	**2.90**
	Gibberella intricans	**−3.27**	**−2.78**	**−2.95**	**−2.19**	–	–	–	**2.14**
	Rhodotorula mucilaginosa	**−3.06**	–	**−2.79**	–	–	**3.69**	–	**2.28**
	Rhodotorula taiwanensis	–	–	–	–	**−2.17**	2.06	–	**2.75**
	Toxicocladosporium irritans	**−3.63**	**−5.83**	−3.26	**−5.29**	**−3.32**	−3.42	**−3.13**	**4.11**
Higher with presence of health outcomes	*Aspergillus sydowii*	–	–	–	–	–	**2.83**	–	**3.15**
	Candida tropicalis	–	–	–	–	–	**2.92**	–	**2.00**
	Curvibasidium cygneicollum	–	–	–	**2.23**	–	–	–	–
	Diutina catenulata	–	–	**2.39**	–	–	–	–	–
	Fusarium acutatum	–	–	–	–	–	**2.20**	–	–
	Naganishia albida	–	–	–	–	–	**2.14**	–	–

- indicates species was not found significant with DESeq and Log 2-Fold Change values > 2 or < -2.
All values have adjusted p-values <0.05.
Bolded values have significant DESeq findings and are in a Weighted Quantile Sum (WQS) regression model with a weight >1%.
WQS regression model weights can be seen in Table S7.

Table 3
Bacterial species that were significantly associated in DESeq with the health outcomes of asthma, wheeze, aeroallergen positivity (+) and rhinitis at ages 7 and 12.

	Indicator Species	Asthma		Wheeze		Aeroallergen+		Rhinitis	
		Age 7	Age 12	Age 7	Age 12	Age 7	Age 12	Age 7	Age 12
		(Log 2-Fold Change)							
Lower with presence of health outcomes	*Actinomyces graevenitzii*	–	–	−2.39	−2.20	–	–	–	–
	Akkermansia muciniphila	–	**−2.49**	–	**−3.38**	–	–	–	–
	Anaerococcus vaginalis	–	–	**−2.60**	–	–	–	–	–
	Cardiobacterium hominis	**−2.35**	–	**−2.92**	–	–	–	–	–
	Coprococcus_2 eutactus	**−4.56**	**−4.10**	**−4.13**	**−3.33**	**−3.22**	–	**−2.62**	–
	Dialister invisus	–	–	**−2.04**	–	–	–	–	–
	Kineosporia rhamnosa	–	–	–	**−2.57**	–	–	–	–
	Lachnoanaerobaculum cf	–	–	**−2.66**	–	–	–	–	–
	Lactobacillus delbrueckii	**−3.03**	–	–	–	–	–	–	–
	Methylobacterium komagatae	–	–	–	**−2.30**	–	–	–	–
	Prevotella nanceiensis	–	–	**−2.45**	–	–	–	–	–
	Sphingomonas daechungensis	–	–	**−2.05**	–	–	–	–	–
	Sphingomonas parvus	**−3.68**	–	–	–	–	–	–	–
	Stenotrophomonas maltophilia	–	–	**−2.15**	–	–	–	–	–
Lower & Higher	*Lawsonella clevelandensis*	**2.28**	–	–	**−2.49**	–	–	–	–
Higher with presence of health outcomes	*Alkanindiges illinoisensis*	–	**2.24**	–	–	–	–	–	–
	Capnocytophaga sputigena	–	–	–	**2.21**	–	–	–	–
	Corynebacterium_1 jeikeium	–	**3.88**	–	–	–	**2.47**	–	–
	Corynebacterium_1 massiliense	–	–	–	–	–	–	**2.49**	–
	Gardnerella vaginalis	–	–	–	–	**3.02**	–	–	–
	Lactobacillus iners	–	–	–	–	2.38	–	–	–
	Massilia brevitalea	–	–	–	–	–	–	**2.19**	–
	Roseburia intestinalis	–	–	–	–	–	**2.47**	–	–
	Staphylococcus aureus	**2.45**	–	–	–	–	2.38	**2.27**	–
	Turicibacter sanguinis	–	–	–	–	–	–	–	–

- indicates species was not found significant with DESeq and Log 2-Fold Change values > 2 or < -2.
All values have adjusted p-values <0.05.
Bolded values have significant DESeq findings and are in a Weighted Quantile Sum (WQS) regression model with a weight >1%.
WQS regression model weights can be seen in Table S8.

15 bacterial species that were lower with the presence of one or more health outcomes, and 10 bacterial species that were higher with the presence of one or more health outcomes. With the presence of age 7 asthma, four species were lower and two species were higher, and for age 12, two were lower and two were higher. *Coprococcus eutactus* was lower with both ages 7 and 12 presence of asthma. For age 7 presence of wheeze, nine species were lower and for age 12, six were lower and one was higher. *Actinomyces graevenitzii* and *Coprococcus eutactus* were lower with both ages 7 and 12 presence of wheeze. For age 7 presence of aeroallergen+, three species were higher and for age 12, two species were higher and one was lower. For age 7 presence of rhinitis, no species were found to be higher or lower, but for age 12, three species were higher and one was lower.

In summary, the bacterial species that were lower with the presence of any health outcome included: *Actinomyces graevenitzii, Akkermansia muciniphila, Anaerococcus vaginalis, Cardiobacterium hominis, Coprococcus eutactus, Dialister invisus, Kineosporia rhamnosa*, *Lachnoanaerobaculum cf., Lactobacillus delbrueckii, Methylobacterium komagatae, Prevotella nanceiensis, Sphingomonas daechungensis, Sphingomonas parvus* and *Stenotrophomonas maltophilia*. The species that were higher with the presence of any health outcomes included: *Alkanindiges illinoisensis, Capnocytophaga sputigena, Corynebacterium jeikeium, Corynebacterium massiliense, Gardnerella vaginalis, Lactobacillus iners, Massilia brevitalea, Roseburia intestinalis* and *Staphylococcus aureus*. One bacterial species was lower or higher, depending on the health outcome: *Lawsonella clevelandensis*. Indicator taxa were compared with previously explored mold and moisture damage indicator taxa (Table S6) and are further discussed in the supplement.

3.5. WQS regression

While the DESeq selected potential indicator species, the WQS analyses utilized a data-driven approach allowing logistic regression to be performed with each of the health outcomes. Fifty-nine fungal and 80 bacterial species were identified in at least 20% of the samples and were subject to WQS analysis. Four regression models (fungi associated with the absence of the health outcome, bacteria associated with the absence of the health outcome, fungi associated with the presence of the health outcome, and bacteria associated with the presence of the health outcome) were developed with specific species weights for each model. Species weights which were determined to be significantly above τ threshold are provided in Tables S7 and S8 (fungi and bacteria, respectively). The generation of τ was the inverse of the number of species in the analysis, which for fungi was 1.67% (1/59) and for bacteria 1.25% (1/80). To simplify this information, the species with WQS weights of at least 10% in one health outcome for each model (fungi or bacteria associated with the absence or presence of the health outcomes) can be seen in Figs. 3 and 4.

3.5.1. Comparison DESeq and WQS weights

The bolded values in Tables 2 and 3 (fungal and bacterial, respectively) show the species that were found to be significantly associated with health outcomes utilizing DESeq and had WQS weights greater than 1%. The specific weights for each of the species are listed in Tables S7 and S8 (fungal and bacterial, respectively). When comparing the species of the DESeq findings with the WQS weights, similar trends can be seen. Some of the strongest weights across all models included fungi *Devriesia strelitziicola* and *Diutina catenulata*, and bacteria *Dialister invisus* and *Gardnerella vaginalis*.

3.5.2. WQS regression logistic models

The odds ratios from the unadjusted univariate regression models utilizing the weights of the four groups of species (fungi associated with the absence of the health outcomes, bacteria associated with the absence of the health outcomes, fungi associated with the presence of the health outcomes, and bacteria associated with the presence of the health

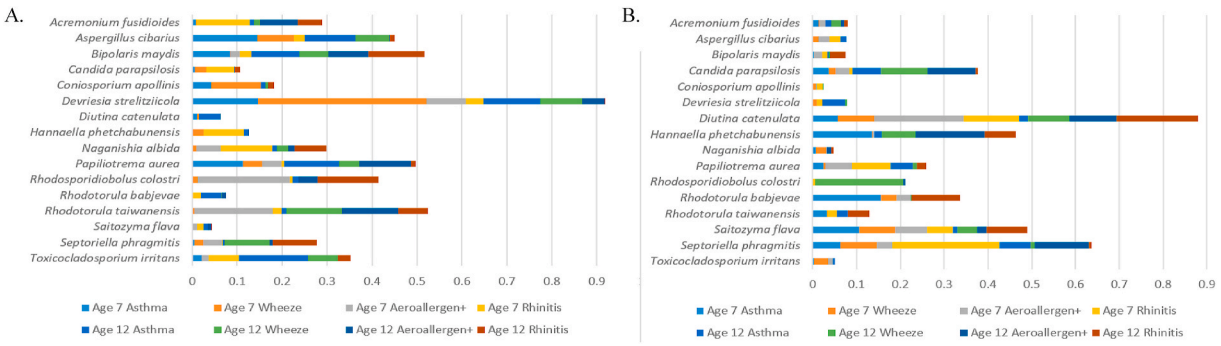

Fig. 3. Fungal species with weighted quantile sum weights of at least 10% in one health outcome in each model (A. fungi associated with the absence of health outcomes and B. fungi associated with the presence of health outcomes).

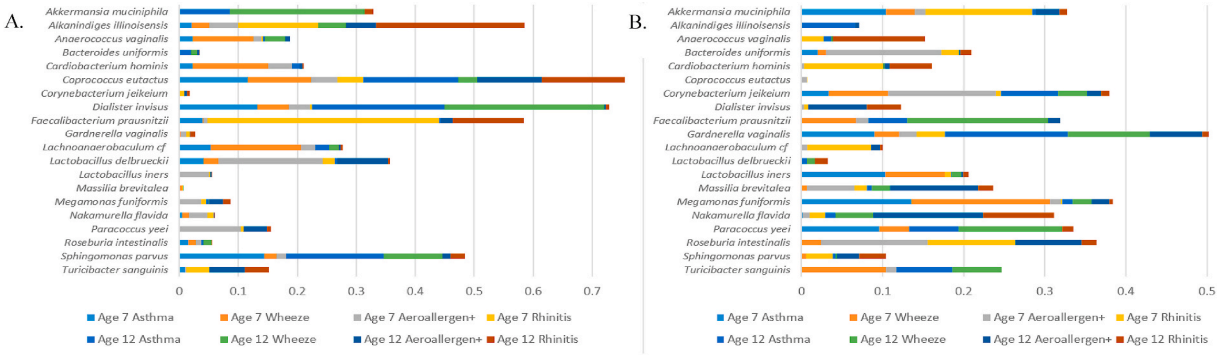

Fig. 4. Bacterial species with weighted quantile sum weights of at least 10% in one health outcome in each model (A. bacteria associated with the absence of health outcomes and B. bacteria associated with the presence of health outcomes).

outcomes) can be seen in Table 4. WQS regression models were adjusted for race, pets, neighborhood socioeconomic status, cockroaches, dust mites, and rodents (Table 5). Adjusted models for fungi associated with the absence of the health outcomes demonstrated significantly lower odds for asthma (age 7 and 12), wheeze and rhinitis (age 7) and aeroallergen+ (age 12), when the dominant species within this model are present. The odds ratios [ORs] of the models for fungi associated with the absence of the health outcomes ranged from 0.69 to 0.88, and p-values ranged from <0.01 to <0.05. Models for bacteria associated with the absence of the health outcomes also showed significantly lower odds for asthma and wheeze (age 7), aeroallergen+ (age 12) and rhinitis (age 7). Models for fungi associated with the presence of health outcomes demonstrated higher odds for all health outcomes (age 7 and 12: asthma, wheeze, aeroallergen+ and rhinitis) when the dominant species within these models were present (OR range: 1.15 to 1.49, p-value range: <0.001 to <0.01). Models for bacteria associated with the presence of health outcomes demonstrated significantly higher odds with aeroallergen+ (age 7 and 12), asthma and wheeze (age 12), and rhinitis (age 7) with ORs ranging from 1.12 to 1.18.

4. Discussion

The regression models demonstrate that a combination of species might influence health in a potentially positive or negative manner.

Table 4
Associations between the unadjusted weights from the weighted quantile sum (WQS) analysis for the four groups of species (fungal and bacterial species associated with the absence or presence of health outcomes) for asthma, wheeze, aeroallergen positivity (+) and rhinitis at ages 7 and 12.

		WQS regression model odds ratios															
		Fungi associated with absence of health outcomes				Bacteria associated with absence of health outcomes				Fungi associated with presence of health outcomes				Bacteria associated with presence of health outcomes			
Age	Outcome	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value
Age 7	Asthma	**0.82**	**0.71**	**0.93**	**<0.01**	**0.76**	**0.63**	**0.90**	**<0.05**	**1.20**	**1.07**	**1.34**	**<0.01**	1.06	0.98	1.13	0.13
	Wheeze	**0.68**	**0.55**	**0.85**	**<0.01**	**0.83**	**0.73**	**0.95**	**<0.05**	**1.27**	**1.13**	**1.43**	**<0.001**	1.09	1.01	1.19	0.06
	Aeroallergen+	0.91	0.83	1.01	0.09	0.93	0.87	1.00	0.06	**1.22**	**1.11**	**1.35**	**<0.001**	**1.11**	**1.04**	**1.18**	**<0.01**
	Rhinitis	**0.87**	**0.78**	**0.96**	**<0.05**	**0.87**	**0.80**	**0.96**	**<0.05**	**1.13**	**1.03**	**1.24**	**<0.05**	**1.09**	1.00	1.19	0.06
Age 12	Asthma	**0.84**	**0.75**	**0.94**	**<0.01**	0.89	0.80	0.99	0.06	**1.24**	**1.09**	**1.41**	**<0.01**	**1.16**	**1.05**	**1.27**	**<0.01**
	Wheeze	**0.81**	**0.68**	**0.97**	**<0.05**	0.83	0.68	1.01	0.07	**1.27**	**1.09**	**1.47**	**<0.01**	**1.16**	**1.05**	**1.28**	**<0.01**
	Aeroallergen+	0.92	0.83	1.02	0.12	0.93	0.85	1.01	0.09	**1.32**	**1.13**	**1.54**	**<0.01**	**1.15**	**1.04**	**1.27**	**<0.05**
	Rhinitis	0.95	0.87	1.05	0.33	**0.86**	**0.76**	**0.96**	**<0.05**	**1.18**	**1.05**	**1.33**	**<0.01**	**1.09**	1.00	1.19	0.06

OR: Odds Ratio; LCI: Lower Confidence Interval; UCI: Upper Confidence Interval; Adjusted p-values.
OR estimates represent the odds ratios of having the health outcome when the WQS index was increased by one decile.

Table 5
Associations between the adjusted[1] weights from the weighted quantile sum (WQS) analysis for the four groups of species (fungal and bacterial species associated with the absence or presence of health outcomes) for asthma, wheeze, aeroallergen positivity (+) and rhinitis at ages 7 and 12.

		WQS regression model odds ratios															
		Fungi associated with absence of health outcomes				Bacteria associated with absence of health outcomes				Fungi associated with presence of health outcomes				Bacteria associated with presence of health outcomes			
Age	Outcome	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value	OR	LCI	UCI	p-value
Age 7	Asthma	**0.83**	**0.72**	**0.95**	**<0.05**	**0.76**	**0.64**	**0.91**	**<0.05**	**1.22**	**1.06**	**1.40**	**<0.01**	1.06	0.97	1.15	0.20
	Wheeze	**0.69**	**0.56**	**0.85**	**<0.01**	**0.84**	**0.73**	**0.96**	**<0.05**	**1.31**	**1.15**	**1.49**	**<0.001**	1.10	1.00	1.21	0.08
	Aeroallergen+	0.91	0.82	1.01	0.08	0.93	0.87	1.00	0.08	**1.25**	**1.13**	**1.39**	**<0.001**	**1.13**	**1.05**	**1.22**	**<0.05**
	Rhinitis	**0.87**	**0.78**	**0.97**	**<0.05**	**0.88**	**0.81**	**0.97**	**<0.05**	**1.15**	**1.05**	**1.26**	**<0.01**	**1.12**	**1.02**	**1.22**	**<0.05**
Age 12	Asthma	**0.84**	**0.74**	**0.94**	**<0.05**	0.91	0.81	1.01	0.09	**1.31**	**1.12**	**1.53**	**<0.001**	**1.16**	**1.04**	**1.30**	**<0.05**
	Wheeze	0.83	0.70	0.99	0.06	0.84	0.69	1.03	0.10	**1.34**	**1.13**	**1.58**	**<0.01**	**1.17**	**1.05**	**1.31**	**<0.05**
	Aeroallergen+	**0.88**	**0.78**	**0.99**	**<0.05**	0.91	0.83	1.00	0.06	**1.49**	**1.23**	**1.81**	**<0.001**	**1.18**	**1.04**	**1.34**	**<0.05**
	Rhinitis	0.94	0.84	1.05	0.26	**0.86**	**0.76**	**0.96**	**<0.05**	**1.23**	**1.06**	**1.43**	**<0.01**	1.09	0.98	1.20	0.12

[1]adjusted for pets, cockroaches, rodents, race, dust mites, and deprivation index.
OR: Odds Ratio; LCI: Lower Confidence Interval; UCI: Upper Confidence Interval; Adjusted p-values.
OR estimates represent the odds ratios of having the health outcome when the WQS index was increased by one decile.

Several heavily weighted species had a strong influence on the models, and therefore, created a microbial community that was positively or negatively associated with asthma, wheeze, rhinitis, and aeroallergen+. Previous indoor microbial techniques had limited the scope to individual species (Cox et al., 2017; Reponen et al., 2012; Vesper et al., 2006). Furthermore, early high-throughput sequencing studies tended to utilize abundance or diversity measures (Dannemiller et al., 2014b; Kettleson et al., 2015; Sharpe et al., 2015), but perhaps did not consider weighting groups of bacterial or fungal species to yield positively or negatively associated effects. With our statistical modeling, this study demonstrates that the community of the environmental microbiota can be positively or negatively associated with health. Models for fungi associated the absence and presence of health outcomes had similarly lower and higher odds, respectively, for asthma and wheeze with both ages 7 and 12. Likewise, models for fungi associated with the presence of health outcomes had similarly higher odds for rhinitis and aeroallergen+ for ages 7 and 12. This indicates that combinations of various fungal species can be associated with the presence of health outcomes such as asthma, wheeze, aeroallergen+ and rhinitis, and alternatively, other combinations of fungal species can be associated with the absence of asthma and wheeze. This was supported by the indicator species (DESeq) findings, showing several specific fungal species were lower with the presence of asthma and wheeze. Bacterial models were less consistent between health outcomes and ages. However, models for bacteria associated with the presence of aeroallergen+ showed higher odds for both ages, and models for bacteria associated with the absence of rhinitis were consistent and showed lower odds at ages 7 and 12.

In contrast to Karvonen et al. (2019), we found that Actinobacteria were more abundant in the homes of asthmatic compared to non-asthmatic children. However, our data on indicator taxa within the phylum Actinobacteria showed 2 species that were lower (*Actinomyces graevenitzii, Kineosporia rhamnosaand*) with the presence of wheeze. In a study by Fu et al. (2020b), two bacterial genera within the class Alphaproteobacteria were found to be protectively associated with asthma severity status in junior high school students. We also found three indicator species (*Methylobacterium komagatae, Sphingomonas daechungensis*, and *Sphingomonas parvus*) within the class Alphaproteobacteria that were lower with the presence of wheeze or asthma; however, they were not in the same genera as in Fu et al. (2020b). Similarly, Fu et al. (2020b) found one positively and one protectively associated genus associated with asthma severity status within the class Gammaproteobacteria. We also found indicator species *(Cardiobacterium hominis, Stenotrophomonas maltophilia*, and *Alkanindiges illinoisensis*) within the class Gammaproteobacteria were higher or lower, depending on the species, when associated with asthma and/or wheeze; however, none were in the same genera as in Fu et al. (2020b). While our study did not have any overlap with positively or adversely associated genera found in Fu et al. (2020b), potential differences could be due to Fu et al.'s limited number of dust samples (n = 21) or geographical dissimilarities between Malaysia and Ohio. When Fu et al. (2021) evaluated an association between bacterial abundance at the genus level in floor dust and asthma symptoms among students in university dormitories, genera that were positively associated with asthma included *Ruminococcus, Blautia, Clostridium* and *Subdoligranulum* in the class of Clostridia, *Geobacillus* in Bacilli, *Megomonas* in Negativicutes, and *Collinsella* in Coriobacteriia. Similarly, in our study, species within the WQS regression models that were associated included *Megamonas funiformis* and *Collinsella aerofaciens* for asthma and wheeze. Our study concurred with Andersson et al. (1999), who found the most common bacterial genera to be *Corynebacterium* and *Staphylococcus*. Similar to Karvonen et al. (2019), our phylum-level data showed that the relative abundance of Firmicutes was greater in the homes of asthmatic children than in the homes of non-asthmatic children. In a related study by Zhang et al. (2016), Firmicutes were also increased in the lower airway microbiota in severe asthmatics compared to controls.

Previous studies have evaluated the relative abundance of bacteria and fungi in various indoor settings (Afshinnekoo et al., 2015; Gangneux et al., 2020; Park et al., 2020). Our study concurred with Pitkaranta et al. (2008), who found that the most dominant species of the fungal flora in indoor dust were members of the class Dothideomycetes. Among fungal species, *Epicoccum nigrum* was the most abundant, which has been a common finding within this region (Cox et al., 2017, 2021). Vesper et al. (2006) found *E. nigrum* was more abundant in homes of non-asthmatic occupants, which agrees with our study for homes of age 12 non-asthmatic children but not for homes of age 7 non-asthmatic children. However, *E. nigrum* was not detected as significantly different when utilizing DESeq between the absence and presence of health outcomes. Dannemiller et al. (2014b) demonstrated that a decreased diversity within the genus *Cryptococcus* was significantly associated with increased asthma risk. While our study did not evaluate the diversity of *Cryptococcus*, we did find *Cryptococcus saitoi* as having significant weighted values with age 12 asthma and wheeze utilizing WQS regression model weights. Similar to Rush et al. (2021), we found that *V. victoriae* (syn. *Cryptococcus victoriae*) concentrations were lower in homes of children with asthma than of those without asthma.

As shown above, many studies have focused on family- or genus-level findings, whereas our study focused on species-level findings. Our results indicate that limited generalizable statements can be made for an entire genus or family in relation to an organism's likelihood to be positively or negatively associated with health outcomes such as asthma,

wheeze, aeroallergen+ or rhinitis. Through analyses at the species level, we have demonstrated a lack of consistent patterns of the association at higher taxonomic levels. It is a mixture of individual bacterial and fungal species that seem to be negatively or positively associated with various health outcomes. Species mixtures that yield positively associated or negatively associated effects are likely dependent on the locality of the dominant species in the area.

The built environment has its own microbial ecology dominated by the occupants and the outdoor environment; therefore, different homes may lack a shared microbiome (Adams et al., 2013; Fahimipour et al., 2018; Maamar et al., 2020). Kirjavainen et al. (2019) found asthma risk decreased as the similarity of home bacterial microbiota composition to that of farm homes increased. The protective effect, however, was independent of richness and total bacterial load. When p-values were adjusted for multiple testing, our study was unable to detect any significant differences in alpha or beta diversity between the absence and presence of the evaluated health outcomes. Similarly, Fu et al. (2020a, 2021) found overall bacterial richness, defined as the number of OTUs in each sample, was not correlated with respiratory tract infections nor, in a separate study, with asthma. A study by Hyytiäinen et al. (2021) suggested that a higher bacterial diversity may reduce the risk of allergic rhinitis later in childhood; however, their findings were not consistent. Furthermore, Dannemiller et al. (2014b) found no clear, consistent differences in fungal beta diversity between asthma and control homes. In a study by Adams et al. (2021), there were several positive associations between particular microbial indicators (diversity, richness, individual taxa) and a respiratory symptom score in Finland, while in the Netherlands, the associations tended to be mostly inverse and statistically non-significant.

In a previous analysis, hydrophilic and mesophilic fungi were shown to have significant dose-related increases with increasing observed moisture damage and mold damage, respectively (Cox et al., 2021). In this analysis, however, we were unable to detect any significant differences in specific fungal groups (xero-, meso- and hydrophilic) with health outcomes.

When to monitor for exposure and what to measure as health outcomes are variable for the aforementioned studies. The primary focus in this study was to evaluate late-childhood indoor microbial exposure with cross-sectional as well as prospective respiratory health outcomes. There is a need to evaluate a variety of exposure times and health assessments in order to provide a fully formed picture of potential childhood exposures and disease. By examining late-childhood exposure and the relationship of individual species and mixtures of species with health assessments, this study has provided additional data regarding the indoor microbiota and its potential influences on childhood health.

4.1. Limitations

Comparisons across studies of the overall microbial composition observed are limited because the selection of primers can vary from study to study. In this study, the ITS fungal rDNA region was selected over the 18s rDNA region because it has been found to be more precise in fungal community analysis (Liu et al., 2015; Schoch et al., 2012). The ITS1 region was selected based on Mello et al. (2011), who determined that using the pair of primers ITS1F/ITS2, for the amplification of ITS1, was more selective, produced fewer non-fungal sequences, and produced a higher number of fungal sequences, than the primer pair ITS3/ITS4 for the amplification of the ITS2 region. However, other studies have illustrated strengths and weaknesses of utilizing other regions, including ITS2 and the full ITS regions (Lindahl et al., 2013; Tedersoo et al., 2015). Another potential limitation in this study may be the lack of generalizability, as a child's eligibility required at least one atopic parent (i.e., had both allergic symptoms and a positive reaction on a skin prick test to at least 1 of 15 common aeroallergens). However, in a study by Arbes et al. (2005), 54.3% of the US population had positive test responses to 1 or more allergens; therefore, we believe it is generalizable to the US population. There could be potential limitations when evaluating absolute abundance values as SE/mg, as the volume of dust in the home and proportion of biomass in dust could be variable between homes. The selection of using SE/mg for absolute abundance was based on previous work (Adams et al., 2021; Cox, 2021; Dannemiller et al., 2016) and does not account for all potential exposure factors, including the volume of dust in the home, the duration of time the child spent in the room, how active that child is, and behaviors that occur in the room. Another potential limitation is the observational nature of a cross-sectional and prospective study. This approach can lead to associations that are challenging to interpret and cannot demonstrate causality.

5. Conclusions

The indoor microbiota is a complex mixture of bacterial and fungal species that can vary greatly from space to space. It is possible that health outcomes could be more influenced by the composition of these various mixtures rather than the absence or presence of individual species. Our investigation considered both the individual indicator species as well as mixtures of fungal or bacterial species. Indicator fungal species were associated with the absence of asthma and wheeze and other fungal indicator species were associated with having aeroallergen+ and rhinitis, whereas bacterial data was less consistent. The utilization of WQS allowed mixtures of species to be evaluated and gave weights to each species that could vary in each model. Our models have shown different mixtures of fungal species and mixtures of bacterial species can have negative and positive associations with childhood asthma, wheeze, rhinitis, and aeroallergen+. This study has demonstrated that the WQS has been a useful tool in evaluating bacterial or fungal mixtures in relation to potential health effects. Future work evaluating the microbiome should consider mixtures, such as by using the WQS approach, as well as potential indicator species in relation to health outcomes.

Credit author statement

Jennie Cox: Conceptualization; Data curation; Formal analysis; Investigation; Funding acquisition; Methodology; Project administration; Visualization; Roles/Writing - original draft; Writing - review &editing. Tim Stone: Formal analysis; Methodology; Software; Validation; Writing - review & editing. Roman Jandarov: Methodology; Validation; Writing - review & editing. Jeff Burkle: Data curation; Formal analysis; Investigation; Resources; Software; Validation. Patrick Ryan: Conceptualization; Data curation; Investigation; Methodology; Resources; Writing - review & editing. Christine Niemeier-Walsh: Data curation; Formal analysis; Investigation; Methodology; Resources; Roles/Writing - original draft. Mark Mendell: Conceptualization; Methodology; Writing - review & editing. Tiina Reponen:Conceptualization; Data curation; Funding acquisition; Methodology; Project administration; Resources; Software; Supervision; Validation; Writing - review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

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