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Fragmented microbial networks and Moraxella-driven airway clustering characterize preschool wheezers across the gut – airways axis

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41

42 **Take home message**

43 Preschool wheezers showed fragmented gut–airway microbial networks and *Moraxella*-
44 associated airway community stratification despite limited differences in overall diversity.

45

Accepted manuscript

Abstract

Early-life wheezing in children has been associated with microbial alterations along the gut-airway axis, yet studies simultaneously investigating bacterial communities in both compartments remain scarce. The aim of this cross-sectional exploratory pilot study (n=25) was to characterize and compare nasal and stool bacterial communities in preschool children aged 1–4 years with recurrent wheezing and healthy controls using 16S rRNA gene metabarcoding.

Across participants, nasal and stool bacteriomes were highly individualized and taxonomically diverse. Overall richness, evenness, and community composition did not differ significantly between healthy children and wheezers in either compartment. However, wheezers displayed markedly higher within-group variability, particularly in nasal communities. Stratification based on microbiome similarity to healthy samples revealed increased *Moraxella* and reduced commensal genera including *Prevotella* spp. and *Veillonella*, along with lower richness and evenness (all $p < 0.001$) in nasal samples with divergent bacterial communities. Stool alterations were more subtle but included trends toward reduced *Bacteroides*, *Faecalibacterium*, and *Alistipes* in wheezers more divergent from healthy controls.

Community assembly in both compartments was largely governed by stochastic processes but accompanied by less complex and more fragmented bacterial interaction networks in wheezing children. Cross-compartment correlations were also altered, most prominently involving stool *Lactococcus* showing stronger and more numerous correlations with nasal taxa in wheezers than in healthy controls. Divergent wheezers exhibited distinct modular network structure and cross-compartment profiles, consistent with a differentiated microbial organization.

Together, these findings suggest compartment-specific differences in microbial interaction patterns across the gut–airway axis in early-life wheezing, despite limited differences in overall community diversity.

Introduction

Human microbiota play a central role in regulating local and systemic immune function. A prominent example of such systemic crosstalk is the axis between the gut and the respiratory system, describing bidirectional communication between intestinal and airway microbiomes mediated by microbial metabolites and immune modulation [1-3]. Increasing evidence indicates that this axis is associated with asthma pathogenesis, particularly during early life when immune development is highly sensitive to environmental influences [4, 5].

The gut microbiome begins to establish at birth and undergoes rapid succession during infancy. In human studies, stool samples commonly serve as a non-invasive proxy for intestinal microbial communities. Early colonization is influenced by mode of delivery, diet, and environmental exposures [6]. During early infancy, *Bifidobacterium* species dominate, particularly in breastfed infants [7, 8]. With the introduction of complementary feeding, microbial diversity increases with greater representation of *Bacteroides* and Firmicutes including *Blautia*, *Faecalibacterium*, and *Roseburia* [7]. By approximately three years of age, the microbiome stabilizes toward an adult-like configuration dominated by *Bacteroides*, *Prevotella*, and various Firmicutes [8, 9].

The airway microbiome develops along a similarly dynamic trajectory. Given the continuity of the respiratory tract, nasal microbiota may provide insight into early upper-airway colonization that contributes to microbial immigration into the lower airways and may influence respiratory immune development [4, 10-12]. In early infancy, nasal communities are typically dominated by *Staphylococcus* and *Corynebacterium* [13], while *Alloiococcus* and *Moraxella* increase during the first year of life [12]. Early colonization with *Streptococcus* and *Haemophilus* has been associated with viral respiratory tract infections, although these genera may also appear during normal microbial succession [12]. With increasing age, airway communities diversify further, with taxa such as *Dolosigranulum* and *Corynebacterium* becoming more prominent by two to three years of age [14]. As in the gut, early microbial composition and diversity in the airways have been linked to immune development and allergic sensitization [15].

Consistent with these observations, early microbial dysbiosis in either the intestinal or airway microbiome has been associated with wheezing illnesses and subsequent asthma development [12, 16-20]. While these findings support the concept of an axis of the gut and the respiratory system, most studies have examined the gut or respiratory microbiome separately, and investigations assessing microbial communities of both organs in parallel remain rare.

To address this gap, we characterized microbial communities of the gut and upper airways in children aged 1–4 years with recurrent wheezing (n=17) and compared them with healthy controls (n=8) in a cross-sectional exploratory pilot study. Stool samples and nasal swabs served as proxies for intestinal and upper airway microbiomes, respectively. Bacterial community composition and diversity were assessed using a metabarcoding approach, enabling parallel profiling of microbial communities in both compartments and facilitating the identification of microbial signatures associated with early-life wheezing and asthma risk.

Materials and Methods

Study design

The ALLIANCE cohort of the German Center for Lung Research (DZL) is a prospective multicenter asthma cohort recruiting in five pediatric specialist centers (Hannover, Luebeck, Munich, Marburg and Cologne) and two adult specialist centers (LungenClinic Grosshansdorf and Research Centre Borstel), registered at ClinicalTrials.gov (pediatric arm NCT02496468, adult arm NCT02419274) and ethically approved across all study centers. Parents of study participants aged <18 years and study participants aged ≥ 18 years gave written informed consent.

Eligibility for this exploratory pilot study required age 1–4 years, availability of paired nasal swab and stool samples, and wheeze status. The wheezer group comprised 19 children from a total of 221 wheezers with ≥ 2 wheezing episodes in the preceding 12 months based on parental reports. Two wheezers were excluded due to absence of bacterial sequencing reads from nasal swabs; corresponding stool samples were also excluded to maintain paired

analyses, resulting in 17 wheezers. Eight age- and sex-matched healthy controls (HC) were recruited from 54 controls if never diagnosed with asthma or preschool wheeze, but irrespective of other allergic diseases. Patient characteristics are summarized in Table 1. Details regarding study design, definitions of parameters, inclusion criteria, and methods are specified in Supplementary Material and published previously [21].

Microbiome analysis

Details on microbiome sample processing and subsequent data analysis are provided in Supplementary Material. Briefly, DNA extraction was performed via ultrasonication (nose) and a phenol-chloroform-based protocol (stool), alongside with blank samples to identify potential contaminants. Amplicon sequencing of the V4 hypervariable region of the 16S rRNA gene was performed on a MiSeq Illumina instrument using the universal eubacterial primers 515F/806R [22, 23]. Sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1499410 (reviewer link: <https://dataview.ncbi.nlm.nih.gov/object/PRJNA1499410?reviewer=okpdiae11ucclqmiq09i436qvh>).

Sequence processing was performed using DADA2 v 1.30 [24], followed by taxonomic assignment via SILVA v138.1. Reads classified as mitochondrial or chloroplast in origin, lacking phylum-level annotation, or detected in blank samples were excluded, resulting in a total of 3,744,773 reads assigned to 2,177 amplicon sequence variants (ASV).

All plots and statistics were performed in R version 4.5.1 (<https://www.R-project.org>). Subsequent bioinformatic analyses were performed to characterize microbial communities in HC and wheezers, including assessments of alpha and beta diversity, core microbiome structure, within-group variability, and microbial biomarkers (ANCOM-BC2). Additional analyses explored associations between microbial taxa, individual variability and host factors, cross-compartment correlations, microbial community assembly processes (β NTI and RCbray framework), and bacterial co-occurrence networks (NetCoMi). Furthermore, wheezers were

stratified into healthy-like and divergent microbiome profiles and their associated features were examined. Detailed protocols are provided in Supplementary Material.

Results

Cohort Characteristics

The study comprised 25 participants at preschool age (17 children with recurrent wheeze and 8 healthy controls (HC)), with baseline data summarized in Table 1. Demographic characteristics (age, gender), early-life and environmental exposures (e.g. mode of delivery, breastfeeding, siblings, daycare, pet contact) and most clinical history variables (allergic and respiratory conditions, prior RSV infection) did not differ between HC and wheezers, while use of antibiotics or steroids (systemic and inhaled) was only prevalent in wheezers. Wheezers exhibited higher thrombocyte and blood basophil counts, while no differences were observed for other hematological or immunological parameters.

Nasal and stool bacteriomes show similar bacterial diversity between groups but higher compositional variability in wheezers

Across participants, nasal and stool bacteriomes were taxonomically diverse but highly individualized. The nasal bacteriome comprised 936 ASV and stool samples 1033 ASV, with overlap but more wheezer-specific variants (Fig. 1a). Group-specific core ASV decreased rapidly with increasing prevalence thresholds in both compartments, indicating limited overlap between individuals (Fig. 1a; Supplementary Table S1). Nevertheless, alpha diversity metrics indicated comparable richness and evenness between HC and wheezers in both compartments (Supplementary Fig. S1a), and NMDS analysis of weighted UniFrac distances showed no group separation (Supplementary Fig. S1b).

Nasal bacterial communities were dominated by *Haemophilus*, *Moraxella*, *Streptococcus*, *Veillonella*, and *Prevotella*-related taxa (Fig. 1b). *Moraxella* tended to be more abundant in wheezers ($p>0.05$) and occasionally dominated individual profiles (Supplementary Fig. S2). Among 11 *Moraxella* ASV, one variant closely related to *Moraxella nonliquefaciens*

predominated across samples (Supplementary Fig. S3) but was more consistently present in HC despite lower relative abundance (Supplementary Table S1). Beta-dispersion indicated greater within-group variability in the nasal bacteriome of wheezers compared with HC ($p=0.054$; Fig. 1c) and subgroup-specific associations with nasal genera. In wheezers, *Neisseria*, *Prevotella* spp., and *Veillonella* were negatively correlated, while *Moraxella* showed a non-significant positive association (Fig. 1d). Comparable non-significant trends were seen in controls.

Stool communities showed higher richness ($p<0.001$) and evenness ($p=0.004$) than nasal samples (Fig. S1) and were dominated by *Bacteroides* and *Faecalibacterium* (>43% of reads; Fig. 1b). Differences between groups were modest, although several commensals like *Roseburia* tended to be reduced in wheezers. Stool within-group dispersion was lower compared with nasal samples ($p<0.001$) but similarly tended to be higher in wheezers (Fig. 1c). This variability was associated with higher *Romboutsia* and *Prevotella_7* and lower *Bacteroides*, *Faecalibacterium*, and *Roseburia*, although not significant after correction for multiple comparisons (Fig. 1d).

Despite substantial inter-individual variability, several host-microbiome associations were observed. In the nose, leukocyte levels correlated negatively with *Corynebacterium*, *Dolosigranulum* and *Moraxella* (Supplementary Fig. S4a). In stool, immune parameters were not associated with bacterial diversity. However, age correlated negatively with *Bifidobacterium*, while *Ruminococcus* and *Agathobacter* were associated with gender. Beta-dispersion, used as a measure of individual variability, was not related with measured host parameters (Supplementary Fig. S4b).

Microbial community divergence identifies distinct wheezer subgroups with divergent nasal communities enriched in Moraxella

To explore heterogeneity among wheezers, samples were stratified into healthy-like and community-divergent based on the Euclidean distance of each sample's bacterial community to the HC centroid in ordination space (90% quantile threshold). This classification reflects

similarity in community structure and was not associated with differences in demographic or clinical parameters (Supplementary Table S2).

In the nasal bacteriome, 9 of 17 wheezers showed a healthy-like bacterial profile, whereas 8 were classified as divergent. Divergent wheezers displayed higher individual variability but lower alpha diversity measures (observed ASV, Shannon index, evenness) (Fig. 2a, Supplementary Table S3), increased *Moraxella* abundance, and reduced abundance of commensals such as *Alloprevotella*, *Prevotella_7*, and *Veillonella* at both genus (Fig. 2b) and ASV level (Fig. 2c).

In stool bacterial communities, 7 wheezers were classified as divergent based on distance from the healthy centroid. Differences between subgroups were less pronounced than in the nose, but divergent individuals tended to show reduced *Bacteroides*, *Faecalibacterium*, and *Alistipes* (Fig. 2b). As in nasal communities, baseline parameters did not differ between stool-based subgroups (Supplementary Table S2).

Partial overlap between compartments was observed: four of the eight nasal-divergent wheezers were also classified as divergent in stool, whereas the remaining individuals showed healthy-like stool profiles. Similarly, six of nine healthy-like wheezers displayed concordant bacterial signatures in both compartments.

Stochastic assembly dominates nasal and stool bacteriomes, while wheezing is associated with fragmented bacterial interaction networks

To investigate processes shaping nasal and stool bacterial communities, we quantified the relative influence of stochastic and deterministic assembly mechanisms using β NTI and RCbray metrics (Fig. 3). Stochastic processes dominated bacterial community assembly in both compartments. In HC, nasal communities were primarily shaped by dispersal limitation (restricted microbial exchange between individuals/environments) and drift (random fluctuations in microbial abundances). Wheezers additionally exhibited deterministic processes (variable selection), suggesting host- or environment-related selection of specific

microbial taxa. Stool communities were also mainly structured by dispersal limitation, with selective processes playing only a minor role in HC but gained importance in wheezers.

Co-occurrence network analysis revealed pronounced differences between HC and wheezers, with similar trends in nasal and stool bacteriomes (Fig. 4). Wheezing was associated with reduced network complexity in both compartments, reflected by fewer nodes, modules, and lower modularity. In contrast, clustering coefficient and centrality measures (degree, betweenness, closeness) were increased. Compartment-specific differences in path length, and proportion of positive edges were observed but less pronounced than the overall pattern of network simplification and reorganization. Notably, the divergent subgroup showed higher modularity and lower clustering and centrality measures than the overall wheezer group, consistent with a distinct microbial configuration. In the nose, network hubs shifted from ASV assigned to commensals like *Staphylococcus* and *Cutibacterium* in HC to *Leptotrichia*, *Fusobacterium*, and *Prevotella* spp. in wheezers. A similar trend was observed in stool, with hubs transitioned from *Veillonella* and *Roseburia* toward Lachnospiraceae and *Ruminococcus*.

Altered cross-compartment correlations between nasal and stool bacterial communities in wheezers

Spearman correlation analysis revealed distinct nasal–stool co-variation patterns between HC and wheezers (Fig. 5), with 42 significant correlations in HC ($|p| \geq 0.97$) and 53 in wheezers ($|p| \geq 0.82$), mostly positive.

In HC, positive correlations were mainly observed among commensals, including nasal ASV assigned to *Prevotella*, *Neisseria*, *Actinomyces*, and *Veillonella* and stool ASV related to *Aggregatibacter*. In addition, stool ASV210 (*Lachnoclostridium*) and ASV69 (*Staphylococcus*) were negatively associated with nasal ASV assigned to *Actinomyces*, *Streptococcus*, and *Prevotella*, suggesting coordinated variation across compartments in HC.

In wheezers, cross-compartment associations differed markedly. Several nasal ASV assigned to *Corynebacterium*, *Dolosigranulum*, and *Moraxella* showed strong positive correlations with stool *Lactococcus* ASV265 ($p = 0.82–0.94$). Additional associations included positive

correlations between nasal *Dolosigranulum* and *Mycoplasma* ASV and stool *Staphylococcus* (ASV69), as well as negative correlations between nasal *Veillonella* and stool *Faecalibacterium* and *Blautia*. Furthermore, stool *Escherichia-Shigella* (ASV20) was positively associated with nasal *Neisseriaceae_u* (ASV97). These correlations were largely absent in the divergent subgroup, showing mainly associations between stool *Escherichia-Shigella*, *Faecalibacterium*, *Rothia* and various nasal taxa (Supplementary Fig. S4).

Discussion

Nasal bacterial diversity in wheezers is characterized by increased variability and Moraxella-associated community shifts

In this cross-sectional study, we analysed nasal and stool bacterial communities in preschool children with recurrent wheezing and HC across the gut–airway axis. While overall bacterial richness and community composition were comparable between groups, wheezers showed higher inter-individual variability in nasal bacterial communities.

Moraxella, a common colonizer of the upper airways, showed a strong but not significant correlation with nasal community patterns. Previous studies have reported contrasting roles of *Moraxella* in respiratory health. Some studies describe it as a typical component of healthy airway communities [13, 25], whereas others link its predominance to respiratory tract infections and wheezing episodes [12, 26]. Our results suggest that the ecological context of *Moraxella* is more important than its mere presence. In HC it occurred at low abundance within diverse communities, whereas wheezers often showed *Moraxella*-dominated profiles associated with reduced richness and evenness. Similar cluster-like community patterns have previously been described in airway microbiota during early-life [26, 27] and at school age [28]. Comparable alterations are seen in chronic airway diseases such as cystic fibrosis and bronchiectasis, where reduced diversity is accompanied by dominance of opportunistic pathogens including *Pseudomonas aeruginosa*, and *Haemophilus influenzae*, and is associated with more severe disease [29, 30]. These observations suggest that *Moraxella* may act as an opportunistic taxon that expands when microbial community balance is disturbed,

potentially following viral infections or changes in local immune responses. Future studies should stratify children by prior infection status and integrate multi-omics data and immunological profiles, to better disentangle host–microbiome interactions driving *Moraxella* expansion.

Stool bacterial diversity remains comparatively stable beyond infancy

In contrast to nose, stool bacterial communities showed only modest alterations between wheezing and HC, consistent with previous evidence that intestinal microbial signatures associated with asthma risk are strongest in early infancy rather than later childhood [17, 19, 20]. Longitudinal studies have demonstrated that intestinal bacterial communities often normalize during the first year of life [16, 18], which may explain why differences in preschool-aged children are less pronounced.

Our data are consistent with the concept of a developmental window during early infancy in which intestinal bacterial colonization has the strongest influence on immune maturation and asthma susceptibility. By preschool age, dietary transitions and environmental exposures may contribute to convergence of intestinal bacterial communities between wheezers and HC. However, antibiotic exposure, a known determinant of gut microbial diversity and maturation in early life, may also act as a potential cofactor influencing these patterns [31, 32]. At the same time, the relatively small cohort size and cross-sectional design limit the ability to detect subtle associations between stool bacterial diversity and wheezing phenotypes. Larger longitudinal cohorts are required to determine whether intestinal microbial signatures associated with early-life wheezing persist or resolve during later childhood, and how compartment-specific microbial patterns relate to asthma development, systemic metabolomic and immune alterations, and their potential reversibility through targeted interventions.

Ecological restructuring of bacterial communities in wheezers

Beyond taxonomic composition, our ecological analyses revealed altered community organization between HC and wheezers. In both compartments, community assembly was

dominated by stochastic processes, consistent with ecological models describing microbial colonization as a balance between dispersal-driven processes and host-mediated selection [33]. However, nasal and stool communities in wheezers showed increased deterministic contribution, potentially reflecting host-driven selection such as airway inflammation, viral infections, or local immune responses [27].

Network analysis further indicated reduced modularity and increased fragmentation of bacterial interaction networks in wheezers, reflecting a reduction in overall network complexity. Such simplification has previously been associated with disturbed microbial ecosystems and lower resilience to environmental perturbations [34, 35]. Cooperative microbial interactions, including metabolic cross-feeding and signalling processes, are thought to stabilize host-associated microbial communities [36, 37]. Their reduction may therefore contribute to microbial ecosystems that are more susceptible to instability or opportunistic expansion of individual taxa. Consistent with this, increased centrality measures indicate that fewer taxa occupied more influential positions, indicating a shift toward greater centralization and uneven distribution of connectivity among the remaining community members. Notably, stratification of wheezers into subgroups revealed distinct modular network structure, suggesting differences in microbial community organization rather than a simple intensification of wheeze-associated dysbiosis.

Finally, cross-compartment correlations between nasal and stool taxa differed between HC and wheezers, suggesting group-specific altered gut-airway covariation. These correlations may reflect shared host or environmental influences rather than indirect microbial exchange across compartments and are consistent with the concept of the axis between the gut and the respiratory system, in which intestinal microbial metabolites such as short-chain fatty acids influence airway immune responses [38, 39]. Nevertheless, the functional mechanisms underlying these associations cannot be addressed by 16S rRNA metabarcoding alone and will require integrative approaches combining metagenomics, metabolomics, and immune profiling.

This study has several limitations that should be acknowledged. These include potential selection bias, cross-sectional design without longitudinal follow-up, and methodological constraints. The small sample size and exploratory pilot design limit statistical power and the generalizability of the findings. Its cross-sectional nature further precludes any conclusions regarding temporal dynamics or causality, and the use of convenience sampling may introduce additional selection bias. While 16S rRNA gene amplicon sequencing provides useful insights into community composition, it offers limited taxonomic resolution and does not capture strain-level variation or functional potential. Future studies in larger longitudinal cohorts, ideally incorporating metagenomics, metabolomics, and immune profiling, will be important to validate and extend these observations.

Conclusions

Taken together, our results indicate that preschool wheezing is associated with increased variability and taxon-level restructuring of nasal bacterial communities, particularly involving *Moraxella*, while stool bacterial diversity remains stable. Altered microbial networks and cross-compartment correlations might further suggest coordinated ecological changes across mucosal sites. These findings highlight the importance of microbial community structure and ecological interactions, rather than diversity alone, when investigating microbial contributions to early-life wheezing and asthma susceptibility. Longitudinal functional microbiome studies will be necessary to clarify whether these signatures contribute to persistent wheezing or asthma development.

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Data availability

The sequence data generated and analyzed during this study have been deposited in the NCBI
 Sequence Read Archive (SRA) under BioProject accession number PRJNA1499410 (reviewer
 link:

<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1499410?reviewer=okpdiae11ucclqmiq09i43>

[6qvih](#)). Associated sample metadata are not publicly available in order to protect participant
 privacy but may be obtained from the corresponding author upon reasonable request.

Ethics Statement

The ALLIANCE cohort of the German Center for Lung Research (DZL) was approved by all
 local ethics committees of the involved centers and registered at ClinicalTrials.gov (pediatric
 arm NCT02496468, adult arm NCT02419274). Parents of study participants aged <18 years
 and study participants aged ≥18 years gave written informed consent.

Author contributions

S. Gschwendtner and M. Schloter contributed to study conceptualization, performed data analysis, and drafted the manuscript. N. Maison, S. Illi, I. Rosenboom, B. Tümmler, and M. Weckmann contributed to the methodology and provided scientific input, including critical review and revision of the manuscript. E. von Mutius, B. Tümmler, G. Hansen, M. Kopp, and A.-M. Dittrich contributed to study conceptualization and provided scientific guidance, including critical review and revision of the manuscript. M. Abdo critically reviewed the manuscript. G. Hansen, F. Brinkmann, B. Schaub, K. Rabe, E. von Mutius, B. Waschki, and M. V. Kopp are principal investigators of the DZL ALL Age Asthma Cohort; they secured funding and contributed to the conception and development of the overarching research goals and aims.

Conflict of Interest

S. Gschwendtner, N. Maison, S. Illi, I. Rosenboom, B. Waschki, M. Abdo, F. Brinkmann, and M. Schloter declare no competing interests.

E. von Mutius reports research support, consulting fees, travel funding, honoraria and patent-related royalties from multiple academic, governmental and commercial entities, including OM Pharma S.A., Elsevier, AstraZeneca and the European Commission.

B. Tümmler reports grants from the Deutsche Forschungsgemeinschaft (DFG), Volkswagen Stiftung, German Center for Lung Research (DZL) and German Center for Infection Research (DZIF); consulting fees from Helmholtz-Zentrum für Infektionsforschung (HZI); honoraria for lectures and educational events from Vertex Pharmaceuticals Germany; and advisory board fees from Vertex Pharmaceuticals Inc.

A.-M. Dittrich reports clinical study funding paid to her institution from Vertex Pharmaceuticals Inc. for the conduct of clinical studies; consulting fees from GSK, Oxford University and Novartis via the c4c consortium; and personal remuneration from Vertex Pharmaceuticals for scientific advice on textbooks and online educational materials.

M. Weckmann reports grants from the German Federal Ministry for Research, Technology and Space (BMFTR) and the German Center for Lung Research (DZL) during the conduct of the study. M. V. Kopp reports payment or honoraria for lectures, presentations, speakers' bureaus,

manuscript writing or educational events from Allergopharma GmbH and Infectopharm GmbH. He also had a leadership or fiduciary role in other board, society, committee or advocacy group, and served as President (2018-2022) and Past president (2022-2025) of the Society of Pediatric Pulmonology ("Gesellschaft Pädiatrische Pneumologie e.V.").

G. Hansen reports funding from the German Center for Lung Research (DZL; BMRTS) with grant payments made to Hannover Medical School, and the German Research Foundation (DFG), EXC 2155 'RESIST', as well as consulting fees from Sanofi GmbH and lecture fees from MedUpdate and AbbVie.

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Support statement

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Figure Legends

Figure 1. Nasal and gut microbiome composition in healthy (n=8) and wheezing (n=17) children.

(a) Shared and unique ASV and core ASV across prevalence thresholds. Top: Venn diagrams showing shared and group-specific ASV in nasal and stool samples. Bottom: Number of core ASV across prevalence thresholds (50–100%), defined as ASV present in $\geq X\%$ of samples within each study group.

(b) Relative abundance of the 20 most abundant genera. No significant differences in genus abundance were detected between groups (ANCOM-BC2; Benjamini–Hochberg-adjusted $p < 0.05$).

(c) Within-group variation (beta-dispersion) calculated from weighted UniFrac distances. Statistical significance was assessed using Levene's test and confirmed using generalized linear mixed-effects models with Benjamini–Hochberg adjustment for multiple testing.

(d) Spearman correlations between beta-dispersion and bacterial genera. Only the 20 strongest correlations, ranked by Spearman's correlation coefficient (ρ), are shown. Asterisks indicate statistically significant correlations (Benjamini–Hochberg-adjusted $p < 0.05$). Correlations were calculated using Spearman's rank correlation test.

Figure 2. Microbial signatures of healthy-like and community-divergent wheezers.

(a) Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis distances, with alpha-diversity metrics (observed ASV, evenness and Shannon index), beta-dispersion and immune variables fitted as environmental vectors. Asterisks indicate vectors significantly associated with community composition (permutation test within the envfit function, $p < 0.05$). Vector length reflects the strength of the association with the ordination, and vector direction indicates the gradient of increasing values across the NMDS space. Blue and red symbols represent subjects classified as healthy-like and community-divergent wheezers, respectively, in both nasal and gut microbiomes; green and orange symbols represent subjects classified

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Figure 3. Community assembly processes in the nasal and gut microbiomes of healthy (n=8) and wheezing (n=17) children. Community assembly mechanisms were inferred using β -nearest taxon index (β NTI) and Raup–Crick Bray–Curtis (RCbray) metrics. Thresholds of $|\beta$ NTI|=2 and |RCbray|=0.95 are indicated by horizontal and vertical dashed lines, respectively.

Figure 4. Bacterial interaction networks in healthy (n=8) and wheezing (n=17) children. Bacterial co-occurrence networks were constructed from core ASV, defined as ASV present in $\geq 50\%$ of subjects, in nasal and stool samples from healthy and wheezing children. Nodes represent ASV and edges represent inferred associations between pairs of ASV estimated using SpiecEasi (green: positive associations; red: negative associations). Modules, shown in different colours, represent groups of highly interconnected nodes. Hub taxa are highlighted in bold, and node size is proportional to eigenvector centrality. The accompanying table summarises key network topological characteristics, including the number of nodes, clustering coefficient, modularity, positive edge percentage (PEP), edge density, robustness, average path length, centrality measures (degree, betweenness, closeness and eigenvector centrality), and the number of modules.

Figure 5. Pairwise cross-compartment correlations in healthy (n=8) and wheezing (n=17) children. Spearman correlations were calculated between individual ASV in nasal and stool

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668 **Table 1. Summary of baseline characteristics of the study cohort**

Characteristic	Overall N = 25	healthy N = 8	wheeze N = 17	p value ¹
Age (year)	2.70 (1.90-3.30)	2.65 (1.90-2.85)	3.00 (1.90-3.40)	0.209
Female	8 (32%)	4 (50%)	4 (24%)	0.359
BMI (kg/m ³)	16.80 (15.50-17.94)	16.50 (15.35-17.30)	16.98 (15.50-18.20)	0.268
Any sibling	16 (64%)	4 (50%)	12 (71%)	0.394
Daycare	23 (92%)	8 (100%)	15 (88%)	>0.999
Pet in household (cat/dog)	9 (36%)	2 (25%)	7 (41%)	0.661
Asthma or recurrent spastic/obstructive bronchitis - DD	2 (8.3%)	0 (0%)	2 (13%)	0.536
Missing	1	0	1	
Hayfever - DD	1 (4.2%)	0 (0%)	1 (5.9%)	>0.999
Missing	1	1	0	
Eczema - DD	2 (8.3%)	0 (0%)	2 (13%)	0.536
Missing	1	0	1	
Food allergy - DD	0 (0%)	0 (0%)	0 (0%)	-- ²
Missing	1	1	0	
Birthmode				0.095
Vaginal delivery	15 (60%)	7 (88%)	8 (47%)	
Primary cesarean section	6 (24%)	0 (0%)	6 (35%)	
Secondary cesarean section	2 (8.0%)	1 (13%)	1 (5.9%)	
Operative vaginal delivery	2 (8.0%)	0 (0%)	2 (12%)	
Any exclusive breastfeeding ever	19 (76%)	5 (63%)	14 (82%)	0.344
Any hypoallergenic formula ever	9 (36%)	1 (13%)	8 (47%)	0.182
Prior RSV infection ever	7 (32%)	0 (0%)	7 (47%)	0.051
Missing	3	1	2	
Antibiotics given for recurrent cough ever	1 (33%)	NA	1 (33%)	-- ²
Missing	22	8	14	
Systemic steroids ever	9 (53%)	NA	9 (53%)	-- ²
Missing	8	8	0	
ICS ever	13 (76%)	NA	13 (76%)	-- ²
Missing	8	8	0	
Hb (g/dl)	11.60 (11.40-12.30)	11.60 (11.30-12.30)	11.90 (11.45-12.25)	0.365
Missing	2	1	1	
Thrombocytes (10 ³ /μl)	374 (328-443)	328 (263-367)	403 (372-443)	0.045
Missing	2	1	1	
Leucocytes (10 ³ /μl)	9.44 (8.23-11.70)	10.00 (5.24-14.00)	9.27 (8.24-11.65)	0.841
Missing	2	1	1	
Neutrophils (%)	40 (33-55)	38 (31-58)	42 (36-55)	0.947
Missing	2	1	1	
Lymphocytes (%)	42 (34-54)	53 (27-60)	40 (36-47)	0.593
Missing	2	1	1	
Eosinophils (%)	1.95 (1.00-3.00)	2.50 (1.00-3.50)	1.50 (0.78-2.40)	0.243
Missing	3	1	2	
Basophils (%)	0.20 (0.00-0.70)	0.00 (0.00-0.20)	0.55 (0.08-1.00)	0.036
Missing	4	1	3	
IgE (IU/ml)	39.0 (9.1-73.8)	47.5 (24.0-61.4)	19.2 (9.0-82.0)	0.583
Missing	2	0	2	
Atopy (spec. IgE to any allergen ≥0.7 kU/L)	8 (32%)	3 (38%)	5 (29%)	>0.999

Values are summarized as median and interquartile range (Q1-Q3) in brackets, or as total numbers per condition level and percentage in brackets.

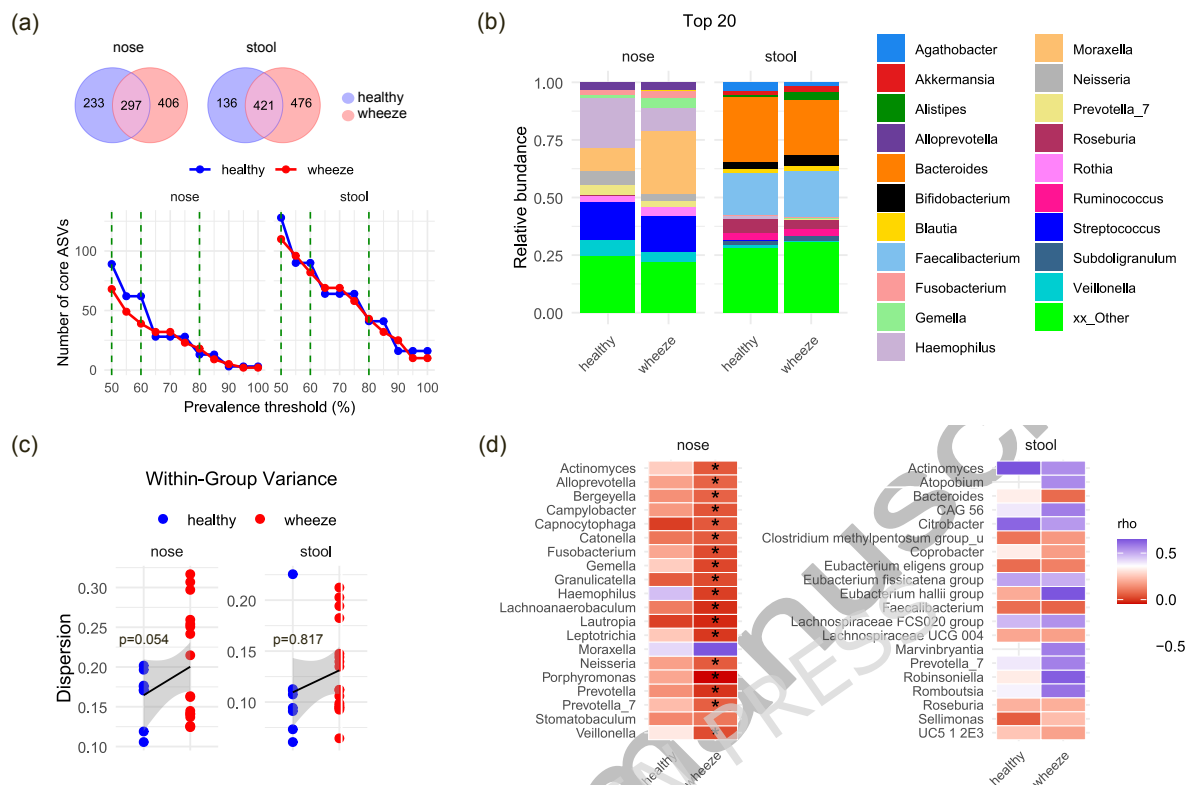
DD = parental report of doctor's diagnosis

¹Kruskal-Wallis rank sum test; Fisher's exact test

²p value not calculated due to insufficient variability (single-level data) and/or missing values

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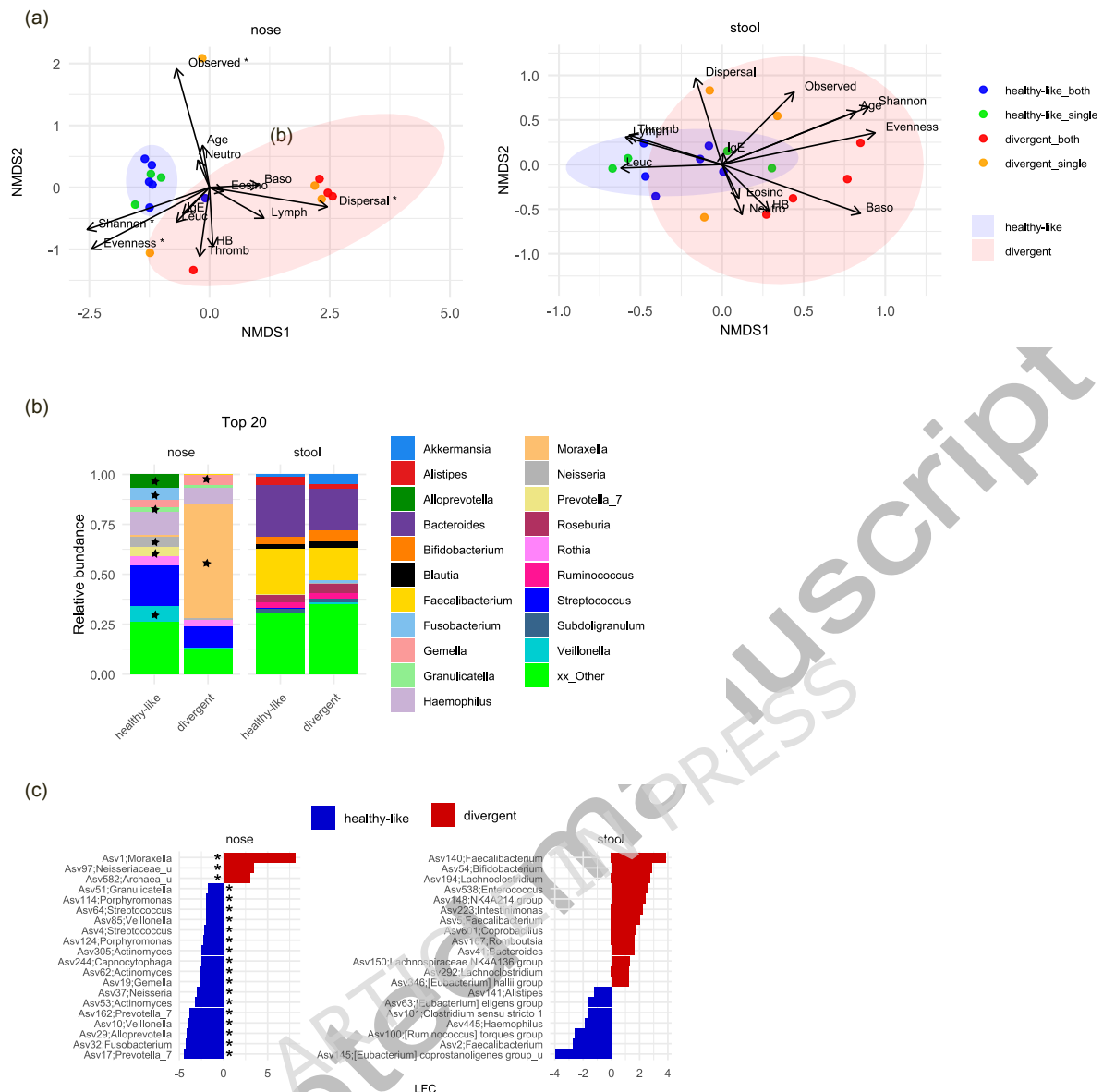


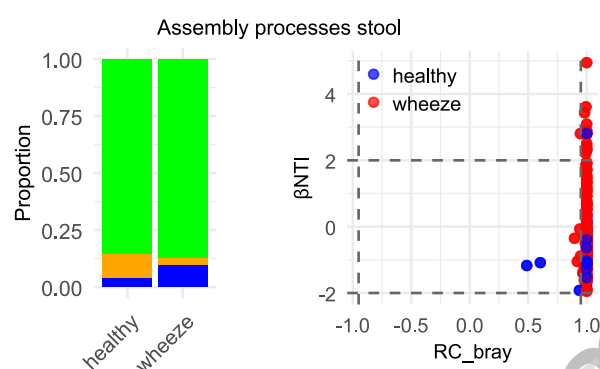
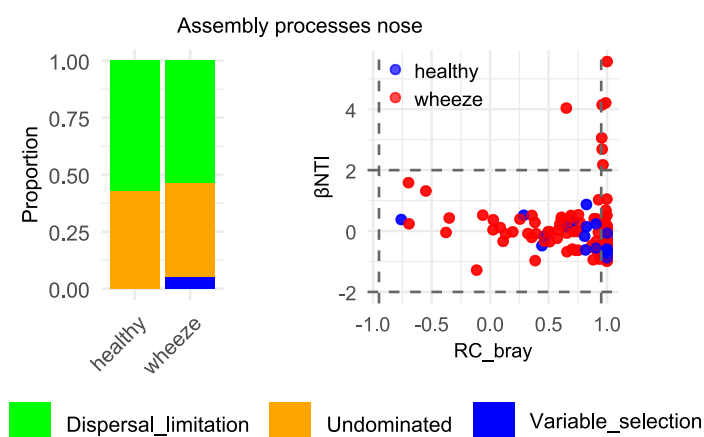
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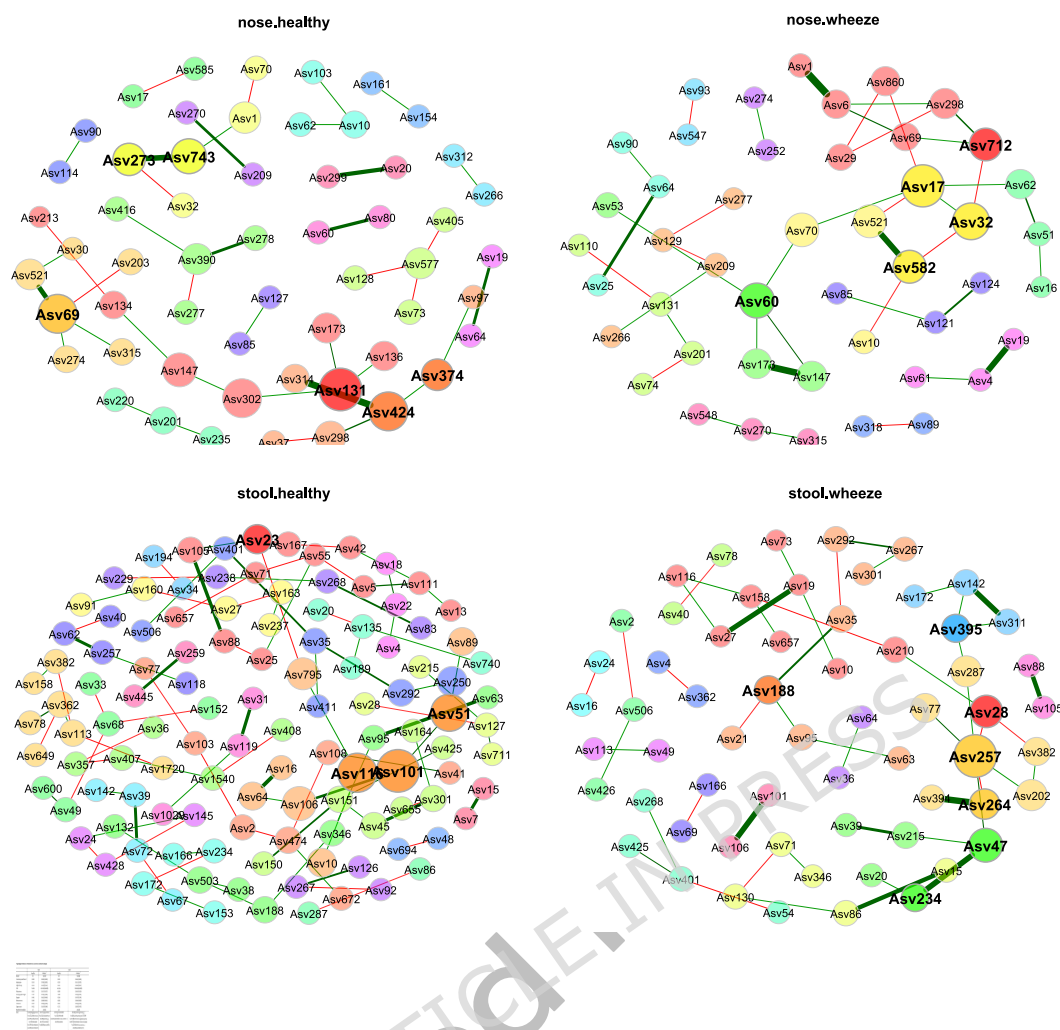
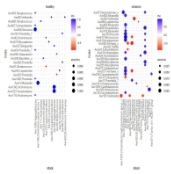


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