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Early-life microbiome trajectories as biomarkers to predict health outcomes

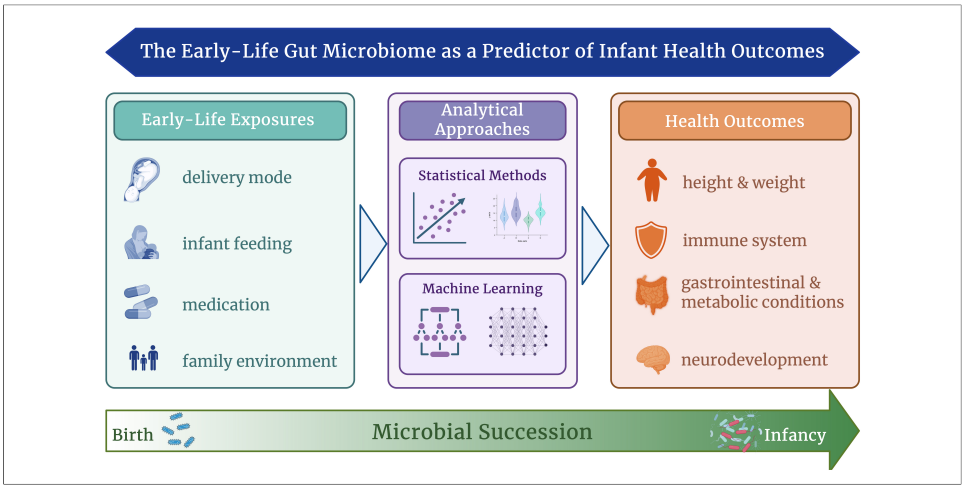
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Keywords:
Early-life microbiome, infant development, machine learning, healthy trajectories, microbial succession, paediatric health

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Abstract

The early-life gut microbiome is tightly linked to different aspects of infant development. Microbial colonisation patterns have been repeatedly shown to play a role in a variety of paediatric outcomes, ranging from metabolism and immune function to neurodevelopment. Concomitantly, the identification of early-life biomarkers is crucial, especially considering that for various conditions, reliable diagnostic tools only emerge in early childhood. As such, microbiome data collected in the first two years of life may offer valuable prospects for early detection, prevention, quantification or even correction of adverse health trajectories. With the increasing availability of high-resolution microbiome data, researchers are leveraging both traditional statistical approaches and machine learning (ML) methods to analyse the evolution of these complex microbial communities. While statistical models are well-suited for identifying associations between microbiome features and health states, ML methods allow for predicting health outcomes from those features. This review explores the role of the early-life gut microbiome in infant health and development, with a focus on how data acquisition and analytical methods can shape current knowledge. We contrast statistical approaches with ML methods, summarising key

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findings on microbial succession and factors influencing it. By addressing current challenges and identifying areas for methodological refinement, we aim to discuss the potential of the microbiome in the assessment of current and future health states of an individual and aid in the development of more robust, clinically-relevant models for paediatric care.

INTRODUCTION

The human gut microbiome - a complex microbial community, its gene collection and metabolic potential - adapts dynamically across a lifetime, yet a critical period is the initial assembly in early life[1-3]. It is initiated from birth onwards, with differential intrinsic and external exposures, including birth mode, feeding patterns and lifestyle factors shaping the colonisation trajectory[4-8]. Mounting evidence suggests that these early microbial communities not only correlate with health conditions in infancy but may also distinguish between divergent health trajectories - current and future[9-14]. While the former findings rely on statistical analysis, which remains indispensable for detecting relationships between microbial communities and host phenotypes, the latter are obtained by employing machine learning (ML), which extends analytical capacity from association toward prediction. ML is a branch of artificial intelligence capable of parsing complex data such as microbial metagenomes for meaningful patterns[15]. Indeed, ML algorithms have been found successful in detecting microbiome-host interactions, predicting host phenotypes as well as potential disease risks based on microbial communities[16-18]. It thereby serves as a tool for translating microbiome data into feasible clinical interventions used for potential microbial biomarker identification.

Several obstacles remain in employing operational microbiome-based predictive models in clinical practice. High inter-individual variability[19], driven by genetic, environmental, and lifestyle factors[20,21], has so far prevented the establishment of a benchmark 'healthy' microbiome[22]. Without such reference points, defining optimal community structures or standardising risk prediction remains difficult. Moreover, while ML can identify prediction patterns linking microbial profiles to health outcomes, prediction does not equate to causality[23], and the opaque nature of many algorithms raises concerns about interpretability[24].

To date, most existing reviews in the field have focused on either microbiome-health associations in early life[25-29], or on machine learning applications in adult populations[16,23,24]. Given the central role of early development in shaping long-term health trajectories, and the limited effectiveness of many diagnostic tools in later childhood[30,31], microbiome-based prediction in infancy holds considerable potential for earlier risk stratification, personalised intervention, and disease prevention. However, the early-life microbiome presents distinct analytical challenges, including rapid temporal dynamics, strong environmental sensitivity, and high inter-individual variability. These considerations in mind, a structured overview of how machine learning models perform in early-life cohorts, and how both methodological and biological factors influence predictive outcomes, is currently lacking.

This review addresses this gap by providing a focused evaluation of machine learning applications in the infant gut microbiome, with an emphasis on predictive modelling from birth to two years of age. In contrast to previous reviews, we critically assess how predictive models are constructed, which outcomes they target, and how performance varies across study designs and analytical frameworks. We further integrate key methodological aspects of microbiome research, including sequencing strategies and pre-processing pipelines, to evaluate their impact on model performance and cross-study comparability. By linking early-life microbial ecology with analytical and computational considerations, this review provides a structured framework for interpreting existing predictive evidence and clarifies the current limitations and requirements for developing robust, generalisable, and clinically meaningful microbiome-based prediction in infancy.

DATA ACQUISITION, PROCESSING AND ANALYSIS IN MICROBIOME RESEARCH

The initial stages of data acquisition and handling are essential for subsequent data quality and analysis. Microbial community profiling typically progresses from sample collection (e.g. saliva, stool or skin swabs) to DNA extraction, sequencing, preprocessing and data analysis. DNA extraction involves breaking open microbial cells using chemical, enzymatic, or mechanical methods to release DNA, followed by its purification from contaminants[32]. Importantly, microbiome data are highly sensitive to variation in these pre-analytical steps. For example, DNA yield varies across stabilisation methods, time to freezing, freeze-thaw cycles, and storage time[33-37]. In addition, DNA recovery can vary substantially based on extraction protocols, particularly due to lysis strategies and efficiency, which disproportionately affects Gram-positive bacteria with more resilient cell walls[38-41].

Such differences in sample handling can introduce significant bias not only to DNA yield but also to inferred microbial profiles, which is especially critical in infant samples, considering their limited microbial community size[37,38,42]. Sequencing requires the preparation of DNA libraries, by either amplifying specific marker regions or sequencing all genetic material[43]. The two main approaches are whole-metagenomic shotgun sequencing (WGS) and 16S rRNA (16S) gene sequencing. The 16S gene, a genetic marker present in almost all bacteria, contains both conserved and variable regions, allowing taxonomic identification of bacteria and archaea typically up to the genus level, but without functional insight[43-45]. For WGS, the genetic material found in samples is sliced into smaller parts (i.e. as if being shot with a gun), and all DNA is sequenced, thereby allowing sequencing of all microbes, including bacteria, archaea, fungi and viruses[43,44]. While WGS enables higher taxonomic resolution and functional analysis, it is more computationally demanding, prone to host contamination and relies on less complete reference databases[20,43,44,46,47]. Although decreasing cost makes WGS more accessible[44], both approaches present challenges in sequence alignment and downstream interpretations.

The consequent processing and analysis of such complex and large data is challenging, both in terms of aligning the genetic sequences with reference databases and investigating associations between genetic material and disease phenotypes. While bioinformatic pipelines dealing with the former are extensively reviewed elsewhere[20,46], we will focus on summarising challenges around the downstream analysis of microbiome data and attempts to combat them. Microbial communities underly principles of biological ecology, manifesting in complex relationships between microbes, and demanding consideration of such relationships in analysis[22]. Moreover, interpersonal variability of compositional microbial profiles based on subjective microbial exposure makes for dataframes with large amounts of columns and zeros, i.e. sparse yet skewed feature distributions, which increases with larger sample sizes[20,43,48]. Equally, large amounts of confounding variables ranging from technical considerations such as read counts or sequencing batches, study design factors including repeated measures, as well as covariates such as lifestyle and diet, further complicate microbiome data analysis[48].

As such, a key feature of microbiome data analysis is to narrow down features, which also improves the interpretability of final outputs[23]. These challenges are currently being tackled by uniquely creative solutions, which can broadly be divided into two categories, as shown in Figure 1: statistical and ML approaches. Statistical methodology aims to use exploratory approaches to answer specific hypotheses, most of which focus on microbiome differences at different developmental stages and between health and disease conditions. As such, statistical methods assess differences between individuals or groups in microbial community diversity or abundances of specific taxa, including compositional features or functional pathways. For the latter, referred to as differential abundance analysis, tests such as the Wilcoxon rank sum test or Fisher's test, as well as a variety of software tools including ANCOM-BC2[49], DESeq2[50], or MaAsLin2[51] are used, employing varying approaches such as mixed linear or binomial models. Lastly, taxa

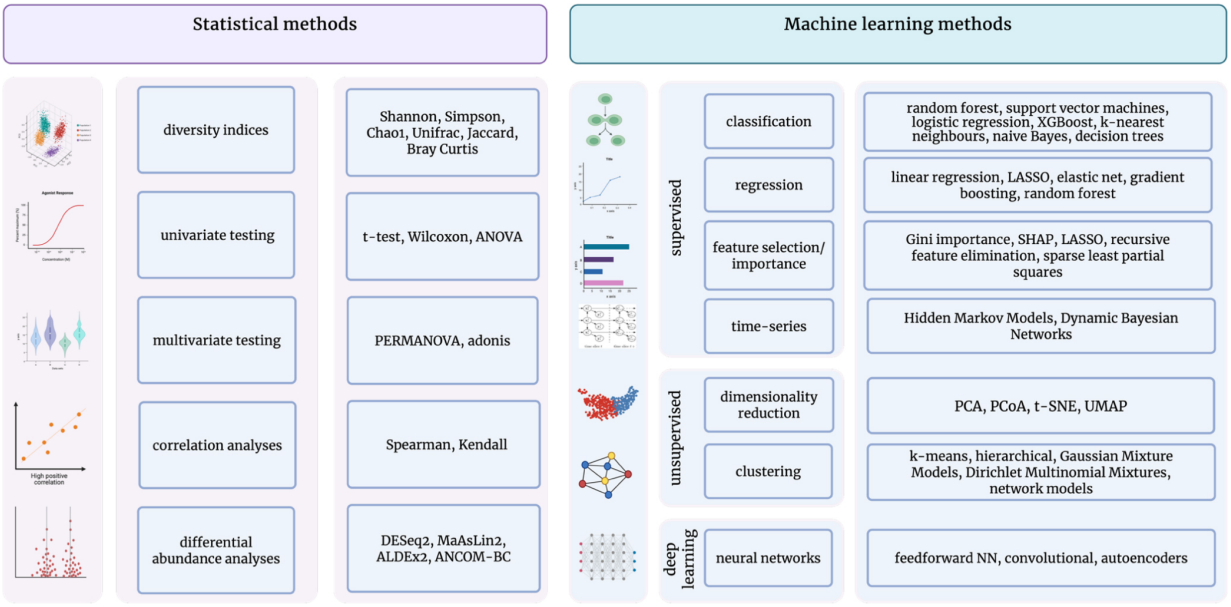


Figure 1. Downstream data analysis in microbiome research; Conceptual schematic summarizing commonly used statistical and machine-learning approaches for the analysis of microbiome data, highlighting methods for quantifying microbial features, modeling community structure, and extracting biologically meaningful insights. Created in BioRender. Lavelle, A. (2026) https://BioRender.com/qwizjk8 ANOVA: Analysis of variance; PERMANOVA: permutational multivariate analysis of variance; SHAP: SHapley Additive exPlanations; LASSO: Least Absolute Shrinkage and Selection Operator; PCA: principal component analysis; PCoA: principal coordinates analysis; t-SNE: t-distributed stochastic neighbor embedding; UMAP, uniform manifold approximation and projection; NN: neural networks.

abundances can be correlated with any given clinical feature, providing a measure of the relationship between them. Taken together, statistical approaches provide interpretable differences between samples, quantifying group-level differences and indicating a degree of relatedness.

ML approaches, in turn, are able to handle greater amounts of more complex data, parsing it for underlying patterns and allowing statements regarding its predictive qualities. They are considered a type of artificial intelligence due to their intrinsic capacity to conduct trial and error processes, adjusting to outcomes automatically[15]. Within ML, models can be categorised as supervised or unsupervised based on whether outcomes or predictors are set a priori[15]. Unsupervised ML methods are predominantly used for dimensionality (i.e. feature) reduction, as well as clustering approaches such as k-means or hierarchical clustering[16,23,24]. The goal of both is to identify how samples or features naturally group together, revealing underlying patterns or structures in the microbiome without prior knowledge or outcome labels.

Supervised ML methods in turn leverage a predefined set of factors to predict a specific continuous (regressor models) or categorical (classification models) outcome, making them a valuable tool for identifying clinical biomarkers or diagnosis[15,23,48]. The respective workflow consists of several steps, starting with feature selection. Besides selecting the outcome variable of interest, a variety of dependent variables are tested for their predictive qualities, a process referred to as the training phase[23]. This process is followed by a testing phase, where the selected features are presented with unseen data, resulting in a variety of model performance metrics[23,43]. Subsequently, the model can be improved by an iterative flow through the previous steps, altering the selected features or model parameters to optimise predictive quality[23].

Taken together, both statistical and ML approaches are essential and complementary in microbiome data analysis. While statistical methods are crucial in data preparation and characterisation, ML methodology allows for the recognition of patterns, clusters or predictive qualities of high-dimensional and non-linear

microbiome data. As such, and especially in the light of the infant microbiome, ML presents an exciting avenue to extend analytical insight from association toward prediction of present and future health states. While prediction does not imply causation, integrating ML with longitudinal data and experimental validation strategies can help to move further towards causal relationships between microbial profiles and diseases.

THE EARLY-LIFE MICROBIOME: MICROBIAL SUCCESSION AND ITS ROLE IN INFANT DEVELOPMENT

The microbial ecosystem in the infant gastrointestinal tract begins to establish itself through a process referred to as microbial succession, whereby different species colonise the body in a relatively ordered yet individualised sequence, gradually building a stable and complex microbial community[1,13,19,52,53]. Universally, infants experience initial colonisation at birth[54], and the transmission process is thought to happen through both genetic predisposition and direct microbial exposure[55]. As such, microbial colonisation is directed by perinatal factors such as individual gestational age and mode of delivery, reflected in vaginal births seeding microbes from the maternal vaginal and gastrointestinal tract, while infants born via cesarean section are primarily exposed to skin- and hospital-associated microbes[1,4,56]. Accordingly, community structures of the two groups differ robustly in the first few months of infancy, with vaginally-born infants displaying higher numbers of species belonging to *Lactobacillus, Bacteroides* and *Bifidobacterium*, while infants born via cesarean section harbour higher amounts of *Staphylococcus, Veillonella* and *Streptococcus* species[1,19,57].

Similarly, various factors, including antibiotic exposure, environmental microbial contact, and maternal health, as well as early-life feeding patterns, play key roles in the individuality of succession[1,19,58]. While in general, microbial profiles of exclusively milk-fed infants are dominated by aerobic and facultative anaerobic taxa of Bifidobacteriaceae and Enterobacteriaceae[8,52,59,60], individual differences based on varying amounts of either breastmilk or formula persist[1,6,13,57,61-64]. Such dissimilarities are thought to trace back to breastmilk containing immunoregulatory compounds[65,66] and promoting more limited amounts of species metabolising high amounts of prebiotic human milk oligosaccharides (HMOs) such as *Bifidobacterium longum* subsp. *infantis*[67], while formula fosters a more diverse but less specialised microbiota[8,19,62,68-71]. The introduction of solid foods, respectively, the stepwise cessation of breastmilk from around six months of age, encourages a major microbial transition[1,8,13,19,72]. As a result, the presence of more anaerobic taxa capable of metabolising complex carbohydrates and proteins, such as Bacillota (formerly Firmicutes), Bacteroidota (formerly Bacteroidetes), and Clostridiales increases[1,13,73]. Continuing throughout toddlerhood, higher abundances of species such as *Faecalibacterium prausnitzii* and *Methanobrevibacter smithii*, as well as increasing diversity and complexity of microbial community, result in mature, adult-like microbiota typically by age three years[1,19,55,74-77]. As such, microbial succession follows predictable phases over the first two years of life, despite the various individual external and internal factors influencing the trajectory[19,53,74].

Building on the understanding of microbial succession in early life, researchers have increasingly investigated how these dynamic microbial patterns relate to infant health outcomes. In most cases, such a role is established by statistically identifying factors associated with an outcome, followed by demonstrating differences in microbial profiles based on the same factors and the outcome, drawing an interactive effect triangle. As such, the early-life microbiome is thought to play a major role in healthy development and associated health and disease conditions, including, among others immuno-mediated diseases such as type 1 diabetes, inflammatory bowel disease, asthma, atopic dermatitis, and food allergies, as well as neurodevelopment, metabolic conditions, including obesity and gastrointestinal conditions such as necrotising enterocolitis (NEC)[27-29,78,79]. Revealing these associations between the early-life microbiome and various paediatric disease states has fuelled interest in discovering microbial signatures that could serve as predictive biomarkers.

THE EARLY-LIFE MICROBIOME AS A PREDICTOR OF HEALTH

Early-life ML studies harness the predictive potential of the infant gut microbiome and can be classified into two broad categories, as summarised in Tables 1 and 2: predicting host phenotypes based on (groups of) individual microbial taxa or based on microbial maturation indices.

Microbial profiles, individual taxa and community clusters as predictors of health and disease states

Studies which predict paediatric conditions based on individual or clusters of microbial taxa have been conducted for a plethora of conditions. They usually include identifying (groups of) features which are correlated or differentially abundant between respective groups, and using these features to build a predictive model, resulting in the identification and ranking of key predictive species and overall predictive performance. A prime example and one of the largest early-life microbiome ML studies to date combined a total of 13,776 global samples collected from 1 to 36 months of 1,956 infants to investigate the emergence of enterotypes during microbial succession[53]. The study found enterotype-specific assembly and diversity patterns, which could be stratified by geographic origin of sample, and differed in terms of birth mode, gestational age and duration of breastfeeding[53].

A number of studies have undertaken the prediction of host phenotypes or early-life exposures. Le Goallec *et al.* predicted host characteristics such as breastfeeding status, sex, antibiotic use, geographic origin and mode of delivery using a variety of different ML methods[77], reporting an area under the curve (AUC), i.e. a model's ability to correctly separate cases from controls, ranging from 0.63 to 0.81. Rahman and colleagues employed Random Forest and gradient boost models to predict antimicrobial resistance genes in infants who received antibiotics *vs.* infants who did not, and found that formula-fed infants predictably harboured higher numbers of class D β-lactamase genes, enzymes which hydrolyse antibiotics and thereby confer AMR[80]. Similarly, other studies reported formula feeding to predict higher numbers of opportunistic pathogenic species belonging to *Staphylococcus* and *Klebsiella*, as well as an increased AMR load[81].

A study conducted by Naspolini employed 966 WGS samples of 525 infants aged three to nine months and a Random Forest algorithm, reaching an accuracy ranging from 0.57 to 0.74 when predicting mode of delivery and breastfeeding status in infants. In line with previous associative studies, breastmilk feeding was found to be a major predictor of microbial composition[58,82], with *Escherichia coli* and *Clostridium perfringens* discriminating strongly for exclusive breastfeeding. *Bacteroides* species were found to be highly discriminatory of mode of delivery, with vaginally-born infants displaying significantly higher relative abundances than infants[57]. These findings, paired with the observation that earlier timepoints (i.e. 1-2 months of age) are more predictive than later ones (3-6 months and 9-12 months), are in line with Vänni[83]. On a similar note, Wang employed 1,024 publicly available WGS samples and a gradient boost algorithm to predict the transmission of microbes from mother to infant, demonstrating higher similarity between related over unrelated mother-infant pairs with an accuracy of 0.69 for shared species[55].

Besides discriminating host phenotypes and exposures, several early-life conditions were found to be accurately predicted when employing microbial profiles. As such, newborns with NEC can be discriminated from their healthy peers with an average accuracy of up to 90%[84-89]. Remarkably, models including only microbiome data had similar, if not better, performance than models containing microbiome and clinical data[86]; however, marked differences in performance were obtained with different ML models[85]. Key predictors were elevated short-chain fatty acids (SCFA) formate in NEC onset, which dissipates during recovery[88], as well as taxa belonging to Enterobacteriaceae[86,88], *Klebsiella*[88,89], Bacillota (formerly Firmicutes) and Proteobacteria[85,86]. Similar success was reached when predicting atopic dermatitis, where an AUC of 83% was observed by a study conducted by Zhang[90]. Specifically, the authors report that lower abundances of *Bifidobacterium* are predictive of eczema in infants. With the same dataset, Ma tried five different

Table 1. Predictive studies using microbial profiles to predict infant phenotypes

Year	Authors	Dataset	Condition	Age	Participants	Samples	Data	Model	Metric	Value
2021	Boutin *et al.*[96]	[96]	Allergies	3-12 months & 5 years	343	545	16S/ITS2	RF	Accuracy	0.81
2022	Casaburi *et al.*[88]	[80,122-126]	Fecrotising enterocolitis	0-2 months	344	1,647	WGS	RF, GB	Accuracy	0.9
2024	Chen *et al.*[127]	[127]	Neonatal jaundice	0 days	196	196	16S	RF, GB, Lasso	AUC	0.91
2017	Dobbler *et al.*[84]	[84]	Necrotising enterocolitis	0-5 weeks	40	132	16S	RF	Accuracy	0.76
2021	Fernández-Edreira *et al.*[92]	[128]	T1 diabetes	0-2 years	33	124	16S	RF, SVM, GLM	AUC	0.8-0.98
2020	Hooven *et al.*[85]	[129]	Necrotising enterocolitis	0-2 months	161	2,895	16S	NN	AUC	0.23-0.9
2020	Korpela *et al.*[98]	[98]	Overweight	0-3 years	212	308	16S	RF	AUC	0.58-0.7
2022	Kortekangas *et al.*[109]	[109]	Environmental enteric dysfunction	6-30 months	610	1,569	16S	RF	Variance explained	0.245-0.277
2020	LeGoallec *et al.*[77]	[1,112,128,130]	Early-life exposures	2-36 months	300	1,570	WGS	RF, GB, EN, SVM, KNN, NB	AUC, R-squared & Mean Class Accuracies	0.63-0.81
2022	Lin *et al.*[86]	[89,129]	Necrotising enterocolitis	0-2 months	261	3595	16S/WGS	NN, SVM, LR	AUC	0.86-0.92
2022	Lou *et al.*[106]	[106]	Autism spectrum disorder	11 months-19 years	1,202	1,202	16S	RF	AUC	0.67-0.86
2020	Loughman *et al.*[94]	[94]	Colics, problem crying & behaviour	0-3 months & 2 years	118	236	16S	RF	AUC	0.65
2022	Lugo-Martinez *et al.*[131]	[131]	Growth faltering	0-1 month	357	2923	WGS	RF, LR, HMM, DMM	AUC	0.59-0.76
2025	Ma *et al.*[91]	[90]	Atopic dermatitis	0-3 years	112	112	16S	RF, GB, SVM, LR	AUC	0.83-0.98
2022	Martin *et al.*[132]	[132]	Allergic proctocolitis	1 week-1 year	160	954	16S	RF	Accuracy	0.76
2021	Masi *et al.*[87]	[87]	Necrotising enterocolitis	0-4 months	48	644	WGS	SVM, RF,	AUC	0.88-0.95
2016	McGeachie *et al.*[133]	[134]	Preterm	0-2 months	58	922	16S	DNB	Mean absolute error	0.01-0.14
2019	Metwally *et al.*[10]	[10]	Food allergy	0-3 years	148	658	WGS	HMM, NN, SVM, RF, LASSO,	AUC	0.44-0.69
2025	Naspolini *et al.*[57]	[57]	Feeding	3-9 months	525	966	WGS	RF	Accuracy	0.57-0.74
2021	Nguyen *et al.*[135]	[64]	Functional microbial capacity	6 weeks-1 year	375	440	16S	RF, EN, SVM, SPLS	R-squared	-0.056-0.118
2019	Olm *et al.*[89]	[89]	Necrotising enterocolitis	0-2 months	160	1,163	WGS	GB, RF, SMOTE	Accuracy	0.64
2022	Pärnänen *et al.*[81]	[81]	Feeding & antimicrobial resistance	1 month	46	46	16S	RF	Feature importance	
2025	Pitekováet *al.*[114]	[114]	Febrile urinary tract infection	0-17 years	35	35	16S	RF	AUC	0.87
2018	Rahman *et al.*[80]	[80]	Feeding & antimicrobial resistance	0-2 months	107	902	WGS	RF	Feature importance	
2024	Sizemore *et al.*[93]	[93]	Neurodevelopmental deficits	0-8 months	88	398	16S	Q-net model, NA	AUC	0.66-0.88

2018	Stanislawski *et al.*[97]	[97]	Overweight	0-2 years & 12 years	165	781	16S	RF	R-squared	0.5
2023	Vänni *et al.*[83]	[7,9,136-144]	Feeding & delivery mode	0-12 months		3,595	16S	RF, GB, RT	AUC	0.72-0.83
2018	Vatanen *et al.*[58]	[58]	T1 diabetes	3 months-15 years	783	10,913	WGS	RF	Error rates	0.45
2021	Wang *et al.*[55]	[1,137,145-150]	Maternal transmission	0-12 months	376	1,024	WGS	RF, GB	AUC	0.53-0.96
2023	Warner *et al.*[95]	[95]	Social & psychological adversity	4 months	121	121	16S/WGS	RF	AUC	0.72-0.87
2021	Xiao *et al.*[53]	[53,56,75,94,136,149-154]	Enterotypes	1-3 years	1,956	13,776	16S/WGS	RF	AUC	0.8
2019	Zhang *et al.*[90]	[90]	Atopic dermatitis	0-3 years	172	229	16S	RF	AUC	0.83

RF: Random Forest; GB: gradient boost; SVM: support vector machines; NN: neural networks; NB: naive bayes; KNN: K-nearest neighbours; EN: elastic nets; DBN: dynamic Bayesian network; LR: logistic regression; HMM: hidden Markov models; DMM: dirichlet multinomial mixtures; LASSO: lasso regression; SPLS: sparse partial least squares; EL: ensemble learning; DT: decision trees; SMOTE: synthetic minority oversampling technique; NA: network analysis; RT: randomised trees; MPPM: multilayer perceptron predictive models; AUC: under the curve.

modelling approaches and found an AUROC of up to 98% when predicting atopic dermatitis status[91].

Fernández-Edreira reported an AUC of 0.8 to 0.98 when using 16S data of the DIABIMMUNE cohort, including 33 infants. In their study, *Bacteroides uniformis*, *Bacteroides dorei*, *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron* were found to be most discriminatory between healthy controls and Type 1 Diabetes (T1D) cases, with abundances being lower in the latter[92]. Vatanen, however, reported a slightly better than random predictive ability with error rates of around 45% when predicting type 1 diabetes in the TEDDY cohort of infants[58]. Remarkably, ML approaches seem to be able to predict behavioural and neurodevelopmental conditions, including autism, with considerably high accuracy[93]. Loughman found microbial profiles, as opposed to clinical covariates, up to three months to be predictive of colic and problem crying in an Australian cohort[94]. Social and psychological adversity in four-month-old infants could be predicted with 72%-87% AUC using Random Forest algorithms, with mothers and infants showing diverse taxonomic profiles[95].

Importantly, some studies also successfully predicted future disease conditions. Boutin used 545 samples obtained from 343 infants between the ages of three and twelve months to predict allergies at five years of age and found an accuracy of 81% when employing Random Forest algorithms[96]. Metwally longitudinally predicted food allergies at age three with an AUC of 0.69 using a deep learning framework, which outperformed various other ML algorithms[10]. Stanislawski used 781 samples, collected from four days to two years, of 165 Norwegian infants in a Random Forest regressor, finding gut microbiome profiles at two years of age to explain more than 50% of variation in BMI z-scores at 12 years[97]. Finally, Korpela *et al.* used the first stool passed by infants, as well as a follow-up sample at one year of age, to predict infant growth[98]. Based on marked meconial differences in taxa belonging to *Bacteroides*, *Staphylococcus* and *Lactobacillus,* their Random Forest algorithm successfully classified overweight at age three with an AUC of 0.7.

In summary, the identification of single-taxon markers offers a simple, feasible way to directly quantify and interpret microbial patterns. They potentially also allow an easy implementation and use in clinical settings, facilitated by low-cost assays such as qPCR[99]. Moreover, many taxa and their mechanistic links to host physiology

Table 2. Predictive studies using microbial maturation indices to predict infant phenotypes

Year	Authors	Dataset	Condition	Age	N	Samples	Data	Findings
2015	Bäckhed *et al.*[1]	[1]	Feeding & birth mode	4-12 months	98	294	WGS	Advanced maturity in formula-fed and C-section infants
2020	Depner *et al.*[73]	[73]	Asthma	2-12 months	618	1,338	16S	Immaturity at 12 months associated with asthma, advanced maturity at 12 months as protective factor, *Blautia* as most age-discriminatory
2025	Fahur Bottino *et al.*[74]	[1,74,112,128,130,145,146,155-159]	Age	2-18 months	1,827	3,154	WGS	Uniform maturation patterns across the globe, *F. prausnitzii* & *Anaerostipes hadrus* as most age-discriminatory
2020	Galazzo *et al.*[82]	[82]	Birth mode, diet & atopic disorders	0-3 years	440	1453	16S	Breastfeeding as main driver of composition that delays maturation; AD related to higher early maturity and later immaturity
2023	Gao *et al.*[12]	[12]	Food allergy	1-12 months	323	~ 900	16S	Higher maturity at 12 months associated with lower odds of food allergy; more siblings were associated with higher maturity at 12 months
2019	Gasparrini *et al.*[75]	[75]	Infections	0-21 months	58	437	WGS	*F. prausnitzii* as most discriminatory of age in healthy infants; accurate MAZ scores in healthy infants; preterm infants show immaturity early on, which levels out around 12-15 months
2020	Hayden *et al.*[110]	[110]	Cystic fibrosis	3-12 months	207	1,157	WGS	Delayed maturation in CF infants, i.e. immaturity at 12 months
2024	Hickman *et al.*[19]	[19]	Health	0-5 years	984	7,211	16S	Microbial maturation highly predictable & predictive of health outcomes at 2 years & 5 years; taxa belonging to *Bifidobacterium* & *Bacteroides* were most influential in first two years
2023	Hoskinson *et al.*[108]	[160]	Allergy	0-5 years	589	1,238	WGS	Immaturity at 12 months related to allergies at 5 years
2019	Kamng'ona *et al.*[161]	[161]	Growth, inflammation	6-18 months	691	1,788	16S	Microbiota diversity and maturity were related to growth in weight from 6 to 12 months, but not to growth in length or head circumference or to growth from 12 to 18 months
2022	Kortekangas *et al.*[109]	[109]	Environmental enteric dysfunction	6-30 months	610	1,569	16S	Higher maturity at 18 months associated with lower EED biomarkers
2022	Lee *et al.*[107]	[107]	Atopic dermatitis	6-36 months	346	346	16S/WGS	*Bacteroides fragilis* and *F. prausnitzii* most discriminatory between AD and controls; advanced maturity in AD at 6 months, immaturity in AD at 12 months
2022	Lou *et al.*[106]	[106]	Autism spectrum disorder	11 months-19 years	1,202	1,202	16S	Immaturity at 18-30 months associated with ASD
2025	Naspolini *et al.*[57]	[57]	Feeding	3-9 months	525	966	WGS	Non-exclusively breastfed infants had higher maturity; EBF infants were closest to their chronological age; birth mode defines starting point: C-section/EBF had lowest maturity, Vaginal EBF the highest
2017	Pannaraj *et al.*[104]	[104]	Feeding	0-12 months	119	255	16S	Erysipelatotrichaceae, Bacteroidaceae and Ruminococcaceae most discriminatory between EBF and non-EBF (more abundant in non-EBF); higher maturity in infants earlier introduced to solids
2023	Robertson *et al.*[103]	[103]	Growth	1-18 months	335	875	WGS	Functional over compositional profiles predict growth; both functional and taxonomy predict age (*F. prausnitzii, Blautia wexlerae* & methanogenesis from acetate, L-tryptophan biosynth);
2018	Stewart *et al.*[13]	[13]	Age	3 months-15 years	903	12,005	16S	Immaturity in breastfed infants early on (3-30 months), but level out later (30-46 months)

2020	Stokholm *et al.*[162]	[9]	Asthma	0-5 years	700	1,784	16S	increased asthma risk in children born by c-section, but only if their microbial maturity at 1 year still showed c-section signature
2018	Stokholm *et al.*[9]	[9]	Asthma	0-5 years	690	1,696	16S	Immaturity at 12 months associated with asthma at 5 years
2014	Subramanian *et al.*[76]	[76]	Malnutrition	0-2 years	126	1, 585	16S	Immaturity in malnourished infants, *F. prausnitzii* most age-discriminatory
2023	Yong *et al.*[11]	[11]	Obesity	1-6 months	349	636	16S/ITS-2	higher early taxonomic and functional maturity (1 month) increased risk for obesity (24 months)
2022	Zhang *et al.*[105]	[105]	Growth	3-12 months	152	152	16S	*Actinomyces* spp most age discriminatory; immaturity in first year of life associated with failure to thrive

WGS: Whole-metagenomic shotgun sequencing; 16S: 16S rRNA gene sequencing; ITS-2: internal transcribed spacer region 2 sequencing for fungal communities; AD: atopic dermatitis; MAZ: microbiota-age-Z-score: EED: environmental enteric dysfunction; ASD: autism spectrum disorder; EBF: exclusively breastfed.

have been identified, i.e. *Bifidobacterium* and immune development[100], highlighting their potential as actionable biomarkers for monitoring early-life health. Importantly, taxa identification is constrained by the limits of microbiome data acquisition and processing, where differences between 16S and shotgun sequencing and gaps in database alignment limit the resolution, completeness and reliability of the resulting microbial profiles[44,101]. Moreover, focusing on individual taxa may oversimplify the highly dynamic nature of microbial ecosystems, overlooking their complex interconnections. As such, community-level classifications, such as enterotype clustering, capture broader ecological relationships and integrate multiple microbial interactions relevant to disease risk[53]. Nevertheless, allowing the emergence of temporally-stable clusters is limited by the aforementioned rapid shifts in early-life microbiomes, as well as highly variable microbial baselines[1]. Consequently, clusters differ between datasets, methodologies and geographies[53,102], raising concerns about their reproducibility and deeming them currently more useful in research as opposed to clinical settings.

Microbial maturation as a proxy for healthy developmental trajectories

Quantifying microbial succession as a process rather than utilising individual or groups of taxa provides a dynamic measure well-suited to the temporal nature of microbiome assembly. These studies use so-called microbial maturation scores, indices that assess the developmental maturity of an infant's gut microbiome relative to healthy peers of the same chronological age. Such indices can be calculated in various ways[19,73], however, the most commonly used indices are microbiota-for-age-Z-scores or MAZ-scores[76]. First coined by Subramanian et al. to study microbial maturity in early malnutrition[76], MAZ scores are calculated by training ML models, often Random Forests, on healthy reference cohorts to predict "microbiota age" based on the abundance and presence of key microbial taxa known to change with age. The MAZ score is then derived by subtracting the child's chronological age from their predicted microbiota age, and dividing this difference by the standard deviation of microbiota ages for that age group. Consequently, MAZ-scores are a proxy for chronological age based on microbial composition, with a value of zero denoting a typical microbiome composition for that age, while a negative and positive score indicate immaturity and advanced maturation, respectively[76].

Importantly, MAZ scores have been used to quantify 'healthy' microbial succession patterns, with studies identifying not only highly-predictable patterns across the globe[19,53,74], but also overlapping age-discriminatory compositional and functional taxa[74-76,103], underscoring the possibility of defining normative trajectories of healthy

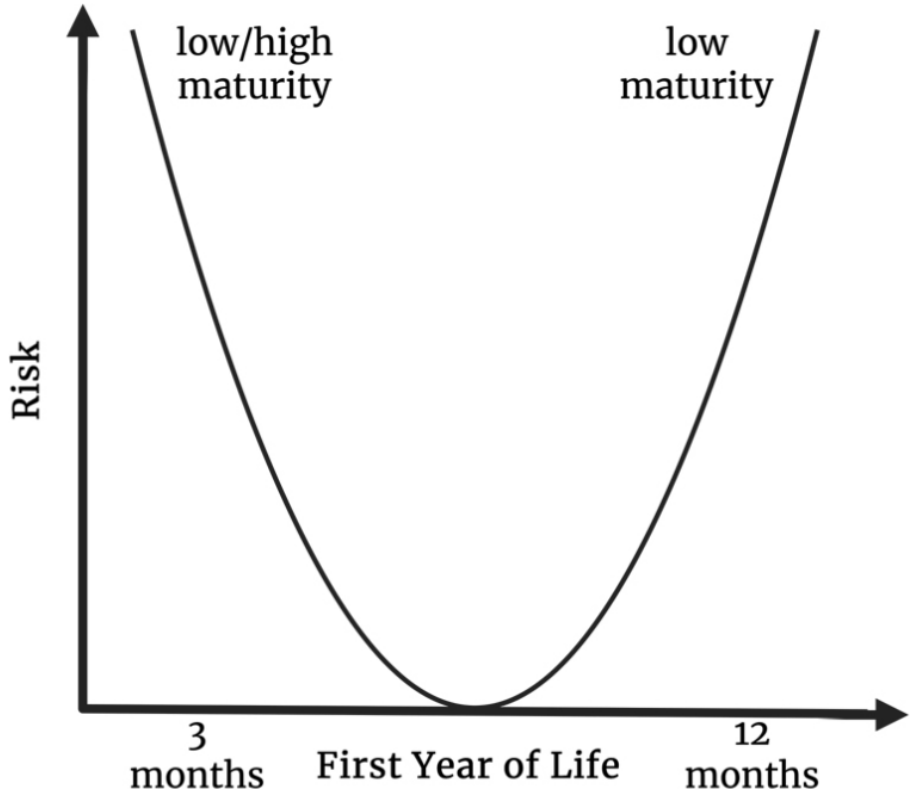


Figure 2. Early-life microbial maturity trajectories and implications for health; Conceptual overview summarising evidence that altered gut microbial maturation during infancy is linked to disease risk. Both unusually low or high microbial maturity in early life (birth - 3 months) and delayed maturation, characterised by low microbial maturity at 12 months, are associated with increased susceptibility to adverse health outcomes. Created in BioRender. Lavelle, A. (2026) https://BioRender.com/sxuv56p

microbiota development and establishing a potential clinical reference framework. These trajectories are thought to be highly influenced by early-life exposures such as family environment and, most importantly, an interactive combination of birth mode and feeding practices[1,12,13,57,76,104]. Bäckhed reported high maturity in formula-fed infants born via cesarean section, while Naspolini found breastfed infants born via cesarean section to have the lowest maturity and vaginally-born breastfed infants to have the highest maturity[57]. Exclusive breastfeeding emerged as the primary factor delaying microbial maturation[1,13,82], yet maintaining maturation scores most closely aligned with chronological age[57], reflecting a normative trajectory.

Deviations from these expected patterns are thought to carry negative implications. Indeed, many studies indicate that both immaturity and over-maturity in early life pose risk factors for adverse health conditions, suggesting a U-shaped distribution as illustrated in Figure 2: Distribution of microbial maturity and associated risk. As such, previous research found early-life gut immaturity to be associated with growth failure[105] and autism spectrum disorder[106], while other studies found advanced maturity in early infancy as a risk factor for obesity[11] and (food-) allergies[12,96]. Strikingly, the majority of studies investigating microbial maturation with health conditions report immaturity at one year of age or later to be predictive of diseases such as dermatitis[107] and other atopic conditions such as asthma[9,73,82] and (food) allergies[12,96,108], environmental enteric dysfunction[109], autism spectrum disorder[106], obesity[11] and impaired growth in cystic fibrosis[110], as well as overall failure to thrive[76,103,105]. In line with these findings, a proof of concept preclinical study implanted faecal communities of varying maturity into germ-free mice and observed clear phenotypic differences, e.g. the gain of lean body mass, bone structure and metabolism[111].

Taken together, studies utilising microbiota age scores attempt to define a 'healthy' developmental baseline; these indices act as a proxy, offering a standard against which deviations can be assessed, capturing delays or accelerated progression that may indicate risk. Unlike single-taxon or community-level measures, maturation indices are sensitive to age-related changes, reflecting the well-established role of time and developmental stage as primary drivers of infant microbiome composition[1,13,74]. They integrate information across multiple taxa and functions, offering a more holistic view of microbiome development, and have been shown to generalise across geographic populations[74], thereby providing the greatest long-term promise as clinically relevant biomarkers that align closely with the biological reality of a developing microbiome. Nevertheless, attempting to create a benchmark of normative trajectories depends heavily on the quality of relative abundance data and representativeness of the reference cohort, demanding large and methodologically homogeneous datasets, sampled from a range of different populations[22]. Moreover, if used in a cross-sectional way, the metric might simplify longitudinal trajectories by assuming a linear development and neglecting baseline differences in samples. Indeed, Hickman reported not time-point specific maturity, but sequential trajectories, which take baselines as well as previous microbial communities into consideration, as predictive of later health and disease states[19], underscoring the superiority of longitudinal approaches over static maturity estimates. Overall, these findings underscore the value of microbial maturation scores as a reference framework for defining healthy microbiome development and identifying deviations linked to disease risk in infancy, with the potential to guide early-life interventions in a clinical setting.

KEY CHALLENGES IN PREDICTIVE MODELLING OF THE EARLY-LIFE MICROBIOME

However, several recurrent limitations across the above-mentioned studies emerge, tempering the strength of their conclusions. Early-life microbiome data introduce a distinct set of analytical obstacles, primarily driven by their variability[1]. Infant microbial communities are highly influenced by peri- and postnatal exposures, including birth mode, gestational age, antibiotic exposure, and rapidly evolving feeding practices[13,112]. Driving substantial inter-individual variability, these factors make it difficult for models to learn stable patterns[23,24]. Few of the studies adjust for a full range of these covariates[74], making it difficult to disentangle microbiome-specific predictive signals from the influence of these wider determinants.

Another concern in prediction studies is overfitting due to the imbalance between high-dimensional microbial features and relatively small sample sizes[23,48,113]. Indeed, several key findings, including predictions of urinary tract infections[114], atopic dermatitis[91], Type 1 diabetes[92], behavioural outcomes[95], and NEC[84], are derived from cohorts with fewer than 150 samples, raising questions about the representativeness of their conclusions. While sample sizes are increasing, and several recent datasets now include thousands of samples, continued expansion of well-powered, multi-cohort studies will be essential to improve generalisability and reduce model variance.

Algorithm choice is another critical factor in infant microbiome modelling, as several of the reviewed studies report differing predictive performances and findings depending on the model applied[10,77,85,89]. Infant microbiome data is compositional by nature, which violates the independence assumptions underlying many standard statistical models and can introduce spurious associations if not properly handled[115]. This challenge is further compounded by the longitudinal nature of early-life sampling, where repeated measurements vary substantially across timepoints and between case-control groups. As a result, careful preprocessing, covariate adjustment, and model selection are essential to reduce noise and improve generalisability. As such, among classical ML methods, Random Forest has consistently demonstrated strong suitability for microbiome data[16,115-118]. Random Forests are particularly advantageous due to their robustness to high-dimensional, sparse, and noisy data, minimal preprocessing requirements, and ability to provide intuitive measures of feature importance[117,118]. Gradient boosting methods, including XGBoost and

LightGBM, offer strong predictive performance by sequentially optimising models, and can capture complex non-linear relationships effectively[77,118]. However, they are more sensitive to hyperparameter tuning, computationally intensive, and may be prone to overfitting in smaller datasets[115]. As a result, their performance is often dataset-dependent rather than universally superior. More complex approaches, including deep learning models such as recurrent and convolutional neural networks, may be better suited for capturing temporal dynamics in infant microbiome data[10,24]. However, these methods require substantially larger sample sizes and are computationally intensive, limiting their applicability in most current early-life studies. In practice, when data are limited, as is often the case in infant microbiome research, simpler ensemble methods tend to outperform deep learning approaches[24]. However, performance differences between ML methods are often dataset-dependent, and no single algorithm consistently dominates across all microbiome applications.

Notably, across all model classes, interpretability remains a key limitation. While Random Forests decision trees provide some degree of transparency, more complex models, including gradient boosting and neural networks, are often considered 'black boxes'[115,119]. This poses a challenge for clinical translation, as identifying which taxa drive predictions is essential for biomarker discovery and potential pharmaceutical use. Post hoc interpretability tools, including SHapley Additive exPlanations (SHAP) values or permutation importance[120,121], can partially address this issue, but they remain underutilised. Taken together, these considerations suggest that infant microbiome data should be handled with methods that account for compositionality, incorporate feature selection, and balance predictive performance with interpretability. Given current data constraints, ensemble tree-based methods represent robust and pragmatic choices, while more complex models may become increasingly relevant as larger, standardised datasets become available.

An additional critical and often overlooked limitation is inadequate validation. Across the studies reviewed, validation is almost exclusively restricted to internal or, in rare cases, external cohort-based evaluation. Internally validated models tend to produce overly optimistic performance estimates, with average intra-cohort performance reaching an AUC of 0.8 or higher, which typically declines substantially in external validation across independent cohorts[16]. This reduction in performance likely reflects both biological variability (e.g. due to differences in geography[52]) and technical heterogeneity, including differences in sample collection, sequencing platforms, and bioinformatic pipelines[115]. Notably, none of the ML-based studies reviewed here incorporated experimental validation, such as *in vivo* or *in vitro* testing of model-derived microbial signatures. Only one study extended beyond prediction by performing downstream mechanistic investigations in necrotising enterocolitis[88]. Casaburi and colleagues experimentally tested metabolite-driven hypotheses derived from metagenomic analyses *in vitro* and *in vivo*, demonstrating dose-dependent epithelial toxicity of candidate metabolites such as formate in rodent and human cell models. While this provides important mechanistic support for microbiome-associated disease signatures, it does not constitute validation of predictive ML models themselves, but rather offers biological plausibility for inferred microbial-metabolic associations[88].

CONCLUSIONS

In summary, current early-life ML studies provide an important foundation for future translational research. The absence of independent and experimental follow-up limits causal inference, making it difficult to determine whether previously identified microbial signatures are drivers of disease or merely correlates of underlying pathology. As larger multi-cohort datasets, longitudinal study designs, and harmonised analytical frameworks continue to emerge, microbiome-derived signals are likely to become increasingly robust, reproducible, and clinically meaningful.

As such, rather than serving as immediate diagnostic tools, current predictive models should therefore be viewed as early-stage frameworks for identifying candidate risk patterns and guiding hypothesis generation. In the long term, integrating computational predictions with experimental validation and mechanistic studies will be essential for refining these signals into biologically grounded biomarkers. With continued methodological standardisation and expanding data availability, the early-life microbiome holds strong potential to support earlier risk stratification, improved disease prediction, and ultimately more targeted preventative strategies in paediatric healthcare.

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Stanton C is an Executive Editor of the journal *Microbiome Research Reports* and Ross RP is a Senior Editor of the journal. They were not involved in any steps of editorial processing, notably including reviewers' selection, manuscript handling and decision making. The other authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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