The Development of the Gut Microbiota in Childhood and Its Distortion by Lifestyle Changes

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Abstract The gut microbiota is established in the newborn period and plays a pivotal role in the development of the mucosal tissue, immune maturation, and host metabolism. Distortions in the assembly and maturation of the microbiota during this critical time-window can therefore have profound effects on future health and the susceptibility to non-communicable diseases.

In this chapter, we provide an overview of the ecological processes involved in the establishment of the indigenous microbial communities during infancy and childhood. Moreover, we summarize the current knowledge on the disruptive effects of lifestyle changes on gut microbiota assembly and maturation. Finally, we highlight important areas for further research in order to identify approaches to revert the deprivation of our microbiota.

Keywords Ecological theory · Microbiota assembly · Birth mode · Breastfeeding · Complementary food · Social interactions · Natural environment · Antibiotics

1 Introduction

The indigenous microbiota of the human gut has long been recognized to contribute to health and disease by influencing gut and immune maturation, host nutrition, and protection against pathogen invasion [1].

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In line with this, perturbations in the development and composition of the gut microbiota have been associated with the development of obesity [2–4], allergies [5–7], inflammatory bowel diseases [8–10], and many other non-communicable diseases [11, 12].

Infancy sets the stage for intestinal microbial assembly, diversification, and maturation. Moreover, this period is a critical time-window during which the microbiota provides a stimulus for the ontogeny of the enteric mucosal tissue and immune system with persistent local as well as systemic effects [13, 14]. Insights into the processes that drive the first inoculum to differentiate into a highly individualized [6, 15–18] and stable microbial ecosystem, as established after the first years of life, will therefore have a direct impact on our ability to manage and maintain human health [19, 20]. To develop successful strategies to restore or maintain a healthy microbiota, we thus need to refine our understanding of the processes driving the inter-individual variation in microbial composition and assembly. It is increasingly being recognized that the principles of ecological theory can help us to understand and predict community variations in the human microbiota [19].

In this chapter we will discuss the development of the gut microbiota during infancy and childhood from an ecological theoretical perspective, and describe how lifestyle changes may distort the natural development of microbial assembly and maturation.

2 Ecological Principles of Microbial Community Assembly

The ecologist Mark Vellend has synthesized various concepts of community assembly by categorizing the underlying processes into four groups: dispersal, selection, drift, and diversification [21, 22].

Dispersal of bacteria from the meta-community is an important process to seed the initially sterile infant gut but is a process that also plays an important role thereafter. The meta-community consists of numerous local communities, some of which are host-related (e.g., maternal skin, gut, and vaginal microbiota), while others are not (e.g., soil, food-born, building environment microbial communities) [23].

In this respect, priority effects describe how the order and timing of dispersal from the meta-community might alter how diversification, drift, and selection affect the assembly of the infant gut microbiota. In other words, the impact that particular species can have on the community assembly in the infant gut would depend on the timing and order in which they arrive (history) [23, 24].

Environmental selection or niche-based interaction is the deterministic process that, based upon the conditions in a given habitat, drives differences in growth and death rates among microbial taxa based upon their fitness and niche-differences [23]. Diet, host metabolites, and the immune system are primary sources that drive environmental selection. From the perspective of environmental selection, the human body can, for example, be seen as a "habitat filter"—a collection of

conditions and resources that nourish the growth of some microbes, but not others. This view implies that the interaction is unidirectional; the host shapes the microbiota. However, in many circumstances of environmental filtering, the host-microbe interactions are bi-directional [19]. The interactions between the host immune system and the microbiota are a classical example, but also the interactions between host metabolites such as bile acids and the microbiota [25] exemplify such bi-directional selection processes. Also, the abrupt shifts in the composition and structure of the microbiota that are commonly observed during an infectious episode or a course of antibiotics are examples of environmental selection processes.

Ecological drift is a completely stochastic (chance-driven) event in which changes in species population size occur regardless of species identity [23, 26]. The effect of drift is expected to be stronger on low abundant species as slight negative changes in their abundance could already push them stochastically to local extinction, unless they have (or gain, e.g., via diversification) a competitive advantage or become replenished by dispersal from outside the local community [19, 27].

It is however almost impossible to distinguish the effect of drift from the effect of other ecological processes except when studied in an experimentally controlled setting [26].

Diversification is the process of the generation of new genetic variants, often as a result of a persistent selective pressure. Highly abundant and dense microbial populations, rapid growth rates, and strong selective pressures, conditions that are all met in the adult human gastrointestinal tract, can fuel microbial adaptation via mutation or recombination (e.g., via horizontal gene transfer) [19]. However, as the selective regimes during infancy frequently shift as a result of, among other factors, a developing host and alterations in feeding regimes, the degree to which diversification is involved in the infant gut during assembly remains largely unknown [23].

Neutral Community Assembly

A theoretical framework of community assembly which assumes that dispersal, diversification, and ecological drift are completely stochastic processes is the **neutral community model** (NCM). According to this model, neither environmental selection nor inherent species differences in their ability to disperse or diversify play a role in the community assembly. Although such models do not account for deterministic factors and make many simplifying assumptions, they have among others successfully been applied to predict the structures of aquatic and respiratory microbial communities [28, 29]. Such models moreover are important for gaining insight into the importance of neutral dispersal in shaping the structure of microbial communities, to identify conditions that lead to divergence from neutral dynamics or to identify microbial taxa that do not assemble in a neutral manner [30]. NCM has also been applied to the assembly of gut microbial communities. In a study on the

(continued)

zebrafish intestinal microbial communities from larvae to adulthood, the importance of non-neutral processes increased as the host matured [31]. A recent study among the Tsimane, an indigenous Bolivian population, also underscored the importance of neutral forces in shaping microbiota assembly in early life and to a lesser extent in adulthood [30]. These observations suggest that a significant amount of diversity in microbial community structures between individuals could be explained by neutral processes of drift and dispersal [31].

Glossary of Terms and Definitions Used in This Review

Terminology	Description	Reference
Community	A group of potentially interacting species that live together in a specified place and time	[21, 27]
Dispersal	Movement of microorganisms across space	[19, 21]
Ecological drift	Stochastic changes in the relative abundance of different microbial taxa within a community throughout time as a result of birth, death, and reproduction	[19, 21, 32]
Environmental selection	Changes in the microbial community structure as a result of deterministic fitness differences between microbial taxa	[19, 21]
In situ diversification	Generation of new genetic variants by mutation or recombination (e.g., via horizontal gene transfer)	[19, 21]
Meta-community	A set of local communities that are linked by dispersal of multiple potentially interacting species	[33]
Neutral assembly theory/ neutral community model	Theory/model assuming that dispersal, diversification, and ecological drift are completely stochastic processes and that neither environmental selection nor species traits play a role in community assembly	[19, 34]
Perturbation	An external event/stressor that causes a distinct selective pressure on the ecosystem, also called disturbance	[35, 36]
Priority effects/historical contingency	The order in which species arrive at local sites (e.g., in the infant gut) dictates the effect of species on one another	[19, 21, 32]
Resilience	The property of a microbial ecosystem that defines how fast, and to what extent it will recover its initial functional or taxonomical composition following perturbation	[35, 36]
Resistance	The power of an ecosystem to remain unchanged upon a perturbation	[35, 36]

3 Establishment of the Microbiome

As compared to the microbiome of adults, which has been suggested to be relatively stable and resilient [37–39], the microbiome in infants is highly dynamic. The microbial richness and diversity gradually increase from early infancy to childhood, while the variation in microbial composition between children decreases [6, 39].

The first colonizers of the infant gut microbiota are typically facultative anaerobes, particularly *Enterobacteriaceae*, but also facultative anaerobic genera within the Firmicutes phylum including staphylococci, streptococci, lactobacilli, and enterococci [6, 40–43]. Although members of the *Enterobacteriaceae* were shown to be specific for the infant gut, most of the initial colonizers are homogeneously distributed across different body sites [44]. During the following months, obligate anaerobes, including *Bifidobacterium*, *Bacteroides*, *Veillonella*, and *Clostridium*, start to dominate [6, 43]. While *Enterobacteriaceae* wane, they still have an important share during the first half year of life after which their abundance further decreases [6, 40, 41, 43]. Under the impact of weaning and transition to an adult-like diet, *Veillonella* and *Bifidobacterium* start to decrease, *Bacteroides* further increase, and members of the *Lachnospiraceae* (e.g., *Blautia* and Roseburia) and *Ruminococcaceae* (e.g., *Faecalibacterium*, *Ruminococcus*) rise to become dominant members of the gut microbiota [6, 41–43]. Many of these bacteria are butyrate-producers, and a concomitant increase in butyrate levels has indeed been observed [45, 46].

Finally, some prokaryotes, including the archaeal genus *Methanobrevibacter* and the bacterial genera *Desulfovibrio*, *Bilophila*, and members of the *Christensenellaceae* family, only colonize after infancy and keep rising in prevalence and abundance beyond the age of 5 years [39]. This indicates that the adequate niche has first to be created before these genera can settle. These niche formations are partly driven by the prior arrival of other microbial taxa for necessary cross-feeding interactions, but also on a fully reduced environment and the availability of complex dietary carbohydrates.

Several studies have aimed to identify distinct phases of microbiome progression [6, 39, 43, 47]. Although the exact period differed between studies, it is evident that most rapid changes in microbiome development occur within the first 6–12 months of life. Microbiome maturation thereafter continues in a less profound manner, but the exact age at which a stable adult-like microbial community structure is reached is still a matter of debate. While it has previously been suggested that stabilization of the microbiome occurs around the age of 2–3 years [47, 48], differences could still be observed in the microbiome of Swedish 5-year-old [39] and Dutch 6–9-year-old children [49] as compared to Swedish and Dutch adults, respectively. A recent meta-analysis on metagenomic data from over 1900 fecal samples from nine studies confirmed that the microbiome could predict a child's chronological age well beyond the first 3 years of life [42]. In part, this controversy can be attributed to the sparseness of data on the microbiome of children beyond the age of 3 years [37].

4 Determinants of Infant Gut Microbiome Assembly and the Impact of Lifestyle Changes (Fig. 1)

4.1 Seeding of Newborn Ecosystem

The microbial species that colonize first play a crucial role in the development of the ecosystem in the gut, potentially influencing the ultimate composition and functionality of the microbiota for life. However, at what stage the first microbes start to seed and colonize the gut is still a matter of debate. In 2014, Agaard and colleagues reported the existence of a microbiome in the placenta of healthy women [50]. Since then, many other human studies detected microbial signals in amniotic fluid, fetal intestine, cord blood, placenta, or meconium samples, thereby suggesting in utero colonization and challenging the concept that seeding of the gut ecosystem initiates at birth [51–54]. In utero dispersal of microbes would have tremendous implications for fetal and neonatal health and development, but objections were raised against
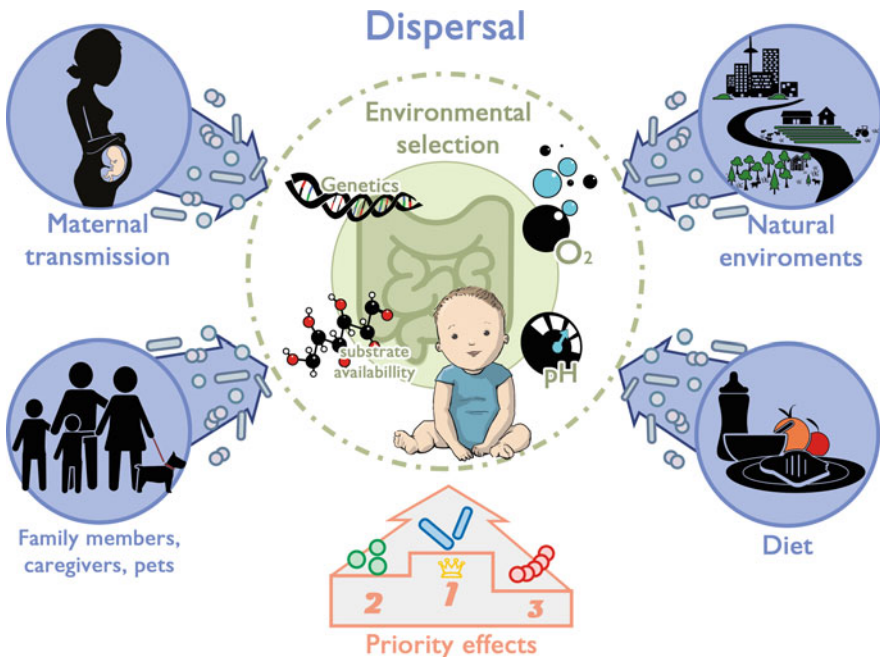

Fig. 1 Infographic depicting the main ecological processes and deterministic factors shaping the microbiota throughout infancy and childhood. Main sources of microbial dispersal to the infant gut comprise the infant's mother and other family members, pets and peers, food sources, and the natural environment (depicted in blue). The order at which microbial taxa arrive in the infant gut may dictate the colonization success of subsequent incoming bacteria (depicted in orange). Additionally, the environment of the infant gastrointestinal tract may select for or exclude specific microbial taxa (depicted in green). This environmental selection is driven by among others the substrate availability, oxygen concentration, pH, and host genetics

most of these studies (see review [52]). First, the majority of studies relied on the detection of bacterial DNA which does not confirm the existence of a living microbial community [55]. Second, no consistent microbial profile was detected across the different studies (i.e., the dominant bacterial taxa varied widely between studies *Micrococcus*, *Lactobacillus*, *Staphylococcus*). Lastly, it appeared that results were biased by contamination, i.e., the detected bacteria in low-biomass samples were also identified in negative controls [56, 57]. As a result, many other studies that appropriately controlled for contamination could not provide evidence of microbial taxa in utero [58–62]. On the other hand, recent studies demonstrated the existence of viable bacteria-like morphology in the fetal intestine and placenta by culture and microscopy [53, 63] albeit debated as well [64, 65]. In addition, oral administration of trackable bacteria (e.g., genetically labeled *Enterococcus faecium*) to pregnant female mice could be recovered from amniotic fluid and meconium of the offspring [66, 67]. Today, the debate still continues, and it is still questionable whether a prenatal intrauterine microbiome exists.

Birth is likely the first step in seeding the newborn gut and definitely the most dramatic one, given the amount and diversity of microbes to which a newborn gets exposed. The passage through the birth canal has long been recognized as the major transmission of first microbes, i.e., *Lactobacillus* and *Prevotella* [68, 69]. However, studies comparing microbial strain profiles of infant fecal to both maternal vaginal and rectal samples revealed that mother-to-child transmission mainly occurs for rectal rather than vaginal strains [42, 70]. In particular, as consistently shown in numerous studies, maternal *Bacteroides* strains are most frequently transferred to the intestine of neonates born via a natural delivery [6, 70–72].

The mode of delivery evidently impacts the dispersal of microorganisms from the mother and results in distinct microbial profiles between infants born vaginally and via Cesarean section (C-section). The microbiota of C-section delivered infants is characterized by delayed colonization of mainly *Bacteroides* and *Bifidobacterium* compared to vaginally delivered infants [40, 43, 47, 70, 71, 73–75]. In a recent study, we longitudinally monitored the establishment of the human infant gut microbiota and further supported that birth mode most strongly affects members of the genus *Bacteroides* [6]. The decreased levels of *Bacteroides* in infants born by C-section (Cesarean section) remained significant after careful adjustment for other confounders and persisted for at least 31 weeks [6]. In addition, other studies demonstrated that this founder effect could even last until 4-years postpartum [47, 76]. Although C-section is accompanied by prophylactic antibiotic administration to the mother, the effect of delivery mode on the infant gut microbiota has recently been demonstrated to be independent of intrapartum antibiotic prophylaxis (IAP) [77]. In this study, IAP was administered only after clamping of the umbilical cord in mothers undergoing C-section, enabling the researchers to examine the impact of the birth mode on the neonatal microbiota in the absence of antibiotic exposure to the baby. The latter study confirmed the lower levels of *Bacteroides* spp. and *Bifidobacterium* spp. in C-section delivered infants. Altogether, this suggests that dispersal of maternal microbes during vaginal delivery is crucial to acquire certain microbial species in early life.

Cesarean section delivery is perhaps one of the factors most commonly linked to distortions in the microbiota establishment in early life [40, 71]. C-section numbers are rising worldwide [78]. Nowadays one out of five babies is delivered by C-section with numbers having almost doubled since 2000, while in Brazil rates have risen up to 45%. Notably, the Netherlands has one of the lowest C-section rates (17%) among developed countries, especially when compared to neighboring countries such as Germany (30%) [78]. The latter might be because pregnant women at low risk of birth complications get unique midwife-led care in the Netherlands which is associated with lower intervention rates [79, 80]. Although in some cases it might be a life-saving procedure, the high numbers of C-sections without medical reason (i.e., elective C-section) are worrisome.

Many efforts have recently been made to reverse the dispersal limitation in C-section delivered neonates by exposing them to the maternal vaginal microbiota immediately upon delivery, a procedure termed "vaginal seeding" or "bacterial baptism" [81]. The recent evidence that maternal fecal rather than vaginal bacteria are depleted in the intestinal microbiota of C-section delivered neonates [42, 70], however, challenges the efficacy of vaginal seeding to abolish the dispersal limitation during C-section. In a recent proof-of-principle study by Korpela et al., fecal microbiota transplantation (FMT) was therefore used to restore the microbiota in infants born by C-section [82]. An enhancement of *Bacteroidaceae* and *Bifidobacteriaceae* in FMT-treated as compared to untreated infants was observed without any short-term adverse effects. Although FMT might be a more successful strategy to restore maternal transfer in C-section delivered infants, concerns remain on the risks and uncertainties of this treatment (i.e., dispersal of pathogens or overstimulation). It is therefore crucial to obtain further insight into the maternal microbial species that C-section delivered infants fail to achieve and the corresponding health implications in order to move towards more controlled attempts to restore the initial microbial colonization of newborns, e.g., in the form of synthetic microbial transplants.

4.2 Infant Diet—Environmental Selection and Dispersal of Milk Microbes

The World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) advocate exclusive breastfeeding (EBF) for the first 6 months of life [83]. Despite these global recommendations, breastfeeding prevalence, particularly EBF, is low in Europe and the Americas with dramatic disparities between countries [84].

Low rates and early cessation of breastfeeding have important adverse public health impacts. Besides nutrients, breast milk contains a wide variety of bioactive factors (e.g., lysozyme, lactoferrin, and immunoglobulins) that support the

development and maturation of the infant gut, the innate and adaptive immunity, and systemic metabolism [85].

Moreover, breastfeeding plays a crucial role in the establishment of the infant gut microbiome both directly, by the dispersal of living bacteria in breast milk, and indirectly, by environmental selection in the form of prebiotic nutrient substrates and bioactive components.

Breastfeeding directly impacts the infant microbiome by dispersal of viable microorganisms in human milk. An exclusively breastfed infant will daily consume the significant amount of approximately 10^5–10^7 commensal bacteria while suckling [86]. Facultative anaerobic skin and throat bacteria, such as members of the genera *Staphylococcus*, *Streptococcus*, and *Cutibacterium*, are the predominant bacteria detected in breast milk by traditional culturing approaches [86, 87]. At lower concentrations lactic acid bacteria, including *Lactobacillus* and *Enterococcus*, members of the gram-negative *Enterobacteriaceae*, as well as obligate anaerobes, such as *Bifidobacterium* and *Veillonella* have been frequently isolated from breast milk [86–88]. The application of culture-independent molecular approaches has revealed a human milk microbial diversity beyond expectancy, including major gut-associated obligate anaerobes such as *Bacteroides* and several members of *Clostridia*, including the butyrate-producing *Faecalibacterium* and *Roseburia*, which are important for colonic health [87]. The origin of the microbes in breast milk is not fully understood, but likely involved the maternal skin, the infant's oral cavity during suckling, and potentially even the mother's gut via the entero-mammary pathway [89]. The exact composition of the human milk microbiome is highly variable and potentially influenced by geography, birth mode, lactation stage, as well as diet, health status, medication use, and genotype of the mother [89, 90]. Numerous studies have shown the importance of breast milk microbiota as an important source of successfully seeding the infant gut. Identical strains of *Bifidobacterium*, *Lactobacillus*, and *Staphylococcus* have been isolated from breast milk and infant feces in mother-infant dyads [91–93]. Transmission of bifidobacterial strains from breastmilk to the infant gut has additionally been demonstrated by a combination of gene marker-based amplicon sequencing, metagenomic shotgun sequencing, and strain isolation followed by genomic analysis [91, 94].

The dispersal of milk microbes to the infant gut obviously contributes to the profound differences in the microbial communities in breastfed as compared to formula-fed infants. The indigenous microbiota of exclusively breastfed infants is low in diversity as a result of the predominance of bifidobacteria, which can account for up to 70–80% of all bacteria in the stools of breastfed infants [6, 95]. In addition, lactobacillus species that are commonly used as probiotics have also been found to be enriched among breastfed infants [73]. Although the absolute bifidobacterium counts in infants receiving formula feeding tend to be as high as in breastfed infants [96–100], the microbiota of formula-fed infants is characterized by a much higher diversity and increased numbers of *Bacteroides*, *Clostridium*, and *Enterobacteriaceae*, including opportunistic pathogens such as *Clostridioides difficile* and *E. coli* [6, 99, 101–103]. Moreover, the *Bifidobacterium* composition at the species level differs according to feeding type with *B. catenulatum*, and

B. adolescentis, species that are commonly found in adults, being relatively more abundant in formula-fed infants [73, 104]. In breastfed infants, bifidobacterial species that thrive on human milk oligosaccharides (HMOs), including *B. longum*, *B. bifidum*, and *B. breve*, prevail [37, 47, 73]. Over 200 different types of HMOs have been known to be present in breastmilk, and the composition is affected by genetic factors, such as secretor genotype, suggesting that the maternal genome can affect the infant microbiota [37]. Indeed, Lacto-*N*-fucopentaose I and 2-′-fucosyllactose, which are dominant HMOs in secretor women, but absent in non-secretors, are associated with the infant microbiota composition [105]. Next to the important selective pressure of HMOs, other bioactive compounds, including lysozyme, lactoferrin, and immunoglobulins, may also impact the infant microbiota [106–108].

4.3   Introduction of Complementary Foods

Weaning is a critical next step in microbiota maturation as the indigenous microbiota becomes exposed to a variety of food components, including plant- and animal-derived glycans. Upon weaning the microbial diversity increases as a result of the complementation and gradual replacement of HMO-utilizing bacteria, such as bifidobacteria, by a more complex ecosystem consisting of specialists such as members of *Bacteroides*, *Lachnospiraceae*, and *Ruminococcaceae* which are more capable of degrading complex plant-derived carbohydrates and starch [37, 73, 109]. The major pectin-degrading enzyme, pectinesterase, has, for example, been shown to be enriched in infants by the age of 1 year, most likely resulting from the increased intake of pectin-rich foods as compared to younger infants [73]. In line with the increased capacity to ferment complex dietary carbohydrates, levels of fecal short-chain fatty acids increase [37, 45]. Additionally, the microbiome shows an increased capacity to produce amino acids and vitamins and metabolize xenobiotics following the introduction of solid foods [37, 73, 110].

The particular effects of complementary foods, however, strongly depend on the geography in line with the major differences in dietary habits around the world. The intestinal microbiota of non-Western populations, known for their high consumption of dietary fibers, has consistently been shown to be more diverse, enriched in *Prevotella* and depleted in *Bacteroides* when compared to populations in Western countries consuming a diet high in simple sugars, starch, and animal fat and protein [111]. Along with other profound differences, this trade-off between *Prevotella* and *Bacteroides* was also reported in a study comparing the microbiota of children living in rural Africa and Europe [112]. However, among children that were still being breastfed, these differences between both populations were not yet apparent, highlighting that both cessation of breastfeeding in combination with the subsequent dietary pattern drive the geographical differences in the intestinal microbiota composition.

Next to the selective pressure of dietary substrate availability, food is also a major source of allochthonous bacteria, ranging from 10^4 to 10^9 bacteria per gram of food with fermented food having the highest bacterial counts [113]. Interestingly, a diet meal plan as recommended by the US Department of Agriculture (emphasizing fruits and vegetables, lean meat, dairy, and whole grains) was found to contain a thousandfold higher numbers of viable bacteria than an average American diet [113]. Reduced consumption of fresh fruits and vegetables and increased consumption of ultra-processed foods (e.g., preserved meats, refined grains, hydrogenated oils) as observed in typical western diets thus substantially reduces the ingestion of food-borne microbes.

Knowledge on the fate of food-borne microbes is largely limited to probiotic bacteria from the genera *Lactobacillus* and *Bifidobacterium* and shows only a transient integration of such bacteria in the intestinal microbiome [113]. However, this may be different for microbial specialists that can colonize intestinal niches that emerge in later infancy or childhood. The archaeon *Methanobrevibacter smithii*, which only colonizes after infancy [39], has, for example, been shown to be more prevalent among school-aged children consuming organic dairy products [114]. Molecular analysis confirmed the presence of *M. smithii* in milk products suggesting that this may be a source of archaea colonization in children.

4.4 Dispersal from Siblings, Peers, and Pets

Social interactions with siblings and peers (e.g., during daycare attendance) may result in dispersal of microbes. Singletons have indeed been shown to have a distinct colonization pattern when compared to infants that grow up together with older siblings. The Canadian Healthy Infant Longitudinal Development (CHILD) cohort showed a lower abundance of *Clostridioides difficile* and its family *Peptostreptococcaceae* in 4-month-old infants with older siblings as compared to singletons [115]. A longitudinal German study reported that exposure to older siblings was associated with an increased diversity as well as increased levels in several genera within the phylum of Actinobacteria (*Bifidobacterium* and *Corynebacterium* at 5 weeks and *Eggerthella* at 21 weeks) and a higher microbial diversity at 31 weeks of age [6]. Several other studies also observed a higher abundance of bifidobacteria among children with older siblings [99, 116]. A higher richness and diversity in children with siblings have been observed in some [117] but not all studies [47, 115]. Within the large TEDDY study, infants with older siblings had a different microbial community structure and accelerated microbiome maturation as compared to infants growing up in the absence of older siblings. Both species level microbial community structure as well as microbiota maturity, however, only started to significantly differ between children with and without siblings after the first months of life [47]. This delayed effect of older siblings might reflect more close interactions and dispersal when infants grow older or the opening of a niche that allows colonization by specific strains from household members. Alternative

explanations for this sibling-effect, such as an altered vaginal microbiota or breastmilk composition in multiparous as compared to primiparous women, appear less likely as the strongest effect would in such cases be expected in earliest infancy.

Companion animals might also have an impact on the infant microbiome development as an altered microbial environment, indicated by different microbial composition of household dust or surfaces in the homes, has consistently been observed among households with indoor pets [118–122]. The influence of pets on the environmental microbiome might subsequently impact both the immune and microbiome development of infants [120]. In particular the intestinal microbial gene content of dogs shows striking similarities with the human gut microbiome [123]. Dogs were the first animals to be domesticated in modern human history and frequently shared food resources with humans, which has likely contributed to the co-evolution of the human and canine gut microbiome. However, the limited studies on the impact of pet exposure on the infant gut microbiome have so far not differentiated between the effect of different pet species. Results from the TEDDY study revealed that infants living with furry pets had an altered microbial community structure and accelerated maturation of the microbiome when compared to infants growing up in the absence of furry pets [47]. In another study, the animal-specific *Bifidobacterium pseudolongum* was detected in a significantly larger proportion of 1-month-old pet-exposed as compared to non-exposed infants [124]. Moreover, increased levels of *Ruminococcus* and *Oscillospira* were observed among infants with furry pets in their households in the CHILD-study [125]. However, not all studies did observe an impact of furry pets on the establishment and maturation of the infant gut microbiota [115], likely due to the more subtle effects as compared to the effects of siblings [47].

Large studies on the identification of determinants of infant gut microbiota development have so far not observed an association between daycare attendance and gut microbial diversity and community structure [47, 115]. A recent study for the first time compared the microbiota of infants before and 4 weeks after entering center-based childcare to that of infants being fully cared for by the parents at home. In line with the previous studies, this study also found the infant gut microbiota not to be affected in a uniform way by center-based childcare [126]. These studies do however not rule out an impact of daycare attendance on an individual level, e.g., by dispersal of microbial taxa between peers. In fact, studies showing the spread of fecal multi-drug resistant *E. coli* strains in daycare centers prove the existence of such dispersal events and underscore the importance of further investigation. Dispersal between socially interacting individuals is further supported by results from the Wisconsin Longitudinal Study in which spouses had a more similar microbiota and shared more bacterial taxa than siblings (adult siblings not living together) and unrelated pairs. Moreover, married individuals, especially those reporting close relationships, harbor microbial communities of greater diversity and richness relative to those living alone [127].

4.5 Dispersal of Microbes from the Natural Environment

The intestinal microbial ecology also differs between infants from various geographical regions. Even within different Western-European countries, differences in the infant microbiota have been observed. Infants from Northern European countries were characterized by an overrepresentation of *Bifidobacterium*, while an increased microbial diversity was found in infants born in Southern European countries [103]. More pronounced differences are observed when comparing the microbiota of infants living in western countries to that of children born in low- and middle-income countries, with the latter group being characterized by a higher diversity and enhanced levels of *Prevotella* and decreased abundance of *Bacteroides* in early life [18, 128–130]. Diet, with a typical Western diet being low in plant-derived carbohydrates and high in animal protein, sugar, starch, and fat as compared to more agrarian societies, is likely the most important driver for geographical variations in microbiota composition via environmental selection of microbes that can benefit from the substrate availability [111]. However, besides selective pressures such as diet and host genetics, the unique natural environment might at least also partly explain geographic variations in the indigenous microbial community structures [131]. According to the biodiversity hypothesis [132], reduced contact with diverse microbiota and macrobiota in our natural environments adversely affects the assembly and composition of the human indigenous microbiota and in turn to inadequate immune stimulation and ultimately increased susceptibility to non-communicable diseases. Several recent studies do suggest that these natural and human microbial ecosystems are indeed interrelated. Most evidence so far stems from studies that show associations between the skin microbiota and living near natural environments [133–135], but the first studies linking living in proximity to natural environments to the gut microbiota also start to emerge. Preliminary results from the Wisconsin Infant Study Cohort (WISC) showed that the microbiota of infants raised in farming versus non-farming environments differed modestly, yet significantly from each other [136]. Within the context of the Canadian CHILD-study, the association between living near to natural environments in the urban context and the gut microbiota in 4-month-old infants was examined. Although proximity to a natural environment was associated with an altered microbiota composition, this could only be observed for formula-fed infants who were exposed to pets in their homes [137]. This suggests that both environmental selection (e.g., breastfeeding prevents the colonization of environmental microbes) and indirect dispersal of environmental microbes via pets as vectors may be involved. In the PASTURE birth cohort, growing up on a family-run farm, and particular farm exposures such as visits to animal sheds and consumption of eggs or milk directly from the farm, influenced the maturation of the gut microbiota during the time window from 2 to 12 months. This accelerated microbiota maturation could moreover explain a substantial proportion of the well-known protective farm effect on asthma. Interestingly, growing up close to green areas (forest and agriculture land) has also been shown to reduce the risk of atopic sensitization, supporting the hypothesis of a strong environmental effect on

the commensal microbiota [138]. The most direct and causal evidence in support of the biodiversity hypothesis, however, comes from intervention studies in which exposure to natural environments is stimulated. In a Finish trial, the environmental biodiversity of urban daycare centers was enriched by covering their yards with forest floor and sod [139]. Not only did the skin microbial (Proteobacteria) diversity increase among participating 3–5-year-old children, also the gut microbiota composition changed with a decreased relative abundance of Clostridiales and an increased diversity after as compared to before the intervention. Another example is the "Play & Grow" program from the University of Hong Kong, a family-oriented early environmental education program aimed to reconnect preschoolers to nature and induce changes in health behaviors and outcomes by having outdoor activities that promote exposure to nature [140]. In a proof-of-principle study of this "Play & Grow" program exposure to nature activities resulted in a decreased Bacteroidetes richness and increased Proteobacteria richness in the gut microbiota of children in the intervention group [141].

   Together these results demonstrate the interrelatedness of the microbial ecosystems of our natural environments and our indigenous microbiota. Reduced contact of people with the natural environment as well as biodiversity loss of our wider environment will thus inevitably impact the biodiversity and composition of our intestinal microbiota.

4.6   Perturbations by Antibiotic Exposure

Broad-spectrum antibiotics, particularly beta-lactam antibiotics (e.g., amoxicillin, amoxicillin/clavulanate, cephalosporins) and macrolides (e.g., azithromycin), are by far the most commonly administered drugs during infancy and early childhood. Prescription rates, however, vary widely across the globe, reflecting differences in medication policies, preferences of health-care providers and mothers, infection rates, access to care, as well as over-the-counter sales of antibiotics [142, 143]. A significant portion of broad-spectrum antibiotics are still being prescribed for upper respiratory infections, which are mostly self-limiting and of viral origin with little evidence for clinical benefit [144, 145]. The negative impact of such misuse and overuse of antibiotics is substantial as it not only drives the development and dissemination of antimicrobial resistance but also results in profound perturbations on the indigenous microbiota [40, 43, 75, 99, 146]. A reduction in microbial diversity and decrease in obligate anaerobes, including *Bifidobacterium* and *Bacteroides*, and increase in Proteobacteria are commonly observed in the fecal microbiota of infants exposed to antibiotics, although perturbations vary according to type of antibiotics administered [147]. While in adults the microbiota shows a high level of resilience upon antibiotic exposure, the infant microbiota is far less resistant and resilient. In previously antibiotic-naive infants, a single course of amoxicillin was found to profoundly disrupt the microbiota composition. Rather than returning to the original composition after the antibiotic course, an accelerated

maturation towards a depletion of bifidobacteria and enrichment of clostridia was observed [147]. This demonstrates that even an antibiotic pulse has a lasting effect on the maturation process of the infant microbial ecology.

Maternally administered antibiotics could potentially also impact the infant microbiota assembly either by altering the maternal vaginal and intestinal microbiota and thus dispersal (limitation) of maternal microbes [148], or by placental transfer of antibiotics [149]. The first route is likely most important for antibiotics administered in the third trimester, while the second route might come into play for intrapartum antibiotic prophylaxis (IAP) to prevent maternal wound and neonatal Group B *Streptococcus* (GBS) infections. IAP is not routinely practiced across the globe, but recent adjustments of international guidelines will lead to a further increase of prophylactic antibiotic administration during delivery and consequently increased antibiotic exposure to the infant [150].

A profound impact of IAP on the infant gut microbiota has been consistently observed across studies, with a decreased diversity and relative abundances of Actinobacteria and Bacteroidetes and concomitant increased levels of Proteobacteria belonging to the most robust findings [150–155]. Far fewer studies have been examining the impact of antibiotic exposure earlier during pregnancy and results are so far contradictory, likely due to the heterogeneity in study designs and potential confounding factors [150].

5 Conclusions

Human lifestyle changes have profoundly affected our indigenous microbiome and depleted microbial diversity at an unprecedented pace [156, 157]. Given the pivotal role of the infant microbiota as a stimulus for the ontogeny of the enteric mucosal tissue and immune system [13, 14], lifestyle changes that distort the assembly of the microbiota in early life can particularly impact future health and disease susceptibility. From an ecological perspective, lifestyle changes could both affect priority effects and other types of dispersal (limitation) as well as environmental selection. Delayed or limited exposure to maternal vaginal or fecal microbes during C-section delivery is a key example of dispersal limitation. Microbial dispersal is further limited as a result of reduced family sizes, reduced contact with the natural environment, and loss of biodiversity in our natural ecosystems. Reduced breastfeeding and increased consumption of ultra-processed foods reduce the dispersal of human milk and food-borne microorganisms. Moreover, changed dietary habits (e.g., more animal fat, simple sugars, and reduced dietary fibers) cause a distinct selective pressure on the indigenous microbial ecosystem of Western populations. These perturbating external events can be classified as pulses which are short-term perturbations (e.g., a course of antibiotics), or as presses which are long-term or continuous perturbations (e.g. dietary habits) [35]. As such, besides our western dietary habits, even relatively discrete, short-term perturbations (pulses), such as antibiotics, can

profoundly and persistently alter the assembly and maturation of the dynamic and low resilient microbiota in early life [147].

Despite the considerable advances in our understanding of the microbiota maturation during infancy over the past decades, a large part of inter-individual variation in microbiota composition remains unexplained. This suggests that either important determinants have so far been overlooked or that other ecological processes such as historical contingency or stochastics effects play a major role [158]. Further research should take these ecological phenomena into account, but also focus on unravelling other so far unknown deterministic factors that might contribute to the variation and acquisition of the microbiota, and thereby the vital biological processes in life.

Many studies are ongoing to examine the impact of targeted treatments, including specific probiotics, prebiotics, and post-biotics (e.g., products of bacterial metabolism) to strengthen the natural development of the intestinal microbiota or restore its disruption. In addition, maternal FMT has recently been shown to be able to overcome the limited dispersal of maternal microbes and to postnatally restore the microbiota of C-section delivered infants. Although promising, data are still scarce and FMT requires careful screening of donor stools. It should therefore not yet be offered as standard care and only be used within the context of well-controlled experimental settings. Ultimately, synthetic multi-microbial substitutes of FMT will likely be an inevitable further development to make this a viable treatment strategy [159].

Next to the abovementioned dietary or clinical interventions, re-connecting to nature might be another approach to stimulate a healthy microbiome maturation. The rapid urbanization of our living environment and reduced exposure to natural environments might have impeded the beneficial health effects including the dispersal of bacteria and the maturation and homeostasis of immunological responses. In contrast, outdoor activity in a natural biodiverse environment may improve the microbial colonization, and in turn decrease the risk of non-communicable diseases and improve children's general well-being. Human intervention studies tackling this concept of "microbiota re-wilding" are scarce, but the few initiatives that have been undertaken so far are promising [139, 141]. To further increase our understanding on the impact of our (natural) environment, observational studies embedding the infant gut microbiota development into a broader framework of environmental exposure are highly warranted [131]. Moreover, additional human interventional strategies should be explored to examine if strengthening connections to nature as part of everyday life indeed positively influences microbiota development during infancy.

Finally, education of the general public, healthcare professionals, and policy makers on the importance of our gut microbiota and the damaging health consequences of distortion of microbiota assembly in early life should be one of the top priorities. Refraining from unnecessary antibiotic use, informing on the negative consequences of elective C-section delivery, stimulating breastfeeding rates, and a diet high in fiber and low in animal fat and protein are all key to prevent the impoverishment of the indigenous microbiota during this critical period of life.

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