Ecology meets human health: studies on human gut microbiota in health and disease

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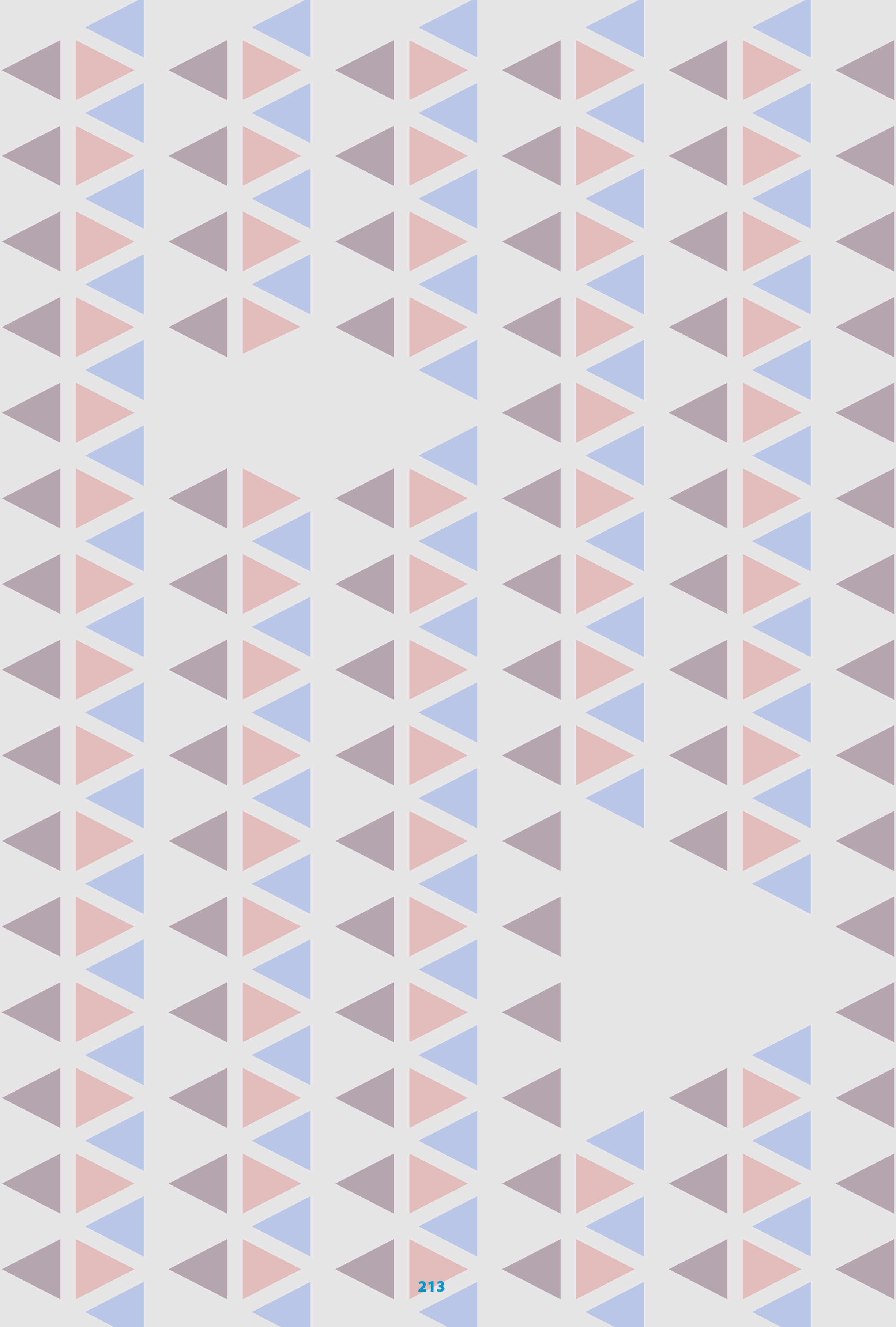

Appendices

Summaries (EN/NL)

English summary

The gut microbiota

The human body hosts countless microorganisms, with a significant portion residing in the digestive system. The bacteria and other microorganisms in our gut, such as fungi and viruses, are collectively referred to as the gut microbiota. A rich and diverse gut microbiota can contribute to good health in its host, for example, by suppressing harmful bacteria. Additionally, these bacteria assist with tasks such as nutrient digestion and training the immune system. The microorganisms constantly influence each other and their human host. Microbes adapt to the specific conditions of their host, with factors such as diet, lifestyle, hormonal regulation, and the immune system playing a role (Figure 1). As a result, the composition of the microbiota frequently changes, for instance, after foreign travel or during antibiotic treatment. Despite these adjustments, it is hoped that the microbiota does not lose its functions, ensuring, among other things, that the integrity of the intestinal wall remains intact.

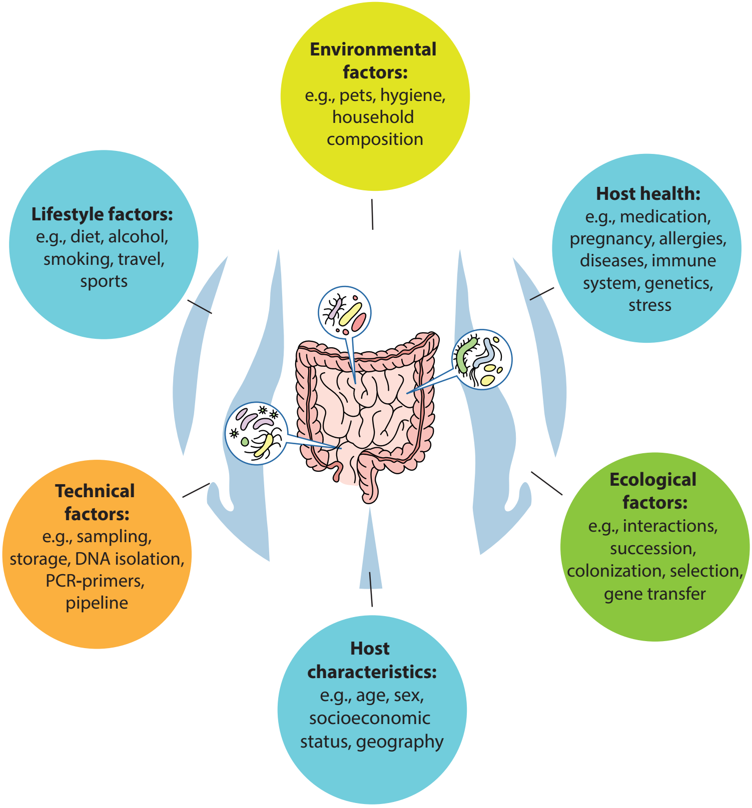

Figure 1. Factors influencing the composition of the gut microbiota.
While technical factors do not directly affect the composition of the gut microbiota itself, they do influence the types and the abundance of microorganisms that can be detected in fecal samples.

The significant role of the gut microbiota in our health has led to extensive research into this ecosystem. Fecal samples are often collected to study the composition and diversity of gut bacteria using DNA analysis techniques such as 16S rRNA sequencing. However, technical factors, such as sample quality, the DNA extraction method used, and the choice of primers, can influence which species are detected and how they are represented in the results (Figure 1). Additionally, incomplete databases, the vast microbial diversity, the dynamic nature of the microbiota, and the limited knowledge about many species make these analyses particularly challenging. The resulting data are complex and require advanced methods for accurate interpretation.

The dynamics and stability of microbial communities, in relation to health and disease, can be studied using techniques such as network analyses and time series models. Network analyses map which bacteria frequently coexist within an ecosystem, providing clues about how microbes might influence and interact with each other. Time series models help track changes in microbial communities over time and uncover patterns. Studying the microbiota is challenging because it cannot be directly observed in the body, and often only a limited number of samples are available. A small number of samples, both in terms of participants and measurement points, complicates the identification of robust associations and makes it difficult to distinguish between individual variation and general patterns.

In the project 'Ecology meets human health', we first examined the reliability of network analyses and alternative methods for mapping relationships between microbes. Next, we combined clinical, microbiological, and ecological concepts to better understand how microbial dynamics are linked to intestinal diseases, specifically Crohn's disease and ulcerative colitis, as well as the success of fecal microbiota transplantation (FMT) as a treatment. In this context, we investigated the ecological factors that influence the gut microbiota and the functioning of this complex ecosystem.

Ecological structure in the gut microbiota

The gut ecosystem consists of numerous species whose presence depends on variations in the environment and functional needs, such as breaking down food, producing vitamins, or combating pathogens. Additionally, interactions often occur between bacteria, which can have positive (beneficial) or negative (detrimental) effects on the species involved. Understanding these interactions is crucial for grasping ecological processes and changes within the microbiota. Correlation methods are often used to map these networks.

In Chapter 2, we investigated the reliability of correlation methods for inferring interaction networks. For our research, we used the generalized Lotka-Volterra (gLV) model to simulate bacterial communities. This model provides insights into microbial dynamics without requiring actual gut microbiota samples and allows for the adjustment of parameters such as bacterial growth rates. Moreover, it enabled us to use a known interaction network as a reference, which is not possible with real samples. We examined the effects of interindividual variation (differences in microbiota composition between individuals) and sample size (the amount of available data) on the accuracy of network reconstructions. While correlations in microbial abundances often indicate ecological interactions, we demonstrated that measurement noise, such as variations in sample processing, complicates the detection of true interactions.

Furthermore, correlations do not differentiate specific interaction types, making laboratory verification necessary to understand these relationships. The gLV model offers valuable insights but also underscores the indispensable role of studies using real data. However, many human microbiota studies are snapshots in time, meaning apparent correlations can be driven by external factors, such as fluctuations in nutrients, without indicating actual interactions between species. Longitudinal studies, which track microbial communities over time, provide a much better basis for understanding consistency and patterns. Such datasets are scarce, likely due to practical challenges, such as repeated sampling, which depend heavily on the host's consent and willingness to participate in research.

In Chapter 3, we demonstrated how the microbiota changes over time and what information these variations reveal about relationships between species. To do this, we analysed time series data from two individuals collected by researcher Caporaso et al. in 2011. These two individuals submitted stool samples almost daily for a year, providing a clear view of the variation within the microbiota. Using wavelet clustering, we uncovered patterns in these data. Wavelet clustering has already been established in ecological and epidemiological studies, and it has also proven particularly suitable for non-stationary microbiota time series, providing greater insight into the collective temporal behaviour of bacteria compared to conventional correlation methods. With wavelet spectra, we constructed 'trees' that depicted relationships between bacterial species. These trees showed significant differences from those based on correlation methods, such as a greater total branch length (indicating higher functional diversity) and distinct subgroups. This highlights that wavelet clustering is more sensitive to subtle differences in community structures than correlation-based methods. Our findings underscore the importance of the method chosen by researchers for analysing microbiota data.

Gut microbiota and inflammatory bowel disease

The interaction between humans and the microbiota is the result of over a billion years of co-evolution, leading to a symbiotic relationship. Our microbes are involved in numerous essential functions, and disturbances in their species composition, known as dysbiosis, have been linked to various diseases. Dysbiosis can weaken the mucus layer in the gut wall, a protective layer that covers the inside of the intestines and helps keep harmful substances and microorganisms out of the body. This can lead to colonization by harmful organisms, an increased risk of inflammation, and metabolic disruptions, putting the host's health at risk (Figure 2).

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are chronic inflammations of the intestinal mucosa. These conditions are associated with an altered composition and diversity of the gut microbiota. In Chapters 4 to 6, we explore the relationships between bacterial dysbiosis and the disease progression in patients with IBD. Although much research has been conducted in this area, findings across studies have often been inconsistent. The differences in findings regarding the involvement of microbes in IBD can likely be attributed to technical variations in research methods and diversity among patients, including variations in disease assessment, medication use, and lifestyle factors. Additionally, the variability of the microbiota in gastrointestinal disorders, such as natural fluctuations in composition, makes it challenging to identify consistent biological signals.

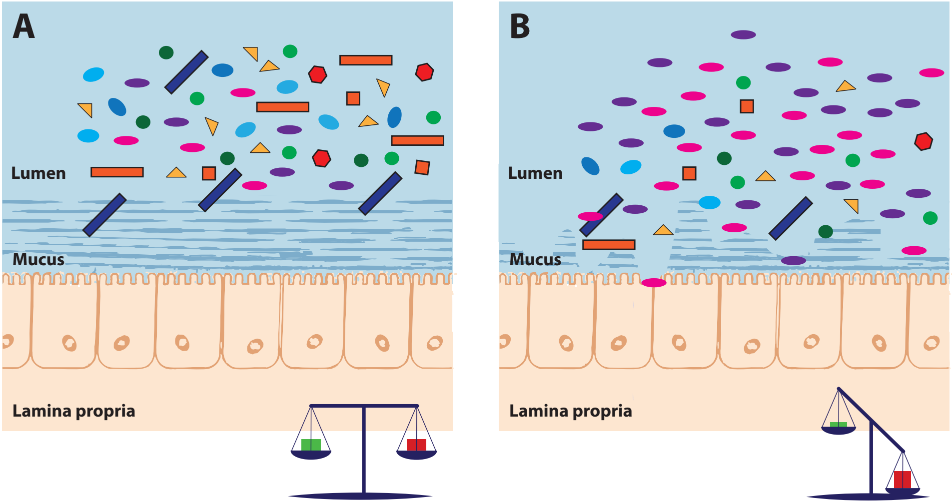

Figure 2. Illustration of the difference between a gut with a healthy microbiota and a disrupted microbiota. A) A healthy gut microbiota is diverse and balanced. The mucus layer provides nourishment for the bacteria while also protecting the gut cells. B) When the balance is disrupted, some bacteria can overgrow and displace other bacteria. Then, the mucus layer can be damaged, allowing bacteria to harm the gut wall.

In Chapter 4, we analysed stool samples from patients with Crohn's disease to describe associations between bacterial abundance and disease remission or exacerbation. We employed a quantile regression model to uncover relationships that go beyond the average response of all patients. Quantile regression allows for a more comprehensive view of the relationships between bacterial abundance and disease. Associations with specific bacterial families may only be observable in a minority of patients. While generic associations can also be identified using methods that focus on the average response, it is also essential to understand less common differences in the microbiota, as these may provide insight into personalized treatment approaches. We also correlated the relative abundance of bacterial families with known biomarkers of disease activity, such as fecal calprotectin and serum C-reactive protein. Our findings revealed significant negative associations between various bacterial families and disease, such as Pasteurellaceae and Ruminococcaceae. When comparing regressions with clinically defined exacerbation, we found that associations with fecal calprotectin were stronger than with other indicators. In summary, our research highlights the heterogeneity of Crohn's disease and its relationship with the gut microbiota.

Ecological determinants of FMT treatment success

Microbiota-related therapies aim to intentionally alter the microbiota of patients to shift it from a dysbiotic to a healthy state. Fecal microbiota transplantation (FMT), commonly known as a stool transplant, is an experimental treatment in which fecal material, including the microbiota from healthy donors, is transferred to the patient to restore the disrupted microbiota. FMT has established itself as a promising treatment for microbiota-related conditions, particularly for the treatment of recurrent *Clostridioides difficile* infection. In ulcerative colitis, the success of FMT is determined by achieving clinical remission.

One of the challenges of FMT is the inconsistency in outcomes regarding the final composition of the patient's microbiota. This means that each person responds differently to certain donor bacteria and that various immune responses can be activated in patients with different conditions. Hypothetical outcomes include a return to the original dysbiotic state, an alternative dysbiotic state, a long-term change with beneficial properties, or a persistent shift to a healthy microbiota with donor species. In our study, we investigated the microbial families associated with the success of FMT treatment in ulcerative colitis. This allowed us to explore the dynamics of the gut microbiota. This longitudinal approach provided insight into weekly changes (Figure 3), a perspective often missing in randomized controlled trials that mainly focus on clinical outcomes.

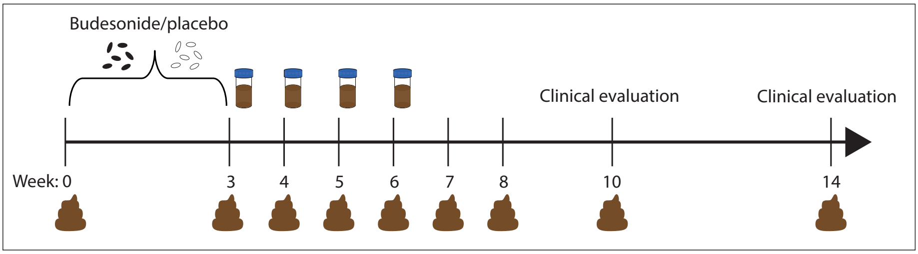

Figure 3. Design of the FECBUD-study (data used in Chapters 5 and 6). Patients were first pre-treated for three weeks with budesonide ($n = 12$), a medication commonly used to reduce inflammation, or a placebo ($n = 12$), an inactive substance with no therapeutic effect. After that, the patients received four consecutive fecal transplants from a healthy donor provided by the Netherlands Donor Feces Bank (NDFB). Treatment evaluation took place after 10 and 14 weeks from the start of the study. A subgroup of the patient group (9 out of 24 patients) achieved a successful combined clinical and endoscopic remission after the FMT treatment. Fecal samples from the patients were collected at the beginning of the study, after the pre-treatment, weekly after the fecal transplant, and at two, four, and eight weeks post-FMT.

In Chapter 5, we studied the associations related to the clinical success of FMT in patients and the development of the microbiota during and after treatment. We used a wide range of analytical techniques to investigate potential associations between bacterial families and clinical outcomes, including ordination analysis, Dirichlet multinomial mixture analysis, and longitudinal modeling. The use of these approaches allowed us to identify significant differences in microbial composition and diversity between patients who benefited from the treatment and those who did not. For example, we found that the success of FMT in patients with ulcerative colitis seems to be associated with limited growth of Prevotellaceae and the presence of the families Lachnospiraceae and Ruminococcaceae. Monitoring the dynamics of these microbial families could potentially provide early insight into the success of treatment during FMT.

It is widely believed that the colonization of donor species in the recipient's microbiota is a key mechanism behind the success of FMT. An interesting finding from our research in Chapter 5 is that we found no indication of a shift in the microbial composition of the recipient towards the donor microbiota among patients with clinical success of FMT. In Chapter 6, we therefore examined whether the donor-centered view of FMT holds true by analysing whether microbiota dynamics are related to achieving remission in patients after FMT treatment.

To do this, we categorized the species based on their origin and temporal presence: already present in the host before FMT, derived from the donor, or species newly introduced during the FMT treatment. We then modelled the number of species per category (host-associated, donor-derived, and novel) for patients who did or did not benefit from FMT. Our results show that patients who benefited from the treatment retained a higher number of host-associated species compared to patients who did not benefit from the treatment. Although donor species initially colonized more extensively in patients who did not benefit from the treatment, this colonization decreased over time, aligning with the level seen in patients who did benefit from the treatment. This suggests that a successful clinical response to FMT may be facilitated by a microbiota that is receptive to colonization without compromising the resident microbiota. We also discovered that host species with higher relative abundances before FMT are better able to persist after FMT.

In conclusion

There are numerous environmental factors and habits (among others, diet and lifestyle) that influence the composition and function of microbes. This complexity can sometimes be overwhelming, but knowledge from various disciplines provides valuable insights. By understanding how systems respond to changes and regain balance, we deepen our knowledge of the complex dynamics within the human gut microbiota. These insights can improve microbiota-modulating strategies such as FMT and stimulate innovation in personalized therapeutic approaches. This leads to a new perspective, viewing the microbiota not just as a battleground against pathogenic microbes, but as a complex ecological community. Management strategies such as habitat restoration play an important role in this. To achieve this, extensive (longitudinal) datasets are needed that include a wide range of individuals with diverse backgrounds and lifestyles. Such data are essential for understanding the mechanisms that influence the dynamics of ecosystems in health and disease. In addition, suitable methods must be available to effectively investigate the complex microbiota data.

The limitations of a 'one-size-fits-all' approach are becoming increasingly evident, particularly due to the diversity, genotypes (genetic composition), and phenotypes (observable traits and characteristics) of the gut microbiota. This underscores the need for a more personalized approach in (clinical) scientific research. For instance, matching donor and recipient based on lifestyle and diet could increase the likelihood that transplanted bacteria successfully colonize and that the resident bacteria receive the right nutrients. This could contribute to a more effective improvement of the recipient's microbiota, tailored to the patient's unique needs. However, to truly understand microbial functioning, we need to keep the ecological perspective of bacteria in mind. This means recognizing that bacteria perceive their environment, resources, and interactions on a much smaller spatial scale, constantly adapting to their immediate surroundings. This implies that even within a single sample, different bacteria can exhibit different behaviours, functions, or interactions. Additionally, within the gut microbiota, various bacteria often have overlapping functions. This means that if a certain species is disrupted or removed, other species can take over that function. This mechanism is crucial for maintaining a healthy gut microbiota and remains an area that has not been sufficiently researched. An ecological approach, grounded in theory, is essential for interpreting the impact of the microbiota on health or disease. This perspective allows us to better understand the complex interactions within the microbiota, which is vital for developing effective therapeutic strategies.