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

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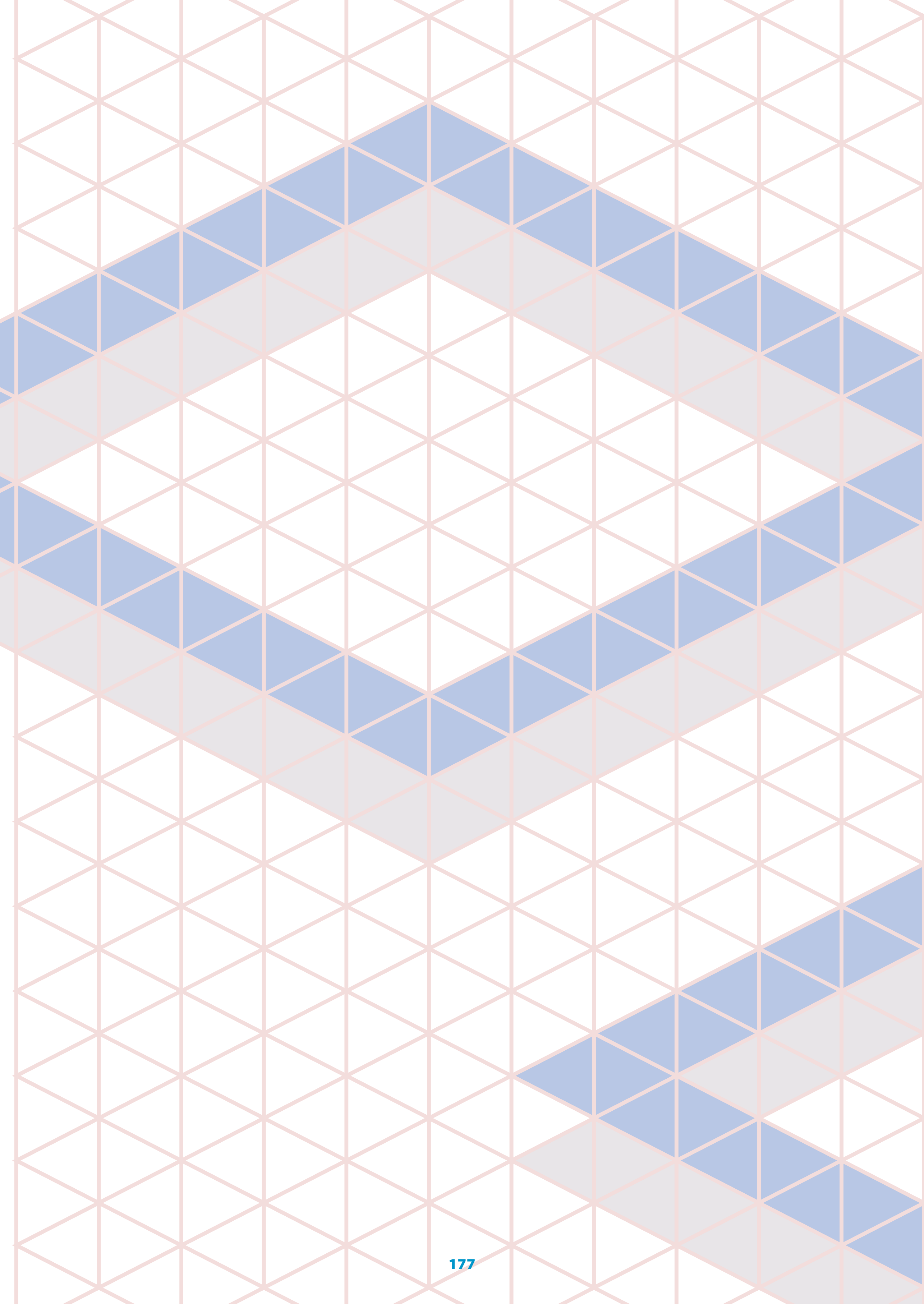

Chapter 7

General discussion and future perspectives

General discussion and future perspectives

Main findings

In this thesis, we applied a theoretical framework and used methodologies derived from the field of ecology to investigate the dynamic properties and characteristics of the human gut microbiota. In this way, we aimed to contribute to a better understanding of the complex microbial ecosystem of the human gut and its association with inflammatory bowel disease (IBD) course (i.e., exacerbation or remission). Additionally, we examined microbial changes following an intervention with fecal microbiota transplantation (FMT). Addressing these aims requires a thorough examination of the human gut microbiome, its dynamics, and the key factors influencing the functioning of this microbial ecosystem. This dissertation contributes to these goals in several ways.

First, we studied the correspondence between correlation-based networks and the underlying network of ecological interactions. Our results demonstrated that correlations could indicate the presence of bacterial interactions, at least in a simulation setting. Interactions were recovered with precision exceeding recall, indicating that the likelihood of missing interactions was higher than the likelihood of finding false positive interactions when using correlations in cross-sectional abundance as their proxy. However, we also showed that asymmetric interaction types cannot be detected and that there are many factors that may worsen these results, such as measurement noise. Unfortunately, biomedical data are always subject to measurement errors, particularly in microbiota studies where data are obtained through sequencing processes.[118] Furthermore, microbiota data are also influenced by host-specific variation in process parameters (process noise) and sampling under various (non-equilibrium) conditions, all of which will influence the inference, though not necessarily in an adverse way.[423] Therefore, while correlations may hint at interactions, independent validation is needed to confirm their presence and to ensure that these correlations represent genuine biological interactions with meaningful implications. Until then, we should continue to refer to these correlations as associations rather than interactions. Moreover, in our second study we showed that wavelet clustering uncovers more diverse community structures compared to analyses based on temporal correlations. We revealed significant differences between these methods and suggested that the correlation-based approaches might overlook certain dynamical aspects of microbial communities. This comparison highlights the potential of wavelet clustering to use the temporal fluctuations and complexity inherent in the human microbiota for characterizing community structure, offering a more nuanced understanding than correlation-based methods alone.

Second, our objective was to describe specific associations between microbial abundances and Crohn's disease (CD), in particular with exacerbation of disease. In doing so, we made the analogy between the gut microbiota in an unhealthy host with an ecosystem under stress. We found that microbial diversity is reduced in the gut of CD patients, and that the process of diversity loss is irregular with respect to specific taxonomic groups. If this process of loss of species continues for an extended period, it may eventually lead to an unhealthy and possibly irreversible state. Moreover, in this study we showed that associations of relative bacterial abundances with CD can be different for subsets of individuals. A practical, though undesirable implication of this finding is that it seems very difficult to pinpoint specific gut microbes as biomarkers or therapeutic targets for CD patients.

Third, we studied bacterial associations with clinical treatment success of FMT in ulcerative colitis (UC) and investigated the succession of the microbiota during and after the treatment. By means of several analytical techniques, such as longitudinal modeling and cluster analysis, we identified potential associations between specific gut microbiota families and clinical outcomes. Our findings suggest that the success of FMT in UC patients may be linked to the control of Prevotellaceae, with potentially beneficial roles attributed to Lachnospiraceae and Ruminococcaceae. Notably, clustering analysis indicated that differences in the gut microbiota between responders and non-responders may manifest early during treatment. Moreover, successful FMT seems to be associated with a resilient gut community that is open to colonization by donor species, while maintaining the original community to some degree. This suggests that a balanced coexistence of host and donor species can induce a shift in which the recipient's microbiota evolves towards a healthier community.

Stability and variability in microbiota dynamics

Over the past 15 years, microbiological research has flourished, driven by technological advancements that have significantly expanded our knowledge concerning the ecology of gut microbiota and its relation to health and disease.[424] The beneficial functions provided by our microbiomes offer potential for improving human health. Therefore, efforts have been made to understand the temporal variations in our microbiota to define 'stable' and '(un)healthy' dynamics.[21, 45, 62] Early attempts to classify the gut microbiota introduced the concept of 'enterotypes', distinct clusters characterized by an enrichment of *Bacteroides*, *Prevotella*, or *Ruminococcus*.[405] However, this early classification was only based on metagenomics from 39 individuals, and much larger studies have challenged the distinctness of these enterotypes, suggesting a more gradient-like distribution with varying levels of *Prevotella* and *Bacteroides*.[233, 412, 425]

The microbiota is acknowledged to be highly specific to individuals, displaying relative stability in adults, with regular fluctuations in the composition over time.[45, 46, 51] These fluctuations suggest that long-term stability of human gut microbial communities is influenced by the tendency of the intestinal ecosystem to maintain internal stability (homeostasis), owing to the coordinated response to any stimulus that disturbs its normal condition.[62] This prompts inquiries into whether fundamental ecosystem 'rules' governing microbiota (group)dynamics can be distilled from a collection of individual microbiota, and to what extent each represents a unique ecosystem with its own host-specific microbial dynamics (Figure 7.1).[426] If microbiota dynamics were independent from the host, then the presence of the same species should result in the same relative proportions of those species, and interventions could be devised to regulate microbial states across different individuals.[284] On the other hand, if the dynamics are strongly host-specific, personalized interventions should be designed, considering not only the unique microbial state of an individual but also the specific host factors of the microbial ecosystem.[46, 55, 59] However, studying this is very difficult due to the presence of latent or unknown parameters (related to lifestyle or diet for example) influencing microbiota composition.[155] The factors contributing to microbiota variation are still not fully understood.[57]

Consequently, comparative analyses between patient and healthy cohorts yield many different dysbiotic states or sets of microbial biomarkers that are dependent on a specific comparison, and the definition of a normal healthy microbiota remains unsatisfactorily answered. Moreover, it is still unclear whether the structure of the gut microbial community shifts gradually within individuals or transitions between distinct community states, and whether such states are consistent among different individuals.[233, 405, 427]

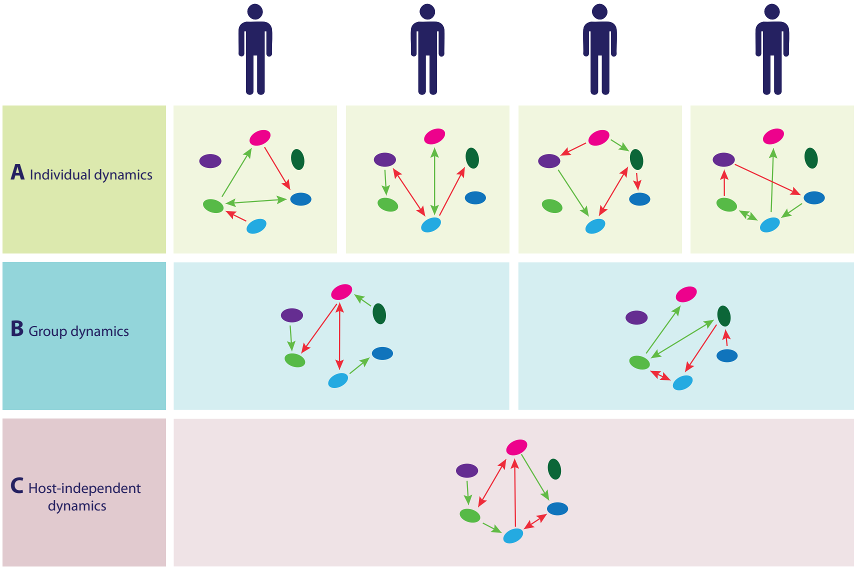

Figure 7.1 - Illustration of microbial dynamics through ecological networks. Microbial dynamics are illustrated through an ecological network, wherein nodes symbolize species and edges depict interspecies interactions (green and red arrows denote positive and negative interactions, respectively). A) The underlying dynamics or networks are unique to each subject. B) Subjects within the same group exhibit shared dynamics or networks, which markedly differ from those of other groups. C) Different subjects display identical underlying dynamics or networks. Note that subjects can also differ in species composition or in the relative abundances of each species. This figure is based on Bashan et al. (2016).[426]

Broader insights from the literature

Part I - Ecological structure in the human gut microbiota

Microbial interactions can yield diverse outcomes, ranging from positive impacts such as mutualism, where species exchange metabolic products to benefit each other, to negative impacts on participating species. These interactions shape community patterns and inhibit the outgrowth of certain species. In Chapter 2, we assessed the reliability of correlation-based methods for inferring microbial interaction networks. Unraveling the network of interactions within ecological systems, particularly in studies of the human microbiome, is challenging. Technical issues in constructing networks from sequencing data, such as compositionality and the predominance of zeros, combined with the influence of often unmeasured environmental factors, make the networks difficult to interpret and susceptible to potential biases.[118] Additionally, data generated from assays may be censored by detection limits, causing species to remain undetected.[203]

Moreover, the presence of a third variable or species (e.g., bacteriophage) can influence the observed correlations, especially if the researcher fails to measure this linked species (Figure 7.2). Correlation-based network analysis typically results in too many spurious edges.[118] Addressing these challenges has led to the development of various co-occurrence methods, such as CoNet, SparCC, and SPIEC-EASI.[237, 253, 428] Interestingly, in evaluations, classical correlation measures often perform just as well as the more sophisticated algorithms.[118, 429]

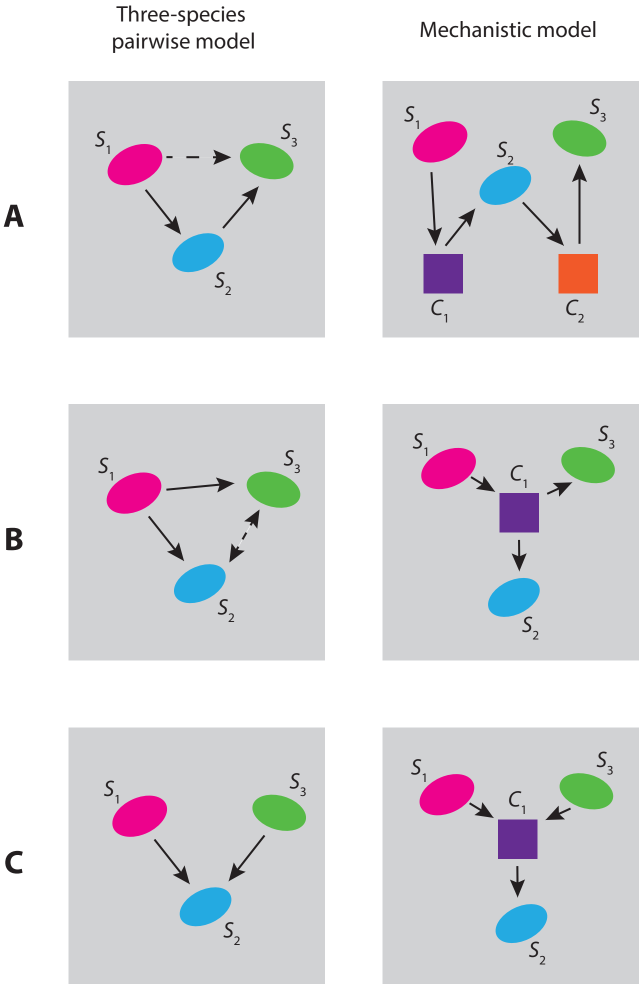

Figure 7.2 - Interaction networks between three species.
Direct interactions are indicated by a solid arrow, indirect interactions are given by a dashed arrow. A) The interactions utilize separate compounds, C_1 and C_2, as mediators. Interaction chain: Species S_1 influences S_2, which in turn affects S_3. B) In this scenario, S_1 initiates a change where S_2 and S_3 interact only when S_1 is present. Modified interaction: Species S_1 influences both S_2 and S_3. Species S_3 consumes mediator C_1, altering the interaction between S_2 and S_3. C) Modified interaction: Both S_1 and S_3 contribute compound C_1, which stimulates S_2. S_1 and S_3 do not directly interact regardless of S_2. This figure is based on Momeni et al. (2017).[240]

To address potential confounding in pairwise interactions, we employed partial correlations in Chapter 2 to infer the correlation network. See Figure 7.3 for a comparison between plain and partial correlations in a real dataset. For most microbes, ecological interactions are poorly understood, necessitating the *de novo* construction of ecological interaction networks without guiding assumptions or a gold-standard set of interactions for validation.[100, 111, 238, 251, 430, 431] Therefore, we used the generalized Lotka-Volterra (gLV) model with simulated interactions to study the correspondence between correlations and interactions.[232] gLV models are widely employed in ecological studies to simulate the dynamics within bacterial communities.[100, 111, 232, 254, 423, 432] This approach enabled us to define the species-species interaction terms and incorporate variations in model parameters to reflect the variability among hosts. The gLV model, while versatile, has drawbacks: it only describes pairwise interactions, disregards immigration and environmental effects, and maintains constant and additive interaction strengths.[49, 57, 100, 232, 240, 433-435] In Chapter 3 we also used an ecological model. Here, we simulated the dynamics of four consumers and four resources to provide an additional dataset to evaluate the accuracy of wavelet clustering in contrast with clustering based on Spearman's correlation.[318-320]

Some scientists tend to approach mathematical models, also the ones used in Chapter 2 and Chapter 3, with skepticism, wary that simplification might sacrifice realism. However, while models may simplify complex systems, they can also serve as invaluable tools for understanding phenomena that are otherwise difficult to grasp.[1] For example, in Chapter 2, we would not have been able to judge the correctness of the correlation matrix without a simulated network that could be used as a ground truth. Models allow scientists to explore hypothetical scenarios, test theories, perform virtual experiments that are impossible or unethical in humans, make predictions, explain complex phenomena, thereby ultimately advance our understanding of the natural world. However, it is imperative to ensure that models are built upon correct assumptions as these can significantly impact model outcomes.

Notably, many studies on microbial communities and their associations with specific disease courses or host conditions heavily rely on a steady-state assumption and the failure to account for non-steady-state dynamics could introduce biases in the findings, leading to an overemphasis on certain taxa while neglecting others that may be important in a non-steady-state context. The microbial interaction network is also likely dynamic, shaped by both negative and positive feedback loops. These feedbacks occur as an organism's metabolic activity alters its environment, influencing its own fitness, and the fitness of competing species, creating ecological niches that drive diversification.[22] Therefore, the niches in the gut might be more comparable to a dynamic river ecosystem than to a more static ecosystem on land, as nutrient flows through the bowel, providing constant resources but also causing constant disturbances and reassembly of microbial communities and interactions.[436]

Future microbiome studies will benefit from larger cohorts, more frequent sampling, and longer follow-up periods to unravel the short- and long-term dynamics of gut microbial communities in real datasets. Longitudinal studies allow for investigating the consistency, or changes, of microbiota patterns over time. Following this, in Chapter 3, we applied a methodology unknown to the microbiome field, namely wavelet clustering analysis. This method clusters time series based on the similarity in their temporal dynamics of microbial communities.

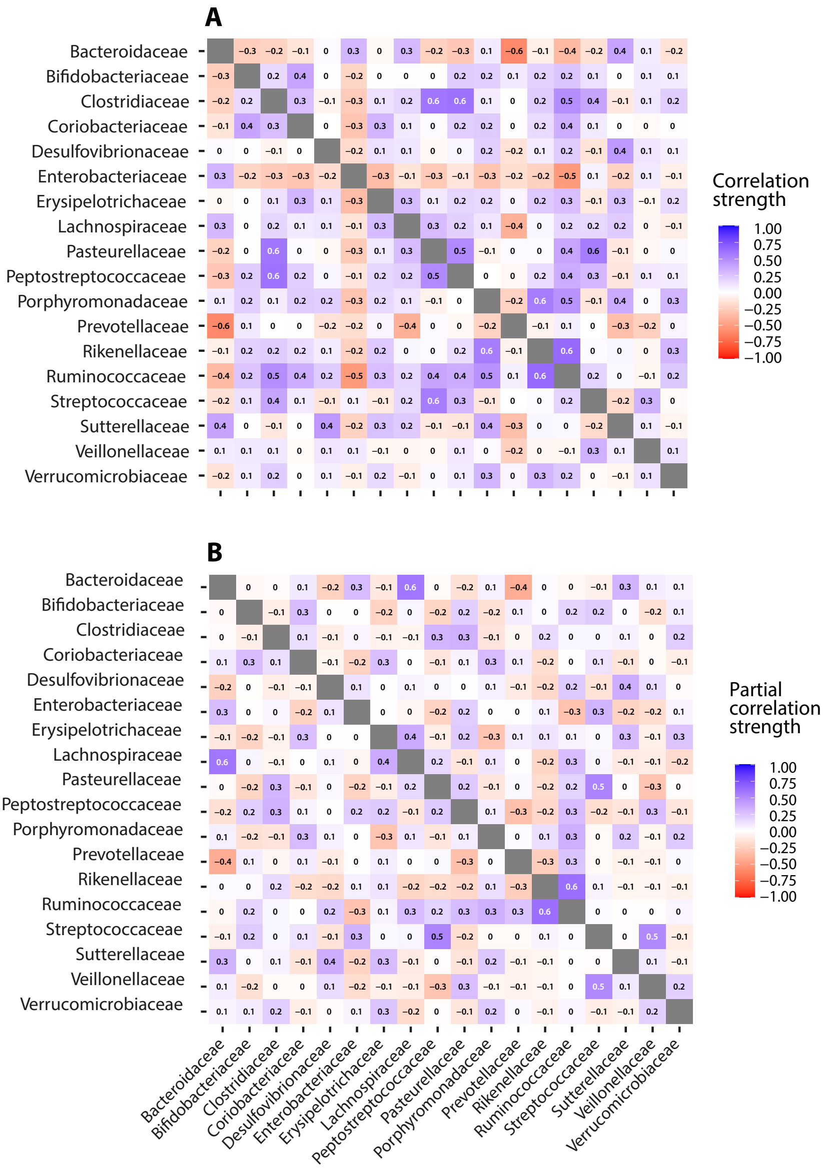

Figure 7.3 - Correlation matrices. Matrices are derived from the dataset presented in Chapter 4 of this thesis. A) Spearman's correlation matrix displaying the pairwise correlations between bacterial families. The correlation matrix provides insights into the linear abundance relationships among variables. B) Spearman's partial correlation matrix illustrating the partial correlations between the bacterial families. Partial correlations help to assess the unique association between bacteria, independent of the interrelated influence of other bacteria. Each cell represents the (partial) correlation coefficient between two variables, with color intensity indicating the strength and direction (e.g., blue is positive and red is negative) of the correlation.

Unlike prevailing co-occurrence methodologies, the novelty of wavelet clustering lies in its ability to characterize community structure based on the collective temporal behaviour of the microbiota, without directly fitting a dynamical model or reconstructing the network of interacting species. While traditional correlation-based methods may offer some, but limited or biased, insights, wavelet clustering enables the extraction of more information on dependencies within microbial communities and can reveal community structures that remain obscured in correlation-based methods.[437] These findings underscore the critical role of longitudinal data and methodological choices in shaping the outcomes of microbiota data analysis.

Mapping ecological networks to predict (temporal) behaviours and discern assembly rules is motivated by the goal of gaining insights into the underlying dynamics that drive microbial ecosystems. Ultimately, this knowledge may be used to establish early warning signals, develop clinical prognostic models, and even engineer stable microbiomes with desired properties.[438-440] The topology of the network often provides insights into the potential explanatory nodes for specific functional properties within the network, allowing for the identification of tightly interrelated modules of variables, such as communities.[3] Additionally, knowledge of the interaction network not only aids in identifying key players within the network (i.e., keystone species) but also facilitates predictions on how microbial communities might respond to diverse stimuli or disturbances, such as alterations in diet or exposure to antibiotics.

Previous research has indicated that correlation-based networks likely capture only a fraction of the interactions occurring in microbiota, with strong symmetric interactions being more readily detected compared to weaker or asymmetric interactions.[216, 235, 273] Correlation-based networks from cross-sectional data are commonly interpreted as representing interspecific interactions.[227] Each significant link in a correlation network suggests a shared process affecting connected nodes; however, we should acknowledge that correlations do not always imply causation or biological meaning.[3, 216] Densities may also vary as a result of an external factor that is not of biological interest.[118] The presence of two species together in one sample, while absent in another, may not necessarily indicate an interaction between them. Instead, they could simply coexist because one sample was taken during a nutrient-rich period that supports the growth of both species independently, whereas the other sample may have been taken at a less favourable time, limiting the growth of both species. Therefore, incorporating additional information about influencing factors can provide a richer, more nuanced picture of the underlying dynamics within the microbiome. Moreover, as most microorganisms form biofilms, i.e., genetically diverse, surface-associated communities embedded in an extracellular polymeric matrix, bacteria primarily interact with others in their immediate neighborhood, with the strength of these interactions diminishing as distance increases.[440, 441] Therefore, the spatial relationships between individual organisms should ideally also be considered in the network, including the nature and strength of their interactions based on their positions within the community.[440] However, before delving into more complex network structures including extensive metadata, it is essential to first gain a thorough understanding of the ‘simpler’ networks to lay a solid foundation for future analyses.

Part II - Gut microbiota and inflammatory bowel disease

Given the involvement of the microbiome in numerous essential functions, it is not surprising that disturbances in microbiota composition (known as dysbiosis) have been linked to the onset and course of various diseases. Many associations found may not always be disease-specific but rather part of a non-specific, shared response to health or disease.[200, 442] Chapter 4 and Chapter 5 of this thesis address the relationships between bacterial dysbiosis and the disease course of CD, which, along with UC, comprises the pathology of IBD. While CD can occur anywhere in the digestive system, UC is limited to the colon. Both diseases exhibit significant distinctions in microbiota compositions from one another, although less strongly than they differ from healthy subjects.[372] However, the findings regarding disease exacerbation among CD or UC patients are often inconsistent and occasionally even contradictory. For example, previous studies have reported both lower and higher relative abundances of *Bacteroides* (Bacteroidaceae) in CD patients compared to healthy individuals.[356-358] This discrepancy can be attributed in part to technical variations between studies such as differences in DNA extraction methods and sequencing depth, but they may also arise from variations in disease assessment or study populations, as well as potential confounding factors, such as medication use or lifestyle factors that remained unidentified.[56, 171, 225, 443] Coupled with the interindividual variability of the microbiome in gastrointestinal disorders, the pursuit of shared biological signals proves challenging. Moreover, while many studies adopt a cross-sectional study design, longitudinal studies are needed for comparing active and inactive disease.[231, 444] The knowledge gap with regards to consistent and specific dysbiosis signatures poses a challenge to reveal the role of gut microbiota in human diseases.

In Chapter 4 we investigated the multifactorial involvement of specific microbial groups with CD compared to healthy individuals. Additionally, we also investigated associations between the relative abundances of specific bacterial families with disease course (remission vs. exacerbation) and disease activity markers (e.g., fecal calprotectin (FC), serum C-reactive protein (CRP), and Harvey Bradshaw index (HBI)) in repeatedly sampled CD patients.[181] Given the variability among CD patients and the complex microbial interactions, associations with disease may only be weak when considering mean responses. Therefore, it requires robust analysis to uncover these associations, and quantile regression is a promising method given that potential relationships may only be apparent in lower or upper quantiles of relative abundances.[361, 363]

We identified several significant associations between bacterial family abundances and CD, particularly when compared to healthy controls. CD patients exhibited distinct microbial profiles, with several families showing predominantly negative associations. While our results confirmed previously identified associations, including Erysipelotrichaceae, Peptostreptococcaceae, Prevotellaceae, Clostridiaceae, and Ruminococcaceae, we also uncovered novel associations with Coriobacteriaceae, Desulfovibrionaceae, Pasteurellaceae, Sutterellaceae, and Streptococcaceae.[171, 177, 181, 356-358] Notably, Coriobacteriaceae displayed a shift in relative abundance across the disease course, with higher values at baseline in patients who later experienced exacerbation. Additionally, Streptococcaceae demonstrated increased abundance over time in patients with exacerbation, compared to both healthy controls and patients in remission. Conversely, Sutterellaceae was consistently lower in patients with exacerbation as well as those in remission compared to healthy controls. Interestingly, associations with disease activity were generally weaker. We also found that FC levels were negatively correlated with the abundance of Porpyromonadaceae and Verrucomicrobiaceae.

Prevotellaceae were among the most heterogeneous across individual patients. The genus *Prevotella*, which belongs to this family, is involved in saccharolytic fermentation and short-chain fatty acid production. *Prevotella* is generally more prevalent in individuals from rural areas compared to urban populations, potentially due to the higher abundance of *Prevotella* phages and a diet lower in plant-derived complex carbohydrates in urban populations.[445, 446] Additionally, *Prevotella* has been linked to inflammation in other diseases; for instance, *Prevotella bivia* is strongly associated with inflammation in bacterial vaginosis and an increased risk of HIV.[447, 448] In Chapter 5, we also observed associations with Prevotellaceae in UC patients undergoing FMT treatment. Non-responders to FMT showed an increase in Prevotellaceae abundance compared to patients who achieved clinical remission after FMT (i.e., responders). However, our data from Chapter 4 and Chapter 5 do not clarify whether these differences are driven by the disease or factors, such as dietary habits, environmental variables, or other unknown factors that could contribute to the outgrowth of Prevotellaceae in these patients.

Interestingly, nearly all significant associations found with quantile regression in Chapter 4 were negative and primarily observed in the lower quantiles of the bacterial abundances. While positive associations in upper quantiles have been linked to unmeasured factors constraining the potential response to positive stimuli,[361] this contrasting trend resembles an ecosystem responding to stress: as the system nears a tipping point, the ability to sustain healthy bacterial abundances gradually diminishes.[97] However, the loss of certain species within the microbial network can be compensated for by others with similar ecosystem functions (functional redundancy). This redundancy enhances resilience, ensuring the continuity of essential functions important to the host, such as butyrate production.[62, 449] Consequently, when solely studying the compositional profile, the actual functional output of a system presumed to be in 'dysbiosis' might be normal, and vice versa; lack of significant differences in abundance doesn't necessarily indicate a healthy state as the species may lack essential functional genes.[450] However, an excessive loss of species may reduce resilience and cause a critical transition to an alternative stable state.[104, 369] A study setup including proteins secreted by the microbiome would provide insights into how dysbiosis is expressed on the functional level. For instance, in a CD case-control study, a lack of species capable of consuming hydrogen sulfide was identified as a key distinguishing microbiome feature of the disease.[451] Other studies showed the role of butyrate, secreted by pathobionts such as *Fusobacterium*. While butyrate is typically beneficial, it may negatively affect the viability of the intestinal epithelium and potentially contribute to IBD pathogenesis.[452, 453]

Note that, from a statistical point of view, investigating numerous bacterial species across multiple patients poses a significant challenge regarding the multiple hypothesis testing problem. To construct a correlation network or investigate significant differences in microbiota composition, adjustments might be necessary to control for false discoveries. The choice between correction methods depends on the research goal; stricter corrections, such as the Bonferroni approach, may be preferred to demonstrate specific associations, while more general impressions may be sought with less stringent corrections, such as the Benjamini-Hochberg (BH) method. However, even the BH approach might still be too strict when applied to microbiota data, because these methods assume independence among bacterial abundances, which is not valid due to (biological) relationships between species (Figure 7.2 and Figure 7.3) and the compositional nature of the data. Ideally, correction methods should account for correlated species to provide more accurate results. However, there is no solution yet available; therefore, conclusions should be based on a comprehensive review of existing literature in addition to study findings and not on *p*-values alone.

Part III - Ecological determinants of FMT treatment success

Concerning the treatment of dysbiosis, since a groundbreaking study in 2013, FMT has emerged as a treatment option for recurrent *Clostridioides difficile* infection (rCDI).[190] However, rCDI remains the only condition for which FMT is widely accepted as a treatment. In all other indications where FMT has shown promise, its use remains experimental or is considered a last-resort option.[454] One of the challenges with FMT is its inconsistency in (microbiota composition) outcomes.[455] This means that every person will react differently to certain bacteria and that diverse immune responses are activated across patients with different diseases.[456] This variability raises significant safety concerns, because the microbiota could also be altered to an even more undesirable state in the recipient's gut.[426, 457] Similarly, other therapies designed to modulate the microbiome, such as probiotics, have also been associated with adverse outcomes. The PROPATRIA study, a Dutch clinical trial conducted from 2003 to 2007, revealed that patients with acute pancreatitis who received probiotics had a higher mortality rate compared to the control group.[458] However, it remains unclear whether the probiotics themselves or other factors contributed to this increased mortality. Therefore, a 'one-size-fits-all' treatment approach does not ensure safety and efficacy against multifaceted diseases, as evidenced by the inconsistent results of FMT trials for IBD and irritable bowel syndrome (IBS).[197, 459-461] The interaction between two microbial consortia (donor and recipient) during FMT can be likened to a complex pulse perturbation. Possibly, the perturbation caused by bacterial components, metabolites, or bacteriophages may also mediate the effects of FMT. Especially as investigations into auto-FMT have also shown promising results in restoring gut microbiome composition.[462, 463] Clearly, there is a need for a deeper understanding of the dynamics underlying the interaction between donor and recipient microbiota during FMT.[464] This could ultimately lead to a safe and controlled modification from disturbed to desired phenotypes in the recipient.[23]

In the studies detailed in Chapter 5 and Chapter 6, we examined stool samples from 24 patients with mild to moderate UC undergoing FMT. Stool samples were collected at nine time points across the study period, allowing for a comprehensive assessment of gut microbiota dynamics during and post-FMT. Our longitudinal approach provided insights into weekly changes, a perspective often lacking in randomized controlled trials (RCTs) that focus primarily on clinical outcomes. Our results in Chapter 5 suggested that there is a potential for predicting clinical success of FMT treatment based on early microbiota analysis in the early phase of treatment, which would make it possible to adapt treatment strategies accordingly. However, developing a reliable predictive model for this purpose will require substantial additional effort.

It is plausible that differences in microbiota related to clinical success become apparent early during FMT treatment. The order in which species arrive can influence community succession (the predictable change in community composition over time), as early-arriving species can modify resources and environmental conditions, thereby affecting the establishment of later-arriving species. These priority effects can lead to varying successional pathways within the gut ecosystem.[83, 93] This concept is akin to plant ecosystems, where pioneer species prepare the environment for subsequent arrivals. For example, while a particular patch may not always host the same grass species, the presence of any grass helps create conditions that are conducive to the establishment of shrubs. Similarly, the growth of taller plants (regardless of specific species) facilitates the establishment of shade-tolerant species.[96] Therefore, to understand how microbial species interactions shape community dynamics during succession after FMT, we need to focus not just on which species are present, but also on the role each species plays within the community.

Several hypothetical outcomes of the FMT treatment are possible (Figure 7.4). First, the host communities may revert to their initial dysbiotic state if the perturbation is too weak and the dysbiotic state too strong. Therefore, the transferred microorganisms fail to change the microbiome or to establish themselves permanently. Second, due to intrinsic host or environmental factors, an alternative dysbiotic state may emerge, wherein the microbial community, although different in composition, possibly continues to perform detrimental ecosystem services. Third, an alternative healthy state may emerge, characterized by a novel microbiota composition with beneficial properties. Fourth, the microbiota changes to resemble the donor state, ideally incorporating the donor's healthy functions.[23]

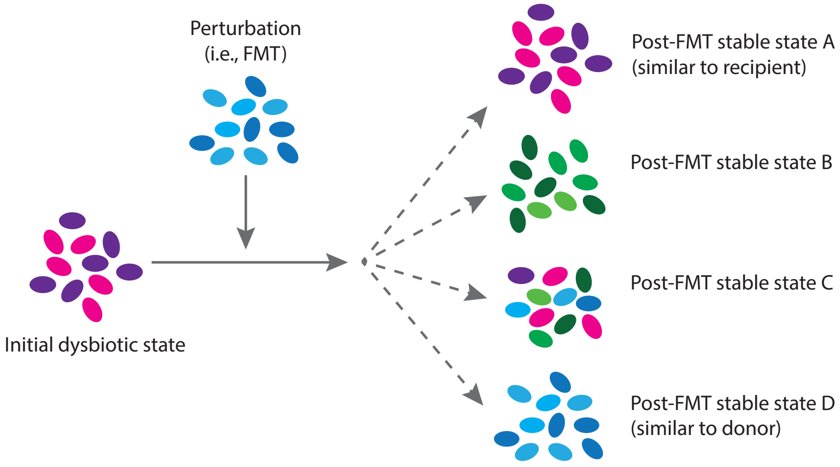

Figure 7.4 - Hypothetical outcomes of FMT treatment on microbiota composition. The interaction between two microbial consortia during FMT treatment may be likened to a complex pulse perturbation intended to transfer the functional properties of a donor microbiota to a recipient. Several potential outcomes can arise. First, one possibility is that the host microbiota returns to its original dysbiotic state (referred to as stable state A), as the introduced microorganisms fail to establish themselves permanently due to an insufficient perturbation. Second, the interaction may lead to the establishment of a completely new microbial community (referred to as stable state B), comprising species neither from the donor nor the original community. This novel community may arise due to a combination of factors such as niche availability, competitive exclusion, and environmental influences. Importantly, this new community could exhibit either beneficial or dysbiotic properties, depending on the specific composition and functional attributes of the newly established species. Third, due to intrinsic host or environmental factors, an alternative state is selected as the outcome (referred to as stable state C), comprising a mix of donor, patient, and new species. Fourth, resilience of the donor community (referred to as stable state D) in the new habitat could lead to a new interaction with long-term transfer of potential beneficial properties. This figure is adapted from Sommer et al. (2017).[23]

In Chapter 6, we applied a methodology inspired by Schmidt et al. 2022 to the same dataset as the one used in Chapter 5 to investigate the extent to which a shift in the patient's microbiota towards the donor microbiota is beneficial for resolving dysbiosis in the patient's gut.[199] Engraftment has long been considered a key mechanism underlying the success of fecal microbiota transplantation.[399] However, insights from earlier studies have raised questions about what happens to all the species involved during the succession phase of the treatment (during and after FMT).[188, 199] Therefore, we categorized species within the recipient's gut microbiota into ecological groups based on their origin and presence over time: those either already present in the host before FMT, derived from the donor, or introduced as novel species (absent in both host pre-FMT and donor samples). Our findings revealed that responders retained more resident species and maintained a more constant level of colonization over time compared to non-responders. This suggests that a favourable response to FMT is facilitated by a microbiota receptive to colonization, without compromising the resident community.

Restoring the microbiota with an FMT treatment is a complex process, as different taxa recover or colonize to varying extents, with some failing to (re)establish entirely.[58, 86, 226, 465] This variability can be influenced by suppression and resource competition between invaders and resident species.[87, 466-468] To mitigate the pressure from the resident species, a bowel lavage was performed prior to the first treatment, allowing for a more conducive environment for donor species to colonize. However, it is likely that the species that successfully colonize the gut after FMT are those closely related to the original inhabitants, as the gut environment provides a suitable niche for their growth.[87, 188, 469] Even if the original species are replaced or supplemented by similar ones, the new microbes may potentially introduce new traits that alter the ecosystem's functionality and metabolic output.[95] Moreover, if donor species may fail to establish, they might still be able to impact the recipient community's functioning and induce autonomous changes through interactions with resident members, for example by horizontal gene transfer or local metabolic activities while passing through.[88, 95, 466, 467] It has been shown that in a fluctuating environment, rapid evolution can destabilize the long-term stability of interactions, potentially enhancing adaptability and resilience or disrupting microbial balance and health.[470] Our study could not determine whether the species that reappear are leftover residents that regrew post-lavage, whether they gained additional functions, or whether they originated from the transplanted donor material if they are identical to the recipient species pre-FMT.

Future directions for microbiome research in health and disease

The recognition of the microbiome's critical role in our health marks a significant shift from traditional clinical perspectives, which often view the body as a battleground between human cells and microbes (i.e., pathogens) to an understanding that embraces the complex ecological community context of the microbiome. A dysbiotic human gut microbiome can be likened to plant or animal communities in a highly disturbed environment, e.g., impacted by overfishing, (abrupt) climate change, habitat loss, ocean acidification, pollution, or an invasive species. Human interventions, such as generic antibiotic use, have demonstrated detrimental effects on both the microbiome and human health, mirroring the irreversible changes observed in disrupted ecosystems where pesticides are used instead of ecological management measures. Therefore, to overcome dysbiosis in complex chronic diseases, we can draw inspiration from strategies such as habitat restoration and targeted removal of invasive species, which have been successfully applied in large-scale biodiversity management. For chronic diseases, an ecological maintenance approach may be more effective than the traditional battlefield strategy.[22, 226]

The limitations of the traditional 'one-size-fits-all' treatment approach, based on broad population averages, have also become increasingly apparent due to the heterogeneity in genotypes and phenotypes of gastrointestinal diseases among human populations. For example, matching donors and recipients by lifestyle and diet could enhance the likelihood of transplanting species that are effective colonizers or providing the resident species with the necessary metabolites that support their growth and function, thereby potentially improving the recipient's microbiome more successfully. Potentially, a better FMT success can also be achieved through the administration of specific prebiotics alongside the microbes. By providing targeted substrates exclusively metabolized by preferred species, prebiotics could create an advantage for them.[471, 472] Such an approach potentially strengthens the recipient's own microbiota and enriches it with species that naturally belong to the same community, leading to a more harmonious and effective community. However, the specific species that are most beneficial and those that are naturally suited to the community still need to be identified before this strategy can become a reality. As our understanding of the microbiome ecosystem advances, doctors will hopefully be equipped with precise disease prevention strategies and more effective treatments in the future.

A recurring theme in microbiome research is the need for large, densely sampled temporal datasets encompassing individuals from diverse backgrounds and lifestyles. Such datasets would be instrumental in unraveling fundamental mechanisms governing ecosystem dynamics in health and disease. Furthermore, studying microbiomes from various geographical regions (e.g., Africa) is important for capturing the global diversity in microbiological research, as most studies to date have focused on the United States, Europe, and Asia.[473] The unique environmental factors, dietary habits, and cultural practices in different regions in the world can significantly influence microbial composition and function.[474] By incorporating diverse microbiomes into our research, we can enhance our understanding of microbial dynamics that could inform health strategies and identify important confounding variables that may influence health outcomes.

Another way forward is to paint a more comprehensive picture of the microbial ecosystem with an integrative ecosystem biology approach that combines multiple omics technologies with host physiological data, and in depth knowledge of bacterial species behaviour and their (chemical) environment.[3, 60, 152, 244, 440, 475] By examining fecal matter in more detail alongside dietary questionnaires or food diaries, we might be able to extract valuable information about the host's diet, offering more insights than what is typically available. Note that the presence of a nutrient in a fecal sample is often assumed to indicate its importance for the microbiome. However, it could also be present because the species have not utilized it, leaving it to be excreted in the feces. Additionally, simultaneous assessment of mediators of reciprocal host-microbe interactions, such as microbial metabolites and immunological parameters, holds promise for identifying causality, discerning what changes first and who or what influences whom at various points in time.[121] At present, a significant question remains unanswered: whether the microbiota differs in various disease states because it causes these states, whether the microbiota differs as a consequence of the patients' disease state, or whether both are caused by the same external factors (for example altered diet or lifestyle). Mixing up association with causality can lead to an overestimation of the clinical relevance and impact of the microbiome on diseases.[214] For example, bacteria associated with unhealthy microbiomes may not necessarily be those directly related to the disease; instead, they could merely be among the few species capable of thriving in a gut environment with reduced diversity (possibly due to chance as described by the neutral theory); or they may play a beneficial role by supporting the host in the restoration of the healthy microbial community.[476]

Future research could also aim to identify not only bacteria, but also other microbes such as Archaea, fungi, and viruses, while exploring their interactions with each other and with bacteria, as well as their potential roles in health and disease. This includes investigating phage therapy as a strategy to target specific bacteria or pathogens, as bacteriophages may regulate intestinal microbiota diversity through mechanisms such as the kill-the-winner principle (which targets the most abundant bacterial species) or by specifically eliminating a species of interest, thereby preventing, for example, the outgrowth of Prevotellaceae in UC patients.[67, 477-479] Cross-domain networks may be important in understanding microbiome dynamics and ecosystem resilience, as there are many correlations with the bacterial microbiome and other domains.[428]

It is important to find a balance between collecting extensive data and maintaining clarity and interpretability. Merely increasing sequencing efforts is insufficient; the analysis pipelines must also continuously evolve to accommodate the influx of new data types and quantities. Moreover, focusing on excessively granular data might lead to a loss of statistical power due to the large number of species or functions relative to the number of patients and the prevalence of rare taxa. Additionally, the fact that different bacterial species can perform the same functional role in different patients may require a much larger sample size or functional assay than is (currently) possible in microbiome studies.[118, 168] On top of that, even the 'simple' networks with only bacteria generated from currently available data are challenging to grasp. Before introducing further complexity, we must step back to reflect on our research designs and develop strategies to effectively comprehend the influx of new information.

Finally, increasing the database of cultured microorganisms and annotated genes is needed for a comprehensive understanding of microbial function and for creating benchmark data to improve the evaluation of tool performance.[118, 480] Without the information about, for example, functional redundancy, dormancy, and phenotypic plasticity, taxonomic data alone offers limited insights into ecosystem processes across space and time.[425, 480, 481] Fundamental research on gut microbiota, including culturing of isolates, remains important for understanding the interspecies interactions and bacterial behaviours and dynamics, as it provides species-specific knowledge.[482] Mechanistic research in wet-lab and (animal) models is also imperative to validate the hypothesized mechanisms of species behaviours, not only for the most abundant ones, as they may not be the most important from an ecological point of view.[483-488] However, replicating complex human gut microbiome interactions (in artificial gut models) poses significant challenges, despite all the current advances in the field, and warrants further improvements.[118, 489-491] Ideally, establishing gold standards for microbiota data analysis and comprehensive reporting of (meta)data would enable more meaningful comparisons across studies, a call made over a decade ago but still largely unaddressed.[492, 493]

Concluding remarks

The journey of microbiome research reveals both the complexities and the promises for enhancing human health. As technology advances, so does our understanding of the microbiome. More fine-grained studies on the (gut) microbiome and its role in human health are needed to provide interpretation and meaning on the differences already found. Sophisticated technologies, such as Artificial Intelligence (AI), machine learning, and network analyses hold potential for identifying patterns within microbiota community data. However, those results should still be considered in light of past discoveries, established methods and models, and longstanding theories from multiple fields. When we combine (mathematical) modeling, theoretical knowledge, and experimental approaches, we gain a more comprehensive understanding of complex biological systems allowing us to validate results, do predictions, uncover underlying mechanisms, and refine our models for more accurate insights, as demonstrated throughout this thesis.

Collaborations across multidisciplinary groups, comprising, among others, (microbial) ecologists, healthcare professionals, complexity scientists, and bioinformaticians will further enrich our research field. Complex systems exist on a spectrum between order and randomness. Although one can get lost in the hairball of a complex network, knowledge from several fields can help. Understanding how systems respond to changes and return to stability enhances our grasp of the complex dynamics within the human gut microbiome. This knowledge can ultimately improve microbiome-modulating strategies and drive innovation of therapeutic strategies. Improved data sharing practices, including publishing raw data in a standardized fashion and statistical code will facilitate higher-quality meta-analyses and the establishment of more robust microbial signatures for diseases.[439, 494] Unfortunately, data accessibility still poses a significant challenge in microbiota research, with researchers frequently withholding study-related data. While concerns about privacy and efforts required for data collection are understandable, limited data sharing impedes scientific advancement.[495-500]

By recognizing that each step brings us closer to harnessing the microbiome's potential to improve human health, we ensure continuous progress and discovery. To truly understand microbial dynamics, it is important to acknowledge that human time is vastly different from bacterial time. Bacteria perceive their environment, resources, and interactions on a much smaller spatial scale. They constantly adapt to their immediate surroundings and rapidly shifting communities. An (microbial) ecological perspective grounded in theory is essential to interpret the impact of the microbiome on our health and disease.