Microbial communities play an important role in human society. They, for instance, clean our waste water and digest our food. To enhance their performance, a better understanding of the relationships and interactions between the community members is required. Therefore, microbial communities and their interactions are intensively studied. However, one of the challenges of studying microbial communities is that most species in an ecosystem are not culturable and therefore, we do not know the metabolic capabilities and potential interactions of most species. Fortunately, sequencing of the full genome of a species allows to identify its metabolic potential without requiring phenotypical data. Nowadays, all DNA from a microbial community is sequenced, the so-called metagenome, and this provides useful insights about a microbial community. The metagenomic data provides a wealth of information, but the work in this thesis was performed in the belief that more information can be extracted from metagenomic data. The way we did this is to integrate the experimental data with genome-scale metabolic models.

Genome-scale metabolic models are created based on the genomic information of a species and contain the full set of metabolic reactions represented as stoichiometries and thereby neglecting the kinetics of these reactions of a species. Though the metabolic models are relatively simple, they are very powerful for the study of pure cultures to, for instance, find targets for metabolic engineering strategies. Since the metabolic models are so successful for pure culture studies, they have the potential to also become an important tool in the study of microbial communities. Therefore, we investigated how useful these genome-scale metabolic models are in the study of microbial communities. We tested different microbial ecosystems to understand what the strengths and weaknesses are of this modeling approach.

In **chapter 1** we give a general introduction about microbial ecology. We also describe what techniques are used to answer the fundamental questions in the field. Additionally, we explain the potential of genome-scale metabolic models to study microbial ecosystems, in particular with the commonly used method of 'Flux Balance Analysis' (FBA). We summarize what questions can be answered with this method and how synthetic ecosystems could help to understand microbial communities.

We explain in more detail in **chapter 2** how we think metabolic modeling of microbial ecosystems could improve our understanding of microbial communities. We argue that the level of detail in the model is dependent on the ecosystem and the type of research question that one wants to answer. We also think that the metabolic models are a useful tool for data integration, especially for the inference of metabolic interactions from experimental data.

One of the advantages of a genome-scale metabolic model is the possibility to test several hypotheses *in silico* that are difficult to test *in vivo*. We tested several hypotheses regarding free energy transduction of the methanogen *Methanosaeta concilii* in **chapter 3**. It is unknown what strategy *M. concilii* uses for its energy conservation and therefore several hypotheses were postulated in the literature. From our *in silico* analysis we concluded that the most likely scenario that *M. concilii* can grow is that it contains more efficient proton and sodium ion translocating proteins relative to other methanogens. We also stressed the importance of the biomass composition in this genome-scale metabolic model for accurate prediction of the growth rate.

We then tested whether it was possible to predict the phenotypic behaviour of *Clostridium acetobutylicum* in a co-culture with a curated genome-scale metabolic model in **chapter 4**.

The interaction between *C. acetobutylicum* and the other species was based on interspecies hydrogen transfer and we observed differences in the metabolism of *C. acetobutylicum* when H_2 was rapidly removed by the other species. This phenotypic behaviour cannot be predicted with a genome-scale metabolic model alone, and additional kinetic parameters had to be implemented into the model of *C. acetobutylicum* to simulate its behaviour in a co-culture correctly. Clearly, and not unexpectedly perhaps, behaviour from monocultures that is used to validate genome-scale metabolic models, cannot always extrapolated to the phenotype of a species in a microbial ecosystem. However, such models can still be useful to explain the phenotypic behaviour of species in a community; they might be useful for the inference of metabolic interactions in a microbial ecosystem. Therefore, we co-cultured *C. acetobutylicum* and *Wolinella succinogenes* during different environmental conditions to investigate the impact on the interspecies hydrogen transfer in **chapter 5**. We integrated the experimental data with the computer models for the inference of the metabolic interactions in the community. We showed with this approach that the nitrogen source affects the rate of hydrogen transfer between the species which could not be concluded from the experimental data alone. This study successfully showed that integration of experimental data with a modeling approach results in the inference of metabolic interactions in a microbial ecosystem.

In previous chapters we worked with synthetic ecosystems where we could design the type of interactions between the species. In **chapter 6** we worked with a more complex and natural ecosystem: a yoghurt co-culture consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Firstly, we conducted an evolution experiment with *S. thermophilus* and *L. bulgaricus* that had no growth history with each other. We investigated whether they would enhance the interactions between the species during the adaptive evolution. The evolved co-culture developed some interesting industrial traits and from the genomic, transcriptomic data and the metabolic modeling of the co-culture we concluded that the evolved co-culture improved the interactions related to amino acid and purine metabolism.

In the last chapter (**chapter 7**) we discuss the impact of genome-scale metabolic models and synthetic ecosystems on the study of microbial ecosystems. We have shown in this thesis that genome-scale metabolic models are very useful to study individual species in a community, investigate the potential interactions between species in a community and infer the metabolic interactions from simple synthetic ecosystems. However, inference of complex microbial communities is more difficult and its success highly depends on the ecosystem and the experimental data that has to be integrated. Overall, genome-scale metabolic models is a powerful tool to study microbial ecosystems.