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Soil bacterial community assembly during succession

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CHAPTER 7

Synthesis

The mounting evidence that microbial diversity affects ecosystem functioning and stability justifies further studies of the mechanisms driving species coexistence and the dynamics of community composition in space and time. The work presented in this thesis studies ecological processes governing the turnover of soil bacterial communities during succession with particular attention to the dynamics of the common and rare biospheres. It also investigates the influence of dispersal mediated by contemporary conditions and historical contingency on community assembly. Soils represent a major organic carbon reservoir and contribute an important habitat for bacteria (among other microorganisms) that are essential for nutrient cycling and the maintenance of below- and above-ground productivity and diversity. This thesis attempts to reveal the mechanisms underlying the community assembly of soil bacterial communities and the turnover of bacterial groups with different abundance distribution patterns during succession by answering the questions presented in **Chapter 1** (see Aims of this thesis and Figure 1.1).

In this chapter, I will discuss the knowledge gathered from previous chapters (see Figure 7.1). In brief, I start this thesis by examining knowledge on microbial succession from literature, as well as the influence of sequencing approaches on quantifying bacterial community turnover and assembly. Next, I discuss aspects of community assembly of the microbial rare biosphere, and a framework to disentangle the mechanisms underlying the coexistence of the different types of rarity. Later, I show the influence of dispersal as promoted by inundation on soil bacterial communities at different successional stages. Here I also discuss the influence of contemporary conditions and historical contingency on the dynamic changes in soil bacterial communities. Last, I describe the outlook of this thesis with an emphasis on the general conclusions, current challenges and potential future directions in this field of research. All my research questions were addressed by studying a barrier island in the Wadden Sea, Schiermonnikoog, the Netherlands. This system presents a unique undisturbed salt marsh chronosequence spanning over 100 years of primary succession.

Microbial community succession

Ecological succession is characterized by the trajectory of species replacement and changes in relative abundance over time. Knowledge of microbial succession helps to understand temporal dynamics of microbial communities and predict how microbial communities develop from newly formed habitats or after disturbance events. In **Chapter 2**, I found consistency in primary succession when synthesizing results from a growing body of literature on microbial succession. For instance, microbial biomass increases over time during primary succession, such as in the glacier retreat chronosequence, the colonization gradient of volcanic deposits, and in the rhizosphere of plants during growth developmental stages. Besides, microbial communities often develop from simplified make-up at the onset of colonization towards complex community structures and arrangements. In contrast, trajectories of microbial secondary succession lack consistency. After disturbance, microbial communities can either recover to the original state or develop alternative states. This directly relates to the resistance and resilience of communities before disturbance, and the type, duration, strength and frequency of disturbance events (Shade et al. 2012).

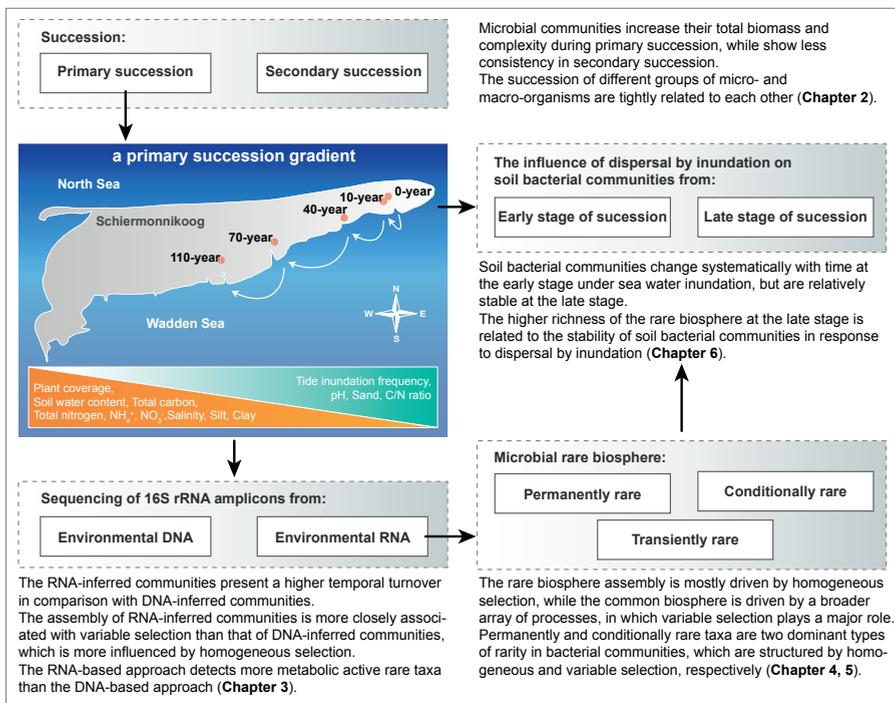


Figure 7.1 Summary of the main results in the corresponding chapters of this thesis. Environmental factors increasing and decreasing along the primary successional gradient are shown in orange and turquoise, respectively (middle left). Keywords in grey boxes relate to the topics presented in this thesis. Main results are listed beside each inset box.

The succession of each group of micro- and macro-organisms is not independent, but tightly associated with each other. For instance, the interaction between plant and soil microbial communities through litter input and root exudates are especially important in early successional stages, which are typically characterized by nutrient-poor soils (Knelman et al. 2012). In comparison with bacterial communities, the development of fungal communities is tightly associated with the development of plant communities in a course of primary succession (Harantová et al. 2017). By focusing on previous studies performed in this system (Schiermonnikoog), I found that communities of micro- and macro-organisms developed together as succession proceeds (**Chapter 2**). Also, both bacterial and fungal communities reach the highest biomass at the intermediate stage of succession (ca. 40–50 years) and remain relatively stable afterward [see Figure 2.1 and 4.1 in (Dini Andreote 2016)]. Even though bacterial communities associated with the rhizosphere and endosphere of the plant *Limonium vulgare* show different distribution patterns along the successional stages (Wang et al. 2015), additional evidence on direct interactions between micro- and macro-organisms are still missing in this system, thus requiring future research efforts.

In addition to the biological components, methodological issues might also influence our ability to study patterns of microbial succession. Amplicon sequencing of the bacterial 16S rRNA gene from environmental DNA is often used to profile community composition during succession. Due to the existence of relic DNA in soil, I explicitly considered how sequencing approaches (i.e. sequencing 16S rRNA amplicons from environmental DNA and RNA transcripts) influence our interpretations of the assembly processes during primary succession in **Chapter 3**. In comparison to the DNA-based approach, the RNA-based approach revealed a higher temporal turnover of bacterial communities in both short-term (bimonthly) and long-term (over 100 years succession). The difference relates to ubiquitous relic DNA in soil and the short half-life time of RNA, as the dead bacterial cells profiled by relic DNA obstruct signals of changes in community composition. Different from the previous findings that relic DNA inflates the estimation of richness (Lennon et al. 2018), in **Chapter 3**, I observed a higher richness in the RNA- than DNA-based dataset. This discrepancy is related to the low-abundance species from the rare biosphere that are metabolically active, which can only be detected by the RNA-based approach rather than the DNA-based approach when using the same sampling effort.

Species turnover during succession is mediated by shifts in community assembly processes. **Chapter 3** shows a distinct interplay of ecological processes in the assembly of microbial communities across successional stages and the increasing influence of deterministic processes as succession proceeds, which is consistent with previous findings (Dini-Andreote et al. 2015). Dini-Andreote et al. (2015) found that stochastic processes prevail at early successional stages, while late stages are strongly affected by deterministic processes. However, **Chapter 3** of this thesis indicates that selection also plays an important role in early successional stages. This difference may be attributed to the variation in sequencing depth between both studies, which is varied from 2,600 to 15,000 sequences per sample in Dini-Andreote et al. (2015) and **Chap-**

ter 3, respectively. Higher detection of rare species, which are primarily structured by selection processes (**Chapter 5**), can be profiled with higher sequencing depth. Therefore, a stronger signal of variable selection was observed at early successional stages in **Chapter 3** compared to what was described in Dini-Andreote et al. (2015). This selection might be a result of temporal variation in soil conditions induced by the tide, such as changes in oxygen and water content. Besides, **Chapter 3** also investigates how sequencing approaches affect the relative importance of each assembly process. The assembly of RNA-inferred community was more closely associated with variable selection driven by environmental heterogeneity, while the assembly of DNA-inferred community was more influenced by homogeneous selection driven by the consistent environmental conditions. This also occurs because RNA is less stable than DNA in the environment. The RNA-based approach encompasses a fewer number of dead microbial cells than the DNA-based approach. This results in a stronger association of community changes with environmental fluctuations (i.e. variable selection).

Assembly of the rare biosphere

The rare biosphere hosts the majority of species diversity in a community (> 90 % of total species) and it is characterized by the long tail in the rank abundance curve (**Chapter 5**). Disentangling the interplay of ecological processes structuring the rare biosphere helps to answer how highly diverse microbial communities maintain such a high number of coexisting species. To understanding the mechanisms controlling rarity and the processes promoting the dynamics of the rare biosphere, in **Chapter 4**, I proposed to link the types of rarity with the relative influence of assembly processes, using the phylogenetic approach coupled with ecological null models to disentangling the processes structuring the rare biosphere.

These ideas were further tested in **Chapter 5** using empirical data from **Chapter 3**, enabling to examine the dynamics of the rare biosphere across both the short (bimonthly) and long (succession over 100 years) temporal scales. Worth mentioning, the five successional stages also served as an environmental gradient across space, as the space-for-time replacement aspect of chronosequence is often assumed to represent the successional stages (see Box 2.1 in **Chapter 2**). I found the rare biosphere assembly to be mostly driven by homogeneous selection (i.e. spatiotemporally constant), while the common biosphere assembly — which consists of abundant species — is driven by a broader array of processes, in which variable selection (i.e. spatiotemporally variable) plays a major role. In other words, both the rare and common biospheres are tightly associated with selection processes. While the rare biosphere is mostly determined by stringent environmental factors, the common biosphere is strongly structured by variation in environmental factors. This finding stands in contrast to the notion that rare species are controlled by stochastic processes, for instance, due to their small population size, rare species are much more susceptible to ecological drift (Vellend 2016). This discrepancy can be attributed to the fact that distinct types of rarity exist, thus leading to an interplay of different ecological processes structuring their occurrence [see **Chapter 4** and (Lynch and Neufeld 2015)].

By partitioning the different types of rarity, in **Chapter 5**, I found homogeneous selection to explain the prevalence of permanently rare taxa, whereas variable selection explained the dynamic of conditionally rare taxa. This suggests environmental conditions that impose selection on permanently rare taxa are constant, while substantially changed on conditionally rare taxa. Conditionally rare taxa stay at low abundances in the rare biosphere to avoid harsh conditions and build up their population sizes once favourable environmental factors emerge, thus reaching up to abundance levels of common taxa. Most taxa in the common biosphere of one community can be found in the rare biosphere of other communities, indicating part of the rare biosphere (about 1/3 of rare taxa) serves as a reservoir for the common species under harsh environmental conditions. The permanently rare species (about 2/3 rare taxa), however, are more prone to perish at low abundances despite environmental disturbances. Last, a small fraction of the rare biosphere accounts for transiently rare taxa. The restricted dispersal process is likely responsible for the existence of transiently rare taxa in the soils. This occurs because some species introduced by limited dispersal are unable to reach high fitness in the new habitat or it is constrained by the resident community, such as pelagic microbes carried by the tide (**Chapter 6**), terrestrial microbes dispersed by the wind from long distance, or microbes hitchhiking on eukaryotic organisms (Grossart et al. 2010, Albert Barberán 2015). Together, this chapter contributes to our understanding of how the relative importance of ecological processes shape the rare biosphere and the dynamic of distinct types of rare taxa, providing an example to be tested in other ecosystems, and potentially across other organismal types.

The influence of dispersal during ecological succession

Dispersal is an important process influencing the assembly of soil bacterial communities in salt marshes. In **Chapter 6**, I evaluated the extent to which soil bacterial communities from different successional stages respond to dispersal by simulating inundation. Here, a microcosm experiment was performed by the application of natural sea water to simulate four levels of inundation frequency, i.e. 2× per day, 1× per day, 1× per 3 days, and 1× per 7 days. Soils collected from early (0-year) and intermediate/late (70-year) successional stages were used, since different successional stages host distinct bacterial species and community composition. Sterile sea water was also applied allowing to distinguish the different influences of biotic (dispersal of microbial cells) and abiotic (physicochemical changes in soil). In brief, I found no significant effect of the overall influence of inundation across the four levels of inundation frequency tested at soils from both successional stages. Sampling time was the most important factor driving the variation in soil bacterial communities. Both α - and β -diversity of bacterial communities changed systematically with time in the early stage, but were relatively more stable in the late stage, which might link to the higher diversity, selection pressure and historical contingency found at this stage.

Soil at late stages is characterized by high nutrient contents, which provides more niches and therefore supports higher bacterial diversity. Together with our finding, this corroborates with the notion that higher diversity leads to higher stability (Wagg

et al. 2018). Bacterial communities at late stages with higher diversity buffer against environmental fluctuation induced by sea water inundation, and also resist the colonization of later arriving species by occupying more niches and leaving less available niches. In contrast, early successional stages with lower diversity are more prone to be affected by inundation by both biotic and abiotic factors. This is mostly a result of changes in members of the rare biosphere, suggesting rare microbes in the early successional stage of salt marsh are sensitive to inundation and can be vulnerable to accelerated sea-level rise. Alternatively, the effect of dispersal might also depend on the selective pressure imposed on local bacterial communities in two successional stages. For instance, sodium concentrations are lower in the soil of early successional stages compared to later successional stages, thus providing little selective pressure there, which allows dispersal processes to more profoundly affect the assembly of bacterial communities. Last, the different responses of these two successional stages to dispersal by inundation indicate succession exerts a cascade of historical contingency effects at which the species at the earlier successional stage influence the successful colonization of species at the next stage during soil formation.

Direction for future research

Bridging gaps between general and microbial ecology

Both empirical and theoretical approaches are needed to effectively evaluate how microbial communities are assembled across multiple spatial and temporal scales. With the advances in sequencing approaches, we are experiencing an explosion in the field of microbial ecology and its integrating with aspects of community ecology. Theories from macroecology have been tested in microbial ecology, even though these theories do not always fit the rules and specificities of microbial systems. As an important gap still persists between the theoretical framework of macroecology and microbial ecology (McGill et al. 2019), it is crucial to find what are the differences between the macro- and microbial ecology, what causes these differences, and what are the general rules applied for both systems.

Disentangling biotic and abiotic selection

In this thesis, I used the four general eco-evolutionary processes, i.e. selection, dispersal, ecological drift and diversification, proposed by Vellend (2010) as a framework to study the community assembly of soil microbes. Despite homogeneous and variable selection are partitioned in this thesis, selection is a general ecological process that relates to the variation of both abiotic and biotic factors. The distinction between these two was not directly partitioned in this thesis. In particular, species utilize a variety of resources or have different environmental optimal conditions. Future studies focusing on which and how environmental factors affect the co-existence and distribution of species are still needed. Besides, divergent types of species interactions (e.g. mutualism, syntrophy, proto-cooperation, commensalism, antagonism/ammensalism, parasitism, predation, competition) play fundamental roles in structuring the ecological dynamics of microbial communities (Faust and Raes 2012, Lv et al. 2019). For in-

stance, protist predation influences the structure of bacterial and fungal communities in the rhizosphere (Gao et al. 2019). Efforts on investigating these interactions within and between different microbial groups (e.g. bacteria, fungi, viruses, protists, algae) are of crucial importance for advancing our understanding of species coexistence and their consequences on ecosystem functioning (Ratzke et al. 2020).

Microbial speciation

The speciation process of microorganisms is often faster than that of macro-organisms. This is mostly due to their shorter generation times and higher ability to exchange genetic materials across far-related phylogenetic groups. Thus, rapid evolution in microbes potentially leads to the convergence of ecological and evolutionary scales. In other words, speciation/diversification is tightly tangled with other ecological processes, such as in the case of biotic interactions being a key predictor of bacterial evolutionary trajectories when species adapt to novel environmental conditions (Scheuerl et al. 2020). Experiments using controlled microbial population in the laboratory can enable to empirically test this evolutionary process, for instance, a team lead by Richard E. Lenski has reported the evolutionary trajectory of 12 *Escherichia coli* populations in 60,000 generations during a >25 years experiment. They found that populations diverge into ecotypes evolving new abilities to exploit previously untapped carbon sources locally available (Lenski 2017). However, due to several challenges associated with measuring diversification *in situ*, we still lack a consensus of how diversification together with ecological processes influence bacterial community structure and dynamics. Hence, future efforts on the direction of eco-evolutionary dynamics and its influence on community assembly and succession are still needed.

Beyond soil bacteria

This thesis seeks to develop a general framework for understanding the ecological processes structuring the rare biosphere across distinct types of rarity. To generalize conclusions on the rare biosphere, future studies require comprehensive investigation of a broad array of microbial groups and different habitats with the application of state-of-art technologies. The high diversity in the rare biosphere not only appears in bacterial communities, but also in other microbial groups. For instance, rare freshwater protists represent the majority (>70%) of total OTUs and phylogenetic diversity (Debroas et al. 2015). Further studies on the rare biosphere of other microbial groups, such as viruses, archaea, fungi and microeukaryotes, will facilitate finding general mechanisms shaping the microbial rare biosphere. Moreover, although in this thesis I focused on bacterial communities in the soil system, bacterial communities are ubiquitous across divergent systems, including 'harsh' or 'poor' (human perspective) environments, such as the deep subsurface environment (Bar-On et al. 2018). Studies across distinct systems are still scarce, especially concerning how the rare biosphere is assembled (Anderson et al. 2015, Yang et al. 2017). In addition, to better understand the dynamics of the rare biosphere, a well-designed study with frequent time-series data collection is also needed, given that the majority of studies based on snapshots in time are unable to capture the temporal dynamics of rare taxa with faster generation turnover rates. Besides, some

ultra-rare species, which are unable to be detected by amplicon sequencing, can only be noticed when they reach high abundances as conditions change (Emerson et al. 2017). Thus, constituting another step forward and dependent on the sensitivity of approaches and new technologies for species detection.

Ecological significance

Recent studies reveal that rare species are often active, and some of them can even perform disproportional functionality in a system. However, the ecological roles of the majority of species in the rare biosphere are still unknown. The recovery of their genomes through culture-independent approaches, e.g. metagenome assembly (Parks et al. 2017, Nayfach et al. 2019) and single-cell sequencing (Woyke et al. 2017, Pachiadaki et al. 2019), can provide novel insights into their ecological roles. For instance, the metagenome-assembled genomes (MAGs) from the rare biosphere showed that a rare bacterium belongs to the candidate phylum Goldbacteria is able to grow on cellulose (Doud et al. 2020), and four rare bacterial populations from the candidate phylum TM7 can only use the pentose phosphate and heterolactic fermentation pathways (Albertsen et al. 2013). Implementing a targeted cell-sorting approach with subsequent single-cell sequencing, Dam et al. (2020) have enriched Chloroflexi species from the rare biosphere in a wastewater treatment plant and obtained their draft genome. Since the microbial rare biosphere contains a large diversity and mostly remains unculturable species, applying the above-mentioned approaches to discover novel gene variants and metabolic pathways from the microbial rare biosphere has a potential implementation for designing biotechnological applications in the future (Pascoal et al. 2020).

In addition to the assessment of uncultivated genomes of rare microbes, infer the ecological traits of rare microbes is of particular importance to unravel the ecological processes maintaining their coexistence with common species. Given that the ecological traits of most rare microbes are still hard to investigate *in situ*, improving cultivation strategies of novel microbes can be an alternative to acquire physiological information of rare microbes (Lewis et al. 2020). Among these attempts, a droplet-based high-throughput cultivation can culture and accurately screen microbes that cannot grow easily in plates due to competition by quorum sensing (Watterson et al. 2020). Approaches like this provide a chance to improve understanding of optimal environmental conditions and niche breadth of rare species, allowing us to answer questions, such as, *whether rare microbes have narrower niche breadth than common microbes?* and *whether niche differentiation plays a fundamental role in rare and common species coexistence* (Sexton et al. 2017)?

Concluding remarks

Understanding the contribution of ecological processes structuring microbial communities and predicting their dynamics are ongoing challenges in ecology. To address these challenges, this thesis combines ecological models with empirical observations from both field sampling and well-designed experimentation. This thesis provides microbial ecologists with advanced insights on how bacterial communities are assembled

during ecological succession, and how the bacterial rare biosphere is structured by a dynamic interplay of quantifiable ecological processes. Many interesting and important questions remain to be investigated in further studies. Tackling these questions will undoubtedly advance our knowledge of community assembly during microbial succession, the importance of the rare biosphere and their role in ecosystem functioning. In doing so, we can advance towards better models to explain and predict the diversity of bacterial communities in the constantly changing world.