General introduction

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The themes of community assembly and ecological succession in soils constitute rather traditional concepts in ecology (pioneered by Clements, 1919; and Gleason, 1927), that are as yet not fully appreciated in soil microbiology. This is due, in part, to methodological impairments associated with observing microbes in nature, and to the different historical paths taken by the disciplines of microbiology and general ecology (Jessup et al., 2004; Prosser et al., 2007). However, over the last two decades, microbial ecologists have witnessed unprecedented advances in their technical abilities to access microbial communities, in particular across a vast range of soil ecosystems worldwide (e.g. Terragenome Project Consortium, Vogel et al., 2009). As a result, long-standing questions in soil microbiology, ranging from the classical ‘who is where?’ and ‘what are they doing?’ to more process-oriented ones, like ‘how will they respond?’, can now be answered on the basis of data obtained at high resolution from experimental or observational studies of the geographic and habitat distributions of microorganisms in living ecosystems (Martiny et al., 2006; Jansson and Prosser 2013).

While community assembly centres on the end state of species diversity and composition of a given community following the creation of an open space, primary ecological succession focuses on the trajectory of species replacements following the initial colonization of a local habitat (Chase and Leibold 2003). The two concepts are intrinsically related, implying that both the ‘static’ measurement of a given community and the understanding of how the interplay of eco-evolutionary processes shapes the systematic changes in community structures with time matter (Figure 1.1). The growing expansion of this field of research has led to confusing terminologies (Nemergut et al., 2013). Along this thesis (with special emphasis on chapter 3), I centre my discussions on a previously developed conceptual synthesis in community ecology (Vellend, 2010). This synthesis stands as an underlying framework to interrogate and interpret how different ecological processes (dispersal, selection, diversification and drift) operate, shaping the dynamic changes in communities in space and time. A detailed overview of these ecological processes is provided in Box 1.

The field of microbial community assembly and ecological succession in soils is still recent, with the majority of the available literature relying on the investigation of community dynamics in receding glacier forelands (e.g. Sigler and Zeyer, 2002; Nemergut et al., 2007; Schmidt et al., 2008), post-mining areas (e.g. Hüttl and Weber, 2001; Kozdrój and van Elsas, 2001), abandoned agricultural fields (Elhottová et al., 2002; Kuramae et al., 2010) or forest fields that have experienced wildfire disturbances (Ferrenberg et al., 2013). A common feature of many of these studies is the examination of the dynamism of the microbial communities across natural trajectories of landscape formation (or recovery, in the case of disturbance). This occurs through the intertwined influence of shifting local abiotic factors and the activity of soil microorganisms. Critical for such studies is the use of so-called chronosequences, as environmental models for temporal soil ecology (Box 2). The inherent assumption of space-per-time replacement of chronosequences allows the interrogation of ecological and potentially physiological changes in the communities across temporal scales — which in the case of microbes can range from hours to months or decades to millennia — in a contemporary manner. In this thesis, I made use of a soil environmental chronosequence that occurs through a natural salt marsh landscape, located at the island of Schiermonnikoog, The Netherlands (details on the system are provided
below). I purposely focused the work carried out throughout this thesis using this model system with a two-pronged perspective: (i) fostering the understanding of how different mechanisms drive community assembly and promote spatiotemporal dynamics through ecological succession of the microbial communities along the chronosequence; here I provide both the phylogenetic and functional aspects of the communities — ‘the ecosystem ecology perspective’; and (ii) explore this natural system for general ecological principles and mechanisms that are potentially applicable across other systems — ‘the microbial ecology perspective’. With respect to the first perspective, the knowledge gained in this thesis provides a better understanding of microbial community variations, connecting these to potential changes in ecosystem functioning. As such, the data are of critical importance for the development of models that aim to better manage salt marsh ecosystems, in particular with respect to monitoring and mitigating anthropogenic and climate change impacts. The second perspective, however, aims at contributing to the continued integration of microbial ecology into the broader field of (theoretical) ecology. I developed and applied concepts from the field of community ecology within microbiology, in an effort to improve our knowledge on the mechanisms that mediate microbial community assembly and successional dynamics. Last but not least, I posit that — given the peculiarities of microbial systems (e.g. growth rates and dynamic responses at relatively short time scales) — microorganisms stand out as very suitable model organisms to explicitly develop and test the ecological principles that are of relevance to theoretical and practical community ecology.

**Figure 1.1.** Schematic representation of ecological succession. The figure displays four different status (‘layers’) of the community undergoing succession that are underlined by the historical contingency. Each box colour represents a different organism that colonizes a niche. The arrows represent the interplay of four classes of processes: dispersal, selection, diversification and drift — see Box 1 for definitions), and their sizes are proportional to their relative influences at each community layer. This representation is schematic and does not intent to fully validate and/or explain the causes and consequences of variations in the relative influence of ecological processes through time. A better view on this subject is provided in chapter 3 (but also see Nemergut et al., 2013 for a synthesis).
The greatest diversity of life lies beneath our feet: a glimpse into the modern era of molecular microbiology of soils

Microorganisms are the major determinants of the soil physical, chemical and biological characteristics (van Elsas et al., 2006; Prosser, 2015; and references therein). It is well-known within the microbial ecology community that soils represent, by far, the most complex and diverse living ecosystem on Earth (Whitman et al., 1998). Perhaps one of the best geological definitions describes soil as a highly complex environment consisting of aggregated particles that create an intricate three-dimensional network of water- and air-filled pores (Oades, 1984). However, a missing link in this definition is the living fraction of soils. Soil constitutes a complex physicochemical matrix that provides a myriad of niches to its inhabitants. A single gram of soil can contain ca. $10^9$ bacterial individuals, encompassing $10^3$ to $10^6$ distinct taxa (Torsvik et al., 2002; Gans et al., 2005; Tringe et al., 2005), in addition to numerous other microorganisms (that is, protozoa, nematodes and fungi). Interestingly, although soils are teeming with life, the fraction of the soil surface area that is covered by soil microbes is only about $10^{-6}$%, which, coincidentally, closely approximates the fraction of the surface area on Earth that humans occupy (Young and Crawford, 2008). The soil-borne microorganisms play fundamental roles in a vast array of ecosystem services, which include the biogeochemical cycling of soil nutrients (nitrogen, carbon, sulphur, phosphorus) (Falkowski, 2001), the sustenance of plant growth, water purification, carbon storage and the maintenance of soil physical structure (Young and Crawford, 2008; Vos et al., 2013). As such, soil must be regarded as a multifunctional, living, intricate system, which unquestionably constitutes the most valuable resource that supports life on Earth.

The study of microbes in soils has undergone a major breakthrough in the past two decades, in which the continuing advances in molecular sequencing technology have enabled to circumvent culturability limitations. The latter has been coined the ‘great plate count anomaly’ — where only a small fraction of the bacterial cells in a soil sample (ca. 1 to 5%) is able to be successfully cultivated under standard lab conditions (Staley and Konopka, 1985). Together with direct nucleic acid extraction methodologies, the breakthrough has allowed the assessment of larger fractions of the soil microbial communities through DNA profiling. In brief, these include the development and application of high-throughput sequencing of conserved genes as phylogenetic markers (i.e. bacterial and archaeal 16S rRNA gene, fungal 18S rRNA gene or ITS region), which is the current standard used to assign identity to hitherto unexplored members of microbial communities. In addition, the application of high-throughput sequencing of total DNA in a given sample (i.e. metagenomics) has also been fundamental to address both the phylogenetic and functional gene repertoires of microbial communities. In spite of the fact that methodological advances inevitably come with inherent limitations, the advances in ‘omics’ technologies have been complementing each other, leading to the so-called ‘multi-omics information pipeline’ (Lamendella et al., 2012). In brief, this promising (yet complex) pipeline aims to integrate DNA profiling data with information about gene expression / activities (metatranscriptomics) and identification of protein profiles (metaproteomics), which collectively aims to corroborate the characterization of metabolites (metabolomics). It is assumed that the joint application of these methods, in spite of the complexity and challenge they pose,
will provide a robust signature of community processes and activities in soil. More discussion on the technical limitations and future perspectives on the application of ‘omics’ in soil microbiology has been provided in the literature (e.g. Temperton et al., 2012; Carvalhais et al., 2012; Delmont et al., 2013; but also see Prosser et al., 2015). Figure 1.2 provides a timeline of advances in ‘omics’ methods and projects on soil biodiversity analysis.

![Figure 1.2. General timeline displaying advances in ‘omics’ methods and projects leading the modern era of molecular microbiology of soils.](image)

Given the enormous progress in our ability to access microbial communities in soils, many outstanding questions in soil biology have started to be answered. For example, the taxon-area relationship has been found to be applicable to microbial communities (Horner-Devine et al., 2004), and the improved understanding of microbial biogeographical patterns has provided evidence for the intuitive contention that microbes are often dispersal-limited (Martiny et al., 2006; Hanson et al., 2012). Additionally, mounting evidence has shown that bacterial communities in soils are largely structured by differences in pH (Fierer and Jackson, 2006; Lauber et al., 2009) and salinity (Lozupone and Knight, 2007), even across continental scales. Moreover, at local sites, a suite of other factors that create environmental variability can exert an influence on microbial populations across different soil systems. These include plant cover and productivity (Berg and Smalla, 2009), animal activity (Singh et al., 2009), wetness (Schimel et al., 1999) and fertilizer application (Fierer et al., 2012). Using these few examples of selected comprehensive studies, it is clear that microbial ecologists face an era of unprecedented transformation. In particular, the integration of the building knowledge of soil microbiology into the general field of ecology, such as the themes of biological diversity, invasion, community assembly, resistance and resilience, is very challenging and promising. Now it is conceivable, through increasingly powerful survey tools, to develop high-resolution field assessments, creative experiments and ecological models that effectively contribute to the continuous use of microbial communities as model entities to study relevant questions into the discipline of community ecology. These developments represent a path towards a better understanding of the eco-evolutionary mechanisms that shape life in soil, leading to the new era of molecular soil microbiology.
Microbial community ecology: definition and properties

The concept of community ecology arose in plant and animal ecology. This discipline seeks to understand how biological communities are assembled, what are their functional interactions and what are the mechanisms that promote community changes in space and time. However, properly defining what constitutes a community is not a simple task. For example, Clements (1916) viewed a community as a ‘supra-organism’, with a well-defined level of organization where organisms interact. The alternative individualistic concept (Gleason, 1926), however, defines community as a collection of species that co-occur in a habitat because they tolerate similar physicochemical conditions and do not necessarily interact with each other. For the sake of clarity, in this thesis I adopted a definition that closely aligns to the one coined within microbial ecology (Konopka et al., 2009). Here, community is defined as ‘multispecies assemblages, in which organisms live together in a contiguous environment and potentially interact with each other’. I consider an assemblage to be the set of taxa (Operational Taxonomic Units — OTUs) or selection of marker genes (chapters 5 and 6) that are inferred to be in a pre-determined spatially-explicit habitat (either local or regional). I base this on data retrieved from high-throughput sequencing methodology (amplicon and/or metagenomics). This is also consistent with the idea that the practical delineation of ‘community’ is determined by the (explicit or implicit) interest of the ecologist rather than by a universally well-accepted concept (Konopka, 2009; Boon et al., 2014).

Community ecologists that study plants and animals conceptually face a delineated set of emergent properties that are intrinsic to complex communities, namely biological diversity, functional redundancy and system stability. These are key general themes that serve as underlying properties from which more refined ecological questions can be raised. In microbial systems, as proposed by Konopka (2009), because microbes possess mechanisms for the horizontal transfer of genetic information and have a distinguished modus operandi (i.e. we often study a system at a larger scale than that of which biological interactions take place, due to analytical bottlenecks), the metagenome may be considered as an emergent community property. As such, the use of community genetic information (i.e. the metagenome) constitutes a valuable piece of information that allows interrogating potential ecological traits as determinants of the successful establishment and dynamics of microbial communities. In line with this, in this thesis soil metagenomes were used to access dynamic shifts in community traits. This was performed at the level of contrasting ecophysiology and niche exploitation (chapter 5) and at that of metabolic redundancy and niche differentiation (that is, the genes driving nitrogen transformations in soil — chapter 6).

Finally, natural microbial communities are compositionally not stable, meaning that they are continuously in a flux (Shade et al., 2013). This dynamics operates at different levels, from the micro- to the macro-scale, and it varies in time. For example, whereas at the microscale ‘endogenous’ dynamics in the relative abundance of community members and their patterns of gene expression operates, at a larger spatial scale, ‘exogenous’ influences exerted by shifting environmental factors prevail. As a result, a clear delineation of what may constitute the proper spatial and temporal scale to interrogate microbial
systems is still a debatable topic (see Vos et al., 2013). In this thesis, I circumvent this conundrum by delineating a framework that takes into account not only the microbial fraction, but also the whole ecosystem succession. Previous studies have contributed to our understanding of the effects of progressive shifts in soil edaphic properties and above-ground (plant and animal) dynamics in the study system (Olff et al., 1997; Schrama 2012, 2013) (more details provided below). As such, I used the previously-gathered knowledge as baseline to interrogate, in a similar fashion, the dynamic changes in soil microbial communities and the properties they carry, ranging from within-successional-stage temporal variations (monthly-base) to a landscape level (~8 km ecosystem scale, spanning over 105 years of succession). Definitions of additional terms used throughout this thesis are provided in Table 1.1.

**Table 1.1. Definitions of terms used in this thesis.**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Alfa-diversity</td>
<td>An inventory metric that expresses the amount of local diversity at the determined spatial scale of analysis</td>
</tr>
<tr>
<td>Beta-diversity</td>
<td>A differentiation diversity metric that refers to community turnover at the determined scale of analysis</td>
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<tr>
<td>Chronosequence</td>
<td>A set of sites formed from the same parent material or substrate that differs in the time since they were formed</td>
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<tr>
<td>Ecological succession</td>
<td>Orderly (and potentially predictable) changes in community composition and/or structure over time</td>
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<tr>
<td>Historical contingency</td>
<td>The effect of the order and timing of past events on community assembly and ecological succession</td>
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<tr>
<td>Soil development</td>
<td>Temporal changes in the abiotic and biotic aspects of soil, including nutrient and water availability, structure, texture and biota</td>
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<tr>
<td>Spatial scale</td>
<td>A delineated size of the study area (e.g. micro, local or regional scales)</td>
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<tr>
<td>Successional rate</td>
<td>The speed at which species/community changes occur</td>
</tr>
<tr>
<td>Temporal framework</td>
<td>A conceptual approach to understand relationships among processes that occur at different temporal scales</td>
</tr>
<tr>
<td>Temporal scale</td>
<td>A delineated timeframe that allows the investigation of environmental and biotic changes in community composition or species distributions over time</td>
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**Integrating microbial community assembly processes into an ecological succession framework**

A fundamental goal in microbial community ecology is to understand the processes that govern the variations in species abundance across space and time (Dumbrell et al., 2010; Stegen et al., 2012). Knowledge from ecological research in macro-organisms recognizes that two distinct classes of processes influence community assembly: deterministic and stochastic. The deterministic class invokes the existence of ecological selection (*sensu* Vellend, 2010) that is imposed by the environment (also termed ‘environmental filtering’) and/or through both antagonistic and synergistic species interactions. In contrast, the stochastic class includes ecological drift (i.e. random birth-death events that cause unpre-
dictable changes in species relative abundances), probabilistic dispersal and unpredictable disturbance events (see Chase and Myers, 2011).

Although there is ample literature examining microbial communities across disparate habitats, the majority of studies generally refrain from analysing whether or not community composition is related to specific environmental factors (for example, Fierer and Jackson, 2006; Lozupone and Knight, 2007). These intrinsically assume that microbial communities are mostly, if not uniquely, structured by deterministic processes. However, by making use of concepts and statistical tools that are commonly applied in macro-organism ecology (for instance, analytical procedures referred to as ‘null models’), evidence has emerged that supports a role for stochastic processes in structuring some microbial systems (e.g. Caruso et al., 2011). It is now a common realization that both stochastic and deterministic processes play roles during microbial community assembly (e.g. Stegen et al., 2012). The challenge lies in the proper disentangling of the mechanisms mediating their relative contributions and how they vary both in space and time.

Several examples from the literature have shown that microbial community assembly can be successfully studied by interrogating the patterns of dissimilarity both within and among habitat types (Caruso et al., 2011; Stegen et al., 2012; Wang et al., 2013). However, I posit in this thesis that an elegant use of plausible conceptual models for this purpose can be best achieved by integrating community assembly into a successional framework. This can be realized by including both spatial and temporal variations in models that access the balance between community assembly processes and mechanisms governing their relative influences along a continuous dynamic trajectory (chapter 3). The major advantage of this approach lies in the fact that, different from across-habitat surveys, ecological succession innately incorporates the community historical contingencies and evolutionary forces (i.e. diversification) as mechanisms that influence community assembly. As such, it simplifies potential biases resulting from the eco-evolutionary mechanisms being divergent across local habitats. Moreover, as succession assumes orderly changes in the relative species abundances, a continuous trajectory allows for the delineation of temporal frameworks to study community assembly and mechanisms imposing them in a multiscalar manner (chapter 3, and see chapter 7 for additional discussion).

**Box 1. Vellend’s conceptual synthesis in community ecology (Vellend, 2010)**

For a long time, the discipline of community ecology has been perceived as a “mess”, given the apparently vast number of processes that govern the many patterns of interest, and the uniqueness of each study system. Inspired by this chaotic reality, Vellend (2010) organized the material of community ecology in a consistent way, in order to clarify the similarities and differences among various conceptual constructs in the discipline. This synthesis systematizes the patterns in the composition and diversity of species (i.e. the subject of community ecology), as being influenced by only four classes of processes: dispersal, selection, diversification and drift.
**Dispersal:** This term is used to define the movement of organisms across space. The influence of dispersal in community assembly depends on the size and composition of the community where the dispersers come from and of those into which they disperse. As such, the consequences of dispersal can only be addressed in relation to the action and results of other processes, in particular selection and drift.

**Selection:** This term is used to describe deterministic fitness, and it implicitly assumes the existence of differences among individuals of different species. In a community context, there are three relevant forms by which selection may operate: *(i)* constant, *(ii)* density-dependent, and *(iii)* spatially-temporally variable. Constant selection occurs when the relative fitness of a species is constant in space and time, independent of the species density but variable across species. In this case, the species with the highest fitness will exclude all others. Density-dependent selection occurs when the individual fitness in a given species depends, at least in part, on the population density of that species, as well as the densities of other species. In brief, the nature of density-dependent selection between pairs of species depends on the type of ecological relationship between them (e.g. competition, predation, mutualism). Finally, whether constant or density-dependent, selection may also vary across space and time (i.e. spatially-temporally variable selection).

**Diversification:** In Vellend’s synthesis, this process was originally coined 'speciation', i.e. the creation of a new species. However, particularly within microbial ecology, this encompasses the role of mutation and horizontal gene transfer, which result in novel ecological types. As such, in this case ‘speciation’ may be more generally interpreted and applied in the context of genetic diversification of organisms rather than purely the creation of species. Studies focusing on how species interactions work out in homogeneous, small-scale localities, often neglect this process (i.e. when the origin of the local species pool does not matter). For comparisons of community patterns across larger scales, diversification/speciation is likely to be an important process to consider. It is also a process of interest for studies focusing on biogeographic patterns that aim to unravel the evolutionary context in which species pools originate.

**Drift:** This term can be defined as the random change in relative species abundances. In other words, because birth, death and offspring production are inherently stochastic processes, changes in any community with a finite number of individuals will also have a stochastic component. The role of drift in community assembly is mostly related to the generation time of species within a community, the community size and factors that alter it (e.g. disturbance) and to its interaction with other processes such as speciation and dispersal.

**Glossary:**

**Species density** – the number of individuals of a given species per unit of space.

**Absolute fitness** – the quantity of offspring produced by an individual organism per unit of time, including survival of the organism itself.

**Relative fitness** – the absolute fitness of a given organism divided by the mean absolute fitness across all individuals in the community.

**Species fitness (absolute or relative)** – the mean fitness (absolute or relative) across all individuals of a given species in the community; for absolute fitness, this is equivalent to the species per capita population growth rate.
Box 2. The use of chronosequences as environmental models for temporal soil ecology

The study of temporal soil ecology is often challenged by setting appropriated time scales in a realistic and feasible analytical manner. This becomes even more complex when the investigated patterns occur over time scales that exceed decades or centuries. In these cases, the most appropriate method relies on the use of environmental chronosequences. The method is based on the study of established sites presumed to represent a sequence of different stages of soil development, with a known initial establishment and a time history afterwards (Walker and Del Moral, 2003). As such, a fundamental assumption regarding chronosequences is that the communities (either micro or macro) and local environmental/ecosystem parameters of the younger soil sites are gradually developing in a temporal manner that resembles how the older sites developed (termed, space-for-time substitution).

Well-known soil chronosequences are those formed by the worldwide recession of glaciers that has occurred over the past 150–250 years, leaving glacier forelands with spatially-ordered sequences of terrain age (Bardgett et al., 2005). In addition, spatially-ordered sequences of terrain can also be found at abandoned fields in the course of several decades, at sand dunes covering centuries, or at areas flanking volcanos, where temporally-distinct events of lava flows promote the chronosequence development. Moreover, in this thesis, the established soil chronosequence was found to concur with a natural salt marsh ecosystem (Olff et al., 1997).

Finally, as chronosequences intrinsically imply the presence of ecological succession (Walker et al., 2010), these model systems stand out as natural experiments that allow testing fundamental questions in temporal soil ecology. A few examples are: (i) 'how do spatial and temporal environmental heterogeneities influence diversity at different scales?'; (ii) ‘What are the relative roles of stochastic and deterministic processes controlling diversity and composition of communities?’; (iii) ‘What are the most appropriate baselines for determining the magnitude and direction of ecological changes?’ and lastly (iv) ‘do the same macro-ecological patterns apply to micro- and macro-organisms, and are they caused by the same processes? These four questions are far from being a complete list, but I purposely select them as the key ones from the ‘Identification of 100 fundamental ecological questions’ (Sutherland et al., 2013), where chronosequences can be applied as a tool. Importantly, these questions permeate the studies and theories unveiled throughout this thesis.

The importance of salt marshes

Salt marshes are ecosystems located along the shores of temperate coastlines around the world. These ecosystems have a low topography, often spanning only a few meters in elevation over thousands of meters of area. They occupy an estimated area of 40 million hectares globally (Duarte et al., 2005) and provide ecosystem services that outshine those of any other coastal habitat (Gedan et al., 2009). These services include several features,
such as shore line stabilization, nursery and breeding areas for commercially important finfish and shell fish species, buffering zones for interception of land-derived contaminants, nutrient removal and carbon sequestration (Valiela et al., 2004). Markedly, the rate of carbon sequestration in salt marsh soils is higher than in many other temperate biomes (Duarte et al., 2005), and these ecosystems contribute 1% to the global loss of fixed nitrogen through microbially mediated denitrification (Seitzinger et al., 2006). In 2007, the value of the ecosystem services provided by salt marshes was estimated at $14,397 ha⁻¹ y⁻¹, of which 66% was attributed to services associated with nutrient removal and transformation (Gedan et al., 2009), much of which occurs as a result of microbially-mediated metabolisms.

Salt marshes now face new regional and global challenges, which are related to the increasing scale of anthropogenic and climate change impacts. Located at the interface between land and sea, marshes are subjected to perturbations from both biomes. It has been estimated that between a quarter and a half of the area of the world’s tidal marshes has already been lost (Deegan et al., 2012). Nutrient pollution and other large-scale anthropogenic disturbances (e.g. land use conversion and species introductions), among other factors (e.g. sea level rise and loss of sediment supply), are known to contribute to marsh loss. Additionally, global climate change certainly also affects salt marsh ecosystems, although the outcomes of specific climate effects remain uncertain. Accelerating sea level rise, which is expected to accompany climate change, will be a universal threat to these valuable coastal ecosystems and the services they provide.

Study system – Schiermonnikoog as a unique field laboratory

Figure 1.3. Map of the island of Schiermonnikoog, the Netherlands (N53°30’ E6°10’). Dot marks represent the location of the five soil successional stages established along the chronosequence in 2012 (that is, stages 0, 5, 35, 65 and 105 — in years of soil development). The below panel displays photographs of each plot taken in July 2012.
The research presented in this thesis was performed using samples collected at the black-barrier salt marsh on the island of Schiermonnikoog, The Netherlands (Figure 1.3). This ecosystem constitutes one of the largest well-preserved salt marshes in Western Europe. From the west to the east, several different stages of salt marsh development can be found adjacent to one another (i.e. chronosequence) (Olff et al., 1997), which span over a century of ecosystem progression. This happens because the main sea currents and winds are directed west-to-east, which leads to the constant deposit of fresh sand at the eastern part of the island, causing it to extend eastwards. On top of the underlying sandflats, there is a progressive accretion of smaller soil particles (i.e. silt and clay) that are carried by the frequent inundation of these sites by the daily tidal regime. This sediment accumulation occurs at the rate of 0.2 to 1.2 cm per year at the young successional stage, which declines gradually towards late successional stages (Olff et al., 1997). This interwovenness of factors modulating the physical structure of the marsh reflects the dynamics by which nutrients are amended and recycled in this system. Nutrient cycling in estuarine systems is often assumed to be largely governed by circulation dynamics, water residence times and sedimentation rates (Bakker et al., 2014). However, a close examination of this chronosequence provides some additional information. In brief, Schrama et al. (2012, 2013) showed that the initial soil sites of this chronosequence are largely under the influence of marine-derived nutrients (termed, the ‘brown food web’). On the other hand, as succession proceeds, soil sites are gradually being transformed into a terrestrial system, which have a more internally driven nutrient dynamics (the ‘green food web’). This knowledge is explored throughout this thesis, as it provides support to our underlying hypotheses and findings regarding the dynamics and potential shifts in organismal/community ecophysiology in this system, representing a marine-to-terrestrial transitional gradient. Additional information on this system is provided along the chapters.

**Aim of this thesis**

The aim of this thesis is to search for general principles, patterns and mechanisms governing microbial community assembly and ecological succession in soil. By making use of a well-established salt marsh chronosequence located at the island of Schiermonnikoog, the Netherlands, this thesis aims to provide the most comprehensive understanding of microbial community dynamics in salt marsh soils to date. General ecological mechanisms developed throughout this thesis and a framework of community succession (emphasis to chapter 3) are broadly applicable to the growing field of community ecology and molecular microbiology of soils.

**General research questions of this thesis**

- What are the drivers of soil bacterial and fungal community dynamics along the investigated salt marsh chronosequence? Do patterns of bacterial and fungal community sizes and diversities differ along the ecosystem progression?
• What is the relative influence of stochastic and deterministic processes mediating microbial community succession? How do different environmental factors govern the interplay between these processes across the environmental gradient?

• How do the patterns in bacterial and fungal community succession in this system shed light on potential ecophysiological, functional and phylogenetic signatures of marine-to-terrestrial transition of microbial communities?

• What is the dynamics of nitrogen (N) cycling microbial communities along this natural gradient? Is there niche partitioning within genes involved in different steps of the N cycle across distinct stages of the salt marsh development?

Thesis outline

Throughout this thesis I sought to elucidate the mechanisms that underpin microbial community assembly and ecological succession in salt marsh soils. For that, I used three main tenets of community ecology: describe, explain and predict. The first tenet was used to describe the distribution of organisms and functions along the environmental gradient and their variation across established temporal scales. The second tenet used the information obtained to search for possible factors (biotic and abiotic) and ecological mechanisms that can explain the observed distribution of organisms and functions, ultimately reflecting the diversity (i.e. species composition and phylogenetic diversity) of patterns and functional capabilities. The third tenet was used to predict the trajectories of communities based on a combination of empirical data, statistical and modelling / simulation analyses.

I start by providing a robust inventory of bacterial community compositions and their temporal dynamics (β-diversities) in soil samples collected along the salt marsh chronosequence (chapter 2). In this initial research chapter, I show that the local turnover in community composition (β-diversity) varies according to successional stage, which is higher at initial stages. I also found that, despite presenting 10-fold lower bacterial abundances, bacterial communities at initial stages unexpectedly presented higher α-diversity than those at late successional stages. This was a remarkable finding that counters general expectations from the macro-ecological theory of community succession, where highest diversities are likely to occur in higher-productivity sites. In order to provide a plausible interpretation of these results, by performing co-occurrence network analysis, I revealed that temporal dynamism at the initial stages is likely to be a major mechanism promoting temporal niche partitioning in ‘species’ occurrence, where continuous environmental change results in the existence of multiple niches over short periods of time, thus promoting biodiversity.

In chapter 3, I showed that the temporal niche partitioning in bacterial communities at these initial soil sites is caused by stochastic rather than deterministic processes. By using a combination of theoretical, empirical and simulation models, I described scale-dependency in the mechanisms controlling microbial community succession in soils. In brief, whereas salinity correlated strongly with the decrease in stochasticity within sites along
the chronosequence, shifts in soil organic matter content were the main predictors of the type and relative influence of determinism at a larger spatiotemporal scale. Throughout chapter 3, I focused on (i) providing a conceptual model that couples the stochastic/deterministic balance to primary and secondary ecological succession, thereby integrating previously isolated conceptual domains; (ii) evaluating this model empirically using the data generated in chapter 2, thus revealing a systematic shift in the type and strength of ecological selection; and (iii) coupling empirical data to a new simulation model to elucidate the underlying mechanisms and characterize their scale dependency. Collectively, the insights and conceptual framework provided here represent a nexus point for the continued integration of microbial ecology into the broader field of ecology.

In chapter 4, I shifted my focus towards the fungal communities in the system. By making use of a similar approach to the one applied in chapter 2, I inventoried the distribution of taxa and dynamics of fungal communities at the level of community β-diversities and shifting organismal ecophysiolgies throughout the ecosystem development. Chapter 4 specifically focuses on the discussion of the ecological roles of fungi acting as major players mediating carbon transformations and storage in the soil chronosequence. In addition, our findings provide initial insight in potential ecological signatures of fungal community transition in a marine-to-terrestrial system, which analogously represents one of the most fundamental shifts in microbial evolutionary history. Moreover, in chapter 4 I provide a meta-analysis that contrasts the shifts in community sizes, α- and β-diversities of both bacterial (chapter 2) and fungal (chapter 4) communities in this system.

Chapter 5 constitutes an investigation of these soil communities at the metagenomic level. I focused on testing the hypothesis that the marine-to-terrestrial gradient reflects functional signatures of the ‘community of genes’ in these soil sites, where functional gene assets conferring ecological advantages differ along ecosystem development by shifting in a progressive manner. I also argue that the eco-evolutionary signatures of microbial adaptation to terrestrial environments resemble the ‘fight’ and ‘flight’ response modus observed in animal behaviour studies. To test for that, metagenomes were annotated with a focus on genes involved in carbohydrate-active enzymes (CAZy), antibiotic resistance genes (ARGs) and metabolic pathways conferring the motile chemosensory behaviour to microbial cells. I further linked the data with local environmental conditions (in particular, soil diffusibility and source of carbon substrates), thus revealing patterns of ecological succession at a functional trait level.

In chapter 6, I gave special attention to the rising body of literature that highlights the increasing threats faced by salt marshes, resulting in ecosystem losses worldwide. In brief, a surge in the literature postulated that the initial recognition of salt marshes as buffering zones removing land-derived compounds (particularly nitrogen – N) has, in fact, deleterious effects on the ecosystem. By making use of this chronosequence, I studied a set of genes involved in the N cycle through data previously obtained by metagenomics (chapter 5) and applied quantitative PCR assays. As a result, chapter 6 provides a unique inventory of systematic changes in genes driving microbially-mediated N transformations in a system that encompasses the natural formation of a salt marsh. I posit that these data are of critical importance for the development of future dynamic ecological models that aim to monitor and potentially mitigate impacts in soils under distinct stages of succession. I also
discuss the importance of historical contingency, ecological succession and niche partitioning within functionally redundant genes in the N-cycle found in the system.

Chapter 7 contains my synthesis and discussion of all results obtained throughout this thesis. I placed these results within the broad context of micro- and macro-ecology. Here I endeavoured to extend these insights towards generalizing their contribution to the field of microbial ecology and community ecology. Future research avenues and potential practical applications of ecological succession in natural systems are also discussed.

References
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