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## **Assemblage and functioning of bacterial communities in soil and rhizosphere**

Yan, Y.

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**Author:** Yan Y.

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## **Chapter 1**

### **General introduction**

Microbial communities in soil are extremely diverse and determine largely the functioning of terrestrial ecosystems (Philippot et al 2013). The soil microbiome is responsible for a range of key ecosystem functions such as decomposition of organic matter and of polluting compounds, and nutrient cycling (Nielsen et al 2011, Nielsen et al 2015). It also has a strong impact on above ground organisms, in particular plants.

Soil ecosystems consist of a wide variety of microenvironments with distinct characteristics of e.g. pH (Lauber et al 2009), salinity (Wichern et al 2006) and moisture (Brockett et al 2012). These specific habitats influence the resident (micro)organisms and recruit specific members of the soil microbiome, what is said to be one of the major reasons for the huge microbial biodiversity in soil (Fierer and Jackson 2006, Kuramae et al 2012).

Biodiversity has been considered as a critical factor influencing ecosystem processes (Butchart et al 2010). Recent studies on the soil microbiome trying to link biodiversity to ecosystems functioning have shown that, indeed, soil ecosystem functioning depends on microbial diversity (Tardy et al 2014, Wall et al 2015). In general, biodiversity is considered to be advantageous to ecosystem functioning because a more diverse ecosystem might be more resilient than a less diverse system (van Elsas et al 2012). Thus, there is a great challenge in assessing the immense diversity of microbial communities and their response to the environment and changes therein.

Several studies have shown that intensification of soil use by agriculture may reduce the diversity of soil organisms dramatically (de Vries et al 2013, Doran 1980, Garbeva et al 2004, Hartmann et al 2015). Consequently, this has triggered increasing concern that the loss of species in soil may impair ecosystems functions, such as nutrients acquisition by plants and resource recycling between above- and below- ground communities. However, the significance of microbial biodiversity loss is challenged by the concept of functional redundancy (Tilman et al 1997, Wall et al 2015) that assumes that (many) different microbial species can have the same functions in a natural ecosystem, and, therefore, the loss of species may not necessarily alter ecosystem functioning (Nannipieri et al 2003).

Until now, studies about the significance of soil microbial diversity are much less common than similar studies on aboveground macroorganisms especially plants (Martiny et al 2006). So there is a need to increase the studies on microbial diversity in different environments and changes therein and how that will impact the role of the microbiome in soil. Recently, the advent of high-throughput sequencing techniques has opened a promising avenue for improving our understanding of microbial biodiversity and community assemblage processes in specific habitats (Costello et al 2009, Franzosa et al 2015, Ofek-Lalzar et al 2014, Prosser 2015). The main goal of the study described in this thesis was to obtain better understanding of the structuring, diversity and functioning of bacterial communities in soil and rhizosphere.

## **1.1 Soil and rhizosphere as habitat of microorganisms**

### *1.1.1 Soil ecosystem*

Soil ecosystems support a large trophic complexity within the soil food web with complex interactions with the abiotic environment (De Ruiter et al 1995, Hunt et al 1987). The structure and function of the soil food web is a primary indicator of ecosystem health because the huge functional diversity that exists among soil organisms is vital to ecosystem services, including plant growth and associated energy input to the system (Wall and Six 2015). For instance, there is mounting evidence that the soil food web, in particular the microbial component, can significantly modify belowground-aboveground interactions by regulating nutrient uptake, carbon storage and direct plant effects (Nielsen et al 2015), which in turn influence vegetation production.

The trophic interactions within the soil food web transfer energy between species and so drive ecosystem processes (Pimm et al 1991). It is widely known that microorganisms, such as bacteria and fungi, are mainly responsible for these ecosystem processes (Six et al 2006). Soil animals such as nematodes and detritivorous organisms mainly facilitate the microbial driven processes such as nutrient mineralization (Postma-Blaauw et al 2005, Setälä and Huhta 1991). Furthermore, the complexity of the soil food web is essential to maintain the resistance and resilience against environmental changes within terrestrial ecosystems (de Vries et al 2012, de Vries et al 2013). Thus, activities that cause

loss of biodiversity belowground may contribute to a reduction in food web complexity and therefore in soil ecosystem functioning.

### *1.1.2 Rhizosphere*

Since German agronomist and plant physiologist Lorenz Hiltner, more than a century ago, coined the term ‘rhizosphere’ as the soil compartment influenced by plant roots (Hiltner 1904), our understanding of this hot spot of biological activity in soil has advanced dramatically. Due to the continuous deposition of energy-rich substrate significant enrichment of organisms, mainly bacteria and other microbes, occurs in the rhizosphere. The release of organic carbon from roots into the surrounding soil, which is often termed rhizodeposition (Jones et al 2004), causes dramatic changes in both the biological and physicochemical nature of the soil. The wide range of rhizodeposition mechanisms includes loss of root caps and border cells, of mucilage, release of soluble root exudates, and of volatile organic carbon, as well as transfer of carbon to symbionts and leakage and destruction of root cells (Jones et al 2009). Numerous studies have revealed that rhizodeposition varies dramatically between different plant species, genotypes and plant growth stage (Bais et al 2006, Dennis et al 2010, Jones et al 2004). Consequently, soil microorganisms are confronted by and feed on a wide and dynamic range of rhizodeposits with strong implications for the structure and functioning of rhizosphere communities (de Boer et al 2006, Garbeva et al 2008, Kowalchuk et al 2002).

Many studies have demonstrated that the microbial communities in the rhizosphere are very different to those of the bulk soil (Costa et al 2006, Garbeva et al 2008, Smalla et al 2001). The dynamic processes between plant roots and microbes are a major force of the assembly and structuring of specific communities consisting of species that have the cellular properties to benefit from plant functioning. The root released materials are utilized by microbes as substrate for growth and energy supply (Vandenkoornhuyse et al 2007), but they may also be used for communication between roots and the biota in the rhizosphere (Kowalchuk et al 2006) or may act as antimicrobials for protection against pathogens (Bais et al 2006, Setälä and Huhta 1991).

There is ample evidence that the significance of the rhizosphere microbiome is critical to health, productivity and overall conditions (Chaparro et al 2012, Mendes et al 2013, Ziegler et al 2013). There is a broad range of interactions between plant and microbes from beneficial to pathogenic involving a wide spectrum of microorganisms: arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria (PGPR), endophytes and (minor) pathogens. The outcome of the interaction between plant and such microbes may vary depending on plant species, soil type and environmental conditions (van der Putten et al 2013). The primary goal of many plant associated microbiome studies is to drive this interaction towards enhanced benefits for plants by promoting beneficial organisms and reducing pathogens.

Studies concerning plant-associated microorganisms are strongly biased towards those individual species that are culturable and thus can easily be traced. These species comprise, however, only around 5% of the total microbial community, thus most of the important interactions are likely overlooked and remain unnoticed (Mark et al 2005, Matilla et al 2007). In order to be able to assess properly the dynamics of microbial communities in the bulk soil and the rhizosphere a comprehensive profile of the soil microbiome is required.

Yet, due to their enormous diversity, the detailed extent of microbial activities in certain niches, such as the rhizosphere, remains largely unclear. One of the major problems to assess the functioning of the microbiome in soil and rhizosphere is the lack of sound approaches to study microbial biodiversity experimentally.

## **1.2 Bacterial biodiversity**

### *1.2.1 Experimental approaches to study microbial biodiversity*

Understanding the assemblage of microbial communities and their associated diversity requires a broad range of approaches, including assessment of the community structure in nature (Lauber et al 2009), manipulation of natural communities in microcosm studies (Barthes et al 2015), and the assemblage of synthetic communities under controlled conditions (Lebeis et al 2015). Natural communities are dynamic and are the result of filtering processes exerted by

abiotic and biotic factors in the environment over a long period of time (Andrew et al 2012, de Ridder-Duine et al 2005). Most studies nowadays have focused on the richness and diversity of taxa to quantify and characterize soil microbial communities at different levels, from species (Mendes et al 2011, Sunagawa et al 2015, Tilman et al 1997) to family or phylum level (Fierer et al 2005). These studies are based often on manipulation of soil-borne microbial populations already present in nature by making changes in important ecological factors such as moisture and/or temperature conditions so to be able to explain the underlying mechanism in soil ecosystem functioning. Compared to the studies on natural communities, the studies on synthetic communities are technically feasible only with a group of species comprising a subset of the local species that are easy to propagate. A recent example of the assemblage of a synthetic bacterial community concerned the recolonization of previously sterilized *Arabidopsis* seed surfaces by easily cultural species (Lebeis et al 2015). However, as mentioned before, only a minority of bacteria has been cultured overall (Pedros-Alio 2006). Thus, it is difficult to extrapolate the result of culture-dependent experiments to the real situation in the field. By contrast, an approach that is often used to assess diversity effects is the so-called dilution method (Franklin et al 2001, Garland and Lehman 1999). The assumption on which this approach is based, is that the biodiversity of a soil microbial community can be reduced in comparison to the diversity of the original natural community after inoculation of sterilized soil with a diluted suspension of that soil and subsequent incubation until similar community size. This approach could provide information on assemblage processes closely related to natural processes. As reported in previous dilution to extinction studies (Franklin and Mills 2006, Garland and Lehman 1999), the regrown microbial communities are the result of assembly processes filtered by abiotic or biotic factors. Compared to the use of synthetic communities, the dilution approach allows for the assessment of the direct effects of the extinction of certain species on ecosystem functions.

Previous studies have reported that the reduction of microbial diversity did not change soil processes such as thymidine and leucine incorporation, nitrification and nitrate accumulation (Griffiths et al 2001). Similarly, the associated functioning groups, i.e. carbon mineralization, denitrification and nitrification did not change after reduction of microbial biodiversity affected by the dilution approach (Wertz et al 2006). However, other studies have indicated



that reduction of rare species modified plant-herbivore interactions (Hol et al 2010) and also that reduction of species diversity affected nitrogen cycling (Philippot et al 2013b). Questions that are claimed to be addressed by the dilution approach are, for instance, related to the significance of microbial diversity in natural ecosystems, to the role of rare species in ecosystem functioning, to the interactions among species or to the relationship between community structure and functionality in natural ecosystems. Referring to the last issue, many studies have addressed the taxonomic content of microbial communities in certain habitats and their interaction with aboveground vegetation within the terrestrial ecosystem (Hol et al 2010, Kardol et al 2006, Mendes et al 2011), but the functional genes involved in the plant microbe interactions have remained largely unclear (Mendes et al 2014).

These whole community approaches are feasible also because of the availability of advanced high throughput sequencing technologies. Recently, advances in next-generation DNA-based or RNA-based sequencing technologies have dramatically reduced costs and substantially increased capacity, resulting in an increasing number of comprehensive characterizations of microbial communities in different habitats. (Bulgarelli et al 2015, Lebeis et al 2015, Lundberg et al 2013, Ofek-Lalzar et al 2014). These metagenome surveys based on phylogenetic gene marker amplicon and/or total DNA not only detected microorganisms and their genetic diversity associated with environmental parameters, but also shed light on the functional attributes that microbes enable to perform important contributions to ecosystems (Mendes et al 2014). Thus, next generation sequencing technologies, together with advanced bioinformatics tools to process the huge data sets may provide new insights into the microbial life in natural ecosystem. In this thesis, I combined sensitive molecular approaches including qPCR and next generation sequencing with the old dilution methodology for deciphering the mechanisms of microbial community assemblage in soil and rhizosphere and the functions relevant for plant-microbe interactions.

### *1.2.2. Functionality of biodiversity*

Although several reports in literature provide information that soil microbial communities are linked to ecosystem functioning (Allison et al 2013,

Vogelsang et al 2006), the common opinion is that there is a large functional redundancy within the soil microbiome preponderating the relationship between diversity and functionality (Nannipieri et al 2003). Functional redundancy across different taxa in microbial communities has been suggested as a buffering capacity for biodiversity loss in the ocean (Sunagawa et al 2015). This is consistent with another study on the human gut in which taxonomical composition of the microbiome varied markedly between bacterial groups but gene abundances were evenly distributed (Yu et al 2012). Together this suggests that ecosystem functioning may be independent of the composition of microbial communities.

The analysis of complex microbial communities has been generally limited by technical approaches and sequencing depth. The advanced developments in high throughput sequencing methods by using 16S rRNA gene marker for bacterial species, such as the Roche 454 pyrosequencing technology (Caporaso et al 2011) and the Illumina technology (Caporaso et al 2012), have facilitated comprehensive surveys of the breadth of microbial communities. In addition to the provision of a profile of microbial taxonomical diversity, such data may also help us to understand the significance of functional genes, for instance those related to plant host colonization.

There is ample evidence that the rhizosphere microbiome influences directly and indirectly the composition and productivity (i.e. biomass) of plant communities in terrestrial ecosystems (Schnitzer et al 2011, van der Heijden 2008). Well known examples are the plant growth promoting rhizobacteria which promote plant growth directly by either facilitating nutrient acquisition (Hawkins et al 2000, Miransari 2011) or by inducing plant hormone levels (Zamioudis and Pieterse 2012), or indirectly by suppressing soil-borne plant pathogens (Mendes et al 2011). In contrast, earlier studies have documented that enemy accumulation could cause negative plant-soil feedbacks processes (Bever 1994, Bulgarelli et al 2015, van der Putten et al 2013). However, there is also evidence that plants may recruit beneficial microorganisms in the rhizosphere to get protection against the pathogens presence (Hawkins et al 2000). For example, a study on sugar beet plants attacked by the root pathogen *Rhizoctonia solani* revealed that plants exploited the soil microbiome for protection against pathogen infections (Mendes et al 2011). The ability to progressively enrich for beneficial microbiota is more effective in successive

generations of plants (Miransari 2011). Hence, the diversity and richness of microorganisms in the rhizosphere have been regarded as frontline for plant development in the terrestrial ecosystems.

Previous studies on plant-microbe interactions and the significance of microbial biodiversity to assess the effects of species loss on plant performance, showed that species reduction, and in particular the loss of rare species, reduced plant biomass production (Hol et al 2010). In contrast, as different species may have the same functions related to plant functioning (Nannipieri et al 2003), the reduction of microbial diversity may not influence plant performance. So, in order to understand the fundamental ecological mechanisms of plant-soil feedbacks, we need to better characterize the assemblage as well as the functionality of the microbiome in soil and rhizosphere.

In this study, I used *Jacobaea vulgaris* as model plant for the assessment of the impact of the rhizosphere microbial community on plant growth. *Jacobaea vulgaris*, is one of the most common weeds in the Netherlands (van der Meijden and van der Waalskooi 1979). *Senecio* spp. usually grows in dry and poor soils, and is considered to be toxic weeds for livestock of cattle and horses when ingested (Hartmann 1999). In a previous study, Joosten *et al.* (2009) found a strong negative plant-soil feedback when a sterilized soil was inoculated with its 'own' microbial community compared to plant growth on sterile soil. This negative feedback effect has been also shown in other plant-soil feedback studies, which showed that *Jacobaea vulgaris* biomass in natural soil from different chronosequence fields was lower than in sterilized soil (van de Voorde et al 2012).

### 1.3 Aim and thesis outline

The aim of this study was to obtain a better understanding of the assemblage of bacterial communities in soil and rhizosphere and the significance of microbial diversity and functional traits for plant-soil feedback processes. The main approach to the assemblage and biodiversity studies was the so-called dilution approach in which the diversity of the bacterial community in soil is manipulated by inoculation and incubation of more or less diluted soil suspensions in pre-sterilized soils.

The main research questions addressed here are:

- 1) Does the dilution approach reduce the diversity of the bacterial community after inoculation and subsequent incubation of soil suspensions in soil? If so, does soil has a selective power during the assemblage process of bacterial communities? (Chapters 2 and 3)
- 2) How do the taxonomical and functional diversity of the bacterial community change in the rhizosphere compared to the bulk soil and what are the relative roles of soil and plants in the assembly process? (Chapter 4)
- 3) What are the main bacterial functional traits that determine the relationship between the bacterial community in soil and plant growth? (Chapter 5)

The experimental approaches to assess the relevance of soil bacterial diversity for the functioning of soil ecosystems are scarce; the dilution method is among the most frequently used. In **Chapter 2**, I revisited this method and shed more light on the assemblage of bacterial communities in soil.

In **Chapter 3** the concept of soil as the driving force for the structuring of the bacterial community was further analyzed by assessing the assemblage of bacterial communities in different soils after inoculation of more or less diluted suspensions in a cross inoculation design.

To further understand the development of the bacterial community in the rhizosphere, *Jacobaea vulgaris* was planted in the incubated soils that were inoculated with the same more or less diluted suspensions as in chapter 2. In **Chapter 4**, I tested which bacterial functional traits were most selected in the rhizosphere. Based on the 16S rRNA gene amplicon sequencing as well as the shotgun metagenome approach the bacterial community composition and its functionality in soil and rhizosphere were compared.

In **Chapter 5**, I report on a study on the impact of the bacterial community in the rhizosphere on plant biomass production linking both species composition and its functionality to plant growth.

The results of the different studies are discussed and summarized in **Chapter 6**.

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