General introduction
Unlike plants and animals, microorganisms are assumed to be cosmopolitan and so they can be found globally across habitats like soils, sediments, lakes and the sea (Hanson et al., 2012). With respect to this, a range of studies have reported endemism in bacteria and fungi, where some taxa are restricted to a distinct habitat (Allen et al., 1995; Lindström and Langenheder, 2012; Oakley et al., 2010). This has been mainly found for, e.g., some extreme environments like hot springs (Bahl et al., 2011; Papke et al., 2003; Takacs-Vesbach et al., 2008; Whitaker et al., 2003). Microbial life in extreme conditions, such as the extremes of temperature, is indeed challenging. With respect to low temperatures, polar regions appear to act as an effective filter that limits colonization of the habitat (Brinkmeyer and Knittel, 2003; Cowan et al., 2014; Cox et al., 2016). Due to their large population sizes, immense phenotypic and genotypic variation, diminutive extinction and high dispersal rates, microbes follow very particular population structuring c.q. biological diversification patterns (Gerstein and Moore, 2011; Kirk et al., 2004). Thus, Fierer and Lennon (2011) suggested that the physiology, life history and ecology of most microbial taxa – even numerically dominant ones - are still understudied and thus remain poorly understood. This is especially true for cold regions such as the Arctic and alpine regions.

Furthermore, the interactions of microbes with plants in arctic and also alpine regions constitute a key ecological phenomenon that has been unexplored. Endophytic bacteria and fungi are ubiquitous in plants (Berg et al., 2014; Elvira-Recuenco and Vuurde, 2011; Hallmann et al., 1997), but the assembly, structure and function of endosphere communities have so far mainly been studied in agricultural plant species or in model plant systems in artificial settings (Badri et al., 2009; Bulgarelli et al., 2013; Compant et al., 2010; Zhang et al., 2006). The factors governing the assembly of the microbiota associated with perennial wild plants in the low temperature and low nutrient soils, such as prevalent in the Arctic, might differ greatly from those of well-fertilized crop plants, as discussed below.

**Origin and evolution of arctic plants**

During the Pleistocene (ca 2.5-0.01 million years before present, a period of repeated glaciations), the circumpolar flora only consisted of about 1,500 species of vascular plants. Plants that survived the advancing and retreating glacial fronts from the Arctic and Alpine regions mixed and migrated back and forth in this period, forming the bulk of the arcto-alpine flora we have today (Quinn, 2008b). Alsos et al. (2007) illustrated that most plant species in the high-Arctic (Spitsbergen) are indeed cold-adapted, being dispersed from surrounding continents on several occasions. Through phylogenetic analyses of
plastid DNA, Wang et al. (2016) then illustrated the origin and circum-arctic distribution of the pioneer arctic plant *Oxyria digyna*, which originated from the Qinghai-Tibet plateau. They postulated that several arctic plants indeed originated in the high mountains of Asia and later spread to the rest of the world. Open landscapes, strong winds and extensive snow (and ice) covers possibly aided in the dispersal of these plants that were or became well adapted to such conditions. The arctic pioneer plant *Dryas octopetala* was found to move along retreating glaciers during the late quaternary (between the last interglacial and glacial period) across Europe, as evidenced by studying plant fossils (Birks, 2008). This general pattern has also been found for other arctic and alpine plants (Quinn, 2008b).

*Plant adaptive responses to cold environments*

Arcto-alpine plants have to withstand stressful environments that are characterized by low temperature, low nutrient availability, severe drought, short growth season (3-4 months) and extremes of atmospheric and soil moisture levels (Billings and Mooney, 1968; Kume et al., 1999; Quinn, 2008b). All of these factors limit plant growth. To overcome the stress posed by these varied factors, plants adapt by various strategies. First, a general reduction of plant size, as clearly observed from the tenet that plant size decreases with increasing altitude/latitude in the Northern hemisphere. Plants in extreme polar desert habitats only measure about 5-10 cm (Quinn, 2008a). Second, plants in these habitats reveal stress-adaptive growth forms like chamaephytes (plants that hold regenerating buds above the soil level), hemicyrptophytes (hold buds at the surface), rosettes (prevent frost damage), cushion forms (maintain temperature raised inside the plants), prostrate shrubs and tussocks (Körner, 2003; Larcher et al., 2010). Furthermore, the allocation of nutrients among different plant parts and their physiological hardening has been observed to prevent frost damage (Margesin et al., 2007; Smallwood and Bowles, 2002). Third, plants secrete the red anthocyanin, especially during the beginning and end of the growth season and convert light to heat when temperatures are low. These anthocyanins are masked by chlorophyll during the summer season. During winter, a majority of arctic plants also synthesize “antifreeze” compounds to protect the tissues from freezing (Quinn, 2008c). Fourth, plants also strictly ‘select’ their microhabitat for growth. Thus *Silene acaulis*, a cushion-forming plant, is usually observed growing at exposed ridges. Finally, reproductive strategies - like high germination rates, early flowering and ability of seeds to survive over winter - along with a dominantly vegetative propagation modus, a low optimal temperature for photosynthesis and a strategic allocation of captured sunlight are other key adaptation strategies of arcto-alpine plants.
General Introduction

Why study arcto-alpine plant-microbe interactions?

Approximately $5.7 \times 10^6$ km$^2$ of the surface of Earth are covered by Arctic regions (Derry and Staddon, 1999), and the arctic habitat is predicted to experience major shifts in vegetation, with potential extinctions of flora and fauna (IPCC 2007). Sturm et al. (2001) reported a progressive (past) increase over time in the dominance of certain shrubs in the Arctic, by comparing photographs of Arctic regions taken with 50-year intervals. The fact that these shrubs displayed a positive response to warming indicated global warming as the cause.

The overall rise in temperature due to global warming is troublesome for the arctic region, as soil defrosting may result in as-yet unpredictable changes in carbon release and storage patterns. Thus, the natural ecosystem in the Arctic may also show potentially undesirable changes in soil nutrient mineralization by the local microbial communities (Zinger et al., 2009). Major questions are on the availability of carbon from thawing permafrost soils, the decomposition rates, the availability of nitrogen, phosphorus and sulfur, and on whether an ecological niche is created on which the emerging plants will rely for their growth and survival (Kirk et al., 2004). A detailed study of microbial functions in arctic/alpine soils is thus needed (Derry and Staddon, 1999).

It is unknown to what extent the aforementioned plant adaptations have affected their interactions with plant-associated microbes. However, it may be assumed that varying interactions, with bearing on plant fitness, may have ensued. For instance, the release of organic compounds by plant roots likely assists in spurring the abundance and diversity of bacteria in the rhizosphere, as compared to those in the corresponding soils (Derry and Staddon, 1999; Tam et al., 2001). In spite of the fact that we currently dispose of a large body of knowledge on the strategies used by temperate-climate plant species to direct, deal with, and potentially make use of, their associated microbiomes, those used by arctic/alpine plants are still largely unknown. Hence, it is important to study the interactions of local microbiomes with these pioneer plant species and their role in the establishment of plants in the new habitats. Hereunder, I briefly discuss plant-microbe interactions from a theoretical perspective, with special emphasis on those specific for the arctic/alpine regions.

Plant–microbe interactions

Microorganisms (bacteria and fungi) have been living in the Earth’s surface layers for over several billions of years. Ever since the (later) emergence of land plants, both beneficial and detrimental interactions with the resident
microbiomes have emerged (Zhang et al., 2006). In theoretical terms, the plant-microbe interactions are differentiated into the following classes:

- **Symbiosis** – as an example, nodulation or mycorrhization of plant parts by bacteria (in particular rhizobia) or fungi
- **Commensal (epiphytic or endophytic) presence** – plant tissues can carry bacterial or fungal cells at their surface or within, without any apparent harm
- **Pathogenicity** – particular bacteria and/or fungi can infest plants and cause plant disease

Of crucial importance is the fact that specialized fungi interact with plants by forming symbiotic relationships named “mycorrhizae”. About 90% of all vascular plant species, including forest trees, crops, grass and tundra plants can form different symbiotic relationships with fungi; i.e., ectomycorrhizal (EM), ericoid mycorrhizal (ECM) and arbuscular mycorrhizal (AM) (Bonfante and Genre, 2010). The ability to interact with fungi is considered to be important for plants in most, if not all, of the ecosystems (Kytöviita 2005). Nutrient acquisition, solubilization of nutrients and minerals, stress tolerance, N and P mobilization and resistance towards plant pathogens are significant benefits provided by mycorrhizae to plants (Finlay, 2007, 2008). Likewise, host plants are necessary for the growth and reproduction of many of the mycorrhiza-forming fungal species (Bonfante and Genre, 2010).

In addition to these associations with clear ecological outcomes, soil bacteria and fungi can also interact with plants yielding less well defined effects, which can be of slightly deleterious, neutral or beneficial nature (Van Elsas et al., 2007).

What connects all of the above interactions is the fact that any successful interaction establishes in phases. To these, a prior phase of recognition and attraction of the microbial partner is primordial. This first interactive phase commonly takes place in the rhizosphere, i.e. the soil surrounding the roots of plants. The microbial communities in soil are attracted to root-exuded compounds, i.e. sugars, amino acids, organic acids, sterols, proteins and/or secondary metabolites. Using such molecules as chemoattractants, they can thus colonize the rhizosphere and rhizoplane (Badri et al., 2009; Compant et al., 2010; Sorensen and Sessitsch, 2007). Some bacteria and fungi from the rhizosphere can subsequently further colonize and invade plant tissues and get established as endophytes. Thus, plants can be thought of selectively attracting and repelling microbes first into their rhizospheres and then placing strong filters on the ones that are admitted to become endophytic.

Although it is attractive to state that the resultant plant-associated microbes can support the colonization of plants in soils that pose harsh
conditions, there is actually only scarce evidence for this tenet when it comes to arctic/alpine plants. Granted, such effects have been revealed in soils with low nutrients, pH or high metal toxicity (Bradley et al., 1981; Mapelli et al., 2011; Sousa et al., 2014). Furthermore, there is extensive knowledge pointing to the fact that specific micro-organisms can provide resistance to plants against plant pathogens, either by outcompeting these or by antagonizing them, and also assist plants in their tolerance to conditions of drought (Miniaci et al. 2007; Hardoim et al. 2008; Compant et al. 2010; Zhang et al. 2006; Sessitsch & Howieson 2002). However, again, we lack knowledge on all of these facets of plant microbiomes for arctic/alpine soils.

We currently know that virtually all plants on Earth harbor microbiomes, that are often partially rhizospheric, partially endophytic (living in the endosphere) (Tan and Zou, 2001). Thus, microbial cells have been found to dwell in the roots, stems, flowers, fruits, tubers, bark and even seeds of diverse plants (Hardoim et al., 2012; Müller et al., 2015; Zhang et al., 2006). Moreover, plants may even ‘select’ their own microbiome from soil via roots secreting signaling molecules (Yergeau et al., 2007). Duineveld et al. (1998), using a direct molecular method, first revealed that plants influence the microbiome of their rhizosphere. Then, Smalla et al. (2001) indicated that different plants attract a different microbiome for the same soil. Moreover, Bragazza et al. (2012) showed that plants [in peat lands] can affect the functioning of the soil microbial communities. These microbial communities differed based upon the plant species, plant physiology and even between different plant parts. Thus, a vision has developed of plants creating specific ecological niches that, consequently, select specific microbial species (Garbeva et al., 2004; Yergeau et al., 2007).

As discussed above, the interactions of plants with microorganisms may give benefits of different kinds to the plant. Moreover, these plant-microbial associations were also observed in perennial trees, herbaceous plants and arctic tundra (Nissinen et al., 2012). Thus, we currently face a situation of emerging evidence for a strong role of microbiomes in the ecology of a range of temperate climate plants, versus a general paucity of knowledge on the diversity, community composition and role of microbiomes in arctic and alpine plants.

Colonization of land by arctic/alpine plants - an invisible help

Arcto-alpine pioneer plants can grow in extremely challenging conditions, for instance in cold rocky areas along receding glaciers. Usually, the soil/moraine forefields of such areas are low in sources of C and N, and they are initially vegetation-free (Miniaci et al., 2007). A logical hypothesis is that local
microbiomes can offer (ecophysiological) help to pioneer plant species. Indeed, a study on bio-weathering of recently deglaciated moraine soils at the Midre Lovénbreen glacier in Svalbard in High Arctic illustrated the role of bacteria in soil formation (Mapelli et al., 2011). Bacteria with high phylogenetic diversity able to drive key biogeochemical processes were shown to play key roles in C and N inputs in these soils. This microbial nutrient deposition contributes to soil fertility, thus assisting early ecosystem development (Mapelli et al., 2011; Nemergut et al., 2007). There is a feedback mechanism as well, as Miniaci et al. (2007), from their studies in alpine glacier forefields, concluded that vegetation patches in the glacier forefield increased microbial activities and biomass. This clearly affected the plants, as a possible payback. However, we know very little about the ecological importance of this “invisible help” and need more detailed studies to understand the role of these early colonizers in ecosystem development. In the upcoming years, freshly deglaciated soils may become hot spots for colonization by plants due to rising local temperatures. Plant–microbe interactions may then play pivotal roles in these events. Several plant factors, like stress tolerance, uptake and processing of nitrogen and phosphorus, survival, early colonization, dispersal by seeds, slow growth rate and survival may be affected or driven by plant-associated microbiomes. To understand the key processes, we need to gather fundamental knowledge on the microbial communities in such habitats and their effects on pioneer plant species.

The microbial diversity in arcto-alpine biomes

**Bacteria** - Several accounts (censi) of bacterial communities from different arctic habitats, i.e. permafrost soils (Bakermans et al., 2014; Gittel et al., 2014; Yergeau et al., 2010), cryoconite holes (Cameron et al., 2012; Edwards et al., 2011, 2013) and tundra soils (Männistö et al., 2007; Neufeld and Mohn, 2005; Schmidt and Bölter, 2002; Wallenstein et al., 2007), have been provided in recent years. The similarities between the catalogues of 16S rRNA gene sequences in soils from Finland and in Alaskan tundra soils have supported the idea of a similar circumpolar distribution of bacteria (Männistö et al., 2007). Likewise, Nissinen et al. (2012) reported that endophytic bacterial communities from Kilpisjärvi, Finland, are host-plant-specific when observed at a high taxonomic level. A tight association of specific *Sphingomonas* spp. with *O. digyna* and *Diapensia lapponica* was observed. In contrast, a study of Antarctic rhizosphere soil communities of *Deschampsia antarctica* and *Colobanthus quitensis* revealed the occurrence of similar bacterial community structures and no influence of plant species (Teixeira et al., 2010). However, in the latter case, the bacterial communities in the respective bulk soils were found to be different from those of the rhizosphere. The authors hypothesized that, even though
vegetation influences the microbial communities, other factors such as differential moisture content or freeze-thaw cycles might have exerted more powerful effects (Teixeira et al., 2010).

**Diazotrophic bacteria** - Nitrogen (N) is considered to be a key limiting factor for both microbial and plant growth in the Arctic tundra and hence the diversity of diazotrophic communities from these (low nitrogen) soils has been relatively well studied. Wallenstein et al. (2009) observed overall increases and decreases in microbial enzyme activities with the presence and absence of soil nitrogen, respectively. Subsequently, a series of N-addition experiments by Sistla et al. (2012) revealed that microbial growth, enzyme synthesis and carbon allocation in tussock tundra soils during the arctic summer are N-limited. A study of a chronosequence along the forefield of the Damma glacier by Brankatschk et al. (2011) reported a very low N content in soils from 10 to 70 years with scarce vegetation. Furthermore, they observed that potential N-fixation activity was undetectable, and denitrification and nitrification rates were extremely low. The lowest $nifH$ gene copy numbers were detected from newly exposed 10-year old soils. In contrast, a densely plant-covered 120-year old soil showed both higher enzyme activities and higher copy numbers of the $nifH$, $nirK$ and $nirS$ genes. This suggested that plants had a strong bearing on the microbial associations and an established increase of nitrification and denitrification activities. A study by Nordin et al. (2004) reported that less than 1% of the experimentally added N was recovered from plants, whereas about 49% was recovered from the soil microbial biomass. The study explains that, even though plants are capable of directly taking up added N ($\text{NH}_4^+$, $\text{NO}_3^-$, amino acids), they compete poorly with microbes. Hence it is clear that microbes constitute an integral part of plant growth in the N limited tundra soils.

**Fungi** - It is known that the majority of plants in tropical and temperate regions form mycorrhizal relationships in order to enhance their capacities to obtain N and P compounds. In line with this, it has been estimated that 61-86% of the plant nitrogen in the arctic tundra is furnished, from bound nitrogen, by ECM and EM fungal communities, a value similar to that in temperate climate regions (Hobbie and Hobbie, 2006). An early report by Högberg (1997) already concluded that soil nitrogen uptake by non-mycorrhizal plants is less than that by mycorrhizal plants. However, the possible dominance of non-mycorrhizal or low-mycorrhizal flora in the Arctic environment is puzzling considering the importance of mycorrhizal symbioses in comparable (nutrient poor) temperate-climate habitats (Kytöviita 2005). Furthermore, very little is known about the role of mycorrhizae in the soil C balance of the tundra ecosystem, in spite of the fact that it contains ca. 11% of world’s C pool (Ludley and Robinson, 2008). The precise roles of mycorrhizae
in the arctic tundra have not been extensively studied and there is very little information about the types of symbioses and fungal adaptations to Arctic conditions. The diversity of root-associated fungi in the arctic tundra has been investigated in a pioneer study by Bjorbækmo et al. (2010). They found that a total of 137 fungal OTUs, mostly representing ECM genera such as *Cenococcum*, *Cortinarius*, *Hebeloma*, *Inocybe* and *Tomentella* were present in the root systems of *D. octopetala*.

With respect to the soil microbiomes, Schadt et al. (2003) reported seasonal variation in the fungal biomasses of tundra soils, with a maximum in fungal activity during late winter under the snow. However, in case of AM fungi, Kytöviita & Ruotsalainen (2007) suggested that the activity decreases with lower temperature and host plants receive thus less benefit from the symbiosis. Also, a decrease in temperature was the single most important factor that limited mycorrhizal growth and colonization rates in the Arctic (Ruotsalainen & Kytöviita 2004; Kytöviita 2005). In a different study by the same group, an increase in temperature from 12 to 17°C benefited the low-arctic host plants *Potentilla crantzii* and *Ranunculus acris* with increased mycorrhizal (AM) association, providing more nutrients (especially phosphorus) (Kytöviita & Ruotsalainen 2007). It was hypothesized that the rising temperature improves mycorrhizal performance, in turn providing more nutrients to the host and thus supporting the persistence of cold-climate meadow plants. Factors such as a change in temperature, soil moisture, freezing-thawing, decomposition of organic polymers and phenolic compounds, and availability of soil carbon, were behind these fungal community shifts. However, the precise effects of each of these factors on the microbial shifts were unclear.

**Sampling regions**

**Mayrhofen, Austria (European Alps)**

The region above the tree-line over the snow-covered mountains (altitude ca. 3000m above sea level) is termed the alpine habitat. The European Alps (43.25-48.25°N, 4.25-16.25°E) constitute a long mountain range in the south of Europe, with the highest peaks reaching 4400 to 4800m above sea level (Casty et al., 2005). The relatively short, snow-free growing season spans 3-4 months. The regional climate is characterized by low temperatures (i.e. annual mean of 2-4°C) and high precipitation rates by mainly snow. Its scarce vegetation reflects the alpine and arctic tundra (Nemergut et al., 2005).

**Kilpisjärvi, Finland (Low-Arctic)**

Kilpisjärvi is located in the northernmost part of Finland, at the borders of Finland, Sweden, and Norway (68°N), 400 km north of Arctic Circle. Its flora
is dominated by mountain birch forest in the valleys and by tundra plant species at higher elevations. The annual mean temperature is about -2.2°C, with a plant growing season of ca. 90-100 days in the valleys. Kilpisjärvi is one of the coldest places in continental Europe (Männistö et al., 2007).

Ny-Alesund, Svalbard (High-Arctic)

Spitsbergen (Norway) is part of an isolated archipelago located in the high Arctic at 74-81°N; most of the land cover is dominated by glaciers and permafrost layers, and the mean annual temperature is -4°C. The soil temperatures have been reported to be below zero for more than 250 days per year ranging from -6°C to -25°C (Coulson & Hodkinson 1995). Any biological activity is restricted to less than 10% of the total land mass coupled with about 3 months of plant growing season. The Svalbard flora is dominated by tree-less tundra and arcto-alpine plant species (Reigstad et al., 2011).

Plant species studied

Oxyria digyna and Saxifraga oppositifolia are among the key pioneer species in the arcto-alpine soils, growing in low-nutrient tundra soils (usually along the rocky patches or snow-melt areas), and were early colonizers of arctic and alpine regions after the ice age. They grow in similar habitats and thrive in tree-less rocky/fell/tundra habitats, but they differ in their mycorrhizal associations and physiological characteristics. Our sampling sites from all the three regions were located in the rocky areas with small soil pockets that held the target plants.

O. digyna (mountain sorrel) is a pioneer plant species and is common in the arctic tundra, with distribution from the high Arctic (80°N. Spitsbergen, Norway) to temperate and alpine habitats. O. digyna usually grows in cold, moist habitats and typically colonizes arctic and alpine stream banks, talus/scree slopes, snow patches and rocky slopes, primarily those facing north. It is also a typical pioneer species in glacier forelands (Robbins and Matthews, 2009) and has been estimated to be one of the first plants to colonize terrain left barren by retreating ice, for instance at the end of the last ice age. O. digyna is a non-mycorrhizal plant species, but it is very successful in colonizing novel habitats and establishing there, in spite of the limited dispersal of its winged fruits (Marr et al., 2008). It is not just a transient pioneer species, but establishes well in novel sites and forms part of the permanent flora.

S. oppositifolia, a semi-woody cushion plant, is a long-lived perennial herb with opposite scale-like leaves, attractive purple-colored flowers and a wide circumpolar distribution. The distribution spans from Spitsbergen, Norway, along Scandinavian Lapland all the way up to 83°N in Greenland, the Siberian
and Canadian Arctic to the alpine regions of Europe, Asia and North America. The habitat range even extends to the polar desert and several sites in Devon Island, Canada. The leaves can tolerate extreme water stress (up to -55 bar in lab) (Teeri, 1973). *S. oppositifolia* is one of the first plant species to colonize soil after glacier retreat. It has also been selected for coordinated observations within the International Tundra Experiment (Kume et al., 1999). It is an ectomycorrhizal species and its dispersal and reproduction is mainly by seeds. Being one of the earliest flowering species (period of 8-9 days after snow melt) among the arctic tundra makes it a strong contender to colonize newly exposed unvegetated soils (Stenström and Molau, 1992).

**Objective of this thesis**

Given the fact that the diversity and factors shaping community structures of plant-associated microbes in the Arctic are poorly known compared to those in temperate climate regions, I decided to make an inventory of the factors (biotic and abiotic) that determine the composition and diversity of soil- and plant-associated bacterial and fungal communities in selected arcto-alpine soils. A key question was how and to what extent pioneer plant species in these habitats make use of their associated microbiomes, and to what extent they select these. Furthermore, I also addressed the diversity of diazotrophic bacterial communities associated with the same plants in the N limited arctic habitats.

To achieve these goals, I characterized the plant-associated microbial diversities in two pioneer arctic plants with differing mycorrhizal association and the soils associated with these plants. The selected plants, *O. digyna* (non-mycorrhizal) and *S. oppositifolia* (EM), are among the early colonizers of the harsh soil habitats and prefer similar growth conditions (Figure 1). The sampling took place in selected low-arctic (Kilpisjärvi, Finland), high-arctic (Ny-Ålesund, Svalbard, Norway) and alpine (Innsbruck, Austria) environments. I associated the local microbial community structures with differences in location, climate, soil properties and plant species (Figure 1). Furthermore, I addressed the putative role of these microbes in the nitrogen cycle in order to shed light on the putative role in plant development.

**Hypotheses**

- Bacterial community structures and functioning in arctic and alpine soils are highly influenced by geographic region, vegetation, pH and soil chemical parameters.
- Plant-associated endosphere and rhizosphere microbial communities are selected from bulk soil communities.
Endophytic bacterial diversity is primarily dependent on plant species, indicating a strong selection by the plants.

Plants select species-specific communities from the soil microbiota in a context (biogeographical zone)-dependent manner. The abundance and diversity of bacterial communities do not correlate with latitude or climate, but community structure does.

Nitrogen acquisition by plants in arctic soils is driven strongly by plant-associated bacteria, and, conversely, plants strongly shape the nitrogen cycling communities.

Outline of the thesis

The aforementioned hypotheses were tested and answered in more detail in the following chapters. The influence of geographic region on bulk soil microbiomes was assessed in chapters 2, 3, 4, 5, the influence of plant species on rhizosphere communities in chapters 2, 3, 4, 5 and the impact of plant species on endosphere communities in chapters 3, 4 and 5.

Specifically, a brief overview of our current understanding of the plant-associated bacterial and fungal communities in arcto-alpine regions is discussed in the introductory chapter 1. Microbiomes may help in initial seed germination, root growth (by producing growth hormones), settlement and adherence of the roots, N fixation and P solubilization. In addition, the pioneer plants may establish proper conditions for the microbes to survive and settle as well and provide an environmental filter for microbial establishment in return for the nutrients received from them. In chapter 2, I used microbial community fingerprintings to investigate the impact of plants or soil physicochemical properties on the diversity and community structure of bacterial (and diazotroph) communities from Kilpisjärvi, Finland (low-arctic) and
Longyearbyen, Svalbard (high-arctic). In addition, Biolog Gen III plates were used to determine the functional diversities of both soil and rhizosphere bacterial communities from the same sites.

In order to understand the key microbial players that determine the impact of geographical region and plant species on the diversity of bacterial and fungal communities, I then used next generation sequencing (NGS) methods for the following chapters. The samples for NGS analyses (chapters 3, 4, 5) were collected from three distinct geographical regions: Mayrhofen, Innsbruck (alpine), Kilpisjärvi, Finland (low-arctic) and Ny-Ålesund, Svalbard (high-arctic). On the basis of NGS, chapter 3 addresses the phylogenetic diversity and community structure of the bacterial communities from bulk soil, rhizosphere soil and the endosphere of *O. digyna* and *S. oppositifolia*. We investigated the key bacterial OTUs that impact the host plant species per geographical region. In addition to determining the impact of region and host plant specificity, we also dig deeper in order to pinpoint the “core” endosphere communities associated with *O. digyna* and *S. oppositifolia* from all three sampling regions. We also hypothesize that “core” endospheres will help in the establishment of the plants in these habitats and play key roles in acquiring N in these low nutrient soils. The diversity and community structures of the fungal communities associated with bulk soil and rhizosphere and endosphere of both *O. digyna* and *S. oppositifolia* are reported in the chapter 4. We also analyzed the impact of soil physico-chemical properties (soil pH, SOM and available P) on the bacterial and fungal bulk soil communities in chapters 3 and 4 respectively. With *nifH* gene as a proxy for diazotrophs, a new nested primer system was developed and used to study the diversity and community structure of potential nitrogen fixing bacterial (PNFB) communities in chapter 5. In addition, the impact of geographic region and host-specificity of the PNFB communities was studied and we found anoxic PNFB enriched in the Arctic regions. In chapter 6, I finally summarize the new knowledge on the relative impact of geographic region and plant species on the plant-associated microbial communities in the (nutrient poor) arctic and alpine regions. Considering the scarce knowledge we have about plant-microbe interactions in the selected arcto-alpine plants, I make some concluding remarks and considerations for the future research in this exciting and ecologically important research area.

References


Chapter 1

of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. *The ISME journal* 4, 1206–14. doi:10.1038/ismej.2010.41.


