Biogeographical diversity of plant associated microbes in arcto-alpine plants
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Microbial diversity in arcto-alpine plants: a synthesis

Manoj Kumar
Background of the study

Microbes (bacteria and fungi) and terrestrial plants have co-evolved since the time higher plants appeared on the Earth (Zhang et al., 2006). Indeed, the association of mycorrhizal fungi with plants may have assisted the colonization of land by plants about 450 Myr ago (Redecker et al., 2000; Simon et al., 1993). In addition, we now know that particular bacteria can also be tightly associated with plants, often occurring inside plant tissue (Kloepper et al., 1992). In addition to the classically known rhizobia, a wealth of other bacteria have been isolated from inside root, stem, leaf, fruit and even seeds (Chanway, 1996). However, the diversity, ecology and functional properties of plant-associated microbes are still not well understood. All the more so, only few studies on arcto-alpine plants have been reported so far (Nissinen et al., 2012; Poosakkannu et al., 2015; Zhang et al., 2015). In this thesis, I set out to analyze the diversity of microbial (bacterial as well as fungal) communities that are associated with two selected pioneer plants, Oxyria digyna and Saxifraga oppositifolia, in arcto-alpine soils. Implicit in this study was the assumption that these plants, for their healthy growth in the cold and nutrient-poor soils, were to some extent dependent on their local microbiomes. In particular, I hypothesized that some of their functions, e.g. nitrogen provision, depended on plant-associated microorganisms. In the light of the overriding effects of edaphic and climatic factors on soil status, I also speculated that bulk soil microbial communities are mainly influenced by geographic region and that, consequently, the plant-associated microbial communities are selected from these, forming sub-communities. I selected the aforementioned two plant species, O. digyna and S. oppositifolia, on the premise that, even though they prefer similar growth habitats, they secrete different compounds in the exudates from their roots and thus influence their associated communities in different manners.

The sparse information that was available at the onset of this study was as follows:

- **Bacterial and fungal diversities in cold soil habitats do not follow similar pattern**
  
  Chu et al. (2010), using next generation sequencing, found that soil bacterial diversity in the Arctic is similar to that in other biomes. In contrast, fungal communities from polar regions (Antarctic and Arctic) were indicated to exhibit endemism in these regions, with cold climate effectively acting as a habitat-specific filter (Cox et al., 2016). Meanwhile, both bacterial and soil fungal communities in the Arctic region were shown to be influenced by soil pH, and so soil pH regulated these communities, albeit to different extents (Männistö et al., 2007; Zhang et al., 2016).
Contradicting statements about host specificity of plant-associated microbes in arcto-alpine soils

Nissinen et al. (2012) observed that endophytes isolated from three arctic plants, i.e. *O. digyna*, *Diapensia lapponica* and *Juncus trifidus*, exhibit host plant specificity. They further identified members of these endosphere communities that closely resembled isolates from other cold habitats. A similar host plant specificity was recently reported for endophytic fungal communities from *S. oppositifolia*, *Saxifraga cespitosa*, *Silene acaulis* and *Cassiope tetragona* sampled from Ny-Ålesund, Svalbard (Zhang et al., 2015). However, other studies indicate fluctuating host specificity for both bacterial and fungal root-associated communities in cold-climate soils (Botnen et al., 2014; Fujimura and Egger, 2012; Teixeira et al., 2010), and so the level of host plant selectivity is controversial and may depend on differing factors, such as plant species and edaphic factors and the niche studied (endosphere vs rhizosphere).

Diazotrophs from cold habitats are similar

Nitrogen is considered to be a major limiting factor for plant (and microbial) growth in arcto-alpine biomes. Deslippe and Egger (2006) analyzed the molecular diversity of *nifH* genes from bacteria associated with high-arctic plants and found a striking similarity between the composition of bacterial *nifH* clone libraries of bulk soils and roots of high Arctic shrubs from Canadian arctic and those from other cold habitats. They postulated that the distribution of nitrogen fixing bacteria can be predicted based on the habitat type (referring to the cold habitats here).

Some other studies - not addressed here - have yielded somewhat erratic results; these are subject of the discussions in the research sections of this thesis.

On the basis of the sparse information available, as depicted above, I took as a strategy to determine the microbiomes of three cold climate regions, including one in the European alps (Mayrhofen, Austria) and two across the northern sphere (Kilpisjärvi, Finnish Lapland [low-arctic] and Ny-Ålesund, Svalbard archipelago; [high-arctic]).

In this study, I have thus been able to pinpoint the unknown/undiscovered plant-associated microbes that are associated with the aforementioned two pioneer plants in this pristine environment, as discussed in the following sections. In the light of the importance of such microbiomes for plant growth and health in soils of other climate zones, I placed a focus on both the bacterial and fungal communities in the samples. Moreover, I also
addressed the (diversity and composition of) diazotrophs given their postulated importance in the study systems. My hypotheses in this study were:

- Bacterial community structure in arcto-alpine soils is highly influenced by geographic region or soil physico-chemical properties.
- Plant-associated microbial communities are primarily dependent on plant species, indicating a strong selection by the plants.
- Nitrogen acquisition by plants in arctic soils is driven strongly by plant-associated bacteria, and the diazotrophic communities are shaped by plant species.

Below, I come to some overriding conclusions on the basis of these hypotheses and the data generated in the research chapters of this thesis.

**Endosphere microbial communities have reduced diversities and are different from (bulk and rhizosphere) soil microbial communities**

There is compelling evidence, from all data generated in chapters 3, 4 and 5, that the diversities and community structures (of bacteria, fungi and potential nitrogen fixing bacteria (PNFB)) in the endospheres of the two selected plant species – in the three geographic regions studied, are different from those in the (bulk and rhizosphere) soil. Expectedly, the endosphere total bacteria (chapter 3), PNFB (chapter 5) and fungal (chapter 4) communities had very low richness and diversity values when compared to those of the (bulk and rhizosphere) soil samples. This generic key finding indicates that the two plant species that were selected act as effective ‘filters’, selecting a restricted set of microbes as endophytes from the highly diverse soil communities. The putative function of such endophytes in these cold-adapted plants has to await further study, but activities with respect to ‘protection’ from frost, low nutrient status and drying/osmotic tension are all possible (Subramanian et al., 2015; White and Torres, 2010).

We thus also identified the key bacterial and fungal taxa that contribute to the apparent ‘endophyte specificity’. The bacterial genera *Clostridium* (*Firmicutes*) and *Leptothrix* and *Burkholderia* (*Burkholderiales*), were selectively enriched in the endospheres, as became apparent from both the bacterial (chapter 3) and PNFB communities (chapter 5) communities. Coupled to the fact that such genera are presumably involved in (non-rhizobial) nitrogen fixation (and that they were also found by using the *nifH* gene proxy), it is logical to posit that they play an important role in the nitrogen acquisition by plants in these N limited soils. The endospheres of the reportedly EM plant *S. oppositifolia* were enriched with dark septate endophytic fungi from the genera *Leptodontidium* and *Phialocephala*. Both *Leptodontidium* and *Phialocephala* have been
reported as fungal endophytes in the high-arctic EM plants *Dryas octopetala* and *Bistorta vivipara* (Bjorbækmo et al., 2010; Mundra et al., 2015). Newsham et al. (2009) reported that the dark septate fungi such as the ones found by us can be present as endophytes in arcto-alpine plants and are predicted to enhance plant performance. The largely non-mycorrhizal *O. digyna* was predominated by the presumably saprotrophic *Varicosporium*. However the functional mechanisms of these fungi-plant symbiosis are unexplored and a potential future prospect.

![Diagram showing interactions of bulk, rhizosphere and endosphere communities of bacteria (red), fungi (green) and potential nitrogen fixing bacterial (orange) communities](image)

*Figure 1* The interactions of bulk, rhizosphere and endosphere communities of bacteria (red), fungi (green) and potential nitrogen fixing bacterial (orange) communities

**Endosphere communities - Host plant specificity**

The bacterial community structures in the endosphere (chapter 3) were influenced by plant species, whereas the fungal (chapter 4) and PNFB (chapter 5) community structures had low host plant specificity (Figure 1). Thus, the two arctic plants may interact differently with respect to the ‘selection’ of bacterial and fungal types into their endospheres. In particular the fungi and the PNFB, as potential nutrient providers, may have been more ‘generically’ attracted, reflecting their potential key roles in plant support. Moreover, it would be intriguing to assess to what extent these groups interact between themselves, in addition to their interactions with the plant. Thus, a detailed study on fungal-bacterial interactions with focus on bacterial nitrogen fixers in the Arctic would constitute a sensible future research scenario.
**Rhizosphere communities – Host plant specificity**

Overall, the influence of plant species on the rhizosphere communities was rather low when compared with that on the endosphere ones (Figure 1). The bacterial communities (chapters 2 and 3) revealed a moderate degree of host plant species specificity, whereas the fungal ones had low host plant specificity (chapter 4). Also the ‘functional profiles’ of the rhizosphere communities of *O. digyna* and *S. oppositifolia* were different. In particular, we observed a raised abundance of antibiotic resistance bacteria in the *O. digyna* rhizosphere, which can be also related to the non-mycorrhizal status of this plant host. On the basis of these collective profiles, we surmised that the provision of different conditions, possibly related to the secretion of different compounds in the root exudates by *O. digyna* and *S. oppositifolia* are at the basis of the establishment of different bacteriomes in the rhizosphere (and endosphere, as discussed in the foregoing).

**Rhizosphere communities of bacteria and fungi assemble via different rules**

Analysis of the bacterial communities (chapters 2 and 3) versus the fungal (chapter 4) and PNFB (chapter 5) ones revealed that the former responded differently from the two latter ones. Thus, specifically, the bacterial rhizosphere communities were more strongly influenced by plants than the fungal or PNFB rhizosphere communities. In several prior studies in temperate climate soils, strong selection of bacterial communities by the rhizosphere of different plants was also found, and most authors have attributed this rather strong effect to the action of plant root exudates on soil bacteria (Duineveld et al., 1998; İnceoğlu et al., 2011; Smalla et al., 2001). We here extend this rhizosphere selective effect fact to two plants in cold-climate soils. We also know, from previous work, that bacteria may show stronger responses to plant exudates than fungi, and so the selection of fungal types by community assessments is often more difficult to discern. Rather, our observations indicate that fungal rhizosphere communities are more strongly influenced by local soil properties. So, generalizing, one may posit that, within the limits of our observations and tools, bacteria appeared as the more avid responders to the two plants, as compared to the fungi.

In addition, both the bacterial (chapters 2 and 3) and fungal (chapter 4) rhizosphere communities had higher richness and diversity values than the corresponding bulk soil communities. This is in contradiction to multifold studies from agricultural soils where bulk soil communities tended to show highest diversity (İnceoğlu et al., 2011; Kowalchuk et al., 2002; Smalla et al., 2001). However, our observation is in support of other data from low-nutrient habitats (Coleman-Derr et al., 2016; Miniaci et al., 2007). It may be attributed to the sparse availability of nutrients in the bulk soil, which I speculate limits
the overall niche-defined diversity. Thus, the plant root exudates as well as other condition in the plant rhizospheres may have been acting as providers of diverse nutrients/conditions and so amplified the number of colonizable niches.

In contrast, the diversity of PNFB (chapter 5) was not raised in the rhizospheres as compared to that in the corresponding bulk soils. Hence, it is possible that the potentially strong selection by the two plants – in this functional group – indeed reduced diversity, as related to the selective role of these organisms. This facet of bacterial life in the rhizosphere of the arctic plants, much like for the endosphere, warrants dedicated future research endeavors.

The influence of geographic region on microbiomes is dependent on compartment

In the light of the differential effect of compartment (chapters 2, 3, 4 and 5), I will discuss the effect of region as per compartment (bulk soil, rhizosphere soil, endosphere), as below:

**Bulk soils**
The assessments of bacterial (chapters 2 and 3), fungal (chapter 4) and PNFB (chapter 5) community structures across the bulk soil samples revealed a strong regional specificity, in particular for the bacterial communities (total and PNFB). In other words, the bacterial and PNFB community structures of the bulk soils were different per region. Moreover, the fact that the diversities of the bulk soil bacterial communities were positively correlated with soil pH (range 4.5 to 8.2) and soil organic matter indicated, in support of work by Fierer and Jackson (2006), that soil pH was a key ‘shaper’ of these communities. The effect of region on the bulk soil fungal communities was much weaker, however their community structures were, again, primarily influenced by soil pH.

Overall, I thus hypothesize that the conditions in the local soils, under which climatic and soil physicochemical (e.g. pH) properties, were the key drivers of all bulk soil communities.

**Rhizosphere soils**
Above, I addressed the selective effect of plants and plant species on the microbiomes, as observed in their rhizospheres. Here, I examine the influence of geographic region. First, the fungal (chapter 4) and PNFB (chapter 5) rhizosphere communities displayed a strong regional selection when compared to the bacterial communities (chapter 3). Clearly, whereas the bacterial communities had a more ‘ubiquitous’ nature, both the fungal and PNFB showed a more endemic one. Concerning the latter, I here speculate that the rhizosphere PNFB communities are strongly affected by the local climatic conditions or soil edaphic factors, and may shape the environment for the
local plants. For example, the cyanobacteria *Nostoc punctiforme* and *Nodularia spumigena* were predominantly observed in the Ny-Ålesund regions. They were reported as key primary producers mediating carbon and nitrogen fixation in early succession soils and also mediating plant establishment in low-nutrient soils (Borin et al., 2010).

**Endosphere**

The endosphere communities of both fungi and PNFB were clearly influenced by region, whereas the total bacterial communities responded to a lesser extent (Figure 1). The strong regional influence on the PNFB communities was remarkable. I relate these to effects of the local climatic conditions (as mentioned above). Clearly, the anaerobic nitrogen-fixing genus *Clostridia* dominated the Arctic region samples, while the genus *Geobacter* (that is capable of fixing nitrogen under both aerobic and anaerobic conditions) dominated the endosphere communities of the alpine region. Indeed, predominantly anaerobic conditions were observed frequently in the soil pockets that held the plants in the Arctic, due to late melting soil and underlying permafrost, whereas such conditions were less frequently found in the alpine region.

Is there a (beneficial) core microbiome that establishes in cold-soil-adapted plant species? The question whether the two pioneer plant species *O. digyna* and *S. oppositifolia* contain a core (trans-species) set of microorganisms (that may yield growth/fitness benefits) was addressed by comparing the key shared members of the endosphere communities (Table 1). Most of the taxa identified in the endosphere bacteriomes (*Clostridium, Bradyrhizobiaceae, Comomonadaceae [Leptothrix]*) were also found in the endosphere ‘PNFB-omes’, and I therefore strongly argue that they play a potentially vital role in nitrogen acquisition. Moreover, in a side project, we also detected *Clostridium, Comamonadaceae* and *Bradyrhizobiae* from the seeds of *O. digyna* (Given et al., in preparation), indicating seed transmission. However, the question whether such core microbes are transported via seeds, thus connecting plant generations, remained unanswered. In other work, such organisms have been found in seeds, which indicates they are vertically transmitted and may be selected by the host plants to be carried off to the next generations (Hardoim et al., 2012; Truyens et al., 2014). A case in point is offered by the seeds of cardon cactus (*Pachycereus pringlei*) which carry endophytes that allow them to establish in barren rocks by weathering these and dissolving the rock minerals (Puente et al., 2009). We suspect similar mechanisms might be involved in the colonization of the non-mycorrhizal *O. digyna* in the nutrient-limited rocky soils that it usually colonizes. Furthermore, several studies, referred to in Table 1, have revealed that, in addition to nitrogen fixation, these key OTUs also have diverse potential functional aspects like enhancement of stress tolerance,
phosphate acquisition, resistance against plant pathogens by secretion of antimycotic compounds, and, finally, promotion of plant growth by synthesis of growth promoting hormones like gibberellins. With reference to this work, I argue here that we are now at a stage where the functional implications of our arctic plant endophytes need to be addressed.

Outlook and recommendations for further work
The study of the interactions of arcto-alpine plants with local microbiomes is relatively new, and the current study has added a large body of information to this area. In the work, I have been able to pinpoint the bacterial, fungal and PNFB communities enriched in the endosphere but I did not yet obtain any information on the details of the functional properties of those communities. It is likely that, in addition to an involvement in nitrogen fixation, the community members have roles in, for instance, frost protection and acquisition of compounds like phosphorus (in addition to nitrogen). Moreover, a possible steering role of plant physiology might be assumed. Future studies on the functional aspects of (members of) the plant-associated microbiomes and why the arcto-alpine plants are in need of these microbial associations for their fitness is vital. I have thus posited that key endosphere specialists assist in the fitness/survival of plants in these cold and nutrient (specially N and P) limited soils (Table 1). For example, some of these endosphere specialists (Table 1) were predominantly present in the endospheres of two plant species across all the regions, and so this indicates they have acquired a niche that potentially also supported their host plant. As I mentioned in my conclusion, there is mounting evidence for the tenet that such plant-supportive endosphere dwellers can be vertically transmitted as they make part of the seed microbiome.

Future work should thus attempt to isolate key plant helper species (both bacteria and fungi), as this will allow studies on behavior and function at the plant. This will also give us vital information on the strategies employed by these pioneer plants in colonizing the low-nutrient mineral-rich deglaciated moraines.

As argued before, a key lack of knowledge concerns the cycles of N and P in the studied habitats. Clearly, the level of N and presumably also of P is the limiting factor for plant growth in the studied arcto-alpine habitats. Studies on the functional involvement of selected microbes in these processes under local conditions is needed. In addition, an investigation into the potential interaction of fungal-bacterial (nifH-endowed) interactions in the rhizosphere, especially in the context of the mycorrhizal associations (considering differential association of O. digyna (non-mycorrhizal) and S. oppositifolia (ecto-mycorrhizal)) will be necessary.
The study of plant-associated communities in arcto-alpine plants is new, and important in an era of global warming; so far only few papers have been published in this area. Thus the mechanistic underpinning of the presumed microbial support of plant growth and health in the challenges posed by the cold (although warming) and nutrient limited soils is in its infancy. Exploring the functional consequences of the microbial diversity in the Arctic, in both bulk soil and associated with plants, will be the “arctic expedition” of this century and I am eager to reach the pole.

References


