JYU DISSERTATIONS 62

Cindy Jittrapan Given

Assembly and Functioning of Endophytic Bacterial Communities in Arcto-Alpine Pioneer Plant *Oxyria digyna*

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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“Doubt is the origin of wisdom”
– René Descartes

ABSTRACT

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Plant microbiomes consist of diverse communities of microorganisms, among which bacteria are highly abundant. The microbiomes are crucial for plants as they rely on their microbial associates for many essential functions. The goal of this thesis was to study the functional diversity and assembly rules of endophytic bacterial communities in different plant tissues of the arcto-alpine pioneer plant species, *Oxyria digyna*. I used high-throughput sequencing and bacterial isolations to characterize the endophytic communities in the leaves and roots of native *O. digyna* plants (wild plants) and micropropagated aseptic plants (bait plants) in the field. Wild plants and tissue-propagated bait plants were shown to harbor endophytic communities with taxonomically similar structures, but with divergent functional profiles. Several plant-associated microbial traits, including nitrogen fixation and phosphate solubilization, correlated with the plant type, as did also the temperature optima of the endophytic isolates. To study endophyte community assembly, I inoculated bait plants via either leaves or roots with bacterial consortia specific for leaves or roots of *O. digyna*. The assembly of endophytic communities in different tissues was primarily limited by the adaptation to plant niche in the leaves, and colonization ability and competitiveness in the roots. Plant inoculation with bacterial consortia originating from different tissues (leaves or roots) restructured the innate endophytic communities, and had divergent impact on the plant phenotype. The observed differences in the plant phenotype and fitness could be explained by direct impact of inoculated bacteria on the plant metabolism (plant-microbe interaction) or indirect impact via altered functioning of the innate endophyte community (microbe-microbe interactions). Taken together, the findings in this thesis demonstrate that the endophytic bacterial communities are tissue-specific and tightly associated with their host plant, but at the same time, are highly dynamic, rapidly adapting to changes in environmental conditions.

Keywords: Arctic; endophytic bacteria; functioning; holobionts; micropropagated plants; *Oxyria digyna*; tissue-specificity.

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Kasvien mikrobiomit ovat monimuotoisia mikrobiyhteisöjä. Elintärkeät mikrobiomit osallistuvat kasvien keskeisiin toimintoihin. Bakteerit ovat runsaslukuinen ja tärkeä osa näitä mikrobiyhteisöjä. Väitöskirjan tavoitteena oli tutkia arktis-alpiinisen pioneerikasvin hapron (*Oxyria digyna*) endofyyttisten eli kasvin sisällä kasvavien bakteeriyhteisöjen toiminnallista diversiteettiä ja bakteeriyhteisöjen rakenteeseen vaikuttavia tekijöitä kasvin solukoissa. Työssä tutkittiin hapron lehdissä ja juurissa esiintyviä endofyyttiyhteisöjä sekä luonnonvaraisissa että mikrolisätyissä kasveissa käyttäen laajamittaista rinnakkaissekvensointia ja bakteerieristyksiä. Luonnonvaraisten ja mikrolisättyjen kasvien endofyyttiyhteisöt poikkesivat toisistaan toiminnallisesti, mutta eivät lajistollisesti. Endofyyttien optimikasvulämpötila sekä niiden kyky sitoa ilmakehän typpeä ja mobilisoida fosforia oli riippuvainen siitä, olivatko bakteerit eristetty luonnonkasveista vai mikrolisätystä kasvimateriaalista. Tutkin endofyyttiyhteisön muodostumiseen vaikuttavia tekijöitä inokuloimalla mikrolisättyihin kasveihin hapron lehtien tai juurten endofyyteistä muodostettuja bakteerikonsortioita joko lehtien tai juurten kautta. Bakteeriyhteisöjen muodostumiseen lehdissä vaikutti pääosin bakteerien kyky sopeutua lehtisolukon oloihin. Juurten endofyyttiyhteisön koostumusta rajoittivat bakteerien kilpailu- ja kolonisaatiokyky. Eri solukosta peräisin olevat bakteerikonsortiot vaikuttivat eri tavoin kasvin ilmiasuun ja muokkasivat voimakkaasti myös kasvin alkuperäistä mikrobiyhteisöä. Kasvien ilmiasussa havaitut erot voivat selittyä joko bakteerikonsortion vaikutuksella kasvin metaboliaan (mikrobi-kasvi-vuorovaikutus) tai vaikutuksella kasvin alkuperäiseen endofyyttiyhteisöön (mikrobi-mikrobi-vuorovaikutus). Väitöskirjan tulokset osoittavat, että kasvien endofyyttiset bakteeriyhteisöt elävät tiiviissä vuorovaikutuksessa isäntäkasvin kanssa, ja että ne ovat erikoistuneet kasvien eri solukoihin. Toisaalta endofyyttiyhteisöt ovat dynaamisia ja mukautuvat nopeasti muuttuviin ympäristöoloihin.

Avainsanat: Arktis; bakteerien toiminta; endofyyttiset bakteerit; holobiontti; mikrolisätyt kasvit; *Oxyria digyna*; solukkospesifisyys.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

I Given C.J., Häikiö E., Kumar M. & Nissinen R. 2018. Tissue-specific dynamics in the endophytic bacterial communities in arctic pioneer plant *Oxyria digyna*. Manuscript.

II Given C.J., Häikiö E. & Nissinen R. 2018. The functional diversity of tissue- and plant type-specific endophytic bacterial community of arctic plant, *Oxyria digyna*. Manuscript.

III Given C.J., Häikiö E. & Nissinen R. 2018. The factors determining the assembly of tissue-specific endophytic bacterial communities in *Oxyria digyna*. Manuscript.

IV Given C.J. & Nissinen R. 2018. Inoculation with endophytic bacteria confers heat stress tolerance to *Oxyria digyna*. Manuscript.

The table shows the contributions to the original papers.

	I	II	III	IV
Original idea	RN, CJG	RN, CJG	RN, CJG	RN, CJG
Data	CJG, RN	CJG, RN	CJG, MK, EH, RN	CJG, RN
Analyses	MK, CJG, RN	CJG, RN	CJG, RN	CJG, RN
Writing	CJG, RN, EH	CJG, RN, EH	CJG, RN, EH	CJG, RN

CJG = Cindy Jittrapan Given, RN = Riitta Nissinen, EH = Elina Häikiö, MK = Manoj Kumar

ABBREVIATIONS

ACC	1-Aminocyclopropane-1-Carboxylate
CFU	Colony forming unit
JBL	Jehkas bait leaf isolate
JBR	Jehkas bait root isolate
JWL	Jehkas wild leaf isolate
JWR	Jehkas wild root isolate
LC	Leaf consortia
LI	Leaf inoculation
L+RC	Leaf+root consortia
m.a.s.l.	Meters above sea level
N	Nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
O. digyna	*Oxyria digyna*
OTU	Operational taxonomic unit
OW	Over-wintered plants
P	Phosphorus
PCR	Polymerase chain reaction
PCA	Principle component analysis
PGPR	Plant-growth promoting bacteria
PO_4	Phosphate
RA	Relative abundance
RC	Root consortia
RI	Root inoculation
UV	Ultraviolet

1 INTRODUCTION

1.1 Plant microbiome

1.1.1 The evolution of plants and plant microbiomes

The first appearance of land plants occurred around 450 million years ago in the Ordovician period (Wellman *et al.* 2003). The fossilized evidence demonstrates that land plants and microbes have co-evolved at least 400 million years (Krings *et al.* 2007, Strullu-Derrien *et al.* 2014).

Currently, all tissues in all plants studied so far are colonized with various microorganisms including bacteria, fungi, and viruses (reviewed by Hallmann 2001, Reinhold-Hurek and Hurek 2011, Hardoim *et al.* 2015, Müller *et al.* 2016). These microbes are collectively known as the plant microbiome (Rosenblueth and Martínez-Romero 2006, Turner *et al.* 2013, Müller *et al.* 2016) (Fig. 1). It has been shown, that the plant-associated microbes play essential parts in the plants' life, fitness and their adaptation to various changes in the environments (reviewed by Bulgarelli *et al.* 2013, Müller *et al.* 2016). Therefore, the plants should not be considered as a separate entity apart from their microbiota, but plant and its microbiome can be seen as one co-evolving unit, a holobiont (Zilber-Rosenberg and Rosenberg 2008, Rosenberg *et al.* 2016). The plant-associated microbiome genome is larger than the genome of the host plant itself, and in line with the holobiont concept, the microbiome's genome can be considered as the plant's second genome (Mercado-Blanco 2015).

The plant microbiome consists of all types of microorganisms. However, in this thesis, I focus solely on the plant-associated bacteria. Most of the information regarding plant-associated bacteria and their interactions with plant originate from studies of plant pathogenic bacteria (Pühler *et al.* 2004, Guttman *et al.* 2014). However, there is considerable overlap between the ecology of endophytic bacteria and pathogenic bacteria. Firstly, a pathogen of one plant species might be endophyte and provide growth-promoting benefits in other plant species (Reiter *et al.* 2002). Second, pathogens and endophytes can gain access into the plants in similar manner (i.e., by entering through the

wounds or natural openings), and must be able to adapt to the physico-chemical environment inside the plant, and sustain plant defenses (Hallmann 2001, Reinhold-Hurek and Hurek 2011, van der Wolf and de Boer 2015). Also, it is still unclear whether some bacteria can live a part of their life cycle as pathogens and another as endophytic bacteria, depending on host plant physiology (Gaiero *et al.* 2013).

1.1.2 Plant tissues offer different niches for plant-associated bacteria

Phyllosphere

The aboveground plant surface or phyllosphere is colonized by epiphytic bacteria (Lindow and Brandl 2003, Vorholt 2012, Bulgarelli *et al.* 2013) (Fig. 1-c). Epiphytic bacteria are mainly acquired via wind, rain, or disseminated by insects (Bodenhausen *et al.* 2013, Leveau 2015). The leaf surface is considered to be an extreme habitat for bacteria (Turner *et al.* 2013). Limited nutrient sources, cuticle waxes coating leaf surfaces, thick-walled epidermal cells, as well as the highly variable environmental conditions and stressors (UV radiation, light, temperature, drought), hinder the bacterial colonization of the leaf surfaces (Hallmann 2001, Lindow and Brandl 2003, Vorholt 2012, Bulgarelli *et al.* 2013). However, various compounds, including amino acids, organic acids, free sugars, pectic substances, phenolic compounds, as well as alcohol sugars are exuded to the leaf surface and serve as attractants for adapted bacteria (Greenaway *et al.* 1992, Mercier and Lindow 2000, Migahed and Nofel 2001). These epiphytes, once they have successfully colonized the leaf surfaces, can gain entrance to the plant endosphere via stomata (Lodewyckx *et al.* 2002, Müller *et al.* 2016) (Fig. 1-b and 1-c) and other natural openings, i.e., hydathodes and micropores on the leaves, flowers, and cotyledons (Hallmann 2001, Lugtenberg 2015). These epiphytic communities are considered to be one of the sources for the endophytic bacteria (Leveau 2015, Mercado-Blanco 2015).

Rhizosphere

The soil surrounding plant roots is known as the rhizosphere, and has long been considered the primary source of the endophytic bacterial acquisition (Hallmann 2001, Lugtenberg 2015, Mercado-Blanco 2015) (Fig. 1-e) due to high nutrient availability (Compant *et al.* 2010, Andreote *et al.* 2014). Plant roots exude various compounds, including sugars, amino acids, secondary metabolites, as well as root cap border cells, that can attract or repel soil bacteria in the rhizosphere (Bulgarelli *et al.* 2013, Turner *et al.* 2013, Lugtenberg 2015, Lareen *et al.* 2016). Root exudates from different plant species differ in chemical composition and quantity. Exudates, combined with biotic and abiotic stressors can shape the composition of the rhizospheric bacteria (Andreote *et al.* 2014, Guttman *et al.* 2014). The prominent entry sites for the rhizospheric bacteria into plant roots include emerging lateral roots, thin-walled surface layer in the apical root region and root hair zone, as well as natural cracks from plant

growth, or wounds caused by soil (micro)organisms (Hardoim *et al.* 2008, Reinhold-Hurek and Hurek 2011) (Fig. 1-d).

Endosphere

Plant-associated bacteria living inside the plant tissues are known as endophytic bacteria. De Barry first coined the term "endophyte" in 1866 (Wilson 1995). The word "endophyte" is derived from the Greek word "endon" meaning "inside or within" and "phyton" meaning "plant," combined as "organism that lives inside the plant." However, a more current definition of the term generally refers to "the bacteria that live inside the plants without causing symptoms or harming the host plants in any way" (Chanway 1996, Rosenblueth and Martínez-Romero 2006, Reinhold-Hurek and Hurek 2011).

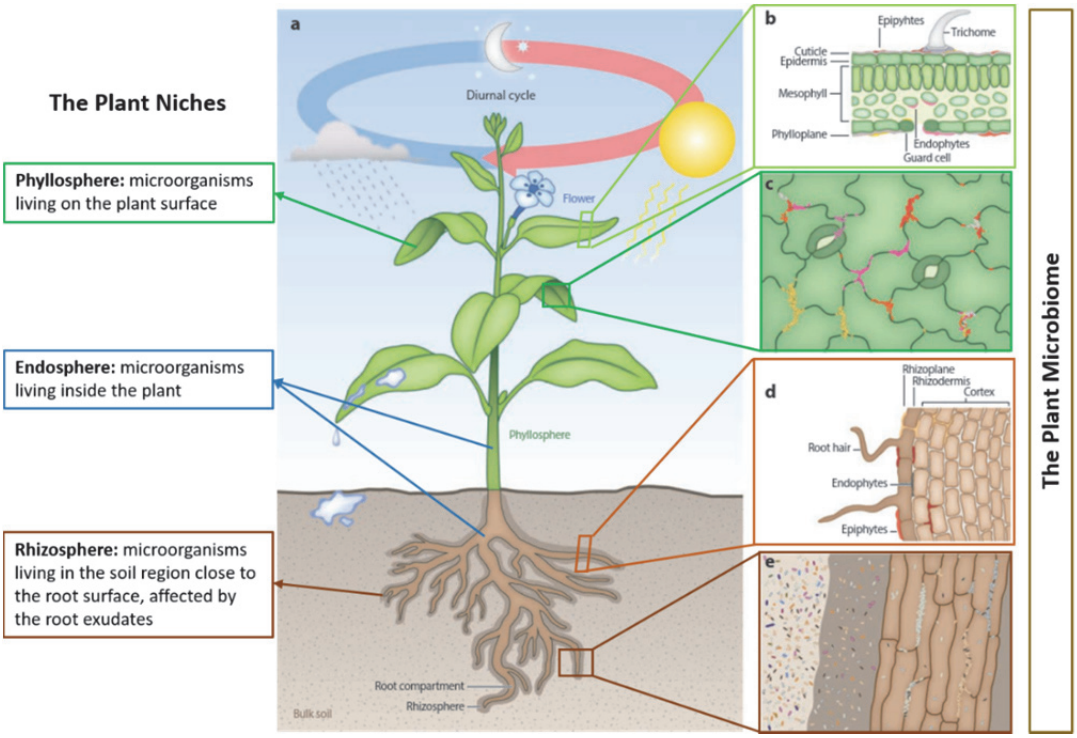

FIGURE 1 Plant and plant-associated microbes that colonize different niches in the soil surrounding plant root (rhizosphere), on plant surfaces (epiphytes) and inside plant tissues (endophytes). (a) Different parts of the plant are exposed to divergent environmental factors. (b) Schematic cross-section of the leaf showing leaf structure and the bacterial colonization. (c) The abaxial (lower) leaf surface showing bacterial colonization along the grooves and near the stomata where epiphytic bacteria can gain entrance to the endosphere. (d) Schematic cross-section of the root showing root structure and the bacterial colonization. (e) Schematic longitudinal-section of the root showing the gradient of bacterial population from bulk soil (light brown background), rhizosphere (dark brown background), and to inner root (modified from Müller *et al.* (2016)).

Endophytic bacteria colonize different plant tissues, including leaves, roots, stems and reproductive organs, including seeds (Fig. 1-b and 1-d). Endophytic bacteria are mostly intercellular but are sometimes also detected inside plant

cells (Pirttilä *et al.* 2000, Compant *et al.* 2005). The endophytes are considered to form the most intimate association with the plant host and have been shown to benefit the plant (see section 1.2). At the same time, these endophytic bacteria also benefit from living inside the plants, as the plants provide a stable environment, nutrients (mainly carbon), and lower exposure to biotic and abiotic stressors (Hardoim *et al.* 2008).

1.1.3 Bacterial colonization of plant endosphere

Bacterial colonization of plant endosphere via roots is currently seen as consisting of two distinct steps: pre- and post-colonization. First, in the pre-colonization phase, the bacteria move towards plant roots via chemotaxis (Hardoim *et al.* 2008, Compant *et al.* 2010) or by an accidental encounter. The bacteria then attach to the roots (reviewed by Hallmann 2001) using various means such as type IV pili, exopolysaccharides or lipopolysaccharides (Mercado-Blanco 2015). Following the attachment, the bacteria start multiplying and forming biofilms or microcolonies on the root surfaces (Lugtenberg 2015). The plant-bacterial recognition process may require at this step for the plant to select the bacteria. Later, the penetration and colonization of the root by endophytic bacteria occur. Some endophytic bacteria can produce cellulolytic enzymes, possibly to help them to penetrate the plants, though how and when the bacteria utilize this enzyme is still unclear (reviewed by Hallmann 2001). The second step of endophytic bacterial colonization (i.e., post-colonization) takes place after the bacteria penetrate and colonize the plant tissues. The bacteria multiply within the plant tissues (reviewed by Hallmann 2001), mostly in the intercellular spaces between epidermal cells (Lugtenberg 2015), in the vascular tissue, or in the cortical regions. The bacteria then spread to various parts inside the plants via xylem (Lodewyckx *et al.* 2002), and establish an association with the plants (Hallmann 2001, Mercado-Blanco 2015). Similar colonization steps happen in the leaves. Leaf epiphytes enter endosphere via stomata, hydathodes, micropores, or wounds (Hallmann 2001, Lugtenberg 2015). The leaf endophytic bacteria usually colonize the mesophyll and can migrate via the apoplastic route to xylem, and systemically spread, and colonize other plant parts (Hurek *et al.* 1994, Shishido *et al.* 1999, Leite *et al.* 2013).

The majority of the plant colonization by endophytic bacteria is suggested to occur via roots due to a high bacterial density in the rhizosphere. Moreover, the adapted bacteria with chemotaxis or mobility features (e.g., fimbriae or flagella) in the rhizosphere can have a high chance of finding the colonization spots by following the plant root exudation.

Vertical transmission via seed is another important pathway for plant colonization by endophytic bacteria. Plants can select and transfer the beneficial bacteria to the seeds to benefit the next generation (Truyens *et al.* 2015), and seeds have been shown to harbor complex microbial communities (Barret *et al.* 2015). These seed endophytes have been shown to increase the seed germination rate and enhance the establishment of the seedlings (Puente *et al.* 2009). Plant genotype and abiotic factors such as soil type can influence the

composition of the seed microbiota (Barret *et al.* 2015). The endophytic bacteria transmitted via seeds have to be excellent systemic colonizers (Hallmann 2001), and possess special characteristics often found only in the seed endophytes (e.g., tolerance to high osmotic pressure, endospore formation, amylase, and phytase activity) (Truyens *et al.* 2015).

The plant microbiome is dynamic, and various biotic and abiotic factors influence the structure and composition of the endophytic bacterial community. The composition, abundance, distribution, and function of the microbiome in a given host plant or tissues can change over time following the environment, e.g., introduction of new bacteria (Bulgarelli *et al.* 2013, Leveau 2015), soil pH and soil properties (Hallmann 2001, Mercado-Blanco 2015, Schreiter *et al.* 2015), plant growth phase and genotypes (Leveau 2015). These factors could also be a limiting factor in shaping the community in a given host plant or tissues (Kroll *et al.* 2017).

1.2 The plant-microbe interactions: Endophytic bacteria and their benefits to the plants

Endophytic bacteria have been shown to provide various benefits to the plants either directly or indirectly (Fig. 2). Plant growth promotion, stress mitigation, antagonism towards plant pathogens, and the induction of plant defense mechanisms are considered as direct benefits (Lodewyckx *et al.* 2002, Ryan *et al.* 2008, Reinhold-Hurek and Hurek 2011, Hardoim *et al.* 2015). The indirect benefits include niche competition with pathogens, which result in plant protection.

Nutrient acquisition

Nutrient acquisition is considered to be one of the essential plant growth-promoting traits by the endophytic bacteria, in particular in low nutrient biomes like the Arctic. In the Arctic, the nutrient mineralization rates are slow due to the frozen soils in the winter and cold soils during the summer, resulting in low availability of plant available minerals. Nitrogen and phosphorus are the most limiting nutrients in the Arctic (Nadelhoffer *et al.* 1992).

Nitrogen (N) is a vital element for all organisms, as it is required for the synthesis of proteins and DNA. Plant roots can take up both inorganic compounds (e.g., nitrate (NO_3^-) or ammonium (NH_4^+)), and organic nitrogen compounds (e.g., amino acids) (Atkin 1996). However, nitrogen fixation by microorganisms is the primary source of nitrogen taken up by plants in the Arctic (Chapin and Bledsoe 1992, Nadelhoffer *et al.* 1992), especially in low organic matter (pioneer) soils. Several endophytic bacteria possess the *nif* genes that encode for the synthesis of nitrogenase enzyme. This enzyme is needed for the biological nitrogen fixation: The process where the atmospheric dinitrogen (N_2) is reduced to ammonia (NH_4^+) (Bulgarelli *et al.* 2013, de Bruijn 2015). The

ammonia (NH_4^+) is then excreted from the symbiotic nitrogen-fixing diazotrophs, and assimilated to the plants (de Bruijn 2015).

Phosphorus (P) is also an important element as it is part of the essential macromolecules (i.e., nucleic acids, ADP/ATP, and orthophosphate ion). Phosphate solubilizing bacteria can increase the availability of phosphates to the plants by converting the insoluble inorganic phosphates into soluble P forms, or by mineralizing organic phosphates for the plants (Barea & Richardson, 2015). Organic P (phytate, phytic acid) is a major storage form of phosphates in the plant seeds (Reddy *et al.* 1982) and also found in the green leaves (Hadi Alkarawi and Zotz 2014). The mineralization of organic phosphates requires the solubilization of the substrates, followed by breaking down the phosphate compounds with phosphatase enzymes (phytases) (Barea and Richardson 2015). The phosphate solubilizing bacteria, mainly reported from genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Bradyrhizobium*, *Enterobacter*, *Panthoea*, and *Erwinia,* can help plants acquire phosphorus from soil via the solubilization of inorganic phosphates (Barea and Richardson 2015).

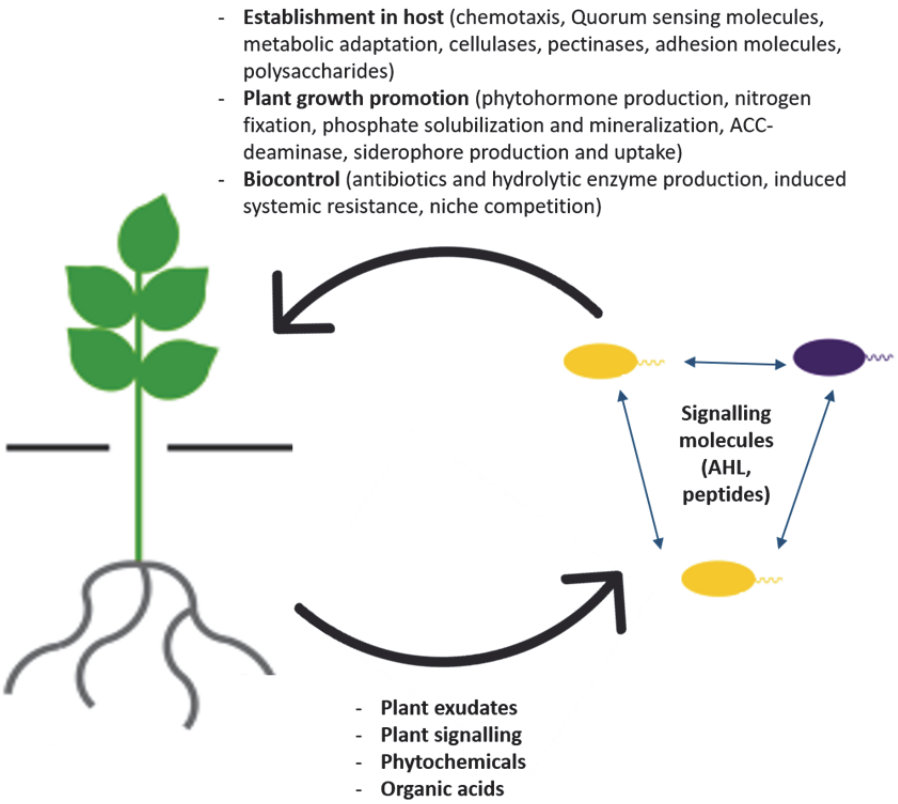

FIGURE 2 Schematic overview of plant-microbe interactions. The members of the microbial communities communicate with each other, as well as with the plants. Plant exudates can attract bacteria to colonize on the surface and later lead to the endophytic bacterial establishment. Bacteria possess various mechanisms to establish in the plant host and provide benefits to the plants in exchange for the safe environment inside the plants (modified from Bulgarelli *et al.* (2013), and Lareen *et al.* (2016)).

Production and processing of phytohormones

The production of plant hormones by bacteria enables the alteration of plant physiology. Diverse plant-associated bacteria can produce phytohormones, including auxins, gibberellins, and cytokinins (Rosenblueth and Martínez-Romero 2006, Bulgarelli *et al.* 2013). Additionally, many soil- and plant-associated bacteria can produce enzyme ACC deaminase, which directly degrades the ethylene precursor, ACC (1-aminocyclopropane-1-carboxylate). The degradation of ACC limits the ethylene biosynthesis and suppresses the plant growth retardation by ethylene, associated with various stresses, thus promoting the plant growth (Hardoim *et al.* 2008, Bulgarelli *et al.* 2013, Glick 2015, Spaepen 2015).

1.3 Arctic vegetation

The Arctic is a demanding environment for plants. The growth limiting factors include short and cold growing season (100–120 days with an average daily temperature of around 5°C), long cold winter and long polar night, fluctuation in temperature and light, water stress (i.e., severe drought, and flooding during snowmelt, resulting in anoxia), and in particular, low nutrient soils (Billings and Mooney 1968, Quinn 2008). The vegetation types and mean July isotherms are means to separate the Arctic from other life zones. Annual plants are very rare, and not present in the high Arctic due to the harsh and unpredictable growing season. Most Arctic plants produce clonal structures and large belowground biomass for overwintering, and to support the plant growth in the short growing season (Chapin III *et al.* 1992).

Glacier forefields and arctic soils in the oroarctic zones above the treeline are usually dominated by mineral soils, characterized by low nutrient levels and patchy vegetation cover (Robbins and Matthews 2009, Schutte *et al.* 2009, Schulz *et al.* 2013). Soils in the valleys and shrub tundra zone are mainly peat soils (organic soils) with higher nutrient levels (Mäkilä and Saarnisto 2008, Tarnocai *et al.* 2009).

This study focuses on a plant species typical of the pioneer plant communities on low organic matter mineral soils.

1.4 Study plant: *Oxyria digyna*

Oxyria digyna (L.) Hill (Mountain sorrel, Wood sorrel, Alpine sorrel, Alpine mountain sorrel, Finnish: Hapro, Sami: Eavru) (Fig. 3a) is a perennial herbaceous plant in the family Polygonaceae. It is a non-mycorrhizal plant species with a circumboreal distribution, commonly found in the arctic regions and high mountainous area of the northern hemisphere (Mooney and Billings

1961) (Fig. 3b). In Finland, the plants are found in the North and North-western part of the country (Fig. 3c). *O. digyna* is a well-studied arcto-alpine pioneer plant species (Russell 1948, Mooney and Billings 1961, Au 1969, Atkin and Cummins 1994, Heide 2005, Holzinger *et al.* 2007), which is able to efficiently colonize pioneer soils with very limited nutrients. It is typically found along stream banks, snow bed sites, waterlogged areas, and moist rocky terrains, as well as in glacier forelands (Robbins and Matthews 2009, Schulz *et al.* 2013).

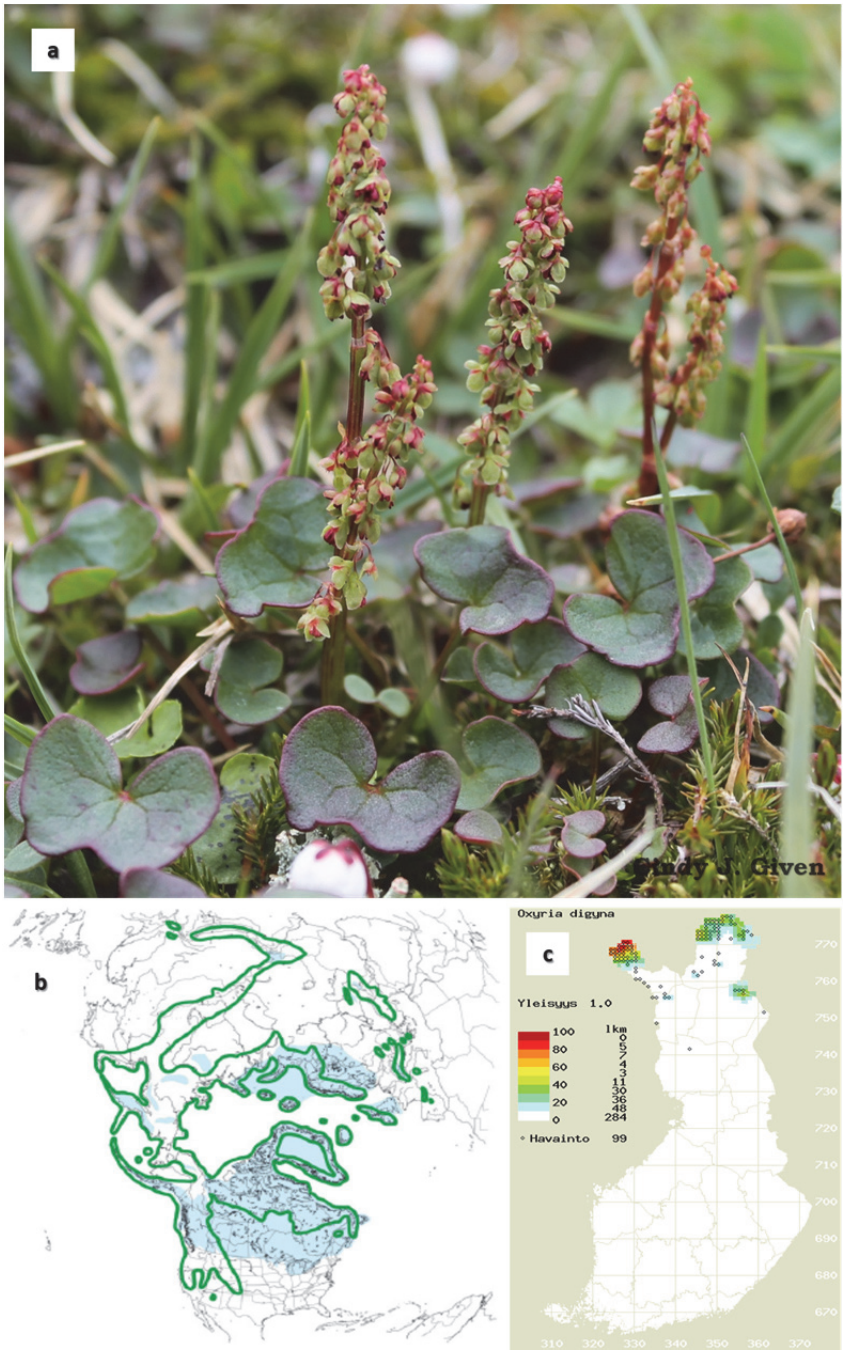

FIGURE 3 (a) *Oxyria digyna* (L.) Hill, (b) The geographic distribution of *O. digyna* worldwide (green outline) and (c) in Finland. Modified from Allen *et al.* (2012) and Kasviatlas Luonnontieteellinen Museo, University of Helsinki (http://koivu.luomus.fi/kasviatlas/maps.php?taxon=40855; access 101017).

This plant species has long tough taproot with long branching side roots and grow in dense tufts to a height of 5 to 15 cm. The leaves are fleshy, kidney-shaped with rosette form, and rich in vitamin C. They are essential food for insects and larger animals in the arctic and alpine regions, as well as for humans. The Sami and Inuit eat the plants to prevent and cure scurvy (Geraci and Smith 1979). The flowers are small and green, and turn red later in the growing season (Fig. 3). The plants form flattened, broad-winged shape seeds, turning red when ripe. The seeds are easy to propagate, and non-dormant (i.e., able to grow in the same growing season that the seeds were produced without the after-ripening step) (Mooney and Billings 1961). *O. digyna* starts flowering in June – August.

The most distinctive character of the northern (Arctic) *O. digyna* population is the rhizome (underground meristems) formation (Mooney and Billings 1961), resulting in the expansion of stems (Fig. 4). This rhizome production could be an additional means to propagate in the unpredictable arctic conditions, in addition to the seed production.

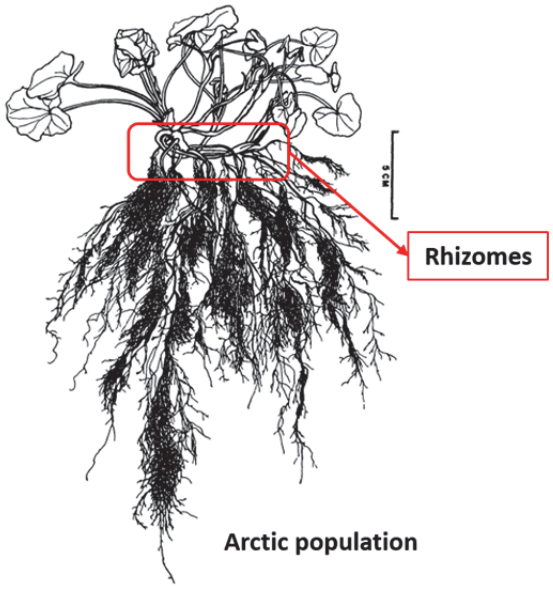

FIGURE 4 Morphological features of the northern (Arctic) population type of *O. digyna* (modified from Mooney and Billings (1961)).

Despite the growing interest in the plant microbiomes, only a few studies have looked into the microbiomes of the cold climate plants. As such, this plant is considered a good target plant to study the plant-microbe interactions, the focus of this study.

1.5 Aims of the study

The aim of this thesis was to study the assembly and functions of the endophytic bacterial communities associated with different tissues of the arcto-alpine plant species: *O. digyna.* This thesis is based on four research questions and four manuscripts (Fig. 5). The four manuscripts (hereafter, referred to with roman numbers I, II, III, and IV) are based on the following four research questions (RQs):

RQ I: Do the taxonomic composition and community structures of the endophytic bacterial communities in *O. digyna* show tissue-specificity? To address this question, I investigated the structure of endophytic bacterial communities in the leaves and roots of *O. digyna*. I also looked at the structure of the endophytic bacterial communities across plants harvested at different time points and from different plant types to investigate the bacterial succession in the plant tissues.

RQ II: Continuing from RQ I, I asked: Is the difference in the phylogenetic structures between leaf- and root-specific communities also reflected in their functional profiles?

RQ III: After detecting tissue-specific communities, I looked further as to how are these tissue-specific endophytic communities formed, and what are the factors determining the assembly in different tissues. Here, I asked, whether the assembly of the tissue-specific endophytic communities is determined by acquisition route (via different inoculation and colonization pathways) or by niche (different plant tissues providing different environment and selective pressures)?

RQ IV: Do the endophytes with different tissue-specificity have divergent impact on plant phenotype? As the endophytic bacterial communities are different between tissues, I investigated their impact on the plant phenotype to see if different tissue-specific communities have a different impact.

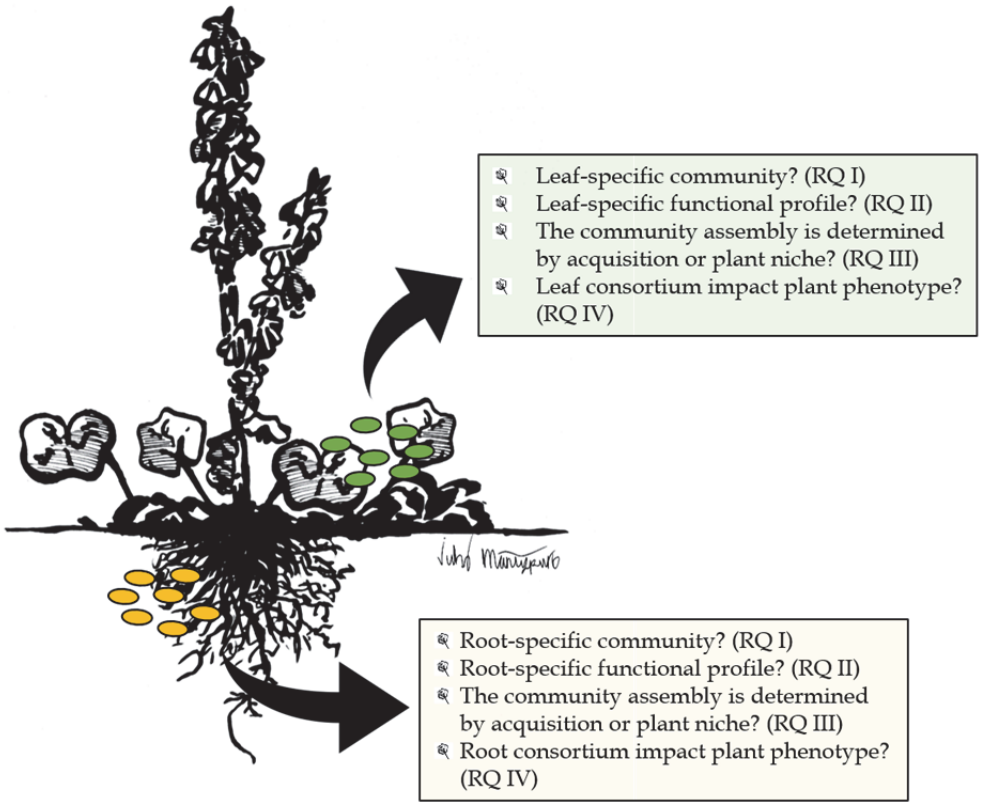

FIGURE 5 An overview of the research questions focusing on the assembly and functions of the endophytic bacteria associated with different tissues of *O. digyna* plants.

2 MATERIALS AND METHODS

2.1 Micropropagated plant material

The *O. digyna* plants used in studies I-IV were micropropagated from surface-sterilized seeds at the University of Oulu Botanical Garden, and were maintained at the Department of Environmental Science, University of Eastern Finland, Kuopio campus (62°53'30.2"N 27°38'04.8"E) (I, II and III), and at the Finnish National Resources Institute (Luke), Laukaa facility (IV). The plants were grown in sterile half-strength Murashige & Skoog (MS) agar medium (Murashige and Skoog, 1962) at 21°C constant temperature, and a photoperiod of 16:8 h of light:dark cycle with low light (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The micropropagated plants were transferred to fresh medium every five weeks.

In the study I and II, the plants were transferred to sterile boxes (containing sterile vermiculite) 45 days before the start of the experiment. The plants were moved to the greenhouse to be acclimated under ambient conditions for seven days (I), with lid slightly ajar, in order to induce the formation of leaf cuticle. After the acclimatization, each plant was transferred into a 7-cm-diameter net pot lined with 15 µ-mesh size plankton net, and filled with sterilized washed sand. The plants were then moved outside as the last acclimatization step before they were transported to the field site in Kilpisjärvi, Finland (I).

The plant acclimatization for study III was slightly different from above (see section 2.2). The micropropagated plants for study IV were not acclimated as the inoculation of the consortia was done only via root.

2.2 Growth chamber experiments

In study III, the micropropagated plants were transferred from the McCown woody plant medium (WPM) into the sterile microcosms boxes (172 x 110 mm) with gas permeable filter lid (63 gas exchanges (GE)/day) (TP1600+TPD1600,

Combiness Microbox). Each box contained sterilized washed sand and sterile 25% Hoagland's liquid nutrient solution (Hoagland and Arnon 1950). The boxes were half-closed in the growth chamber for the acclimatization of the plants three days before the experiment start. The plants were then inoculated with endophytes consortia (see section 2.3) and were harvested after five weeks in the closed microcosm boxes inside the growth chamber (20°C/10°C), light intensity of 200 µmol $\cdot m^{-2} \cdot s^{-1}$ and 20 µmol $\cdot m^{-2} \cdot s^{-1}$ with 16:8 h light:dark photoperiod) (Fig. 6). More information can be found in the original paper III.

Experiment IV composed of two separate experiments (IV-a and IV-b). The plantlets were inoculated with bacteria (see section 2.3) and transferred into the sterile microcosm boxes, like in experiment III. The growth chamber settings for experiment IV-a were 20°C/10°C, 17:7 h light:dark photoperiod with light intensity of 200 µmol $\cdot m^{-2} \cdot s^{-1}$ and 50 µmol $\cdot m^{-2} \cdot s^{-1}$ for the ambient temperature, and 35°C/20°C, 17:7 h light:dark photoperiod with light intensity of 200 µmol $\cdot m^{-2} \cdot s^{-1}$ and 75 µmol $\cdot m^{-2} \cdot s^{-1}$ for the elevated temperature. For experiment IV-b, the growth chamber was set to ambient temperature setting similar to experiment IV-a. However, due to a malfunction, the temperature in the growth chamber was increased to 35°C–42°C for 48 hours before it was set back to that of the ambient temperature setting. The plants were grown in the closed microcosm boxes for ten weeks before harvesting. More information can be found in the original paper IV.

FIGURE 6 The micropropagated *O. digyna* in the microcosm box before harvesting (III). Photographed by Cindy Given.

2.3 Consortia

Bacterial consortium (also known as a synthetic community, SynCom) is the assemblage of two or more defined microbial isolates in a controlled

environment. Usually, the isolates are selected to represent the natural ecosystem of interest (De Roy *et al.* 2014, Großkopf and Soyer 2014). Consortia are used to gain insight into the fundamental principles of the system of interest, for example the factors shaping the ecosystem, the interaction of the community members or the function of the complex systems (De Roy *et al.* 2014, Großkopf and Soyer 2014). The use of consortia has gained much interest due to its simplicity and controllability.

The consortia used in the experiments III and IV consisted of the endophytic bacterial isolates selected from the endophytic bacterial isolate collection from study II (see original paper II). The selected isolates represented the bacterial taxa that showed specific enrichment in either the leaves or the roots in study I. Each selected strain was tested for their plant-associated traits (II). Leaf consortium (LC) included eight strains of the leaf-specific bacteria (III, Table 1), while root consortium (RC) consisted of five strains of the root-specific bacteria (III, Table 2) (III and IV). Additionally, a consortium containing both leaf- and root-specific bacteria (leaf+root consortia; L+RC) was also used (III). These consortia were used to test for their ability to colonize *O. digyna* (III), as well as their impact on the plant phenotypes under normal condition, and under heat treatments (IV). The preparation of LC, RC, and L+RC inocula was described in the original paper III and IV. The consortia were prepared in potassium phosphate buffer (50 mM, pH 6.5), which was also used as control inoculum in these experiments (III and IV).

The leaf inoculation process is explained in the original paper III. The process for the root inoculation was different in the studies III and IV and is explained in the original papers III and IV.

2.4 Field site

The field site is located in Kilpisjärvi, northwestern Finnish Lappland, 400 km north of the Arctic circle (69°1′N, 20°50′E) (Fig. 7). The growing season in Kilpisjärvi lasts approximately 90–100 days with the annual mean temperature of –1.9°C (1981–2010) and precipitation of 487 mm/year (1981–2010) (data retrieved from Finnish Meteorological Institute).

The study site in fell Jehkas was located in the oroarctic zone, at the elevation of 925 meters above sea level (m.a.s.l.), next to a snowmelt stream. The soil in the site is classified as a Leptosols soil (i.e., a shallow soil over hard rock or calcareous materials with large amounts of gravels) (Jones *et al.* 2009), and contains low levels of soluble phosphate and nitrogen (1.11 mg N/kg soil as soluble NO_3^-, 0.33 mg N/kg soil as soluble NH_4^+, and 1.56 g/L soil as soluble PO_4) (Kumar *et al.* 2016). The vegetation is patchy, and the site is covered by snow patch typically until early July. The soil temperature at a depth of 10 cm at the field site varied between 11.5°C (July) and -4.5°C (February). The average air temperatures in June-August 2013 were 13°C (data retrieved from the Finnish Meteorological Institute).

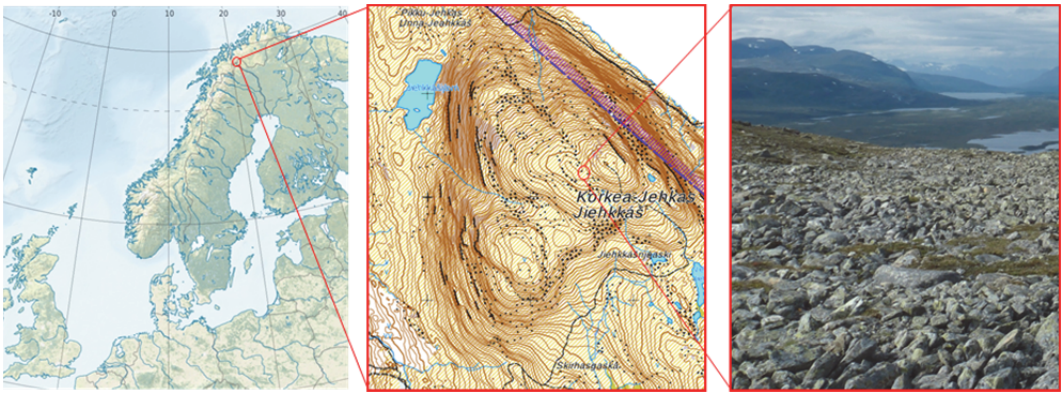

FIGURE 7 The location of the study site; fell Jehkas, Kilpisjärvi, North-western Finland Map retrieved from National Land Survey Finland (https://karttapaikka.fi).

2.5 Sampling schemes

2.5.1 Study I

The micropropagated plants (see section 2.1.) were transported from Kuopio to Kilpisjärvi, and planted in the field sites on July 2nd, 2013. Before shipping, six sterile, acclimated plants were harvested, surface-sterilized and stored at -80°C (see section 2.6) and are referred to as "starter plants." The rest of the plants were transported and transplanted into the field site next to the native *O. digyna* (Fig. 8–9) (I).

FIGURE 8 The transplantation of micropropagated *O. digyna* plants in the field site. The plots are next to the native *O. digyna* plants and were covered with metal cages to protect the plants from reindeer grazing. Photographed by Cindy Given.

Twenty plants were harvested at the late growing season the same year (late-August; "August bait plants") along with ten native *O. digyna* plants growing adjacent to the experimental plants (referred to as "wild plants"). The rest of the bait plants remained in the field site. Twenty-seven plants were harvested at the beginning of the following growing season (mid-July 2014) (referred to as "over-wintered bait plants"). No wild plants were harvested at this time point (Fig. 9) (I).

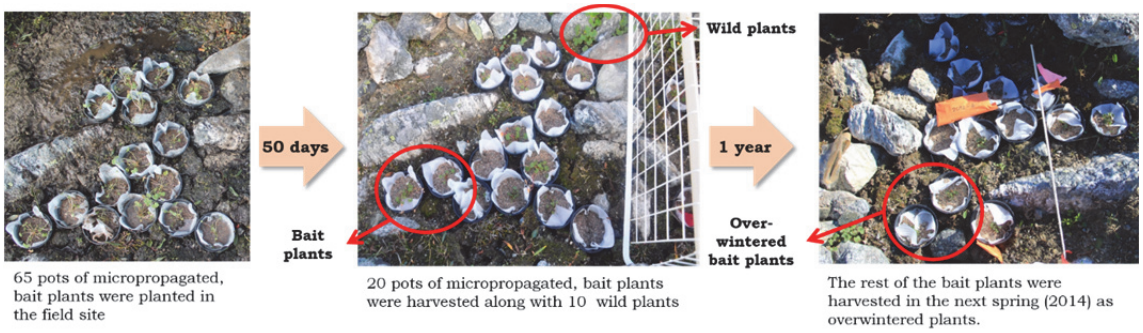

FIGURE 9 The sampling of the micropropagated and wild *O. digyna* for the study I and II. Photographed by Cindy Given.

All plant samples were carefully removed from the pots and separated into leaves and roots before surface sterilization (see section 2.6) within 48 hours after harvesting. Around 100 mg of the surface-sterilized tissue samples, used for the molecular analysis, were stored at -80°C until the DNA isolation (I).

Leaf and root tissue samples from four plant groups: starter plants (5 replicates), August bait plants (10 replicates), over-wintered bait plants (10 replicates), and wild plants (10 replicates) were used for analyses.

2.5.2 Study II

After the surface sterilization (see section 2.6), one leaf and about 1-cm-length of root were taken from each plant. Twenty leaves and twenty roots from August bait plants were pooled and formed bait leaf samples (hereafter, referred to as "JBL") and bait root sample (hereafter, referred to as "JBR"), respectively. Likewise, leaves and roots from wild plants were pooled and formed wild leaf sample (hereafter, referred to as "JWL") and wild root sample (hereafter, referred to as "JWR"), respectively, and were used for the isolation of endophytic bacteria. More information can be found in the original paper II.

2.5.3 Study III

The study included eight treatments (III, Table 3). Each treatment consisted of 12 plants. The plants were harvested, and the photosynthetic activity and the fresh biomass of the plants were measured (III). Dry weight was measured from shoot and root separately after drying at room temperature for seven days. Simultaneously, one leaf and 1-cm-length root from the plants in all treatments

were collected for the DNA-based community analyses. More information can be found in the original paper III.

2.5.4 Study IV

In this study, 12 plants were used for each treatment. The plants were harvested after ten weeks in a growth chamber (IV). Leaf and root dry weight were measured in addition to the analysis of root-shoot ratio (IV).

2.6 Plant tissue surface sterilization

The plant samples were surface sterilized as described in Nissinen *et al*. (2012) with slight modifications, described in the original paper I. The surface sterilization was used with the samples from study I, II, and III. The surface-sterilized samples were used for isolation of culturable endophytic bacteria or were stored in sterile Eppendorf tubes at -80°C for DNA isolation. The sterility check was done by plating the last rinse water to ensure the success of the surface sterilization procedure. More information can be found in the original paper I.

2.7 Culture-dependent methods: Endophytic bacterial isolation, identification, and characterization

2.7.1 Endophytic bacterial isolation

The surface-sterilized plant tissue samples were homogenized in 50 mM potassium phosphate buffer, pH 6.5, in sterile, clear stomacher bag by gently but firmly pounding with a stainless steel hammer. Ten-fold serial dilutions of the aliquots were prepared down to 10^{-3} for the leaf samples and 10^{-6} for the root samples (II). The colony forming units (CFU) were counted and bacterial densitied were calculated as CFU per gram of fresh weight (CFU/g). Morphologically different colonies were picked at various time points as described in the original paper II. Individual colonies selected were sub-cultured to acquire pure bacterial cultures for further identification and characterization (II).

2.7.2 Bacterial genomic fingerprinting by BOX-PCR

Bacterial strains were genotyped by molecular fingerprinting using BOX-PCR, a repetitive element palindromic PCR (rep-PCR) targeting the BOX repetitive elements in the bacterial genome. A single colony of pure bacterial culture was used directly as a template for the PCR. BOX-A1R primer (Table 1) was used in

a final reaction volume of 30 µl/reaction. The amplification was performed with C1000™ Thermal Cycler (Bio-Rad), following the PCR program reported in the original paper II. The final PCR products (10 µl) were resolved by electrophoresis in 1% (w/v) agarose gel at 80V for approximately four hours (II). The genetic fingerprints of each isolate were photographed and verified under a UV transilluminator (Fig. 10). The representative isolates of each unique fingerprint pattern were used for bacterial identification. Culture stocks were prepared in 30% glycerol and stored at -80°C. More information can be found in the original paper II.

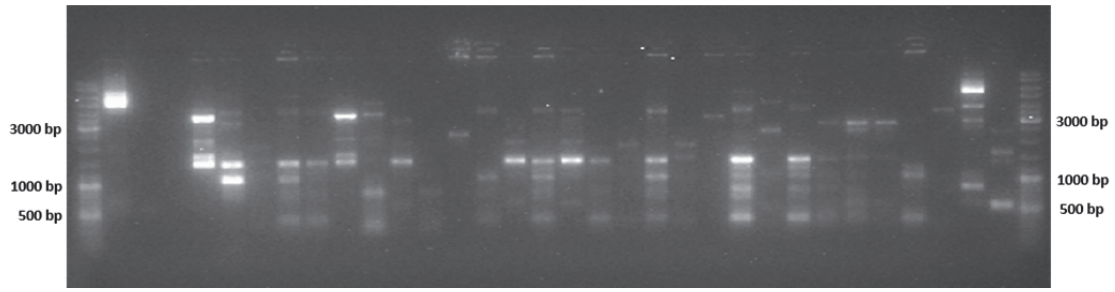

FIGURE 10 Genetic fingerprints of endophytic bacterial isolates from the leaves and roots of *O. digyna* after BOX-PCR were visualized by transilluminator. Isolates with identical BOX-PCR patterns were considered to be clonal. Photographed by Cindy Given.

TABLE 1 Names and DNA sequences for the primers used in the study.

Name	Sequence	References
BOX-A1R	5′-CTA CGG CAA GGC GAC GCT GAC G-3′	Versalovic *et al.* (1994)
27F	5′-AGA GTT TGA TCC TGG CTC AG-3′	Lane (1991)
1492R	5′-GGY TAC CTT GTT ACG ACT T-3′	Chelius and Triplett (2001)
799F	5′-AAC MGG ATT AGA TAC CCK G-3′	Chelius and Triplett (2001)
M13-1062F	5′-TGT AAA ACG ACG GCC AGT – GTC AGC TCG TGY YGT GA-3′	Ghyselinck *et al.* (2013), Mäki *et al.* (2016)
1390R	5′-ACG GGC GGT GTG TRC AA-3′	Zheng *et al.* (1996)
Barcode-M13	5′-[BC1-48] – TGT AAA ACG ACG GCC AGT-3′	Mäki *et al.* (2016)
1390R-P1	5′-CCT CTC TAT GGG CAG TCG GTG AT – ACG GGC GGT GTG TRC AA-3′	Mäki *et al.* (2016)

2.7.3 Bacterial isolate identification by partial 16S rRNA gene sequencing

A single colony was used directly as a PCR template by using universal primer pair 27F and 1492R (Table 1) targeting the bacterial 16S rRNA gene. PCR mixture without DNA template was included as a negative control of the PCR. The details of PCR conditions and amplification program are reported in the

original paper II. The amplification was performed using C1000™ Thermal Cycler (Bio-Rad), and the final PCR product was confirmed by gel electrophoresis (II). For the isolates that failed to show PCR product by the colony PCR method, the DNA was extracted using the Genomic DNA extraction kit (QIAGEN), followed by PCR using 27F-1492R PCR protocol with the same conditions described above. The sequencing for bacterial identification was done using the PCR amplicons as template and was performed according to the protocol used with ABI Prism® 3130xl sequencer described in the original paper II. The sequences were aligned with the reference sequences in Ribosomal Database Project (RDP Release 11) database for the bacterial identification (Cole *et al.* 2014) (II).

2.7.4 Bacterial characterization by activity plate assay

2.7.4.1 Inorganic and organic phosphate solubilization

Two bacterial culture media with a different source of inorganic and organic phosphate were used to test the phosphate solubilization ability of the strains. The National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal 1999) was used for the inorganic phosphate solubilization ability, while the phytase screening medium (PSM) (Jorquera *et al.* 2011) was used for the organic phosphate (phytate) solubilization activity. The test was done in triplicate and plates were incubated at room temperature for seven days. Phosphate solubilization activity of the bacteria was detected as a clear halo surrounding the colony (II).

2.7.4.2 Cellulose and starch hydrolysis

Cellulose and amylase activity of the strains were tested on M9 minimum medium, with carboxymethyl cellulose (CMC) and soluble starch added as a sole carbon source for cellulase and amylase activity media, respectively. The test was done in three replicates and plates were incubated at room temperature for seven days. The unhydrolyzed cellulose from cellulose hydrolysis was stained by 0.1% (w/v) Congo red solution, while 0.2% Lugol's solution was used for the staining of starch. The cellulose and starch hydrolysis activities were detected as clear haloes around the colonies (II).

2.7.4.3 Chitin hydrolysis

The chitin agar medium was prepared using colloidal chitin as a carbon source according to Souza *et al.* (2009) with slight modifications. Ten pure isolates were inoculated per 30 mm petri dish plate and were incubated at room temperature. Each strain was tested in triplicate. After seven days, clear zone around the colony indicated the chitin hydrolysis activity (II).

2.7.4.4 Nitrogen fixation activity

The qualitative analysis of the nitrogen fixation ability of the isolates was done using nitrogen-free JNFb semi-solid medium (Baldani *et al.* 2014). Pure isolates were inoculated into 13 mL culture tube containing 8 mL of the medium with

10 µl inoculation loop. The tubes were incubated at room temperature for five weeks. The nitrogen fixation activity was detected as color change from light yellow to blue of the medium (Fig. 11) (II).

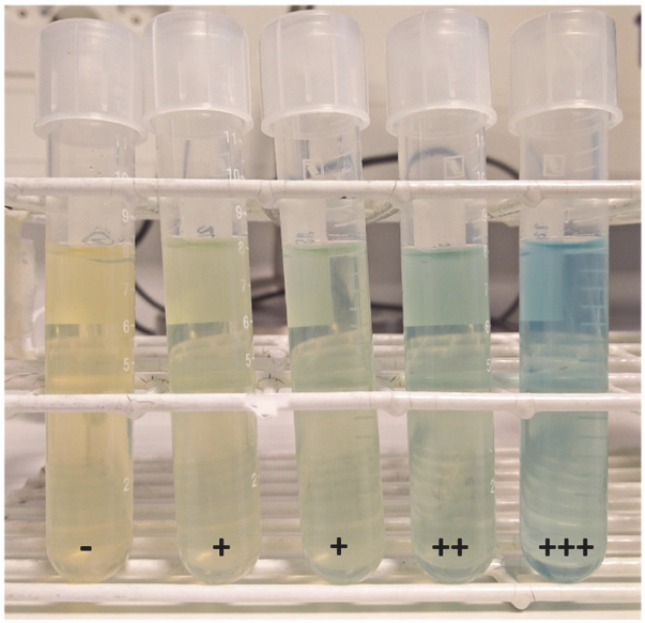

FIGURE 11 Nitrogen fixation activity; - no activity, + low positive activity, ++ medium activity, and +++ strong activity. Photographed by Cindy Given.

2.7.4.5 Growth at 4°C, 20°C, and 37°C

The isolates were diluted in 5 mM potassium phosphate buffer, pH 6.5 to OD_{600} = 0.3. Thirty pure isolates were inoculated onto large format (10x10 cm) solid R2A (pH 6.5) plates by dropping 10 µl of bacterial suspension on the plate. Plates were incubated at 4°C, 20°C and 37°C, and were prepared in triplicates for each temperature. The visible growth at different temperatures was recorded after three days (II).

2.8 Sequence-based community analyses

2.8.1 Metagenomic DNA extraction

The plant samples were homogenized dry and frozen by bead-beating using the combination of metal and glass beads for disruption of plant tissues and bacteria, respectively. Metagenomic DNA was isolated with Invisorb® Spin Plant Mini Kit (Stratec Biomedical) following the manufacturer's instructions (I). The samples were kept frozen during homogenization until suspension into isolation kit lysis medium in order to prevent the damage of the eubacterial DNA by the plant enzymes released during pre-homogenization. Original paper I describes the process in full details.

2.8.2 Library preparation by the M13-PCR method

The M13-PCR method developed by Mäki *et al.* (2016) was used to prepare the amplicon libraries for the next-generation sequencing. The method is a three-step nested PCR approach. Primer pair 799F (Table 1) and 1492R (Table 1) were used in the first round of PCR to target the v5–v9 regions of 16s rRNA gene and to prevent the amplification of plant chloroplast ribosomal gene (Chelius and Triplett 2001). The second round of PCR was done using primer pair M13-1062F (Table 1) and 1390R (Table 1) in order to produce an amplicon of proper size for the sequencing. The third round of PCR with primers Barcode-M13 and 1390R-P1 (Table 1) incorporated the sample-specific barcodes (48 barcodes) for sample identification and P1-adaptor for sequencing. PCR products were purified, quantified, pooled equimolarly, and size-fractionated to remove plant mitochondrial amplicons prior to sequencing on the Ion-torrent platform. The PCR mixture and amplification conditions for each PCR step are explained in the original paper I. All PCR procedures were done in C1000™ Thermocycler (Bio-Rad).

2.8.3 Next-generation sequencing on Ion-torrent platform

The final samples at 400 ng of pooled eubacterial DNA amplicons were sequenced in the University of Oulu (Finland) sequencing facility (I), or the University of Jyväskylä with Ion-torrent platform (III). The samples were sequenced on Ion-torrent PGM using Ion PGM Hi-Q view sequencing kit.

2.9 Bioinformatics

All of the bacterial isolates, as well as clone library sequences, were assigned taxonomy utilizing the Ribosomal Database Project (RDP Release 11) database (Cole *et al.* 2014). The RDP classifier function (Wang *et al.* 2007a) was used to classify the sequences, and the RDP seqmatch function was used to identify the closely-related sequences from the database. BioEdit software version 7.2.5 (Hall 1999) was used mainly for the trimming of the low quality 5′ and 3′ end of the sequences. Mega6 (Tamura *et al.* 2013) was used for the multiple sequence alignments, for phylogenetic analyses, and phylogenetic tree construction. Neighbor-joining method and Kimura 2-parameter model for the distance matrix calculations were used for the construction of phylogenetic trees. The phylogenetic tree was combined with phenotyping data using Interactive Tree of Life tool (iTOL) (Letunic and Bork 2016).

The open-source bioinformatics pipelines Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso *et al.* 2011) and UPARSE (Edgar 2013) were used to process raw DNA sequencing data obtained from the next-generation sequencing. The pipeline for the 16s rRNA gene data analysis developed by Pylro *et al.* (2014) was used with slight modification in the quality

filtering step (I). In study III, CLC software suite (QIAGEN) was used for the NGS data processing.

PRIMER 6+PERMANOVA software package (www.primer-e.com; Quest Research Limited) was used for the Univariate Diversity Indices analysis to obtain the species richness, evenness and Shannon diversity index of the samples using the tool DIVERSE. The analysis of similarities (ANOSIM) and Similarity Percentages Species Contribution (SIMPER) were used to compare the differences between groups of community samples and to determine which species were the major contributors to these differences, respectively.

2.10 Statistical analyses

To compare the diversities of endophyte communities in different plant tissues and plant groups in the study I, Two-way ANOVA (SPSS Statistics, IBM) was employed. The differential abundance analyses of the community members (I and III) were performed with Kruskal-Wallis test using log-transformed relative abundance data in RStudio statistical software (version 1.0.136) and SPSS version 16 (SPSS Statistics, IBM). The differences in the bacterial community composition between treatments (I) and community manipulations, inoculation pathways, and plant tissues on bacterial community structures (III) were examined using Permutational Manova (PERMANOVA) and visualized with principal component analysis (PCoA) ordination, both incorporated into PRIMER 6+PERMANOVA (PRIMER-E, Quest Research Limited) (Anderson 2017).

ANOVAs with Tukey test as a post-hoc were also performed to assess the statistical difference of the photosynthesis rate (III), plant dry weight, and the root-shoot ratio between treatments (III and IV) using the program SPSS version 16 (SPSS Statistics, IBM).

3 RESULTS AND DISCUSSION

3.1 Assembly of the endophytic bacterial communities associated with an arcto-alpine plant species: *Oxyria digyna*

3.1.1 Tissue is the primary factor shaping the endophytic bacterial community (I and III)

In study I, I examined the assembly of the endophytic bacterial communities in the leaf and root tissues of *O. digyna* in order to find out, if the endophytic bacterial communities in *O. digyna* show tissue-specificity. Micropropagated plants with low initial bacterial load were used as bait plants and were transplanted in the field site in Kilpisjärvi (Fig. 11; materials and methods) next to wild *O. digyna* population. The communities in different tissues were examined at the end of the growing season (after six weeks; August bait plants) and after winter (1 year; over-wintered bait plants) in the field site. The August bait plants, over-wintered bait plants, and wild plants are considered as the plant groups. The community composition of endophytic bacteria from the bait plants was also compared to wild plants growing adjacent to the plot in order to investigate whether the bait plants were able to acquire root and leaf endophytic communities similar to native plants or not.

The species richness and community diversity of bacterial communities were significantly higher in the roots than in the leaves in all plants in the field (I). These findings are in agreement with Robinson *et al.* (2016) and de Souza *et al.* (2016) who also detected significantly higher species richness in the roots than in the leaves in winter wheat (*Triticum aestivum*) and sugarcane, respectively. However, Bodenhausen *et al.* (2013) showed that the species richness and diversity did not differ between leaf and root of the model plant, *Arabidopsis thaliana*. The type of plant and residence time could be the factors impacting the species richness and diversity of the communities in the leaves and roots. Our study plant, *O. digyna*, sugarcane, and winter wheat have a longer life cycle than *A. thaliana*, which is a relatively short-lived annual plant

with a complete life cycle of only six weeks. The longer life cycle in *O. digyna* could mean the longer time to show the difference in the diversity between leaf and root endophytic bacterial communities. The difference in the diversities in leaf and root endophytic communities between *O. digyna* and *A. thaliana* could also be due to the different surface sterilization procedures used in the different studies, as well as plants' different growth forms. *A. thaliana* leaves are formed at the base of the plant, close to the ground. This growth from combined with the inefficient surface sterilization procedure used (sonication and washed with 70% ethanol twice) (Bodenhausen *et al.* 2013) may have resulted in insufficient removal of the epiphytic bacteria, possibly obtained from the soil. In contrast, *O. digyna* leaves are formed on the stalk above the ground, and the surface sterilization procedure used with the plants was more stringent.

In addition to community diversity, tissue type was also the main determinant of the structures of endophytic bacterial communities in our studies I and III, as the communities clustered according to tissue type, regardless of plant origin (bait vs. wild plants) (I) or the type of bacterial treatments (III). Similar results from two separate experiments under different growing conditions (I and III), one in the field next to native *O. digyna* plants (I) and another in controlled, aseptic conditions in the growth chamber (III), suggest that tissue is the strongest factor structuring the endophytic bacterial communities. Leaf and root endophytic bacterial community structures were significantly different also in winter wheat, sugarcane and *A. thaliana* (Bodenhausen *et al.* 2013, de Souza *et al.* 2016, Robinson *et al.* 2016).

Different sources of bacteria and different conditions in different plant tissues (niche) could be the factors, which result in divergent endophytic bacterial communities in leaves and roots. Root endophytes originate mainly from the rhizosphere. Plant roots secrete various sugars, amino acids, organic acids, and secondary metabolites into soils (Hardoim *et al.* 2008, Bulgarelli *et al.* 2013), resulting in the nutrient-rich zone around plant roots which accommodates dense and diverse bacterial communities in the rhizosphere. In contrast, limited bacterial inoculum sources (e.g., air, aerosols, rain, and insect vectors) (Bulgarelli *et al.* 2013) combined with low nutrient levels, highly variable and extreme habitat in the phyllosphere, limit the number of candidates for the leaf endophytic bacterial community. After the rhizospheric bacteria and phyllospheric bacteria enter the plant tissues, they also face different conditions and need different adaptive properties. Endophytic bacteria colonizing in the leaf have to tolerate the highly oxidative conditions and reactive oxygen species (ROS) which are features of the leaf niche (Delmotte *et al.* 2009, Vorholt 2012). The endophytic bacteria in root tissues, in turn, must be able to efficiently metabolize different carbon sources (Sood *et al.* 2011), compete with other bacteria and fungi, and sometimes cope with anoxic conditions (Hardoim *et al.* 2012, Iversen *et al.* 2015). These factors may explain the differences in the community diversity and structure between the endophytic bacterial communities in leaves and roots.

3.1.2 The differences of tissue-specific community structures were detectable at the phylum level, as well as in the lower taxonomic levels (I and III)

Phylum Firmicutes, especially class Bacilli was highly abundant in the leaf communities of all plant groups (starter, August bait, over-wintered bait, and wild plant) in study I, while β-Proteobacteria and Bacteroidetes were relatively more abundant in the roots (I). In agreement with my results, Firmicutes (as well as Actinobacteria) were reportedly more abundant in the leaves of winter wheat, while Proteobacteria and Bacteroidetes dominated the root communities (Robinson *et al.* 2016). In contrast, Proteobacteria and Bacteroidetes dominated the leaf communities in both perennial wild mustard (*Boechera stricta*) and *A. thaliana*, while Actinobacteria were more abundant in the roots (Bodenhausen *et al.* 2013, Wagner *et al.* 2016).

The majority of the OTUs (operational taxonomic unit, used as a proxy for bacterial genus) in study I were shared between leaf and root samples, but the relative abundances of these OTUs were different in different tissues (I). OTUs representing bacterial orders Bacillales, Pseudomonadales, Sphingomonadales, and Enterobacteriales were significantly more abundant in the leaves, while the abundances of Burkholderiales, Rhizobiales, Flavobacteriales, and Xanthomonadales were higher in the root samples. Similar to our observations, de Souza *et al.* (2016) reported higher relative abundances of Pseudomonadales and Enterobacteriales in the leaves and enrichment of Rhizobiales (in addition to Saprospirales and Rhodospirillales) in the roots of sugarcane plants grown in native soil in the greenhouse.

These results suggest that although tissue is the primary factor shaping the endophytic bacterial community in various plant species, there is no distinct general pattern to determine specific leaf- or root-community member in different plant species. The tissue-specific endophytic bacterial communities seem specific to plant species, along with environment and their habitats.

In study III, conducted in growth chambers, *O. digyna* leaf communities were dominated by *Sediminibacterium* sp. (Sphingobacteriales, Bacteroidetes) and *Sphingomonas* sp. (α-Proteobacteria, Proteobacteria). *Aeribacillus* sp. (Bacillales, Firmicutes) which was the dominant taxon in the leaf communities of all plant groups in the study I, was present only in low relative abundances in the leaves in study III, conducted in growth chambers. *Aeribacillus* sp. was also detected in small relative abundances in *O. digyna* leaves in a study conducted the following year (2014) in the same field sites (Kumar *et al.* unpublished), where *O. digyna* leaf endophytic communities were dominated by α-, and β-Proteobacteria and Actinobacteria and Firmicutes (class Clostridia) were highly abundant in the root communities (Kumar *et al.* unpublished). These results suggest that the sampling at different times could also influence the difference in the major community members in different tissues as the community is dynamic and may change year after year.

3.1.3 Plant origin influences the composition of endophytic bacterial communities and the communities change in the field gradually in a tissue-specific manner (I)

The species richness or diversity of the leaf endophytic communities were not impacted by plant origin or plant group (starter plants, August bait plants, over-wintered bait plants, or wild plants, I). However, plant group did have a small but significant impact on the structures of leaf endophytic bacterial communities (I, Table 4 and 5), although the leaf communities from different plant groups clustered together in PCoA ordination (I, Fig. 6b). The isolation of endophytic bacteria was done to check the population density of the culturable endophytic bacteria. There were remarkably less culturable endophytic bacteria detected in the leaves of the bait plants than in the wild plant leaves (II). Conjointly, the leaves of bait plants contain endophytic bacterial communities with similar diversity and community structure than wild plants, but at lower population densities. Since bacterial isolation from the bait plant was done after six weeks of transplant in the field, the difference in the population density could be the result of that limited time in the field.

In root samples, the effect of plant group on species richness and diversity, as well as community structures of bacterial endophytes was clear. The starter plants had endophytic bacterial communities with significantly lower richness and diversity than the wild plants, with the communities in the starter plants differing most from the wild plants. This was expected, as the starter plants were aseptically propagated and acclimated outdoors only ten days prior to the sampling. The period for the endophytic bacterial acquisition of the starter plants was thus significantly shorter than other plant groups, partially explaining the lower bacterial richness and diversity compared to the bait plants in the field and wild plants. The starter plants were propagated in sterile sand and nutrient solution, were exposed to the environment in the greenhouse and air of central Finland (Kuopio) which is warmer and possibly has different bacterial inoculum for the plant acquisition when compared to the environment in the field site. These factors might explain the differences in the community structures between starter and wild plants. Many bacterial taxa detected in wild plants were not detected in the root communities of the starter plants, including OTUs representing order Myxococcales. In contrast, several OTUs representing bacterial genus *Flavobacterium* were present in high relative abundances in the roots of starter plants but not in the wild plants. *Flavobacterium* species are common in roots, soil, and water (Bernardet and Bowman 2015).

Interestingly, the endophytic bacterial community structures in bait plants gradually changed towards the wild plant-type community structures (I, Fig. 3-b and 3-c). The successive change in the community structure in the leaves was more subtle than in the roots. Investigation at the OTU level revealed that the shift of the endophytic bacterial community structures towards that of the wild plant type was caused by the change in relative abundances of many OTUs, together with the acquisition of additional OTUs in the field (I). No loss of any major OTU was detected. The OTUs acquired by the bait plants in the field

were mainly identical to those present in the wild plants. This suggests a strong, specific selection by the plants. This result also showed that the longer the bait plants were in the field, the more their endophytic communities resembled those in the wild plants. Similar findings were reported by Dombrowski *et al.* (2016) in arcto-alpine perennial plant species, *Arabis alpina*, where the residence time of transplanted plants in the soil was the major determinant of the root microbiota structures. Similarly, Wagner *et al.* (2016) reported, that the microbiome structures of a perennial plant *Boechera stricta* changed after transplantation in the field, and became similar to those in wild *B. stricta* plants. These communities were different from the greenhouse control plant endophyte communities, thus showing microbial succession (Wagner *et al.* 2016). They also showed that the leaf community stayed quite stable as the plant aged while the root microbiome was highly dynamic (Wagner *et al.* 2016). Therefore, my study is in agreement with the observations above, suggesting that the endophytic bacterial community is dynamic, especially in the roots.

Despite the acquisition of many bacterial taxa from the field by the bait plants, several taxa present in the wild plants were not acquired by the bait plants even after a year in the field (I), for example, several OTUs in the order Myxococcales. Several OTUs representing Myxococcales have been previously found to be consistently enriched in the *O. digyna* roots and are considered to be a member of the core microbiome of *O. digyna* (Kumar *et al.* 2017, Nissinen unpublished). The failure of the bait plants to acquire these putatively important endophyte taxa may suggest that certain conditions are needed for the acquisition, for example, longer time in the field or acquisition only at the early plant developmental stage. Germinating seeds secrete exudates that attract surrounding bacteria to colonize the developing seedling (Nelson 2004, Truyens *et al.* 2015). These bacteria can play a role with the seedling establishment and growth, especially in extreme, low nutrient habitats (Puente *et al.* 2009, Lopez *et al.* 2012). Our micropropagated plants (bait plants) were obtained from surface-sterilized seeds, and the initial explants were sterilized to ensure contaminant-free plantlets. Moreover, these plantlets were maintained in the sterile, nutrient-rich environment, and might therefore, had no need for these beneficial bacteria to assist in the nutrient acquisition and growth of the plant. In contrast, *O. digyna* seeds in the field germinate in low nutrient soils and are likely to recruit additional endophytic bacterial members from the surrounding soils that can support the plant's survival in the high-stress environment.

Alternatively, the inability of our bait plants to acquire key bacterial endophytes from their natural environment could be due to plant developmental stage: Yuan *et al.* (2015) sampled *A. thaliana* roots subjected to soil slurry, and demonstrated, that the plants selected root-associated bacteria specific to different plant developmental stages. For example, phylum Firmicutes was found to be significantly more abundant in the seedling stage while Proteobacteria was prominent in the vegetative and bolting stage (Yuan *et al.* 2015). Chaparro *et al.* (2014) also observed a similar phenomenon in *A. thaliana* grown in soil. It is likely that the composition of root exudates in

different plant developmental stages could lead to the selection of different bacteria from the rhizosphere, and our micropropagated bait plants were unable to acquire the taxa present in the wild plants, because they were transplanted at the late vegetative stage with mature root systems. This phenomenon, where some essential endophytic bacterial taxa are not acquired after the transplantation of plants in a new location could have implications for viti- and horticulture: Often seedlings and saplings of trees and woody plants in viti- and horticulture are raised in greenhouses and transplanted in a well-developed stage. Further, plant material is often imported from a location with different climatic conditions and harbor microbial communities specific to the environment they were growing before transported. Combining with the effect of the developmental stage at the transplantation of the plants as discussed above, they may not be able to acquire the local bacteria from a new location, which could have been beneficial in growth and survival in the new habitat.

3.2 The acquisition of the endophytic bacterial community in *Oxyria digyna*

3.2.1 The impact of acquisition route on tissue-specific community formation (III)

In study I, I demonstrated that plant tissue is the main factor shaping the endophytic bacterial communities. In study III, I examined how these tissue-specific communities are formed, and tested two possible explanations for the observed divergence. First, different plant tissues (niches) offer different environment and different selective pressures for the bacteria: in leaves, bacteria are exposed to oxidative and dynamic conditions (Delmotte *et al.* 2009), while in the roots, the conditions are more stable, but microbes face high competition pressure (Rosenblueth and Martínez-Romero 2006, Compant *et al.* 2010). Alternatively, the ability to colonize particular tissue might determine tissue specificity of different bacteria: In the leaves, bacteria must be able to cross the protective cuticle into leaf endosphere, while in the roots, bacteria need to be able to penetrate the root outer layers and compete or co-exist with other bacteria (Hallmann *et al.* 1997, Verma *et al.* 2004, Edwards *et al.* 2015, Müller *et al.* 2016). To test, whether the niche or the colonization ability impact the assembly of tissue-specific communities, I inoculated micropropagated *O. digyna* plants via leaves or via rhizosphere using bacterial consortia consisting of endophytic isolates representing leaf- or root-specific taxa (leaf consortium or root consortium). These bacterial strains were isolated from the same plants used in study I (II) and were assigned tissue specificity by their enrichment in root or leaf tissues. Bacterial consortia strains are listed in Table 1 and 2 (III). I analyzed the community structures in the leaves and roots and tested the impact of bacterial consortia used for the inoculation (leaf, root or leaf+root) and inoculation route (via leaf or root) for community assembly.

The leaf-specific taxa were detected in both leaf and root tissues when the inoculation of the leaf-specific consortium was done via leaf. When the leaf-specific consortium was inoculated together with the root-specific consortium (leaf+root consortium), the leaf consortium members were able to colonize the leaf endosphere only via leaf inoculation. The colonization of leaf-specific taxa (in leaf consortium and leaf+root consortium inoculation) via root was not efficient, as the isolates could not get into the leaves. Based on this result, the leaf-specific endophytic community members seem to colonize the plant via phyllosphere, and are likely originate from air, aerosols, or insects, but not easily colonize leaves from the rhizosphere via plant root tissues. One of the leaf-specific isolates used in the leaf consortium, *Sphingomonas* sp. (JWL29), is closely related to *Sphingomonas* sp. isolated from cloud water at puy de Dome, France (1465 m.a.s.l), suggesting possible acquisition from rain. With scanning electron microscopy (SEM), I found *Sphingomonas* sp. on the *O. digyna* phyllosphere mainly clustered around secretory trichomes (Fig. 12). Plant leaf secretion can attract these bacteria, specialized at utilizing plant secondary metabolize on leaf surfaces, where they can gain entrance to the internal tissues via stomata located nearby (Vorholt 2012). *Sphingomonas* spp. are frequently found in *O. digyna* samples, and are highly enriched in the leaves, especially in the wild plants (I, Kumar *et al.* 2017, Kumar *et al.* unpublished). The genus *Sphingomonas* is frequently found as an epiphyte, carried by wind, rain, and clouds (Mano *et al.* 2007, Vaïtilingom *et al.* 2012, Dees *et al.* 2015). Taken together, this information supports our finding that the acquisition of some members of the leaf-specific community, at least *Sphingomonas* sp., was likely restricted to the leaf.

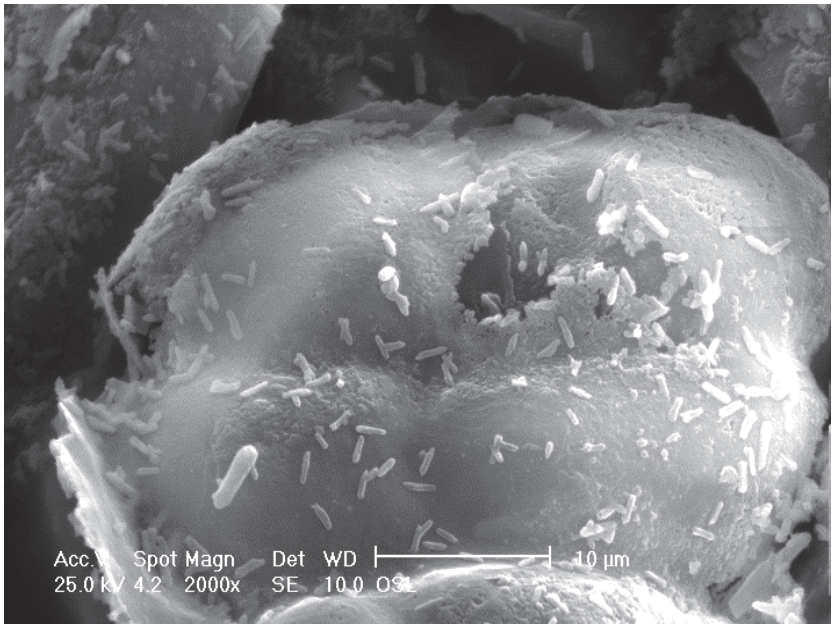

FIGURE 12 *Sphingomonas* sp. on secretory trichome of the leaf of *O. digyna*, observed by SEM. Bar: 10 µm. Photographed by Cindy Given and Riitta Nissinen.

In contrast, the root-specific bacterial taxa, either inoculated just as root consortium or as the leaf+root consortium, were detected significantly enriched in the roots, regardless of the inoculation route. Root isolates were considered a strong root colonizer even with the leaf inoculation, especially J5H36, identified as unclassified Microbacteriaceae.

Based on my results, different factors limit the assembly of tissue-specific communities in different tissues, plant niche in the leaves and colonization ability in the roots. The root-specific community was not restricted by the colonization pathway indicating the role of plant niche in determining the assembly of root endophytic bacterial community – the bacteria in root consortium were robust colonizers and competitors, as expected, but were likely not equipped for long term survival in the leaf tissue. In contrast, the leaf-specific consortium members were unable to reach leaf tissues via root inoculation, suggesting the ability to colonize the rhizosphere and root, and compete with other endophytes are the main factors limiting the assembly of the leaf endophytic bacterial community.

Bai *et al.* (2015) reported that the synthetic communities consisting of leaf-derived isolates and root+soil-derived isolates could colonize both their respective plant tissue (cognate tissue) and in the other tissue (ectopic) in *A. thaliana*. Similarly, in my study, the leaf- and root-specific consortia were detected in their respective tissues after the inoculation, and the ectopic colonization was also found in some of the leaf and root isolates.

3.2.2 Impact on the resident endophytic community (III)

The plant material used in the experiments was prepared from surface-sterilized seeds and maintained under aseptic conditions. However, I did detect endophytic bacteria in the uninoculated micropropagated plants used as the negative control (III). Several of the OTUs detected showed 100% identity to bacterial strains used in the consortia (III). These OTUs were also identical with the OTUs detected in the starter plants in the study I. Moreover, the data from study I showed that the majority (82.5%) of the OTUs detected in starter plants were shared with all plant groups including wild plants. Taken together, the bacteria detected in the micropropagated control plants were tightly associated with *O. digyna.* This resident endophytic bacterial community detected had low diversity, was present at very low abundances, and was not readily culturable, as most test isolations from tissue culture plants yielded no colonies (Nissinen, personal communication).

Similar to our findings, the endophytic bacteria found to be colonizing tissue culture plants has also been reported for many other plant species (Partida-Martinez and Heil 2011, Esposito-Polesi *et al.* 2015, Orlikowska *et al.* 2017). These bacteria detected in tissue-culture plants should no longer be considered as contaminants as they are found to be tightly associated with the plants (Abreu-Tarazi *et al.* 2010, Lucero *et al.* 2011, Quambusch *et al.* 2016), sometimes providing benefits to the tissue culture plants (Quambusch *et al.* 2014, Orlikowska *et al.* 2017).

I found that the inoculation of our experimental consortia strongly impacted the resident endophyte community structures (III, Fig. 3c–3e). Similarly, Wagner *et al.* (2016) reported, that acquisition of endophytic bacteria from the soil resulted in the change of the resident endophytic bacterial community, especially in the roots of the tested plants, *B. stricta*. Oliveira *et al.* (2017) also showed that the inoculation of *Pseudomonas* sp. strain SPN31 could influence the diversity of the endophytic bacterial community in *Halimione portulacoides*. Additionally, Ardanov *et al.* (2016) showed that the bacterial inoculation induced the shift in the endophytic bacterial community structure of potato (*Solanum tuberosum* L.).

Sediminibacterium sp. was highly dominant in the leaves of our control plants (III). When plants were inoculated via leaves, regardless of the bacterial consortium used, *Sphingomonas* sp. replaced *Sediminibacterium* sp. in the leaf communities, and the relative abundance of *Sediminibacterium* sp. decreased to near zero. This negative correlation may result from direct antagonism or could be via plant signaling as shown by Innerebner *et al.* (2011) where *Sphingomonas* sp. had a role in the defense mechanism diminishing the growth of pathogen *Pseudomonas syringae* pv. tomato DC3000 in *A. thaliana.* However, the suppression of the disease was only obtained by the plant-colonizing *Sphingomonas* sp. and not by *Sphingomonas* strains isolated from air, water, or dust (Innerebner *et al.* 2011) which suggests that plants can distinguish plant-associated bacteria from pathogenic or other bacteria in the surrounding environments, possibly via a signaling pathway that involves the bacterial invasive molecules, e.g., microbe-associated molecular patterns (MAMPs).

3.3 Functional diversity of the endophytic community in *Oxyria digyna* does not follow the taxonomic diversity (II)

In study II, I asked if the functional profiles of the endophytic bacterial communities show tissue-specificity, like their taxonomic profiles were shown to do (I). To address this, I analyzed the functional diversity of the culturable endophytic bacteria isolated from the leaves and roots isolated of the same plants I used for the molecular community analyses in the study I. As standard isolation medium (R2A, pH 6.5) was used for isolation, only a part of the total community was captured in the culture collection. Taxonomically, the isolate collection was dominated by Proteobacteria.

High proportion of the isolate collection from the leaves were positive for solubilization of organic and inorganic phosphate. The result was unexpected, especially with inorganic phosphate, since the plant phosphorus uptake mainly occurs in the roots, and endophytic bacteria are postulated to contribute to phosphate mobilization. In contrast to our study, Croes *et al.* (2015) found that the inorganic phosphate solubilizing endophytic bacteria were predominantly present in the roots of field-grown *Brassica napus* L. The ability to solubilize

organic phosphate was more prominent among the leaf isolates from both plant groups (bait and wild plants) than among the root isolates. Phytate or phytic acid (inositol hexaphosphate) is the major storage form of phosphates in the seeds and functions as the energy stores for the germination of seeds (Reddy *et al.* 1982), and phytase enzyme is important in the seed germination (Asada *et al.* 1970). Poosakkannu *et al.* (2015) showed that the great majority (92%) of endophytic bacteria isolated from seeds of *Deschampsia flexuosa*, especially bacteria in genus *Pseudomonas* (*P. graminis)*, were able to solubilize organic phosphate. *Pseudomonas* sp. with the ability to solubilize both organic and inorganic phosphate were also found in our isolate collections from both leaves and roots. In addition to seeds, phytic acid is also present in some green leaves (Hadi Alkarawi and Zotz 2014), and could explain the abundance of phytate solubilization activity among our *O. digyna* leaf isolates.

Ability to degrade starch (amylase activity) was more common among the root isolates than in the leaf isolates from both bait and wild plants. High levels of starch and sugars have been detected in the roots of *O. digyna* at the beginning and the end of the growing season (Russell 1948). Due to the short growing season in the Arctic, and the annual leaves in *O. digyna*, rapid development in early spring depends on the large reserves of carbohydrate (stored as starch) in the roots before the plants can photosynthesize. Thus, the starch hydrolysis ability among the root isolates could be the adaptation to plant niche.

Unlike the amylase activity and solubilization of organic and inorganic phosphate, cellulose degradation had different tissue-specificity in bait plants and wild plants. Cellulolytic activity was more common among root isolates of wild plants (42.8%) than leaf isolates (20%) while it was slightly more common among leaf isolates of bait plants (33.3% of the isolates) than root isolates (26.5% of the isolates) (II, Fig. 4). Nitrogen fixation was more common among isolates from wild plants, in particular from wild plant leaf sample, than among bait plant isolates. Micropropagated plants have been provided with all the macronutrients and trace elements crucial for the growth of plants, resulting in altered nutrition dynamic as the leaves become major carbon reserve with limited photosynthetic capacity and roots become non-functional in the in vitro plants (Abreu-Tarazi *et al.* 2010). This could also be the case in our bait plants and would explain the functional difference between bait and wild plant isolates. Both our study and Abreu-Tarazi *et al.* (2010) use the same half strength MS medium (Murashige and Skoog 1962) containing 3% (w/v) sucrose for micropropagated plants. The wild plants, on the other hand, grow in nutrient poor environment. The wild plants in the Arctic have a short growing season (80–100 days) where the leaves are produced annually before senescing at the end of the growing season and survive as rhizomes along with the roots in winter. Even though the micropropagated bait plants were later transplanted into the field, the difference in the physiology and growing condition, between bait and wild plants might influence the difference in the functional profiles between plant types rather than between tissues.

Bai *et al.* (2015) reported findings similar to ours, based on genomic sequencing of endophyte bacterial isolate collections from leaves and roots of *A. thaliana*: Plant tissue was the major factor shaping the taxonomic structures of the endophytic bacterial communities, but the functional diversity was weakly affiliated with the tissues. Further, no clear separation of the functional traits of bacteria was detected based on the niche (leaf, root or soil), suggesting shared or overlapping core functions of the (currently known) endophytic bacterial communities (Bai *et al.* 2015).

The growth profiles measured at three temperatures were drastically different between bait and wild plant isolates, with no distinction of the host tissue. All bait plant isolates were able to grow at 37°C compared to only 23% of the wild plant isolates. On the other hand, 88% of the isolates from wild plants grew at 4°C while 55% of the bait plant isolates could grow well at 4°C. The difference in the growth temperature profile could result from the selection of endophytic bacteria by bait plants. The isolates obtained from wild plants that grew nearby did not show a similar growth profile. Therefore, it was highly unlikely the bait plants may have selected the mesophilic bacteria (optimum growth between 20°C and 37°C) after they were transplanted in the field. These mesophilic bacteria may have been acquired when the bait plants were acclimatized in the greenhouse in Kuopio before being transplanted in the field. Another possible explanation is that they are part of the resident bacterial community of the micropropagated plants. This minimal bacterial community survived through the micropropagation and was maintained with the plantlets in a culture medium under an optimal condition (21°C) for several years, gradually adapting to higher temperatures. The last explanation is also supported by observations of conserved resident community detected in plants by molecular methods (I and III).

The difference in the growth temperature profiles also correlated with the phylogeny of the isolates. Although wild and bait plant isolates were closely related, on the fine phylogenetic scale, 80% of wild plant isolates showed highest sequence similarity (98–100%) to bacterial isolates from other cold environments including glacier, polar seas, subnival plants, and cryoconite holes, based on the 16S rRNA sequence alignments against public database (RDP) sequences. Sheng *et al.* (2011) reported that, among the isolates retrieved from alpine-subnival plants of the Tianshan Mountains, 46.4% of the isolates were highly similar to the bacteria from other cold environments. Nissinen *et al.* (2012) also reported that 40% of the isolates from three arctic plants were most closely related to bacterial strains from other cold environments. These consistent findings indicate the presence of the plant-associated bacterial lineages endemic to the cold climates.

The results from this study also demonstrate the limitations of current, widely used molecular approaches for community characterization of prokaryotes. Most of the OTUs (clustered at 97% sequence identity) in study I were shared between bait and wild plants. However, analysis of isolate collection from the same samples revealed that these OTUs consisted of several different bacterial species (II). For example, in study I, two OTUs representing

genus *Pseudomonas* were detected, both being present in leaf as well as root samples of both bait and wild plants (I). However, sequencing of the isolate collection from the same plants resulted in 22 unique *Pseudomonas* isolates, which clearly clustered into two separate, tissue-specific clusters. The leaf-specific pseudomonads showed higher nitrogen fixation ability when compared to the root-specific pseudomonads (II, Fig. 5). Moreover, I also found a difference in the growth temperature between pseudomonads isolated from bait plants and wild plants where all the bait plant pseudomonas grew at 37°C, but only 15% of wild plant pseudomonads grew at that temperature. As such, the use of a polyphasic approach gives many advantages, as more in-depth information regarding the endophytic bacterial communities of the plants and their functions might be revealed by combining methods of molecular and culturable bacterial analysis.

3.4 Impact of the endophytic bacteria on the plant phenotype (III and IV)

As endophytic communities in different tissues of *O. digyna* were taxonomically distinct, I could hypothesize, that they would have a divergent impact on plant phenotype. I tested this hypothesis in studies III and IV, using plant tissue-specific endophytic consortia (leaf- or root-specific (III and IV), or combination of both, described in study III), both under ambient (III) and elevated temperatures (III and IV).

Inoculation of the plants with endophytic bacterial consortia, regardless of the consortium type and inoculation route, increased the rate of photosynthesis compared to uninoculated control plants (III, Fig. 5). Inoculation of combined leaf+root consortia increased the root dry weight, total biomass, and root-shoot ratio, significantly (III, Fig. 4b – 4d). Moreover, all consortia treatments impacted the root morphology, as the treated plants had denser and bigger roots with a thicker mucous layer on the root surfaces than the control plants (IV, Fig. 1 and 2). Similar impacts were reported by Marcos *et al.* (2016), who observed, that inoculation of sugarcane plants with bacterial consortium improved the photosynthesis and nitrate reduction rate in plants, and also changed plant morphology, but did not impact the shoot and root dry weight. However, Mishra and Sundari (2015) reported a significant increase in total biomass and shoot dry weight of *Sorghum bicolor* after inoculation with a mixture of plant growth promoting bacteria, in contrast with our and Marcos *et al.* (2016) results.

When *O. digyna* plants in study IV were exposed to temperatures between 30°C and 42°C for 48 hours, the majority of the plants died immediately. None of the control plants or plants inoculated with root consortia (RC) or with individual root consortia isolates survived. In contrast, the plants inoculated with leaf consortium (LC) or with of individual leaf consortium isolates (isolates

JWL5 (*Pseudomonas* sp.), JWL15 (unclassified Pseudomonadaceae), JWL29 (*Sphingomonas* sp.), JWL33-3 (*Janthinobacterium*), JBL9 (*Paenibacillus* sp.), or JBL14 (*Sphingomonas* sp.)) survived the severe heat stress (IV, Fig. 3). Possible explanation for this phenomenon is by linking to what is known from study II and III. The bait plant isolates (JBL) can grow well at 37°C but not the wild plant isolates (JWL) (II). I also perceived that the inoculation of consortia had a strong impact on the resident community in our micropropagated plant (III). Hence, I speculate that the increased survival of the plants inoculated with leaf consortium (members) may be due to the impact of the inoculation on the plant resident community, either by the effect on plant hormone production or directly on the endophytic community. Similar phenomenon of increased plant fitness by the change in endophyte community structure after bacterial inoculation was reported for potato by Ardanov *et al.* (2016). It was also possible that these endophytic bacteria prime the plants for several abiotic stressors as has been reported for cold, salinity and drought (Hardoim *et al.* 2012, Daffonchio *et al.* 2015). Analysis of functional potential of *A. thaliana* endophytes by whole-genome sequencing (Bai *et al.* 2015) revealed that bacterial families differ in their functional diversity: Family Microbacteriaceae was functionally least diverse while Pseudomonadaceae and Oxalobacteraceae showed highest functional heterogeneity. In relation to this, both our leaf and root consortia had *Pseudomonas* spp. isolates, which may have diverse functional traits. The leaf cluster pseudomonads may possess the ability of plant protection under severe heat stress while the root pseudomonads may not. Further study is required to confirm these results. Furthermore, the molecular analysis to investigate if plants select a different set of bacteria according to different stresses and the possible functional traits based on their genomes might provide more information on the function of the endophytic bacteria under various stresses. In the present scenario of global warming and shifting weather patterns, heat stress is considered important to the Arctic plant species, but also for global agriculture.

3.5 Endophytic bacteria conserved to *Oxyria digyna* (I, II, and III)

Endophytic bacteria associated with *O. digyna* have been studied since 2012 (Nissinen *et al.* 2012), and since then, biogeography, community structure, assembly, and functions have been addressed in various studies. In addition to molecular data (Nissinen *et al.* 2012, Kumar *et al.* 2017, Studies I, and III in this thesis), endophytic bacteria have been systemically isolated from the leaves, roots and seeds of *O. digyna* in different geographical locations (Nissinen *et al.* 2012, Study II in this thesis, Nissinen, pers. comm., Given *et al.* unpublished). Cross-comparison of all these data reveals several bacterial species that are consistently associated with *O. digyna.* These highly conserved endophytes represent bacterial genera *Microbacterium*, *Sphingomonas*, *Pseudomonas*, and bacterial families Comamonadaceae and Oxalobacteraceae (Table 2).

OTUs classified as *Janthinobacterium* (Oxalobacteraceae), and unclassified Comamonadaceae have been identified as members of highly conserved core community of *O. digyna,* associated with the host plant in three climatic regions (Kilpisjärvi; low Arctic, Svalbard; high Arctic, and Mayrhofen; European Alps, Kumar *et al.* 2017). In our study, OTUs and isolates classified as members of family Oxalobacteraceae including genera *Janthinobacterium, Herbaspirillium*, *Rugamonas* and *Duganella,* a group of unclassified Oxalobacteraceae were detected in the leaves, roots (I, III), as well as in the seeds and seedlings of *O. digyna*. Detection of these bacteria in the seeds indicates vertical transmission of these endophytes via seeds. Several Oxalobacteraceae isolates were able to mobilize organic and inorganic phosphate and were positive in the nitrogen fixation test (II). These properties could be highly beneficial for *O. digyna*, which as a pioneer plant species, is able to colonize very low nutrient soils. Seed association of these endophytes could enhance seedling establishment in the low nutrient soils in the Arctic (Truyens *et al.* 2015), similar to phosphate solubilizing diazotrophic bacteria in the seeds of cardon cactus (*Pachycereus pringlei*) (Puente *et al.* 2009), also a pioneer plant species.

TABLE 2 Endophytic bacterial taxa conserved in *O. digyna* and their functions.

Taxon	Compartment	Phenotype (II)	Study I	Study II	Study III	Seed clone libraries	Seed culture collections	Nissinen *et al.* (2012)	Kumar *et al.* (2017)**
***Janthinobacterium* sp.**	Leaf, Root, Seed	inorg. P, org. P, N-fixation	Yes (10.7% RA)	Yes (3 isolates)	Yes (4.1% RA)	Yes (1 clone)	No	No	Yes
***Pseudomonas* sp.**	Leaf, Root, Seed	C, A, inorg. P, org. P, N-fixation	Yes (2.2% RA)	Yes (22 isolates)	Yes (27.2% RA)	Yes (68 clones)	Yes (8 isolates)	Yes (17 isolates)	No
***Sphingomonas* sp.**	Leaf, Root, Seed	C, inorg. P, org. P, N-fixation	Yes (3.8% RA)	Yes (9 isolates)	Yes (5.5% RA)	No	Yes (3 isolates)	Yes (21 isolates)	No
***Microbacterium* sp.**	Leaf, Root, Seed	A, inorg. P, org. P, N-fixation	Yes (0.65% RA)	Yes (23 isolates)	Yes (0.5% RA)	No	Yes (1 isolate)	No	No
unclassified Oxalobacteraceae	Leaf, Root, Seed	A, inorg. P, org. P, N-fixation	Yes (3.3% RA)	Yes (9 isolates)	Yes (1% RA)	Yes (37 clones)	Yes (1 isolate)	No	No
family Comamonadaceae	Leaf, Root, Seed	C, N-fixation	Yes (15.5% RA)	Yes (5 isolates)	Yes (5.5% RA)	Yes (9 clones)	No	Yes (12 isolates)	Yes

*A = amylase activity, C = cellulose activity, inorg. P = inorganic phosphate solubilization, org. P = organic phosphate solubilization, N-fixation = nitrogen fixation
*Core microbe was defined by the presence in 4/7 databases as listed in the table, >1%RA and observed in at least 40/71 samples based on study I
*Seed clone libraries and isolate collections were obtained from five geographical locations (fells Jehkas and Saana in Kilpisjärvi, Utsjoki and Kaldoaivi (Eastern Lapland), and Longyearbyen (Svalbard)
** Based on the study by Kumar *et al.* (2017), I considered only the core members reported in the study.

4 CONCLUSIONS

Plant tissue type was found to be the main factor shaping the taxonomic structures of endophytic bacterial communities (I and III) in *Oxyria digyna*, with distinct bacterial taxa enriched in the leaves and the roots. Functional profiles of the isolates, however, did not correlate with plant tissues, but rather with the plant type (bait plant or wild plant) (II). This suggests that tissue is a strong determinant of the community composition but is not necessarily a determinant for the functions of bacteria that occupy these niches. The co-adaptation of bacteria and their host plants under different conditions might be the driver of the difference between the functional profiles of endophytic isolates in different plant types. Furthermore, the co-adaptation might also explain the finding that taxonomically highly similar bacteria, but with different functional profiles and growth temperature requirements were observed in different plant groups (II). These findings demonstrate the limitation of the purely DNA-based next-generation sequencing approach and demonstrate that multiphasic approach is a more suitable choice for studies on plant-microbe interactions.

Experiments with endophytic bacterial consortia demonstrated, that even though the composition and functional profiles of consortia from different tissues did differ, all consortia types resulted in similar plant-growth promotion when compared to the controls (III). However, small but significant differences in morphology (under both ambient conditions (III) and under heat stress (IV)) were detected between leaf consortia- and root consortia-treated plants. Thus, it appears that, through the plant's recognition of the beneficial bacteria, possibly via microbe-associated molecular patterns (MAMPs), the interactions between plants and beneficial microbes commonly result in plant-growth promotion disregarding the origin of bacteria and the functions of individual bacteria. Alternatively, it is possible that the bacteria in the consortia used in this thesis share important functions, which were not measured in this study.

Inoculation of plants with bacterial consortia shaped the resident microbial community in the plants (III). This indicates that, in addition to plant-microbe interactions, we should also regard microbe-microbe interactions when seeking explanations to plant phenotype modification by microbial treatments. Reshaping of the resident endophytic community by microbial inoculation could

explain the survival of the plants inoculated with the leaf consortium and the individual leaf-specific endophytic isolates under the severe heat stress (IV). Plant-microbe and microbe-microbe interactions are dynamic and constantly shaped by plant metabolism, reflecting the environmental conditions the plant is challenged with. The change in the structure of the endophytic bacterial community may affect the functioning, possibly via the signals of both parties.

In respect to the changes in the endophytic bacterial community composition, the root communities of *O. digyna* were more dynamic, and the microbial succession was evident, while the leaf communities were stable and showed very little structural changes during the experiment (I). The factors governing the leaf and root endophytic bacterial community assembly were different in different tissues: Plant niche seemed to be the major factor shaping the assembly of the root-associated endophytic bacterial community, as the bacteria were able to colonize their target tissue, regardless whether they were acquired via leaves or roots (III). In contrast, the leaf-specific endophytes were able to reach their target tissue only if they were acquired via leaves, suggesting that competition or colonization potential determined the establishment of these bacteria in plants.

This study also detected several endophytic bacterial taxa that were highly conserved in *O. digyna*, including isolates representing bacterial family Oxalobacteraceae and genus *Sphingomonas*. The endophytic bacteria from wild *O. digyna* were most closely related to bacteria from other cold climates, supporting the existence of plant-associated bacterial lineages endemic to cold climates. These bacteria could offer a source for novel and diverse secondary metabolic products functional at low temperatures.

The findings of the co-adaptation of plant and its associated bacteria to cold climates, and the highly conserved core endophytic bacterial community in *O. digyna* detected in this study, collectively support the holobiont theory, where plant and its microbiome are considered as a co-evolving meta-organism (Zilber-Rosenberg and Rosenberg 2008, Rosenberg *et al.* 2016).

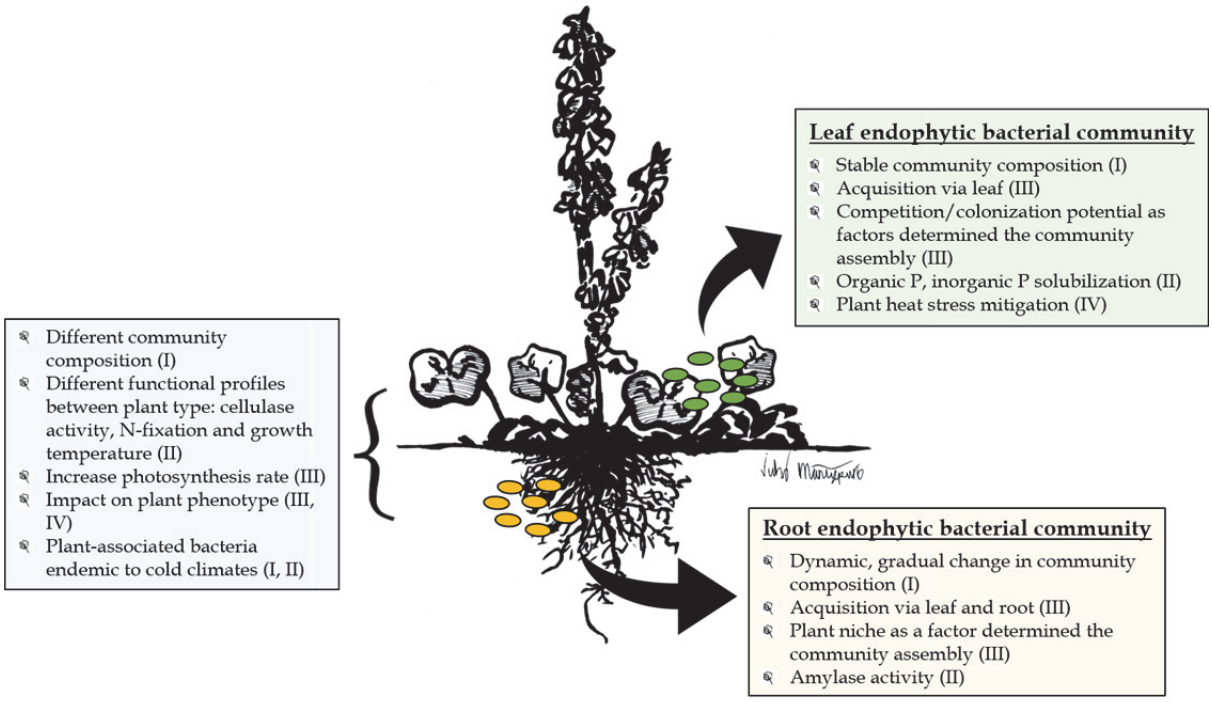

FIGURE 13 Summary of the findings on the endophytic bacterial community structures, the acquisition and the functional profiles from the endophytic bacteria isolated from leaves and roots of bait and wild plants of *O. digyna*. Roman letters I, II, III, and IV indicate the respective manuscript numbers.

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"Being deeply loved by someone give you strength,
while loving someone deeply gives you courage"
– Lao Tzu

REFERENCES

Abreu-Tarazi M.F., Navarrete A.A., Andreote F.D., Almeida C. V, Tsai S.M. & Almeida M. 2010. Endophytic bacteria in long-term *in vitro* cultivated 'axenic' pineapple microplants revealed by PCR-DGGE. *World J. Microbiol. Biotechnol.* 26: 555–560.

Allen G.A., Marr K.L., McCormick L.J. & Hebda R.J. 2012. The impact of pleistocene climate change on an ancient arctic-alpine plant: Multiple lineages of disparate history in *Oxyria digyna*. *Ecol. Evol.* 2: 649–665.

Anderson M.J. 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). *Wiley StatsRef Stat. Ref. Online*: 1–15.

Andreote F.D., Gumiere T. & Durrer A. 2014. Exploring interactions of plant microbiomes. *Sci. Agric.* 71: 528–539.

Ardanov P., Lyastchenko S., Karppinen K., Häggman H., Kozyrovska N. & Pirttilä A.M. 2016. Effects of *Methylobacterium* sp. on emergence, yield, and disease prevalence in three cultivars of potato (*Solanum tuberosum* L.) were associated with the shift in endophytic microbial community. *Plant Soil* 405: 299–310.

Asada K., Tanaka K. & Kasai Z. 1970. Formation of phytic acid in cereal grains. *Ann. N. Y. Acad. Sci.* 165: 801–814.

Atkin O.K. 1996. Reassessing the nitrogen relations of Arctic plants: A mini-review. *Plant, Cell Environ.* 19: 695–704.

Atkin O.K. & Cummins W.R. 1994. The Effect of Nitrogen Source on Growth, Nitrogen Economy and Respiration of Two High Arctic Plant Species Differing in Relative Growth Rate. *Funct. Ecol.* 8: 389–399.

Au S.-F. 1969. Internal Leaf Surface and Stomatal Abundance in Arctic and Alpine Populations of *Oxyria digyna*. *Ecology* 50: 131–134.

Bai Y., Müller D.B., Srinivas G., Garrido-Oter R., Potthoff E., Rott M., Dombrowski N., Münch P.C., Spaepen S., Remus-Emsermann M., Hüttel B., McHardy A.C., Vorholt J.A. & Schulze-Lefert P. 2015. Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature* 528: 364–369.

Baldani J.I., Reis V.M., Videira S.S., Boddey L.H. & Baldani V.L.D. 2014. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. *Plant Soil* 384: 413–431.

Barea J.-M. & Richardson A.E. 2015. Phosphate Mobilisation by Soil Microorganisms. In: *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, pp. 225–234.

Barret M., Briand M., Bonneau S., Préveaux A., Valière S., Bouchez O., Hunault G., Simoneau P. & Jacques M.-A. 2015. Emergence Shapes the Structure of the Seed Microbiota. *Appl. Environ. Microbiol.* 81: 1257–1266.

Bernardet J.-F. & Bowman J.P. 2015. *Flavobacterium*. In: *Bergey's Manual of Systematics of Archaea and Bacteria*, pp. 1–75.

Billings W. & Mooney H. 1968. The ecology of arctic and alpine plants. *Biol. Rev.* 43: 481–529.

Bodenhausen N., Horton M.W. & Bergelson J. 2013. Bacterial Communities Associated with the Leaves and the Roots of *Arabidopsis thaliana*. *PLoS One* 8: 1–9.

Bruijn F.J. de. 2015. Biological Nitrogen Fixation. In: *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, pp. 215–224.

Bulgarelli D., Schlaeppi K., Spaepen S., Themaat E.V.L. van & Schulze-Lefert P. 2013. Structure and Functions of the Bacterial Microbiota of Plants. *Annu. Rev. Plant Biol.* 64: 807–838.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich K., Gordon J.I., Huttley G. a, Kelley S.T., Knights D., Jeremy E., Ley R.E., Lozupone C. a, Mcdonald D., Muegge B.D., Reeder J., Sevinsky J.R., Turnbaugh P.J. & Walters W. a. 2011. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7: 335–336.

Chanway C.P. 1996. Endophytes: they're not just fungi! *Can. J. Bot.* 74: 321–322.

Chaparro J.M., Badri D. V. & Vivanco J.M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* 8: 790–803.

Chapin D.M. & Bledsoe C.S. 1992. Nitrogen Fixation in Arctic Plant Communities. In: Chapin III F.S., Jefferies R.L., Reynolds J.F., Shaver G.R., Svoboda J. & Chu E.W. (eds.), *Arctic Ecosystems in a Changing Climate: an Ecophysiological Perspective*, Academic Press, pp. 301–319.

Chapin III F.S., Jefferies R.L., Reynolds J.F., Shaver G.R. & Svoboda J. 1992. Arctic Plant Physiological Ecology in an Ecosystem Context. In: Chapin III F.S., Jefferies R.L., Reynolds J.F., Shaver G.R., Svoboda J. & Chu E.W. (eds.), *Arctic Ecosystems in a Changing Climate: an Ecophysiological Perspective*, Academic Press, pp. 441–451.

Chelius M.K. & Triplett E.W. 2001. The diversity of archaea and bacteria in association with the roots of Zea mays L. *Microb. Ecol.* 41: 252–263.

Cole J.R., Wang Q., Fish J.A., Chai B., Mcgarrell D.M., Sun Y., Brown C.T., Porras-Alfaro A., Kuske C.R. & Tiedje J.M. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* 42: D633–D642.

Compant S., Clément C. & Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* 42: 669–678.

Compant S., Reiter B., Sessitsch A., Nowak J., Clément C. & Barka E.A. 2005. Endophytic Colonization of *Vitis vinifera* L. by Plant Growth-Promoting Bacterium *Burkholderia* sp. Strain PsJN. *Appl. Environ. Microbiol.* 71: 1685–1693.

Croes S., Weyens N., Colpaert J. & Vangronsveld J. 2015. Characterization of the cultivable bacterial populations associated with field grown *Brassica napus* L.: An evaluation of sampling and isolation protocols. *Environ. Microbiol.* 17: 2379–2392.

Daffonchio D., Hirt H. & Berg G. 2015. Plant-Microbe Interactions and Water Management in Arid and Saline Soils. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 265–276.

Dees M.W., Lysøe E., Nordskog B. & Brurberg M.B. 2015. Bacterial Communities Associated with Surfaces of Leafy Greens: Shift in Composition and Decrease in Richness over Time. *Appl. Environ. Microbiol.* 81: 1530–1539.

Delmotte N., Knief C., Chaffron S., Innerebner G., Roschitzki B., Schlapbach R., Mering C. von & Vorholt J.A. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *PNAS* 106: 16428–16433.

De Roy K., Marzorati M., Van den Abbeele P., Van de Wiele T. & Boon N. 2014. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ. Microbiol.* 16: 1472–1481.

de Souza R.S.C., Okura V.K., Armanhi J.S.L., Jorrín B., Lozano N., da Silva M.J., González-Guerrero M., de Araújo L.M., Verza N.C., Bagheri H.C., Imperial J. & Arruda P. 2016. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Sci. Rep.* 6: 1–15.

Dombrowski N., Schlaeppi K., Agler M.T., Hacquard S., Kemen E., Garrido-Oter R., Wunder J., Coupland G. & Schulze-Lefert P. 2016. Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *ISME J.* 11: 43–55.

Edgar R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10: 996–998.

Edwards J., Johnson C., Santos-Medellín C., Lurie E., Podishetty N.K., Bhatnagar S., Eisen J.A. & Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *PNAS* 112: E911–E920.

Esposito-Polesi N.P., Andrade P.A.M. de, Almeida C.V. de, Andreote F.D. & Almeida M. de. 2015. Endophytic bacterial communities associated with two explant sources of *Eucalyptus benthamii* Maiden & Cambage. *World J. Microbiol. Biotechnol.* 31: 1737–1746.

Gaiero J.R., McCall C.A., Thompson K.A., Day N.J., Best A.S. & Dunfield K.E. 2013. Inside the root microbiome: Bacterial root endophytes and plant growth promotion. *Am. J. Bot.* 100: 1738–1750.

Geraci J.R. & Smith T.G. 1979. Vitamin C in the diet of Inuit hunters from Holman, Northwest Territories. *Arctic* 32: 135–139.

Ghyselinck J., Pfeiffer S., Heylen K., Sessitsch A. & Vos P. De. 2013. The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PLoS One* 8: 1–14.

Glick B.R. 2015. Stress Control and ACC Deaminase. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 257–264.

Greenaway W., May J., Scaysbrook T. & Whatley F.R. 1992. Compositions of Bud and Leaf Exudates of Some *Populus* Species Compared. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* 47: 329–334.

Großkopf T. & Soyer O.S. 2014. Synthetic microbial communities. *Curr. Opin. Microbiol.* 18: 72–77.

Guttman D.S., McHardy A.C. & Schulze-Lefert P. 2014. Microbial genome-enabled insights into plant–microorganism interactions. *Nat. Rev. Genet.* 15: 797–813.

Hadi Alkarawi H. & Zotz G. 2014. Phytic acid in green leaves. *Plant Biol.* 16: 697–701.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95–98.

Hallmann J. 2001. Plant interactions with endophytic bacteria. In: Jeger M.J. & Spence N.J. (eds.), *Biotic interactions in plant-pathogen associations.*, CAB International, pp. 87–119.

Hallmann J., Quadt-Hallmann A., Mahaffee W.F. & Kloepper J.W. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43: 895–914.

Hardoim P., Nissinen R. & Elsas J.D. van. 2012. Ecology of Bacterial Endophytes in Sustainable Agriculture. In: Maheshwari D.K. (ed.), *Bacteria in Agrobiology: Plant Probiotics*, Springer, pp. 97–126.

Hardoim P.R., Overbeek L.S. van, Berg G., Pirttilä A.M., Compant S., Campisano A., Döring M. & Sessitsch A. 2015. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol. Mol. Biol. Rev.* 79: 293–320.

Hardoim P.R., Overbeek L.S. van & Elsas J.D. van. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* 16: 463–471.

Heide O.M. 2005. Ecotypic Variation among European Arctic and Alpine Populations of *Oxyria digyna*. *Arctic, Antarct. Alp. Res.* 37: 233–238.

Hoagland D.R. & Arnon D.I. 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* 347: 1–32.

Holzinger A., Wasteneys G.O. & Lütz C. 2007. Investigating cytoskeletal function in chloroplast protrusion formation in the arctic-alpine plant *Oxyria digyna*. *Plant Biol.* 9: 400–410.

Hurek T., Reinhold-Hurek B., Montagu M. Van & Kellenberger E. 1994. Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *J. Bacteriol.* 176: 1913–1923.

Innerebner G., Knief C. & Vorholt J.A. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl. Environ. Microbiol.* 77: 3202–3210.

Iversen C.M., Sloan V.L., Sullivan P.F., Euskirchen E.S., Mcguire A.D., Norby R.J., Walker A.P., Warren J.M. & Wullschleger S.D. 2015. The unseen iceberg: Plant roots in arctic tundra. *New Phytol.* 205: 34–58.

Jones A., Stolbovoy V., Tarnocai C., Broll G., Spaargaren O. & Montanarella L. 2009. *Soil Atlas of the Northern Circumpolar Region*.

Jorquera M.A., Crowley D.E., Marschner P., Greiner R., Fernández M.T., Romero D., Menezes-Blackburn D. & La Luz Mora M. De. 2011. Identification of β-propeller phytase-encoding genes in culturable *Paenibacillus* and *Bacillus* spp.

from the rhizosphere of pasture plants on volcanic soils. *FEMS Microbiol. Ecol.* 75: 163–172.

Krings M., Taylor T.N., Hass H., Kerp H., Dotzler N. & Hermsen E.J. 2007. Fungal endophytes in a 400-million-yr-old land plant: Infection pathways, spatial distribution, and host responses. *New Phytol.* 174: 648–657.

Kroll S., Agler M.T. & Kemen E. 2017. Genomic dissection of host – microbe and microbe – microbe interactions for advanced plant breeding. *Curr. Opin. Plant Biol.* 36: 71–78.

Kumar M., Brader G., Sessitsch A., Mäki A., van Elsas J.D. & Nissinen R.M. 2017. Plants Assemble Specific Bacterial Communities from Common Core Taxa in Three Arcto-Alpine Climate Zones. *Front. Microbiol.* 8: 1–15.

Kumar M., Männistö M.K., van Elsas J.D. & Nissinen R.M. 2016. Plants impact structure and function of bacterial communities in Arctic soils. *Plant soil* 399: 319–332.

Lane D.J. 1991. 16S/23S rRNA Sequencing. In: Stackebrandt E. & Goodfellow M. (eds.), *Nucleic Acid Techniques in Bacterial Systematic*, John Wiley and Sons, New York, pp. 115–175.

Lareen A., Burton F. & Schäfer P. 2016. Plant root-microbe communication in shaping root microbiomes. *Plant Mol. Biol.* 90: 575–587.

Leite H.A.C., Silva A.B., Gomes F.P., Gramacho K.P., Faria J.C., Souza J.T. De & Loguercio L.L. 2013. *Bacillus subtilis* and *Enterobacter cloacae* endophytes from healthy *Theobroma cacao* L. trees can systemically colonize seedlings and promote growth. *Appl. Microbiol. Biotechnol.* 97: 2639–2651.

Letunic I. & Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 2006: 1–4.

Leveau J.H.J. 2015. Life of Microbes on Aerial plant Parts. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 17–24.

Lindow S.E. & Brandl M.T. 2003. Microbiology of the Phyllosphere. *Appl. Environ. Microbiol.* 69: 1875–1883.

Lodewyckx C., Vangronsveld J., Porteous F., Moore E.R.B., Taghavi S., Mezgeay M. & Lelie D. van der. 2002. Endophytic Bacteria and Their Potential Applications. *CRC. Crit. Rev. Plant Sci.* 21: 583–606.

Lopez B.R., Tinoco-Ojanguren C., Bacilio M., Mendoza A. & Bashan Y. 2012. Endophytic bacteria of the rock-dwelling cactus *Mammillaria fraileana* affect plant growth and mobilization of elements from rocks. *Environ. Exp. Bot.* 81: 26–36.

Lucero M.E., Unc A., Cooke P., Dowd S. & Sun S. 2011. Endophyte Microbiome Diversity in Micropropagated *Atriplex canescens* and *Atriplex torreyi* var *griffithsii*. *PLoS One* 6: 1–12.

Lugtenberg B. 2015. Life of Microbes in the Rhizosphere. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 7–15.

Mäki A., Rissanen A.J. & Tiirola M. 2016. A practical method for barcoding and size-trimming PCR templates for Amplicon sequencing. *Biotechniques* 60: 88–90.

Mäkilä M. & Saarnisto M. 2008. Carbon accumulation in boreal peatlands during the holocene – impacts of climate variations. In: *Peatlands and climate change*, pp. 24–43.

Mano H., Tanaka F., Nakamura C., Kaga H. & Morisaki H. 2007. Culturable endophytic bacterial flora of the maturing leaves and roots of rice plants (*Oryza sativa*) cultivated in a paddy field. *Microbes Environ.* 22: 175–185.

Marcos F.C.C., Iório R. de P.F., Silveira A.P.D. da, Ribeiro R.V., Machado E.C. & Lagôa A.M.M. de A. 2016. Endophytic bacteria affect sugarcane physiology without changing plant growth. *Bragantia, Campinas* 75: 1–9.

Mercado-Blanco J. 2015. Life of Microbes Inside the Plant. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 25–32.

Mercier J. & Lindow S.E. 2000. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Appl. Environ. Microbiol.* 66: 369–374.

Migahed F.F. & Nofel A.M. 2001. Leaf exudates of *Vicia faba* and their effects on *Botrytis fabae* and some associated fungi. *Mycobiology* 29: 198–204.

Mishra N. & Sundari S.K. 2015. Native PGPM Consortium: A Beneficial Solution to Support Plant Growth in the Presence of Phytopathogens and Residual Organophosphate Pesticides. *J. Bioprocess. Biotech.* 5: 1–8.

Mooney H.A. & Billings W.D. 1961. Comparative Physiological Ecology of Arctic and Alpine Populations of *Oxyria digyna*. *Ecol. Monogr.* 31: 1–29.

Müller D.B., Vogel C., Bai Y. & Vorholt J.A. 2016. The Plant Microbiota: Systems-Level Insights and Perspectives. *Annu. Rev. Genet.* 50: 211–234.

Murashige T. & Skoog F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* 15: 473–497.

Nadelhoffer K.J., Giblin A.E., Shaver G.R. & Linkins A.E. 1992. Microbial Processes and Plant Nutrient Availability in Arctic Soils. In: Chapin III F.S., Jefferies R.L., Reynolds J.F., Shaver G.R., Svoboda J. & Chu E.W. (eds.), *Arctic Ecosystems in a Changing Climate: an Ecophysiological Perspective*, Academic Press, pp. 281–300.

Nautiyal C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* 170: 265–270.

Nelson E.B. 2004. Microbial Dynamics and Interactions in the Spermosphere. *Annu. Rev. Phytopathol.* 42: 271–309.

Nissinen R.M., Männistö M.K. & van Elsas J.D. 2012. Endophytic bacterial communities in three arctic plants from low arctic fell tundra are cold-adapted and host-plant specific. *FEMS Microbiol. Ecol.* 82: 510–522.

Oliveira V., Gomes N.C.M., Santos M., Almeida A., Lillebø A.I., Ezequiel J., Serôdio J., Silva A.M.S., Simões M.M.Q., Rocha S.M. & Cunha Â. 2017. Effects of the Inoculant Strain *Pseudomonas* sp. SPN31 *nah*+ and of 2-Methylnaphthalene Contamination on the Rhizosphere and Endosphere Bacterial Communities of *Halimione portulacoides*. *Curr. Microbiol.* 74: 575–583.

Orlikowska T., Nowak K. & Reed B. 2017. Bacteria in the plant tissue culture environment. *Plant Cell. Tissue Organ Cult.* 128: 487–508.

Partida-Martinez L.P. & Heil M. 2011. The Microbe-Free Plant: Fact or Artifact. *Front. Plant Sci.* 2: 1–16.

Pirttilä A.M., Laukkanen H., Pospiech H., Myllylä R. & Hohtola A. 2000. Detection of intracellular bacteria in the buds of Scotch pine (*Pinus sylvestris* L.) by *in situ* hybridization. *Appl. Environ. Microbiol.* 66: 3073–3077.

Poosakkannu A., Nissinen R. & Kytöviita M.-M. 2015. Culturable endophytic microbial communities in the circumpolar grass, *Deschampsia flexuosa* in a sub-Arctic inland primary succession are habitat and growth stage specific. *Environ. Microbiol. Rep.* 7: 111–122.

Puente M.E., Li C.Y. & Bashan Y. 2009. Rock-degrading endophytic bacteria in cacti. *Environ. Exp. Bot.* 66: 389–401.

Pühler A., Arlat M., Becker A., Göttfert M., Morrissey J.P. & O'Gara F. 2004. What can bacterial genome research teach us about bacteria-plant interactions? *Curr. Opin. Plant Biol.* 7: 137–147.

Pylro V.S., Roesch L.F.W., Morais D.K., Clark I.M., Hirsch P.R. & Tótola M.R. 2014. Data analysis for 16S microbial profiling from different benchtop sequencing platforms. *J. Microbiol. Methods* 107: 30–37.

Quambusch M., Brümmer J., Haller K., Winkelmann T. & Bartsch M. 2016. Dynamics of endophytic bacteria in plant *in vitro* culture: quantification of three bacterial strains in *Prunus avium* in different plant organs and *in vitro* culture phases. *Plant Cell. Tissue Organ Cult.* 126: 305–317.

Quambusch M., Pirttilä A.M., Tejesvi M. V., Winkelmann T. & Bartsch M. 2014. Endophytic bacteria in plant tissue culture: Differences between easy- and difficult-to-propagate *Prunus avium* genotypes. *Tree Physiol.* 34: 524–533.

Quinn A.J. 2008. Introduction to Arctic and Alpine Biomes. In: *Arctic and Alpine Biomes*, pp. 1–32.

Reddy N.R., Sathe S.K. & Salunkhe D.K. 1982. Phytates in legumes and cereals. *Adv. Food Res.* 28: 1–92.

Reinhold-Hurek B. & Hurek T. 2011. Living inside plants: bacterial endophytes. *Curr. Opin. Plant Biol.* 14: 435–443.

Reiter B., Pfeifer U., Schwab H. & Sessitsch A. 2002. Response of Endophytic Bacterial Communities in Potato Plants to Infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl. Environ. Microbiol.* 68: 2261–2268.

Robbins J.A. & Matthews J.A. 2009. Pioneer vegetation on glacier forelands in southern Norway: emerging communities? *J. Veg. Sci.* 20: 889–902.

Robinson R.J., Fraaije B.A., Clark I.M., Jackson R.W., Hirsch P.R. & Mauchline T.H. 2016. Endophytic bacterial community composition in wheat (*Triticum aestivum*) is determined by plant tissue type, developmental stage and soil nutrient availability. *Plant Soil* 405: 381–396.

Rosenberg E., Zilber-Rosenberg I., Manwani D., Mortha A., Xu C., Faith J., Burk R., Kunisaki Y., Jang J. & Scheiermann C. 2016. Microbes Drive Evolution of Animals and Plants: the Hologenome Concept. *MBio* 7: e01395-15.

Rosenblueth M. & Martínez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. *Mol. plant-microbe Interact.* 19: 827–837.

Russell R.S. 1948. The Effect of Arctic and High Mountain Climates on the Carbohydrate Content of *Oxyria digyna*. *J. Ecol.* 36: 91–95.

Ryan R.P., Germaine K., Franks A., Ryan D.J. & Dowling D.N. 2008. Bacterial endophytes: Recent developments and applications. *FEMS Microbiol. Lett.* 278: 1–9.

Schreiter S., Eltlbany N. & Smalla K. 2015. Microbial Communities in the Rhizosphere Analyzed by Cultivation-Independent DNA-Based Methods. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 289–298.

Schulz S., Brankatschk R., Dümig A., Kögel-Knabner I., Schloter M. & Zeyer J. 2013. The role of microorganisms at different stages of ecosystem development for soil formation. *Biogeosciences* 10: 3983–3996.

Schutte U.M., Abdo Z., Bent S.J., Williams C.J., Schneider M.G., Solheim B. & Forney L.J. 2009. Bacterial succession in a glacier foreland of the High Arctic. *ISME J.* 3: 1258–1268.

Sheng H.M., Gao H.S., Xue L.G., Ding S., Song C.L., Feng H.Y. & An L.Z. 2011. Analysis of the composition and characteristics of culturable endophytic bacteria within subnival plants of the Tianshan Mountains, Northwestern China. *Curr. Microbiol.* 62: 923–932.

Shishido M., Breuil C. & Chanway C.P. 1999. Endophytic colonization of spruce by plant growth-promoting rhizobacteria. *FEMS Microbiol. Ecol.* 29: 191–196.

Sood S., Rahi P., Thakur R., Chauhan S. & Chawla I. 2011. Diversity Analysis of Diazotrophic Bacteria Associated with the Roots of Tea (*Camellia sinensis* (L.) O. Kuntze). 21: 545–555.

Souza C.P., Burbano-Rosero E.M., Almeida B.C., Martins G.G., Albertini L.S. & Rivera I.N.G. 2009. Culture medium for isolating chitinolytic bacteria from seawater and plankton. *World J. Microbiol. Biotechnol.* 25: 2079–2082.

Spaepen S. 2015. Plant Hormones Produced by Microbes. In: Lugtenberg B. (ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, Springer, pp. 247–256.

Strullu-Derrien C., Kenrick P., Pressel S., Duckett J.G., Rioult J.P. & Strullu D.G. 2014. Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant-fungus symbioses. *New Phytol.* 203: 964–979.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6 . 0. *Mol. Biol. Evol.* 30: 2725–2729.

Tarnocai C., Canadell J.G., Schuur E.A.G., Kuhry P., Mazhitova G. & Zimov S. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem. Cycles* 23: 1–11.

Truyens S., Weyens N., Cuypers A. & Vangronsveld J. 2015. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environ. Microbiol. Rep.* 7: 40–50.

Turner T.R., James E.K. & Poole P.S. 2013. The plant microbiome. *Genome Biol.* 14: 209.

Vaïtilingom M., Attard E., Gaiani N., Sancelme M., Deguillaume L., Flossmann A.I., Amato P. & Delort A.M. 2012. Long-term features of cloud microbiology at the puy de Dôme (France). *Atmos. Environ.* 56: 88–100.

Verma S.C., Singh A., Chowdhury S.P. & Tripathi A.K. 2004. Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter. *Biotechnol. Lett.* 26: 425–429.

Versalovic J., Schneider M., Bruijn F.J. de & Lupski J.R. 1994. Genomic Fingerprinting of Bacteria Using Repetitive Sequence-Based Polymerase Chain Reaction. *Methods Mol. Cell. Biol.* 5: 25–40.

Vorholt J.A. 2012. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* 10: 828–840.

Wagner M.R., Lundberg D.S., Rio T.G. del, Tringe S.G., Dangl J.L. & Mitchell-Olds T. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat. Commun.* 7: 1–15.

Wang Q., Garrity G.M., Tiedje J.M. & Cole J.R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73: 5261–5267.

Wellman C.H., Osterloff P.L. & Mohiuddin U. 2003. Fragments of the earliest land plants. *Nature* 425: 282–285.

Wilson D. 1995. Endophyte: The Evolution of a Term, and Clarification of Its Use and Definition. *Oikos* 73: 274.

Wolf J. van der & Boer S.H. De. 2015. Phytopathogenic Bacteria. In: *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*, pp. 65–77.

Yuan J., Chaparro J.M., Manter D.K., Zhang R., Vivanco J.M. & Shen Q. 2015. Roots from distinct plant developmental stages are capable of rapidly selecting their own microbiome without the influence of environmental and soil edaphic factors. *Soil Biol. Biochem.* 89: 206–209.

Zheng D., Raskin L., Elm E.W. & Stahl D.A. 1996. Characterization of universal small-subunit rRNA-targeted oligonucleotide hybridization probes. *Appl. Environ. Microbiol.* 62: 4504–4513.

Zilber-Rosenberg I. & Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiol. Rev.* 32: 723–735.

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