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Soil microbial communities in agroecosystems and natural habitats contributing to resistance and resilience of the soil environment

Půdní mikrobiální společenstva přispívající k rezistenci a resilienci půdního prostředí v agroekosystémech a na přírodních stanovištích

Ph.D. Thesis

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Declaration

The thesis was conducted at the Laboratory for Epidemiology and Ecology of Microorganisms, Crop Research Institute in Prague from 2012 to 2019.

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Obsah

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Summary

The control of common scab of potatoes (CS) includes resistant varieties (cultivars), precise fertilization, increase of soil moisture, and chemical treatments. Yet, these management practices do not have common or reproducible results at differing sites. A monitoring study was done in 32 sites to evaluate the relation between CS and biological/chemical soil parameters. Correlations were observed between scab severity and content of nutrients such as Fe, N, and Ca in soil and periderm, and between disease severity and abundance of actinobacteria and total bacteria, together with the pathogenicity determinant, *txtB* gene (biosynthetic gene of thaxtomin) in both soil and periderm of potatoes. The findings led to novel conclusions, which can help to understand relationships applicable in scab control.

Peat and DTPA chelated iron were supplemented to pots filled with soil conducive for CS in order to determine the effects of soil organic matter, iron and pH on CS development. The results were compared with data obtained for a suppressive soil from a nearby field with naturally low CS severity. Both peat and iron supplements decreased CS and the combination of the two supplements reduced CS the most effectively. Moreover, the bacterial community changed towards its composition in the suppressive soil after the combined peat and iron treatment.

To assess cultivar resistance × soil suppressiveness interactions, one resistant and one susceptible cultivar were grown in conducive and suppressive fields. The results showed that communities of bacteria, archaea and micro-eukaryotes differed between resistant and susceptible cultivar and between suppressive and conducive soil. In bacteria, cultivar effects were the most important and highest diversity was found in tuberosphere of the resistant cultivar. In archaea and micro-eukaryotes, differences were between suppressive and conducive soils.

Interactions between 21 actinobacterial strains isolated from potato rhizosphere and the pathogen (*Streptomyces scabiei*) were studied *in vitro.* The results showed that several strains could suppress *S. scabiei* in vermiculite media and may be further tested as soil inoculants for biological suppression in fields.

In conclusion, the studies demonstrated how potato plants and soil microbial communities interact in CS control and it was showed that the plant – soil interfaces (tuberosphere, rhizosphere) are the most important compartments for further CS studies. The plant-microbe interaction is influenced by the properties of both soil and

cultivar. Therefore, nutrient supplementation, and choice of resistant cultivar or suppressive soil can be used as an accessible way to suppress the CS.

Souhrn

Regulace obecné strupovitosti brambor (CS) zahrnuje rezistentní odrůdy (kultivary), hnojení, zvyšování vlhkosti půdy a chemická ošetření. Přesto tyto postupy nedosahují na různých lokalitách obdobných nebo reprodukovatelných výsledků. Na 32 lokalitách byla provedena monitorovací studie za účelem vyhodnocení vztahu mezi CS a biologickými / chemickými parametry půdy. Byly pozorovány korelace mezi závažností onemocnění a obsahem živin Fe, N a Ca v půdě a peridermu, a mezi závažností onemocnění a množstvím aktinobakterií a celkových bakterií, spolu s determinantem patogenity, genem *txtB* (biosyntetický gen thaxtominu), rovněž v půdě i v peridermu brambor. Tato zjištění vedla k novým závěrům, které mohou pomoci porozumět vztahům využitelným při kontrole strupovitosti.

Přídavky rašeliny a železa chelatovaného DTPA do půdy s vysokým výskytem CS (konduktivní) byly použity v nádobovém pokusu pro zjištění účinků půdní organické hmoty, železa a pH na rozvoj CS. Výsledky byly porovnány s údaji získanými pro půdu z blízkého pole s přirozeně nízkým výskytem CS (supresivní). Přídavky rašeliny i železa snižovaly CS, a kombinace těchto dvou ošetření snižovala závažnost onemocnění nejúčinněji. Kromě toho jejich kombinace způsobovala změnu složení bakteriálního společenstva směrem k jeho složení v půdě s přirozeně nízkým výskytem onemocnění.

Pro hodnocení interakcí mezi rezistencí kultivaru a supresivitou půdy byly pěstovány jeden rezistentní a jeden citlivý kultivar na polích s konduktivní a supresivní půdou. Výsledky ukázaly, že společenství bakterií, archaea a mikroeukaryot se lišila mezi rezistentním a citlivým kultivarem i mezi konduktivní a supresivní půdou. U bakterií byly nejvýznamnější účinky kultivaru a nejvyšší diverzita byla zjištěna v tuberosféře rezistentního kultivaru, zatímco u archaea a mikroeukaryot byly nalezeny rozdíly mezi konduktivní a supresivní půdou.

Interakce mezi 21 aktinobakteriálními kmeny izolovanými z rhizosféry brambor a patogenem (*Streptomyces scabiei*) byly studovány in vitro. Výsledky ukázaly, že několik kmenů bylo schopno potlačit *S. scabiei* ve vermikulitových médiích a mohou být dále testovány jako půdní inokulanty pro biologickou ochranu na polích.

Závěrem tyto studie prokázaly, jak rostliny brambor a půdní mikrobiální společenstva interagují při regulaci CS, a ukázalo se, že rozhraní rostlina - půda

(tuberosféra, rhizosféra) jsou nejdůležitějšími kompartmenty pro další výzkum CS. Interakce rostlin a mikroorganismů je ovlivněna vlastnostmi půdy i kultivaru. Z tohoto důvodu lze jako proveditelný způsob potlačení onemocnění obecnou strupovitostí použít dodání živin a výběr rezistentního kultivaru nebo supresivní půdy.

List of Abbreviations

ANOVA	**an**alysis **o**f **va**riance
b	**b**ulk
CS	**C**ommon **S**cab,
CTAB	**c**etyl **t**rimethyl **a**mmonium **b**romide
EDTA	**e**thylene**d**iamine**t**etraacetic **a**cid
G	**G**auze
H	**h**igh
L	**l**ow
P	**p**eat
P	**p**hosphorus
PCA	**p**rincipal **c**omponent **a**nalysis
PCR	**p**olymerase **c**hain **r**eaction
qPCR	**q**uantitative **p**olymerase **c**hain **r**eaction
r	**r**hizosphere
txtB	one of the genes encoding the first step in thaxtomin biosynthesis

1. Aims of the thesis

The aim of the Ph.D. Thesis was to explore the mutual interaction between soil chemical conditions and microbial community in the development or suppression of CS, and to evaluate the interaction of the soil environmental factors with potato variety in shaping the community inhabiting potato rhizosphere.

Consequently, specific aims included (i) broad scale screening of soil chemical properties and potato-associated bacterial communities covering maximum variability of soil types and environmental conditions, (ii) evaluation of impact of added peat with and without available iron on the disease development as well as on soil pH and communities of total bacteria and actinobacteria community in potato tuberosphere and periderm, (iii) disentangling the relative effects of soil suppressiveness and resistance of potato cultivar on the structure of microbial communities (bacteria, archaea, and micro-eukarytes) in the soil in contact with potato tubers, and (iv) developing a system suitable for *in vitro* assessment of antagonistic properties of isolated bacterial strains towards the potato common scab pathogens for selection of potential antagonists for subsequent testing *in vivo* with potato plants to develop inocula for efficient biological control of potato common scab.

2. Introduction

2.1. Preface to potato common scab

Potatoes belong among the fourth most common crops in the world together with wheat, rice and corn. Also, potatoes have the highest rank of consumption among vegetables in the US (Brown *et al.,* 2010). It's a low-caloric, nutritious and healthy food, which provides most of the main nutrients to a diet (Potatoes production guideline, 2013). Common scab of potato (CS) is one of the four most serious potato diseases. It causes reduction of marketable yield and has an impact over the quality of potato based products (Callum Wilson, 1996). Since CS disease has a worldwide importance and potatoes are an essential crop, this disease has an economic impact, particularly on the exportability and marketability ratio of the crop. In order to be certified as "U.S.NO.1", potatoes must be scab-free and defined as "practically no skinning" which means that not more than 5% of tuber surface can be affected (Eckwall and Carl, 2000).

Potato scab is caused by three groups of microbes. Potato tubers can be infected by cercozoan *Spongospora subterranea*, which causes the powdery scab. Symptoms of powdery scab include small lesions in the early stages of the disease, progressing to raised pustules containing a powdery mass. These can eventually rupture within the tuber periderm (skin) (Hernandez *et al*., 2015). Serious erumpent or pitted corky symptoms characterize CS, while superficial reticulations are called russet or netted scab. The netted scab is caused by *Streptomyces aureofaciens*, occurs on stolons of potato and is reported in different areas in the world (Kreuze *et al.,* 1999). Russet scab and netted scab have, however, been shown to differ in several characteristics such as varieties susceptibility, root attack, and optimum soil temperature and are, therefore, considered to be different diseases and can probably be caused by several *Streptomyces* spp. (Scholte *et al.,* 1985).

In contrast, the CS drastically affects tuber quality because of superficial and pitted lesions that form around the site of infection on potato periderm. CS disease, which is caused by several *Streptomyces species*, is common in potato growing areas all around the world. Symptoms of CS disease emerge as superficial, raised or deep pitted, brown to dark-brown corky lesions on potato tubers. Lesions can turn up as single and isolated, round and coalesced (5-8 mm in diameter) or erumpent and

expand over the entire tuber surface. In some cases, lesions are also found on the roots and stolons. The CS can also be found in virgin fields. It is only during tuber initiation and enlargement that the potato plant is susceptible to CS infection because with the expansion of the newly developed tubers, the CS lesions develop and spread, but there is no additional disease development during harvesting and storage. Also, no above-ground symptoms are found (Potatoes production guideline, 2013). The same indications are also described on beet, carrot, parsnip, radish, rutabaga and turnip, where the symptoms appear as randomly distributed shallow, raised or deep-pitted corky lesions. Their size and color are quite variable, but lesions typically are brown with a diameter of a few millimeters (Lerat *et al.,* 2009; Bouchek *et al*., 1998).

2.2. Management strategies in use and the necessity of new studies

The irrigation (avoiding low soil moisture at tuber initiation) and decreased soil pH were shown traditionally to be useful for control of CS and were examined in various fields, however they were difficult and costly and, moreover, can also lead to conditions favorable for other diseases (van der Wolf *et al.*, 2007, Dees *et al*., 2012). Selection of disease-resistant potato varieties was also essayed, however, currently there is no available commercial potato variety completely resistant to CS (Larkin *et al*., 2017, Potatoes production guideline, 2013). Application of soil- and seed-applied pesticides were also examined; however, those methods took a lot of time and provided variable results (Braun *et al*., 2017; Dees *et al*., 2012; Bailey and Lazarovits, 2003). The fields were manipulated by introducing organic amendments and crop residue management, which showed desired results to some extent, but yet those approaches need more detailed development. The crop rotation with different plants such as red clover, mustard, rapeseed, sudangrass and rye followed by potato was assessed in previous studies (Honeycutt *et al*., 2017, Potatoes production guideline, 2013). Crop rotation provides multiple benefits to crop production, and can reduce soil-borne diseases that can demolish potato crops grown in consecutive years through a variety of mechanisms, including changes in soil microbial communities and it may be implemented as full-season harvestable crops, cover crops, or as green manures. Yet, different types of rotation crops have very different effects and their complex outcomes are not known (Larkin *et al.*, 2012). Finally, the effects of micronutrient

amendments to control of CS and the resulting impact of different potato varieties on bacterial community in favor of scab suppressiveness were shown but only in relatively small case studies (Klikocka, 2009; Mishra and Srivastava, 2004 and Kristufek *et al*., 2015).

Out of those practices, the cropping system strategy had considerable and lasting impacts on soil microbial community and soil borne diseases (Honeycutt *et al*., 2017). However, all the mentioned practices such as selection of resistant varieties, reducing seed-borne inoculum, modifying the soil pH, crop rotation, changing the temperature and time of harvest, adding chemical and biological amendments have provided results with high variability between years and places (Dees *et al*., 2012). Consequently, CS is still an unresolved problem in the world and needs more vast and comprehensive studies to find the suitable management.

2.3. Description and taxonomy of causal agents of CS

Over 900 species of *Streptomyces* have been described but only around a dozen of them are recognized as plant pathogens (Bignell *et al.*, 2010). The first known reference to CS of potatoes comes from 1825, but it was not initially thought to have a biological cause (Millard, 1923). Isolates of an organism that causes CS of potato were first isolated in 1890 and the primary strain was described as *Oospora scabies*. The original culture was not maintained (Lambert *et al.,* 2007, 1989). In 1914 the species *Actinomyces scabies*, was renamed, noting that *Oospora* was an incorrect classification since the disease was not caused by a fungus (Loria *et al.,* 1997*,* Gussow, 1914). *Streptomyces* genus was first proposed in 1943 (Loria *et al.,* 2003). Most species of *Streptomyces* are saprotrophic feeding off dead matter with relatively few being pathogenic. In 1948, the name *Streptomyces scabies* was used to describe the species and this name was revived in 1989 by Lambert and Loria, who bought together 12 different strains that formed one homogeneous group (Lambert *et al.,* 1989). In 1997, the name was changed to *Streptomyces scabiei* following a grammatical convention as set out in Rule 12c of the *International Code of Nomenclature of Bacteria* (Truper *et al.,* 1997). In 1979, Elesawy and Szabo proposed it be assigned to the Diastatochromogenes cluster along with *S. neyagawaensis*, *S. bottropensis*, *S. diastatochromogenes*, *S. eurythermus* and *S. griseosporeus*, which

was later confirmed by other authors based on morphological and genetic analyses (Bukhalid *et al.,* 2002).

The most widespread species other than *S. scabiei* are *S. turgidiscabies* and *S. acidiscabies*, which can be distinguished based on their morphology, the way they utilize carbon sources and their 16S rRNA sequences (Lerat *et al.* 2009). The most symptoms of CS: the superficial, raised or pitted lesions that form on tuber surfaces, are caused by the best characterized pathogenic streptomycetes, *Streptomyces scabiei, Streptomyces turgidiscabies*, and *Streptomyces acidiscabies* (Bignell *et al.,* 2010, Kreuze *et al*., 1999). *Streptomyces acidiscabies* tend to produce superficial lesions, whereas *S. scabiei* often produce raised and/or erumpent lesions. The phenotypic responses may be pathogen-specific (Hiltunen *et al*., 2005, Tashiro *et al*., 2012). In 2003, three other species of *Streptomyces* that cause CS symptoms were isolated and named *S. luridiscabiei*, *S. puniciscabiei* and *S. niveiscabiei* (Park *et al.,* 2003*,* Park *et al.,* 2003) *S. ipomoea* causes a similar disease on sweet potato tubers (Clark, 1987) Leiminger *et al.*, for the first time presented the pathogenic strains of *S. europaeiscabies and S. stelliscabies* (Leiminger *et al*., 2013). Finally, the relatedness between strains of *Streptomyces* pathogenic to potato was most recently described using a microarray including probes specific to genes of *S. scabiei* and *S. turgidiscabies*, pathogenic strains, classified as *Streptomyces* species based on morphological criteria, were subjected to comparative genomic hybridization (CGH) (Dees *et al*., 2012) Bouchek-Mechiche, *et al.,* has explained DNA relatedness between strains of *Streptomyces* pathogenic to potato using DNA-DNA hybridization, 16S rRNA sequence and biochemical test (Bouchek *et al*., 2000).

There are various unique aspects of *Streptomyces* as a pathogen: Only the developing periderm of underground stems, stolons and tubers is susceptible to CS (Dees *et al*., 2012). The genes coding for biosynthesis of pathogenicity determinants causing CS are carried on a pathogenicity island (PAI). Lateral transfer of pathogenicity genes among different scab-causing streptomycetes lead to emergence of novel pathogenic species in various agricultural systems (Hogenhout and Loria, 2008, Loria *et al*., 2003). It is suggested that for a successful root infection, the ability to penetrate plant tissue is important as there are a few natural openings in roots. Thaxtomin, a phytotoxin which is produced by plant pathogenic *Streptreptomyces* causing CS seems to aid in the penetration of developing plant tissues by preventing

the development of primary cell wall (Loria *et al*., 2003). Cellobiose and cellotriose of plant cells, incite thaxtomin production of pathogen. Pathogens causing scab upregulate the production of thaxtomin in response to cellobiose and cellotriose unleashed from thaxtomin-sensitive growing plant tissue (Johnson *et al*., 2007).

2.4. Thaxtomin and other pathogenicity determinants

Pathogenic *Streptomyces* spp. produce "thaxtomin A" - a cyclic dipeptide with a nitrated tryptophan moiety composed of 4-nitroindol-3-ylcontaining 2,5-dioxopiperazines, which is the most known pathogenicity determinant that suppresses cellulose synthesis in spreading plant tissues. However, the mechanism and complete pathway of its biosynthesis has not yet been defined. Thaxtomin A is produced by the action of two non-ribosomal peptide synthetase modules (*txtA* and *txtB*) and a supplement of modifying enzymes (Johnson *et al*., 2007, 2009). Increased resistance of potatoes to CS is related to the reduced sensitivity to thaxtomin (Nathalie *et al*., 2013). Hiltunen *et al.* proposed an elimination of CS sensitive progeny from a potato breeding population using thaxtomin A as a selective agent in the field (Hiltunen *et al.,* 2011).

The production of thaxtomin increases in response to cellobiose, a plant cell wall component, and occurs at the host-pathogen interface indicating induction by host signals. Besides cellobiose, thaxtomin production is stimulated by oat bran broth proposing that specific carbohydrates may affect thaxtomin biosynthesis. Oat bran includes high grades of xylans and glucans as well as xylans and these carbohydrates stimulated thaxtomin A production. Consequently it was concluded that complex carbohydrates function as environmental signals to plant pathogenic *Streptomyces,* which allows thaxtomin production and enables bacteria to colonize its host (Wach *et al*., 2007).

The two other important pathogenic determinants are *nec1* encoding a protein inducing necrosis in plant tissue, and *tomA* encoding a virulence factor, which is homologous to tomatinase, found in plant pathogenic fungi, proposed to have a role in the interaction of plant-microbe (Bukhalid et al. 1998; Seipke and Loria 2008, Ryan *et al*., 2008, Joshi *et al*., 2007). There is a direct relation between *nec1* gene copy number

in *Streptomyces scabiei* detected by q-PCR and occurrence of CS disease (Koyama *et al*., 2006).

The *txtA, txtB*, *tomA*, and *nec1*, genes in 325–660 kb of large mobile region PAI (pathogenic island) were detected in streptomycete isolates, capable of inducing necrosis on tuber disks and stunting of radish seedling. Among them, 50% of isolates included all the four pathogenicity genes, 33% had an atypical combination of PAI marker genes and 17% did not include any of the genes and there were no hits for any of the virulence factors among the genomes. The missing of the thaxtomin biosynthetic genes (*txtA, txtB*) was confirmed by whole-genome sequencing of two representative strains of this group. These results suggest a participation of other virulence factors in pathogenicity mechanism of some strains causing CS (Lapaz *et al.,* 2017).

To detect the other virulence factors, Joshi (2007) evaluated differential expression of *ipt* located in *fas* locus, that is an isopentenyl transferase and the key enzyme for cytokinin (phytohormones that promote cell division) biosynthesis in phytopathogenic bacteria, which identified by sequence homology and by biochemical approach. Inactivation of the *fas* locus, which is directly involved in cytokinin production, leads to a complete loss of phytopathogenicity (Crespi et al., 1992). Interestingly, cellobiose upregulates the *ipt* gene. Besides the thaxtomin, there are other secondary metabolites (organic compounds produced by bacteria, aid a host in important functions such as protection, competition, and species interactions), which are generated by various phytopathogenic bacteria such coronafacoyl as a phytotoxin (an important family of plant toxins) that are also produced by potato scab pathogen *Streptomyces scabiei* (Bown *et al.,* 2016).

Concanamycin is a microbial secondary metabolite, which acts as inhibitor of ATPases in prokaryotic and eukaryotic cells (Drose and Altendorf, 1997), and has weak necrosis-inducing activity (Natsume *et al*., 2017). Another phytotoxin which was defined as a 16-membered macrolide, FD-891 produced by *Streptomyces species*, can also induce apoptosis in human cells (Natsume *et al*., 2005). In addition, *Streptomyces scabiei* produces a molecule, which is previsied to resemble the *Pseudomonas syringae* coronatine (a multifunctional phytotoxin) and such contributes to the development of seedling disease symptom. Other identified *Streptomyces* phytotoxic secondary metabolites include borrelidin, (an 18-membered polyketide macrolide shows

antibacterial activity by acting as an inhibitor of threonyl-tRNA synthetase), which caused deep-pitted lesions (Natsume *et al*., 2017). The genome sequence of *S. scabiei* 87-22 was recently completed, and comparative genomic analyses with other sequenced microbial pathogens have demonstrated the appearance of additional genes that may take part in plant pathogenicity. Such analyses led to the identification of 4259 protein-coding genes that are absent from the nonpathogenic species and are either shared among two or more pathogenic species or are unique to a specific pathogen. Several genes were identified that are predicted to encode secreted proteins, transcriptional regulators, membrane transporters, and enzymes for secondary metabolite biosynthesis, which could potentially contribute to pathogenicity in these organisms (Bignell *et al*., 2010).

2.5. Horizontal transfer of mobile genetic elements

The pathogenic elements of CS can be mobilized and transferred to nonpathogenic relatives, during conjugation (transfer of genetic material between bacterial cell), which leads to appearance of new pathogenic streptomycetes (Loria *et al*., 2006, Kers *et al*., 2005). *Streptomyces* sp. strain 96-12 harbors the PAI that is almost identical to the PAI in *S. scabiei* 87-22, despite noticeable variations in their genome sequences. That proposed a direct or indirect *in vivo* transfer of the PAI among *S. scabiei* and nonpathogenic *Streptomyces* species. *S. scabiei,* the earliest qualified *Streptomyces* pathogen, could be the source of a PAI responsible for the appearance of novel pathogenic species in various areas (Zhang *et al*., 2016). In the pathogenicity island, many mobile genetic elements were revealed by a partial DNA sequence of this PAI (Zhang *et al*., 2016). In *S. turgidiscabies*, two regions were reported on PAI: ‘colonization region’ (CR) and ‘toxicogenic region’ (TR) which may undergo independent evolution (Aittamaa *et al*., 2010). In *Streptomyces scabiei* the thaxtomin biosynthetic cluster is placed within a mobile and TR divided into two sub regions TR1 and TR2. The compound pathogenicity island (PAI) formed by TR1 and TR2 is dynamic and unstable in newly described pathogenic species (Zhang and Loria, 2010).

2.5.1. The first region on PAI

The first region is called the 'toxicogenic region' with GC content of 68%. All genes defined to be associated with thaxtomin biosynthesis are found in this region. The region which can be transfered, contains coding genes: *txtAB*, *txtC, nos* and *txtR* (an *AraC/XylS* family member of transcriptional regulators) and they code for a nonribosomal peptide synthase (cyclization of the dipeptide), a cytochrome P450 monooxygenase (hydroxylation of the cyclic dipeptide), a nitric oxide synthase (nitration of the tryptophan moiety is essential for the toxicity of thaxtomins) and a cellobiose-binding regulatory protein, respectively (Joshi and Loria, 2007). Virulence was almost removed in the *txtR* deletion mutant of *S. scabiei* (Joshi *et al*., 2007).

The *S. scabiei* 87.22 genome was analyzed in order to survey the potential mobility of the TR. Attachment sites (*att*), short homologous sequences that delineate integrative and conjugative elements (ICEs), were defined at both extremities of the toxigenic region. An internal *att* site was also discovered, proposing that the toxigenic region has a mixture structure consisting of two regions TR1 and TR2. Thaxtomin biosynthetic genes, important for pathogenicity, were established in TR1, while candidate genes potentially acting in recombination, replication and conjugal transfer were discovered in TR2. Excision of the TR1 or TR2 subregions or of the whole TR region was perceived, however, the excision frequency of TR was low. Though, the excision frequency was significantly enhanced in the presence of either mitomycin C or *Streptomyces coelicolor* cells. A composite TR structure was not perceived in all *S. scabiei* and *S. acidiscabies* strains tested. From ten strains analyzed seven missed TR2 and no TR excision event could be recognized in these strains, thus proposing the implication of TR2 in the mobilization of *S. scabiei* was not conclusive (Chapleau *et al*., 2016).

2.5.2. The second region on PAI

The second section of the PAI, which is called the 'colonization region' contains genes coding for necrotization factor (*nec1*) and tomatinase A (*tomA*), which are not essential to pathogenicity but play an important role in virulence. Nec1 and TomA participate in infection through the appeasement of plant defenses (Lerat *et al*., 2009). The *nec1* and a neighboring transposase pseudogene, ORF *tnp*, are absent from nonpathogens but conserved among pathogenic streptomycetes. An atypical high GC

content and an IS element (insertion sequence: a short DNA sequence that acts as a simple transposable element) adjacent to the 3' end of *nec1* in *Streptomyces scabiei* propose that it was acquired through horizontal transfer events (Healy *et al*., 1999). There are four open reading frames demonstrating similarities with transposes observed in close vicinity of the *nec1* gene. As extracellular esterase activity was measured in *S. scabiei* and some esterases are thought to play a role in penetration in periderm by degradation of potato suberin (an inert impermeable waxy substance present in the cell walls of corky tissues) (Lerat *et al*., 2009).

Since previous studies have demonstrated high sequence conservation of the PAI-associated *nec1* gene and *Streptomyces* PAI is expected to be horizontally transferred as a unit, it could be predicted that all identified PAI genes are harbored by *txtAB*+ isolates and species. Yet, many *txtAB*+ isolates missing the *nec1* gene have been proposed to be pathogenic. With an estimated size of more than 500 kbp, *Streptomyces* PAI is exceptionally large for a PAI, and the *nec1* and *txtAB* genes are placed at opposite ends of this long region. A saponinase like gene (*tomA*), also characteristic of the PAI, evidently lies outside of *nec1*, in even more distant region from *txtAB*. These two genes were traced in tomatinase genes from the putative pathogenicity island were missed by some pathogenic isolates; several missed the *nec1* gene, and one was lacking the most reliable pathogenicity determinant *txtA* gene, which encodes thaxtomin biosynthesis. Variations in disease symptoms and intensity composed with lack of known pathogenicity determinants (*txtA*) or factors (*nec1*) propose that there may be pathogenicity factors in addition to thaxtomin (Wanner, 2004; Wanner, 2009). *Streptomyces scabiei* 87-22 possess a functional tomatinase with high homology to the gene encoding tomatine-detoxifying enzyme tomatinase recognized in fungal tomato pathogens. Yet, tomatinase is not essential in pathogenicity on tomato plants but conservation of *tomA* on pathogenicity island in *S. acidiscabies* and *S. turgidiscabies* proposes a role in the plant-microbe interaction (Ryan et al., 2008). Partial *tomA* gene sequence did not vary among *tomA*+ *Streptomyces* spp. isolates and strains in contrast to *txtA* and *txtC* gene sequences suggesting that this gene is highly conserved among *Streptomyces* spp/ inducing CS (St-Onge et al., 2011).

There is a wide genetic variation of PAIs between strains of *S. turgidiscabies* and the reigion of the pathogenicity island, which indicated that PAI is made up of a

mosaic of regions which may undergo independent evolution (Aittamaa *et al*., 2010). Disease occurrence and variety differ among places and years; that is due in part to diversity in the environment (weather) and genetic shifts in potato varieties (Wanner *et al*., 2006). In the US some pathogenic isolates missed one or more genes characteristic of the PAI, however all had genes for biosynthesis of the pathogenicity determinant thaxtomin.

2.6. Research methodology to study CS

2.6.1. Phenotypical methods

There are many phenotypical and molecular methods to identify potato common scab agents in soil and infected tubers. Streptomycetes are found in soil everywhere in large amounts (10^6 to 10^7 colony forming units -CFU- per gram of soil). They are easily identified in culture isolation due to specified morphology.

The organism inducing CS was characterized by creamy colonies on yeast malt extract (YMA) and also by aerial mycelium, which turned brown in time. The organism is Gram positive, non-motile, utilizes L-arabinose, D-fructose, D-glucose and rhamnose. It reduces xylose and starch by producing melanin on peptone yeast extract agar-iron (PYI). And the type strain of *S. scabiei* could be characterized by morphology of spores born in spiral chains (sporophores), production of melanin pigment on tyrosin-containing medium (peptone iron agar), and utilization of all the diagnostic sugars recommended by the International *Streptomyces* Project (ISP) for identification of *Streptomyces* spp. *S. scabiei* does not grow at pH below 4.5 (Bencheikh and Setti, 2007).

Isolates of streptomycetes from scabby potato plants differ in morphology and pigmentation. Scab lesions vary in appearance and severity. So it is difficult to use phenotypic traits in order to differentiate pathogenic streptomycetes from nonpathogenic ones exactly. In return, none of the nonpathogenic isolates could be confused with *S. scabiei* regarding to their 16S rDNA sequences. This indicates that most of the saprophytic streptomycetes preventing CS lesions belong to species other than *S. scabiei* (Doumbou *et al*., 2001).

Using the methods described before (Wenzl and Demel, 1967; Rasocha *et al*., 2006) Occurrence of tuber diseases was studied immediately after the washing and

during potato storage. From diseases, presence of CS was evaluated on a 9-point scale, where 1 is the health tuber and 9 is very strong infection (1: no scab, 3: skin scabbed to 10% of tuber surface, slight scabbed, 5: skin scabbed on 11–20% of tuber surface, intermediate scabbed, 7: skin scabbed on 21–50% of tuber surface, strong scabbed and 9: skin scabbed on more than 50% of tuber surface, very strong scabbed).

Andrade *et al*., 2019 proposed a Standard area diagrams (SADs) were developed to aid the experimental visual assessment of common scab severity in potato tubers, increasing the accuracy, precision and reliability of disease severity estimates more than other methods evaluated. To create SADs, images were obtained of tubers inoculated used to define the intervals between CS classes. The evaluators recognized and delimited disease symptoms and located severity values between the SAD intervals. The CS severity of total images in the SADs varied from 0% to 96% and exhibited patterns typical of the disease, from small initial lesions to coalesced lesions covering the entire tuber surface (Andrade *et al*., 2019)

The analysis of cellular fatty acid profiles was used in order to distinguish among introduced pathogen- suppressive strains and indigenous strains of *Streptomyces* spp. (Bowers *et al.,* 1996). The studies showed significant differenses in the level of some fatty acids in the pathogenes compared to suppressive strains. Pathogenic strains had significantly smaller quantities of the fatty acids: 15:0 *anteiso*, 17 *anteiso* and 17:1 *anteiso* C and significantly greater quantities of the fatty acids 14:0 *iso*, 16:0 *iso* and 16:1 *iso* H compared to the suppressive strains (Ndowora *et al*., 1995, Paradis *et al*., 1994).

2.6.2. Molecular methods

Identification of species is not possible based on morphology of pathogenic *Streptomyces* (mycelium colour, sporulation and pigmentation) (Flores-González *et al*., 2008), therefore several molecular methods were used to survey of *Streptomyces* strains isolated from scab lesions and infected soils.

The usefulness of a whole-genome oligonucleotide microarray was demonstrated (citation of the Norwegian paper). Whole-genome microarray analysis, based on 12 766 probes designed for 8848 predicted open reading frames (ORFs) of *S. scabiei*, showed that the 14 strains were different from *S. scabiei.* They were subsequently identified to be *S. europaeiscabies* based on the internal transcribed

spacer (ITS) sequences of the rRNA genes. That was the first report of the occurrence of *S. turgidiscabies* and *S. europaeiscabies* in Norway. The putative 762 genes exhibiting the highest sequence differences between strains of *S. europaeiscabies* and *S. scabiei* according to microarray analysis were concentrated in relatively few gene ontology (GO) categories, including 'symbiosis and mutualism through parasitism', 'cell death' and 'responses to biotic stimulus', whereas genes related to primary metabolism appeared to be more conserved. Microarray data and 16S rRNA gene phylogeny showed, consistently, that there were two genetically distinguishable groups of *S. europaeiscabies* on the basis of differences in 131 genes. The results provide novel information about the genetic variability of *S. europaeiscabies* and the gene-specific variability between the genomes of *S. europaeiscabies* and *S. scabiei* (Dees *et al*., 2012).

The limited number of 16S rRNA gene sequences in databases or insufficient 16S rRNA gene polymorphism caused the incomplete coverage of actinomycetes by a genus specific probe (Kyselkova *et al*., 2008).

In another study a microarray tool was extended with probes focusing on CS pathogens. A 16S rRNA gene-based taxonomic microarray, representing 19 bacterial phyla at different taxonomic levels was used to assess soil samples from potato fields.Twelve probes targeting the genus *Streptomyces*, as well as *S. scabies* and relatives were added to the probe set (1033 probes). The samples from suppressive and conducive soils were significantly distinguished by the signals of 22 probes. The 22 probes targeting various bacterial taxa discriminated between suppressive and conducive soils, and 65 probes did between resistant and susceptible cultivars. Signals of probes targeting the CS pathogen were detected in the tuberosphere of the susceptible cultivar grown in conducive soil only. The results showed that microbiome features differed when comparing suppressive vs conducive soil as well as resistant vs susceptible cultivar (Kopecky *et al*., 2019: in review)

A DNA microarray (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Each DNA spot contains a specific DNA sequence, known as a probe. Probes are a short section of a gene or other DNA element used to hybridize a DNA or RNA target. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid

sequences in the target (Taub, 1983). Many bacterial pathogens blackleg and soft rot (*Pectobacterium atrosepticum, Pectobacterium carotovorum,* and *Dickeya* spp.), ring rot (*Clavibacter michiganensis* subsp. *sepedonicus*), scab (*Streptomyces scabiei* and *Streptomyces turgidiscabies*) and brown rot (*Ralstonia solanacearum*), which could infect potato directly from tuber instances were readily detected by specific microarrays. However, the rate of microarray's analytical sensitivity under the tested situation was not enough to determine bacteria directly from tubers. As a result, in addition to the cost and organizational complications, for establishing the platform for routine detection of potato bacterial pathogens from tuber samples was not recommended (Degefu *et al*., 2016). Another method was provided, which uses the "Biotype-100" identification system for description of *Streptomyces* strains based on the assimilation of carbon sources (Bouchek-Mechiche *et al*., 1998). The PCR (Polymerase chain reaction, a method used to make many copies of a specific DNA segment) is used to show relation between *Streptomyces* spp. (Keinath *et al*., 1989; Lazarovits *et al*., 2007). A PCR-based diagnostic method was developed to amplify a fragment of the *txtAB* (*txtA* and *txtB*) genes, which are the most reliable pathogenicity determinants in the primary pathogenic *Streptomyces* species, together with carbon source utilization and repetitive BOX profiles in order to directly detect tuber lesions of pathogenic *Streptomyces* causing CS (Flores-González *et al*., 2008). The pathogens were identified by PCR using 16S rRNA-specific primers and PCR-RFLP of the 16S–23S internal transcribed spacer (ITS) region with Hpy99I at the genetic level. Pathogenic strains of *S. europaeiscabies, S. stelliscabies, S. acidiscabies, S. turgidiscabies* and *S. bottropensis* reported and *Streptomyces europaeiscabies* was the predominant species found (Leiminger *et al*., 2013).

Composed real-time PCR (TaqMan) approach has been applied for the simultaneous determination and discrimination of potato powdery and CS diseases and pathogens. Based on the DNA sequence of the ribosomal RNA ITS2 region, real-time PCR primers and a probe for *Spongospora subterranea* were designed. Primers and a probe for pathogenic *Streptomyces* were designed on the basis of the DNA sequence of the *txtAB* genes. This multiplex real-time PCR is a rapid, cost efficient, specific and sensitive tool for the simultaneous determination and discrimination of the two pathogens on infected potato tubers when optical symptoms are indeterminate or not present (Xinshun *et al*., 2010). Also a SYBR Green quantitative real-time polymerase chain reaction (PCR) assessment was developed using primers designed to anneal to

the *txtAB* operon of *Streptomyces* in order to quantify pathogenic bacteria populations in potatoes and soil. The real-time PCR assessment was particular for pathogenic *Streptomyces* strains. Cycle threshold (Ct) values were linearly associated with the target DNA concentration (correlation coefficient R2 = 0.99) and were not influenced by the appearance of plant DNA extracts, demonstrating the efficiency of the assessment for quantitative analyses of the pathogenic bacteria in plant tissues. Using primers designed from the *txtAB* operon, this real-time PCR assessment enables rapid, precise, and cost effective quantification of pathogenic *Streptomyces* strains in potato tubers and in soil. 1 g of scab lesion tissue and also 1g of peel tissue was used for DNA extraction in regard to symptomatic tubers (Qu *et al*., 2008).

A new assay based on HybProbes chemistry for rapid and accurate determination of the CS pathogens was reported to create and validate a HybProbes-based real-time PCR assay aiming the *trpB* gene) a housekeeping gene involved in tryptophan biosynthesis) for specific determination of *Streptomyces scabiei* and *Streptomyces europaeiscabies.* Since the HybProbes chemistry needs two probes for positive determination, the assay is taken to be more specific than conventional PCR or TaqMan real-time PCR. The resulted assay is a useful tool with high potential in early diagnosis and identification of CS pathogens of potatoes in infected plants or for surveillance of potatoes grown in soil environment (Xu *et al.*, 2016).

A multiplex PCR with a specific primer set for detecting 16S rDNA and 16S-23S ITS regions (internal transcribed spacer) comprising of universally conserved regions, is a rapid and easy tool to detect of multiple species of potato scab pathogens in a vast range of environmental samples including the soil and plant tissues (Tagawa *et al*., 2008).

The *rpoB* gene based method can be applied in order to complement other genetic methods such as 16S rRNA gene analysis or DNA–DNA hybridization to phylogenetically differentiate *Streptomyces* spp related potato scab (Mum *et al*., 2007). Same as above, most likely it can discriminate streptomyces taxonomically but not functionally.

In conclusion, new diagnostic tools are offering the means for exact recognition and identification of pathogenic *Streptomyces* spp. These will now enable researchers to dissect the microbial interactions happening in soils with a view to

better understand the mechanisms of individual management measures and their efficient integration to agriculture practice.

2.7. Strategies for control of common scab

2.7.1. Chemical methods and environmental factors

2.7.1.1. Chemical treatments

Agrochemicals used in agricultural lands in order to protect plants from various diseases, may possess health and environmental side effects. Therefore, they represent a controversial environmental health risk factor (Stamati, 2016).

Chemical based treatments include: 2.4-dichlorophenoxyacetic acid (2,4-D), mercuric chloride, formaldehyde, copper sulphate, Burgundy mixture and boric acid. Application of 2.4-dichlorophenoxyacetic acid (2,4-D) has been demonstrated to be an efficient control method for CS, not directly by effect on pathogen, but via amelioration of thaxtomin A toxicity (Thompson *et al.*, 2013; Thompson *et al.*, 2013; Tegg *et al*., 2008).

Disinfection of seed tubers is done before planting by solutions of mercuric chloride, formaldehyde, copper sulphate, Burgundy mixture and proprietary organic mercury compositions. The most acceptable method was dipping of the tubers in solutions of proprietary organic mercurials (Cairns *et al*., 1936).

Boric acid solution and sulphur solution as seed treatments have been indicated to give considerable control of disease occurrence on potato CS. Boric acid treatment recorded the lowest disease occurrence and disease index, with greatest healthy and total tuber yield (Asif Ur *et al*., 2003; Gharate *et al*., 2016).

It seems that using 2,4-D, auxin, boric acid and organic mercurials are good choices of chemical treatments however the most chemicals lose effectiveness as the plant develops (Abram, 2009).

2.7.1.2. Effects of temperature and culture conditions

Culture conditions (pH, Ca^{2+}, posphate, and temperature) on thaxtomin production and aerial mycelium formation were examined for two strains of *S. scabies* and *S. acidiscabies*. Thaxtomin production was reduced at pH 7.6 and enhanced at 15°C in *S.*

scabiei and increased at 30mM phosphate in *S. acidiscabies.* The higher the pH, Ca^{2+} concentration and temperature was, aerial mycelium formation in *S. scabiei* was greater, whereas that in *S. acidiscabies* was unaffected (Natsume *et al*., 2001). Enhancing soil temperature increased toxicity, but toxicity was unaffected by soil texture or organic carbon content (Bailey and Lazarovits, 2003).

2.7.1.3. Soil pH

Potatoes are more resistant to low pH than most other crops. Occurrence of CS tends to be less of an issue where soil pH is lower than 5.4. The disease is often controlled by maintaining soil pH in the range of 5.0 to 5.4 for varieties that are susceptible to CS. Although, pathogenic streptomycetes such as *S. acidiscabies* are adapted to low soil pH so they are resistant to its decrease. Moreover, enhancing soil pH in not recommended because it will rapidly limit iron availability, which may increase disease susceptibility (Hoskins, 2012; Stead and Wale, 2004).

Thaxtomin production decreased at pH 7.6 and increased at 15°C in S. *scabiei* and increased at 30mM phosphate in *S. acidiscabies.* With higher pH, Ca^{2+} concentration and temperature aerial mycelium formation in *S. scabiei* was greater, while it was unaffected in *S. acidiscabies* (Natsume *et al*., 2001). Also, the effect on CS development appeared to be seasonal. However, the pH alone may not be a driver for the development of CS symptoms (Wiechel and Crump, 2010). It is worth mentioning that using peat leads to reducing soil pH and consequently leads to enhancing the availability and absorbance of iron.

In conclusion, probably CS decrease occurres at low concentration of exchangeable cations in soil. CS was not detected on potatoes grown in soil with composed exchangeable Ca, Mg and K at 12 cmolc/kg or less. A strong relation was found between soil pH and these exchangeable cations, particularly calcium (Lacey and Wilson, 2001).

2.7.1.4. Solarisation, irrigation and climate

Rainfall would seem to be the most important climatic factor, which changes the air content of the soil and therefore, controls the development of the CS pathogenic organisms, which are strongly aerobic. The effect is most marked in clay soils, where CSis almost totally inhibited by a wet season (Millard, 1923). *S. scabiei* causes more

disease with enhanced aerobic situation and temperature in soil, thus, keeping high soil moisture during the early stages of tuber formation by irrigation can be applied as a method for controlling CS caused by *S. scabiei* (Lapwood *et al*., 1973; Adams & Lapwood, 1978, Davies *et al*., 1976; Hiltunen *et al*., 2005). High soil moisture rates may protect the pathogen from colonizing because of increase inaccessibility of antagonist species or competition from other microorganisms. Therefore, irrigation at the beginning of tuber formation is often an efficient control measure (Eckwall and Carl, 2000; Wilson *et al*., 2001). Also irrigation scheduling during the early stages of tuber initiation can also deter infection (Potatoes production guideline, 2013; Hiltunen *et al*., 2005).

2.7.2. Biological methods and the role of nutrients

2.7.2.1. Site selection and reducing seed-borne inoculum

To provide potato crop with the greatest opportunity to grow to its full potential free of disease, utilizing certified seed is an essential principle. Pathogens may be moved on seed-tubers that provide inoculum for disease in the subsequent crops, and are evaluated in potato seed-tuber certification schemes all over the world. Seed lots are typically certified by visual subjective assessments of disease but lately, it's done by developed quantitative polymerase chain reaction (qPCR) tools. A comparison of both visual and qPCR assessments of potato seed lots was done, visual assessment, in most conditions provides an exact measure of tuber seed health for certification; although qPCR had the ability to identify ranges of pathogen DNA existing on symptomless tubers. In addition, it was indicated that planting tubers with high pathogen inoculum loads derived to increased progeny disease in compare with planting 'certified' tubers (Tegg and Wilson, 2016).

Streptomyces scabiei lives in soil or on the surface of tubers; it is a saprophyte which may over-winter and can serve as next year's inoculum source. Inoculum could also be spread by water and wind-blown spores. *S. scabiei* may survive indefinitely once present in the soil (Driscoll, 2007). Therefore, in site selection for potato growing, fields with a history of scab and also soils with light texture favor scab infection and should be avoided (Kirkwyland and Thomas, 2013). Soil treatment of infected fields can lead to insufficient management because the effectiveness of any treatment is distinguished by soil type (texture and structure) and pH. One possibility

for control is addition of elemental sulphur or an acid based fertilizer e.g. ammonium sulphate. Application of lime to raise soil pH can enhance disease occurrence, while the addition of gypsum has no influence on soil pH and is a favorable practice. Water irrigation may be efficient however, the evaporation rate must be monitored closely because too high water levels may also increase disease severity (citation). Crop rotation of 3 to 6 year cycles with non-host crops is suggested. This practice would lead to reduction of soil inoculums but will not eradicate the pathogen. Wheat, rye, oats, soya bean, sorghum and lucerne are appropriate rotation crops. Finally, green manuring with brassica crops (e.g. mustard, cabbage), rye, clover, bean or grasses can decrease CS occurrence.

A perfect association (r =1.00) was observed between the population densities of pathogenic *Streptomyces* in the root zone and daughter tuber disease occurrence demonstrating that evaluation of such populations in the field can serve as an excellent predictor of scab disease (Wang and Lazarovits, 2005). The necessity of both seed and soil-borne inoculum control in the epidemiology of CS disease was clearly indicated. Applications of chemical seed dressing treatments to visibly clean seed failed to considerably diminish disease rates below that observed on untreated seed. Preliminary investigations of some chemical soil treatments gave dissatisfying levels of control (Wilson *et al*., 1999). The use of peat and iron treatment concurrently can increase the yield and the proportion of large tubers (Sarikhani *et al.,* 2017).

2.7.2.2. Using resistant varieties of potatoes

Development of potato varieties with a high level of scab resistance has been a goal for most potato-breeding programs (Loria *et al.*, 2006; Haynes *et al.,* 2009). Unreliable CS control has been produced by cultural practices such as fumigation, altering soil pH and controlling soil moisture during tuber development. The best CS control method is the application of disease-resistant varieties. However, breeding programs have made little process in developing resistant varieties due to high location to location and year to year diversities that decrease selection effectiveness (Navarro *et al*., 2013; Bouchek *et al.,* 2000).

Recently, the potato cultivar (variety) ‘Mountain Gem Russet’ was tested in several regions in the USA on different soil types and indicated a complete resistance to the CS pathogen (Stark *et al.,* 2016). However, screening methods to evaluate

susceptibility of scab in breeding programs take a lot of time and can provide variable results because there is a great range of differences in resistance among potato varieties and there are other requirements to the quality of potato varieties as well. Many public research programs are done to breeding for scab-resistant varieties and methods for evaluating management. This new breeding strategy based on clone crosses with cultivated potato, allow the introgression of resistance genes into advanced breeding and would allow breeders to incorporate recessive traits into varieties, for instance a diploid F2 population derived from a cross between the cultivated potato (*Solanum tuberosum)* clone US-W4 and the resistant wild relative (*Solanum chacoense*) clone 524–8 that showed previously to have the genomic regions associated with resistance to CS, exhibited high levels of resistance to the scab (Braun *et al.,* 2017). Scab resistance was reported to be located on two independent loci, one dominant and one recessive (Alam, 1972). Murphy *et al*. were successful in transferring scab resistance from the diploid parent to the tetraploid offspring using 4x-2x crosses (diploid and tetraploid, stand for two and four sets of chromosomes in a cell, and hence the number of possible alleles for autosomal genes) (Murphy *et al*., 1995); However, Haynes (1990) showed that it is not possible to predict how successful this population might be in transmitting resistance to CS to the tetraploid level (Haynes, 1990). The same author also showed in 2009, that resistance to CS is not a simply inherited trait because of reasons such asinherent genetic differences in the population and differences in breeding strategies. That research showed the levels of resistance to CS cannot be improved by breeding in the diploid population and suggested it may be feasible to transfer the high levels of resistance of that population to the tetraploid one via 4x-2x crosses (Haynes, 2009). The most of new varieties are as resistant to CS as the moderately resistant standard ‘Russet Burbank’. Some clones, however, behave better in some locations than in others. The breeding clones with stable resistance across a wide range of conditions is suggested (Haynes, 2010). In Wisconsin, a complete breeding program and evaluation of breeding behavior of resistance to CS has been performed. Recently, no commercially significant varieties are immune to infection by *S. scabiei.* Current reports of clones with resistance to scab include Russet Nugget, Genesee, AC Chaleur, Krantz, AC Novachip, Ontario, Andover and Pike (Jansky, 2000). The average of the infection of CS in variety ‘Teele’ was low, which significantly differs from the standard varieties ‘Maret’ and ‘Anti’ (Tahtjarv, 2016).

In botanical potato species, resistance to CS exists in *S. chacoense, S. commersonii,* and *S. yungasense* (Hawkes, 1990); as well as some cultivated relatives, such as Group Phureja (Maine *et al*., 1993). Wild potato species Solanum chacoense, Solanum commersonii, Solanum yungasense, Solanum bukasovii, Solanum canasense and Solanum multidissectum belong to a suite of genetic resources possessing resistance to common scab (Bradshaw and Mackay, 1994; Hosaka *et al*., 2000).

A study on 44 potato varieties of the Czech Republic and European showed that the less severity and incidence of CS was belong to the varieties: Mozart, Samantana, Satina, Laura, Ornella, Flavia, Velox, Adella, Belana, Vineta, Granola and Asterix. The factor variety and year had a statistically significant effect on infection and the effect of environmental conditions was responsible for the variability of severity and incidence in individual years (Sedlakova *et al*., 2013).

2.7.2.3. Other biological approaches to potato protection

Suberin.

Previously, disease resistant potato clones were acquired by cell selections against the pathogen's toxin. These clones had broad-spectrum resistance to various pathogens invading tubers. The mechanism of increased disease resistance was unknown. The soma clones resistance to disease reacted to both pathogen and toxin by generating more cork layers in the tuber periderm, and acquiring higher suberin polyphenols in these tissues. The resistance phenotype is due to infusion of enhanced periderm cell layers and suberization (make the suberin) of the tuber periderm, which restricts infection. The soma clones offer a valuable resource for future examination of suberization responses and its genetic control (Thangave *et al*., 2016).

Suppressive microbial communities.

Some varieties that stimulate disease inhibition can increase populations of specific bacteria with antagonistic activity in their rhizosphere toward their pathogens. Methods which transform resident microbial communities in a way, which impacts natural soil suppressiveness have potential as components of environmentally sustainable systems for management of soil borne plant pathogens (Mazzola, 2002).

Actinobacterial population densities on tuber surfaces reflect variations between the susceptible and resistant potato varieties (Rozenzweig *et al.* and Meng *et al*., 2012). Larger numbers of actinobacteria were isolated more frequently from the periderm of the scab-susceptible varieties than from the moderately resistant varieties in England. In a greenhouse study, the treatment with the lowest scab occurrence also had the lowest density of actinobacteria in the soil adhering to tubers. The density of total actinobacteria may be a demonstration of a favorable environment for pathogenic actinobacteria development. The positive correlation of disease with soil populations early in the growing season could be useful in a predictive model for scab occurrence or severity (Keinath and Loria, 1989).

The combination of bacterial communities in the rhizosphere of potato plants was indicated to be dynamic and affected by the plant growth development level, the year and the site. The taxonomic combination of potato rhizobacteria and the way their abundance was influenced by the plant genotype or the site are still largely unknown. The OTUs affiliated to *Streptomycetaceae*, *Micromonosporaceae*, *Pseudomonadaceae* or *Enterobacteriaceae* found in potato rhizosphere have imputations not only for biocontrol but also for phytopathology (Weinert *et al*., 2011).

Crop rotation.

Following barley, canola, and sweet corn rotation, soil population of culturable bacteria and overall microbial activity tended to be at highest level, and it was lowest with continuous potato. Potato rotations tended to have lower substrate richness and variation. A higher proportion of bacteria was related to fungi for potato rotation than for other rotations (Larkin, 2003). It is not recommended to rotate with CS hosts such as spinach, turnip, parsnip, radish, beet, and carrot; rotate with alfalfa, rye, soybeans and corn.

Favorable conditions such as warm, dry weather and sustained cropping with potato or other susceptible crops (e.g. beet, sugar beet, carrot, turnip, and groundnut) induce disease development. Cultivation of spinach and the incorporation of red clover as cover crop can also lead to a growth in disease occurrence. The best biofumigation method was incorporating of mustard residues. Glucosinolates (GLN) are found in the leaves, stems and roots of Brassica crops, and are unleashed when plant parts are chopped up and worked into the ground. The GLN gasses are introduced into the soil

during the biofumigation process and inhibit the CS soil pathogens. It also enhances the levels of good soil organisms (Gouws, 2013).

Understanding of detailed genetics, physiology and interactions between pathogens and plants.

New molecular genetics tools indicate greater understanding of the genetics of CS resistance and the regulation of thaxtomin production and contributory pathogenicity factors for better control over the disease. (Wanner and Leslie, 2014; Kotiaho *et al*., 2008). *Streptomyces* isolates vary significantly in aggressiveness, and there are specific plant genotype-pathogen isolate interplays. Significant variations among experiments, between isolates and among varieties were found (Wanner and Haynes, 2009). However, based on thaxtomin, the mechanism of pathogenicity is likely conserved in all pathogenic species. High variability in disease symptoms among tubers from a single plant, ranging from no CS to severe pits, makes it difficult to phenotype the susceptibility of CS (Driscoll *et al*., 2009). Resistant varieties which can be enhanced through breeding, should be effective in many different environments (Eckwall and Carl, 2000).

Resistant potato varieties may have smaller lenticels and also they may have a higher rate of suberization than susceptible types. A critical period for pathogen infection is early tuberization hence, studies of host gene expression responses during this developmental stage can be essential to determine putative resistance genes. In an examination of infection with a highly susceptible and a relatively resistant potato variety transcription profiles were acquired by RNA sequencing at two developmental stages: the early hook stage and the early tuber formation stage. A considerable down-regulation of genes included in ribosome biogenesis was evidenced for the infected resistant variety at the early hook stage, which demonstrated an allocation of resources that favors the expression of defense-related genes (Dees *et al*., 2016).

Biological treatments.

Bacterial biocontrols. Suppressive soils are described by a very low rate of disease development even though a virulent pathogen and susceptible host are available. Suppressive soils are differentiated in “long-standing suppression” and “induced suppression”. Long-standing suppression is a biological situation naturally correlated

with the soil, with unknown origin, and it seems to remain in the absence of plants. Induced suppression is initiated and sustained by crop monoculture or by addition of inoculum of target pathogen. In many examples, the microbial community structure of soil is bearing the suppressiveness of plant pathogens. Suppressiveness is very likely to be associated with microbial community structure based on antagonistic functions (Garbeva *et al.*, 2004). The organisms that are operative in pathogen suppression do so via different mechanisms containing competition for nutrients, antibiosis and infusion of host resistance. Some varieties that stimulate disease suppression can increase populations of specific bacteria with antagonistic activity in their rhizosphere toward their pathogens (Mazzola, 2002; Kopecky *et al*., 2018).

Biocontrol isolates of bacteria can suppress the disease by their antagonistic activity and production of enzymes, antibiotics, siderophores, and induce plant growth hormones. Bacterial species from the genera *Bacillus* and *Pseudomonas* were great compositions of those rhizoflora communities antagonistic to *S. scabiei.* The development of rhizobacterial communities that created secondary metabolites with antibiosis ability, against *S.scabiei* stimulated by soil acidification with +SO4 treatments (Sturz *et al*., 2004).

The proportion of isolates with antagonistic activity was highest against *Streptomyces* sp. Almost all *γ-Proteobacteria* and also *Xanthomonas*, indicated biocontrol activities against *Streptomyces scabiei.* The existence of the phytopathogen may have infused the colonization of suitable antagonists (Sessitsch *et al*., 2004). Moreover, nonpathogenic antibiotic-producing *Streptomyces*, such as Strains 346 and K61, prohibited the pathogenic strains (Eckwall *et al*., 2001; Hiltunen *et al.,* 2009) *S. diastatochromogenes* PonSSII (nonpathogenic) or *S. scabiei* PonR (weak pathogen) and *S. albogriseolus* as suppressive strains can decrease the intensity of scab (Weller, 2002; Rosenzweig *et al*., 2012; Kyselkova and Moenne-Loccoz, 2012). Although, even nonpathogenic strains of *Streptomyces turgidiscabies* indicate antagonism against *S. scabiei* (Hiltunen *et al*., 2009). Other molecules (including aromatic amino acids and some secondary metabolites) demonstrate inhibitory effects on the toxin production (Lerat *et al*., 2009).

Pseudomonas sp. LBUM223 is able to produce phenazine-1-carboxylic acid affecting the growth of *Streptomyces scabiei,* the expression of thaxtomin biosynthesis

genes and the biological control potential against CS of potato (St-Onge *et al*., 2011; Arseneault *et al*., 2013; Arseneault *et al*., 2016).

Bacillus amyloliquefaciens BAC03, *Bacillus subtilis* GB03 and *Rhizoctonia solani* hypovirulent isolate Rhs1A1, can be a suitible biological management agent for potato CS (Gomez *et al*. 2013; Larkin and Tavantzis, 2013; Meng and Hao, 2017). The biocontrol microbes such as *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus sp. Sunhua* enhance the release of nutrients from fertilizers, aid to generate rooting and supply the plant defense against infection. The plant roots are colonized by the live bacteria added to the fertilizer, which feed the bacteria with carbon-rich root exudates: in contrast, the microbes enhances nutrient uptake, making fertilizers more efficient, and produce metabolites that stimulate healthy growth and repress disease as a side effect. The microbes get stronger and more active as the plant grows. There have also been noticeable reductions in CS after this experiment (Han *et al*., 2005; Larkin and Tavantzis, 2013; Abram Mike, 2009).

The Phages. The composition and diversity of bacterial population is affected by actinophages, thereby, it can be applied as biological control. Two specific phages against *S. scabiei* were isolated from various potato fields in several locations in Giza, Egypt. Results offer a useful data for designing a control strategy against potato scab disease (AlKhazindar *et al*., 2016). McKenna *et al.,* applied a polyvalent *Streptomyces* phage to get rid of infesting *Streptomyces scabiei*-infected seed potatoes *in vivo*. That was the first *in vivo* study that has applied *Streptomyces* phage to considerably disinfect seed potatoes of *Streptomyces scabiei* and thus decrease contamination of soil from seed-tuber-borne inoculum and decrease infection of daughter tubers (McKenna *et al*., 2011).

The role of plant defense. Plant has several defense mechanisms to come over the disease because during evolution, plants necesitated to acquired mechanisms to define their aggressors and defend themselves. There are two pathways phenylpropanoid and oxylipin metabolisms, which both are included in plant resistance at different levels: by synthesizing an array of antibiotic compounds, by supplying building units of physical barriers against pathogen invasion, and by generating signals implicated in the mounting of plant resistance (Camera *et al*., 2004). Moreover, there are two 13-

AOSs (allene oxide synthase) in potato, and one 9-AOS (AOS3) which is highly specific for 9-hydroperoxides and leads *in vitro* to a- and g-ketol formation, AOS3 is expressed in sprouting eyes, stolons, tubers and roots ending to a-ketol formation *in vivo*, in below ground organs (Wasternack, 2007).

Plant hormones play essential roles in controlling developmental processes and signaling networks included in plant responses to vital and abiotic stresses. Important progress has been made in identifying the key element and comprehending the role of salicylic acid (SA), jasmonates (JA) and ethylene (ET) in plant responses to vital stresses. New studies demonstrate that other hormones such as abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK), brassinosteroids (BR) and peptide hormones are also implicated in plant defense signaling pathways. Defense in response to microbial attack is controlled through a complicated network of signaling pathways that include three signaling molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene. The SA and JA signaling pathways are reciprocally antagonistic. This regulative cross talk may have erupted to let plants to fine-tune the infusion of their defenses in response to various plant pathogens (Kunkel and Brooks, 2002; Howe, 2004). The CS resistance is associated with state of two MYB and three bHLH genes (as the large transcription factor families), demonstrating that they might be included in the adjustment of the defense response of potato against the CS pathogen (Tai *et al*., 2013). Proteins are infused in *Streptomyces scabiei* by potato suberin, a lipidic plant polymer also appeared to affect plant protection through modifications of secondary metabolism (Lauzier *et al*., 2008).

Bacterial communities and nutrients can help the plant to decrease the pathogen efficiency. Ferritin, a universal intracellular protein that stores iron and releases it in a controlled fashion may be a protective molecule for plant cells, can restrain the generation of reactive oxygen species by scavenging the intracellular iron. Ferritin gene (*StF1*) has enhanced tolerance to viral and fungal infections. These records recommend the idea that ferritin can be part of host defense responses triggered during infection (Expert *et al.*, 2012).

2.7.3. Macro and micro nutrients

2.7.3.1. The role of solubility of nutrients on plant uptake

Nutrient uptake in soil is determined by cation exchange, in the way root hairs pump hydrogen ions (H^+) out of the plant and into the soil by the proton pumps. These hydrogen ions displace cations attached to negatively charged soil particles so that the cations are available for uptake by the root. The soluble forms of cations are free to the plant and easily available, however, the most cations in soil are tied up on the exchange complexes and not readily available (Cole *et al*., 2016). The roots can only take up nutrients that are dissolved in a water solution, they cannot take up the solid nutrient form (Solomon *et al*., 2013; Wolf, 1943).

Soil minerals like any other chemical compound exhibit some solubility in water. They are mostly sparingly to very slightly soluble compounds. Solubility of solids vary considerably, for example, sodium salts are generally soluble, while salts of Fe and Al are much less soluble (Strawn, 2015). Increased pH can decrease the solubility of cations. An increase in soil pH, decreased amounts of water-soluble Ca, Mg, Na, K, NH_4-N, and P, while NO_3-, N and Cl increased. A change in soil pH by acid rain, fertilizer, and lime inputs affects cation and anion solubility (Sharpley, 2008), Moreover, soil organic matter and subsequently also peat as a source of organic matter decrease soil pH and influence nutrient solubility (Reykjavík, 2005). Among the micronutrients, Fe, Mn, Cu, Zn, and Ni are taken up by plants in their cationic forms, and B, Mo, and Cl are taken up by plants in their anionic forms. Plant availability of both Fe and Mn is greatly reduced in calcareous soils (pH > 7) due to the extremely low solubility of Fe and Mn oxides and of Mn carbonates (Singh, 2015), also the studies showed that bacteria can increase the solubility of soil minerals (Gramss *et al.,* 2005).

Bacteria can increase the solubility of soil minerals (Gramss, 2005). The plant roots are colonized by the live bacteria added to the fertilizer, which feed the bacteria with carbon-rich root exudates: in contrast, the microbes enhances nutrient uptake, making fertilizers more efficient, and produce metabolites that stimulate healthy growth and suppress disease as a side effect (Abram Mike, 2009; Souza *et al*., 2015).

2.7.3.2. Nutrients in rhizosphere and bulk soils

The population density of microorganisms on the root surface and in the rhizosphere is multiple times greater than that in bulk soil (Huber *et al*., 2011). The relative enhance in the number of microorganisms is described as the R/S ratio, in which R is the numbers per gram of soil in the rhizosphere and S in the bulk soil. The ratios differ greatly, between 5 and 50, for example, depending on microbial species, plant age, plant species and nutritional status of plant (Marschner, 2011).

Under field situations, fertilizers influence the performance of plants directly via their impacts on plant nutrition and indirectly by converting the vital and abiotic environment, which impacts pathogen and survival and function of pest (Huber *et al*., 2011). Plants in return induce biochemical responses, such as release of reducing and chelating compounds and acidification of rhizosphere, which can increase the availability of Fe, Mn, and other micronutrients (Singh and Schulze, 2015). Similarly, the root associated bacteria can increase the solubility of soil minerals (Gramss *et al*., 2005). Therefore, soil microbes play an essential role in nutrient turnover and thus, nutrition of plants by decomposing and mineralizing organic material and releasing as well as changing inorganic nutrients by solubilization, chelation and oxidation/reduction is modified by microbial activities. Nutrient cycling at a particular site is, however, also influenced by grazing of predators, which unleash nutrients from plants and microbial biomass and increases turnover at all trophic levels (Sagova-Mareckova *et al.* 2019, in rev.). Rhizosphere microorganisms may also impact plant nutrient uptake indirectly by increasing root growth (Marschner, 2011).

2.7.3.3. The role of Iron in plant growth

Potato plants were grown in refined sand at variable iron ranging from 0.001 to 2.0 mM in order to investigate the effect of iron on biomass, economic yield . Exposure of potato plants to Fe stress (i.e. a Fe concentration different from 0.1 mM) indicated a retarded growth, reduced chlorophyll concentration and Hill reaction activity, induced changes in enzyme activities and condensation of Fe and Mn. The visible symptoms of iron deficiency emerged on day 15 at 0.001 mM Fe as chlorosis of young leaves. The increase of iron (at >0.1 mM Fe) appeared later, after 25 days and the chlorosis was perceived on older leaves. Both deficiency (0.001 mM) and increase (>0.1 mM) of iron decreased the tuber yield, deteriorating its quality by reducing the condensation of

sugars, starch and protein nitrogen and enhancing the aqcuisition of non-protein nitrogen and phenols in tubers (Chatterjee *et al*., 2006).

2.7.3.4. Organic matter and Suppressive bacteria

The major activities of soil microbes in soil involve the decomposition of organic materials, nitrogen fixation, mineralization of nutrients, suppression of crop pests, protection of roots and also preventing parasitism and harm to plants. The occurrence and severity of root diseases is an indirect assay of soil health for specific commodity/soil use (Abawi and Widmer, 2000). Experiments with unrehearsed non inhibitory mutants of *Streptomyces* biocontrol strains and unrehearsed pathogen mutants resistant to at least one antimicrobial produced by the biocontrol strains showed that both antibiosis and competition contributed to detention of pathogenic strains. Streptomycetes soil subcommunity can be changed considering the type of organic soil amendment, proposing that the biocontrol potential of preventive indigenous strains could be increased via appropriate selection of farming practices (Kyselkova and Moenne-Loccoz, 2012). Modification in the farming practices such as adding organic matter or micronutrient can change the combination of *Streptomyces* soil sub community and increase biocontrol potential of preventive indigenous strains.

2.7.3.5. Organic matter ammendments and disease

The indicated facts concerning the incidence of CS on light soils, its comparative lack on peat soils, the cure of the disease by green-manuring may be explained by the preferential food hypothesis. According to this theory, the scab organisms, which are mainly saprophytic living on vegetable remain in the soil stay until their natural food supply is exhausted and develop their parasitic tendency only under the stress of hunger. Thus, if the soil contains sufficient amount of organic matter, which can aslo add other nutrients to soil and/or increase their availability due to low pH may lead to keeping the pathogen on the surface of tubers only as saprophytic bacteria without going to the virulence stage (Millard, 1923).

Organic matter ammendments may help in development of disease suppressive soils but the benefits will be acquired only over long time because enhancing soil health and modifying its structure is a complex process. Cereal crops and green manures decreased CS occurrence. The impacts of summer green manures on those

crop parameters were greater than those of cereal crops and fall green manures (Adrien *et al.,* 2013). Meat and bone meal, soy meal, and poultry manure at rates of 37 T ha−1 incorporated to a 15 cm depth, considerably decreased the occurrence of verticillium wilt, CS, and plant parasitic nematode populations. Animal manures have been involved in enhancing the occurrence of CS disease of potato and most directions suggest avoiding the application of fresh manure on soils destined for potato production. Yet, in experiments with liquid swine manure amendments in one potato field, found decreased occurrence of wilt and CS, and a lowering in the amount of plant parasitic nematodes for 3 years after a single manure application. Although, when soil pH was increased from 5 to 6.5, all activity against the pathogen was eliminated. Therefore, soil pH is essential to the activity of the amendment (Bailey and Lazarovits, 2003).

Drastic disease occurrence has been reported on fields that were fertilized with animal manure (Poatoes production guideline, 2013). In other studies the plots with compost amendment had substantially greater CS than non-treated control plots with the more severe erumpent form of the disease compare to russet form (Larkin and Tavantzis, 2013). Although, Treating CS have been done using fertilizer treatments. It has proposed that the usage of fertilizer amendments can alter rhizobacterial communities that are associated with a varying degree of antibiosis against *S. scabiei* (Driscoll, 2007; Sturz *et al*., 2004). Finally, peat is a natural product generated from the progressive acquisition of plant and moss residues decomposed in waterlogged situations. Although, peat generally loses its suppressiveness during decomposition, different effects of peat and manure amendment on scab were reported with peat making significant changes to the soil microbial community (Bonanomi *et al*., 2010).

2.7.3.6. The effects of different nutrients on the scab severity

Researches showed different effects of organic matter and nutrients on the scab severity (Lazarovits *et al*., 2007; Lambert *et al*., 2005; Davis *et al*., 1976). However, the effects of nutrient additions differ by locations and situations (Bailey *et al*., 2003; Lazarovits *et al*., 1999; Lazarovits *et al*., 2010; Soltani *et al*., 2002). The reduction of scab after application of ammendments is soil-specific and varies from year to year (Abbasi *et al*., 2006). The data indicate that the correlation between scab severity and

soil chemical components is very complex (Lazarovits *et al*., 2007; Conn and Lazarovits, 2013).

Potassium: The impact of potassium on scab severity varied in different studies. The potassium measured in tuber periderm, was not related to scab occurrence or severity (Kristofek *et al*., 2000) yet, in another study the scab was less in soils with relatively high composed exchangeable K, although combined exchangeable cations, such as K, varied between locations (Lacey and Wilson, 2001). Disease intensity correlated with soil factors such as available K content for one studied area but not for another one (Lazarovits *et al*., 2007). An application of potassium to soil was not considered to have any influence on CS (Stead and Wale, 2004).

Copper: The studies showed that the copper measured at harvest in periderm, may not be related to scab occurrence or severity (Kristufek *et al*., 2000). In another study the use of Cu in soil reduced the tuber infection level and intensity by *Streptomyces scabiei* (Klikocka, 2009).

Zinc: The concentration of Zn, measured in periderm, was not related to the scab severity (Kristufek *et al*., 2000) and Zn was not considered to have any influence on CS (Stead and Wale, 2004); however the use of micronutrients Zn in soil reduced tuber infection level and intensity by *Streptomyces scabiei* (Klikocka, 2009).

Magnesium: In several studies assessing magnesium in soil, the tuber scab decreased and occurrence of *Streptomyces scabiei* and scab disease was not detected on potatoes grown with composed exchangeable Mg (Lacey and Wilson, 2001; Klikocka, 2009). It was demonstrated that suppressive soils had higher content of Mg compare to conducive soils (Sagova-Mareckova *et al.*, 2015). The same author showed in 2017 that the number of *txtB* gene copies in potato periderm was positively correlated to a higher content of Mg in periderm (Sagova-Mareckova *et al.*, 2017). Moreover, the resistant variety had a higher Mg content in periderm compared to sensitive varieties (Kopecky *et al*., 2018). In the recent study, we showed that there were the positive correlation between CS severity and amount of Mg in soil and periderm. Although, a negative correlation between thaxtomin copy numbers in soil and Mg in periderm was seen but there was a positive correlation between thaxtomin copy numbers and Mg in periderm.

Iron: Different effects of iron content in soil and periderm on scab severity were observed. It was suggested that plant pathogens including *S. scabiei* may use

chelating compounds for iron uptake, and those can act as essential virulence determinants (Seipke *et al*., 2011). Potato varieties Binella and Agria (as sensitive varieties to scab) were the most Fe dependent varieties in comparison with other varieties (Adamski *et al*., 2011; Ozturk *et al*., 2011). Availability of iron may be important in order to produce biocontrol metabolites by suppressive bacterial community (Kyselkova and Moenne, 2012). In suppressive soils, potatoes had lower CS severity in soils with more available iron (Sagova-Mareckova *et al.*, 2015), while in another study, number of *txtB* gene copies in periderm was positively correlated to a higher content of Fe (Sagova-Mareckova *et al.*, 2017). In the last study, we showed that enrichment of field with available iron and/or peat ammendments can suppress the scab efficiently (Sarikhani, *et al*., 2017).

In our study, it seems that the aggressiveness of pathogen for competition on iron source with the plant could be stopped by soil enrichment by sufficient available iron. The iron supplement supported plant defense while both iron and peat additions changed the bacterial community in favor of CS suppression (Sarikhani *et al*., 2017).

Phosporus: High soil phosphorus content was seen in the CS suppressive fields (Sagova-Mareckova *et al.*, 2015). Also, number of *txtB* gene copies in periderm was positively correlated to a higher content of P in periderm (Sagova-Mareckova *et al.*, 2017). Evaluating the mineral components of potato periderm, the CS intensity was negatively correlated to phosphorus content (Kristufek *et al*., 2015). However in other study, the application of P was not considered to have any influence on CS (Stead and Wale, 2004). In our recent study, the negative correlation between CS severity and P content in soil was observed; moreover between there was a positive correlateoin between soil *txtB* and soil P as well as between *txtB* and P in periderm.

Calcium: High calcium levels, in the absence of changes in pH, may induce CS intensity. CS was positively correlated with calcium content of tubers and negative correlated with composed exchangeable Ca of soil (Lacey and Wilson, 2001; Horsfall *et al.,* 1954, Davies *et al.,* 1976). The Ca was less in soils with low CS severity and the suppressive fields had lower Ca in periderm. (Sagova-Mareckova *et al*., 2015). Yet, a different study showed that the application of Ca, was not considered to have any influence on CS (Stead and Wale, 2004). In higher Ca^{2+} concentration, the aerial mycelium formation in *S. scabiei* was greater (Natsume *et al*., 2001). In *Arabidopsis*

thaliana, the Ca^{2+} influx impelled by thaxtomin A is essential for cell death (Errakhi *et al*., 2008).

Nitrogen: Nitrogen had contrasting effects on potato scab. In some studies, nitrogen levels did not seem to directly impact CS but many nitrogenous fertilizers have an indirect effect becuase they acidify soil (Stead and Wale, 2004). Also, the use of organic amendments, manures and composts which are rich in nitrogen, may decrease soil-borne diseases by unleashing allelochemicals engendered during product storage or by subsequent microbial decomposition (Bailey and Lazarovits, 2003).Other studies showed that adding N rich soy meal, meat and bone meal to soil leads to increase of ammonia, nitrite, nitrate, pH, and bacterial quantity, and can suppress CS (Lazarovits *et al*., 1999). However, oligotrophic conditions of low soil C and N remain potentially associated with suppressivity to CS and the less content of N was seen in other soils with low CS severity. Possibly, the pathogens favor soil conditions of high C and N (Sagova-Mareckova *et al.*, 2015). The observed differences in N impact on CS may be partially attributed to different contents between bulk soils and rhizosphere and different utilization by potato varieties.

Sulphur: The application of sulphur or gypsum (calcium sulphate) reduced the tuber infection level and intensity of *Streptomyces scabiei* (Klikocka, 2009; Davies *et al.,* 1974). Elemental sulfur and ammonium sulfate significantly decreased CS (Klikocka and Glowacka, 2013). The use of S, reduced the tuber infection level and occurrence of *Streptomyces scabiei* (Klikocka, 2009). Sulphate fertilizers can enhance biodiversity and antibiosis ability of root zone bacteria against *Streptomyces scabiei.*

Previously, soil S was also found to be related to CS severity, the elemental sulfur and ammonium sulfate significantly decreased CS (Klikocka and Glowacka, 2013). In other experiments (Pavlista, 2005), scab reduction also occurred with elemental sulfur or ammonium sulfate but the results were inconsistent. Similarly, sulfur treatments interacted with irrigation and calcium content in the periderm in CS suppression (Davis *et al*. 1976; Lazarovits *et al.*, 2008); but in another study the positive effect of elemental sulfur and kieserite fertilization on resistance against *S. scabiei* was attributed to reduce soil pH. It was demonstrated that soil S is connected to disease but not to pathogen abundance, and sulfur-containing defense compounds including elemental sulfur, H_2S, glutathione (an importnt antioxidant in plant) phytochelatins (a detoxificator of heavy metals), can play a good role in suppression

(Klikocka *et al.,* 2005). It is important to note that various secondary metabolites and sulfur-rich proteins are crucial for the survival of plants under biotic and abiotic stress (Rausch and Wachter, 2005). Also results of our study indicated that the CS severity was negatively correlated with soil S content and a negative correlation was seen between soil *txtB* and S content in soil (Sagova-Mareckova *et al.*, 2017).

Aluminium: The effects of Al on CS showed to be varied in different fields. It has been demonstrated in Japan that even when the pH decreased in the examined soils, higher concentrations of water-soluble aluminum were found in fields that had less scab disease than fields which were highly conductive. They suggest including aluminum could be applied to manage the disease. Although, this study showed that, the value of required aluminum in the field in order to control disease was too great to be economically feasible (Eckwall and Carl, 2000). In another study it was demonstrated that conditions present during the infection period cannot be reflected by concentrations of Al, measured at harvest in tuber periderm, and consequently may not be related to CS occurrence or severity (Kristufek *et al*., 2000). Accordingly, disease intensity was anticipated by soil factors such as organic matter and Al for one studied field but not for another one (Lazarovits *et al*., 2007).

Carbon: Compost amendments from different sources enhanced total and marketable tuber yield, but CS was substantially higher in plots with compost amendment than in no treated plots of control, and was most drastic in plots treated with the peat compost (Larkin and Tavantzis, 2013).

In another study farmers used compost of cattle or pig slurry implementation to soils with suboptimal C levels for at least 4 years, to enhance C content in the top, without infusing greater N and P leaching. There was no impact on disease intensity caused by *Streptomyces* after a potato cropping (Hose *et al*., 2016). In some studies the low soil C, N, and C/N was associated with suppressivity to CS at sites, it seemed that lower content of organic matter and oligotrophic conditions, are favorable to disease suppression because C, N, Ca and Fe were less in soils with low CS severity, in other hand, the correlation of number of soil *txtB* gene copies to soil C, may be showed that the pathogens favor soil conditions of high C and N (Sagova-Mareckova *et al.*, 2015).

Manganese: The effect of manganese on CS was not consistent but some studies have demonstrated a decrease (Stead and Wale, 2004). In another study, the use of Mn, reduced the tuber infection level and intensity of *Streptomyces scabiei*

(Klikocka, 2009). The occurrence of CS infection of potato tubers by *Streptomyces scabiei* is repressed either by decreasing the soil pH or by using Mn. The repressive effect on Mn is possibly because of enhanced resistance of the tuber tissue to the pathogen and prohibition of the vegetative growth of *S. scabiei* before the beginning of infection (Huber *et al*., 2011).

2.8. Future research requirements

Many management practices such as keeping pH levels below 5.2, crop rotation, resistant varieties, seed selection, irrigation, organic matter management, production of potato scab-suppressiveness soils, were suggested and used to manage the CS. All have advantages and disadvantages and no complete way to control potato CS is available at present. Some strategies must be changed field by field and year by year. A new suitable suggestion comes from our results showing the effect of peat and available iron on decrease of CS. In the vast monitoring study, we also found a new relationship between the disease and several factors and nutrients. The content of nutrients such Fe, Mg, N, C, Ca, P, in soil and periderm can play a predictive role for potato scab severity and the *txtB* copy numbers in soil (vs in periderm) could be a good predictive factor for disease suppression. Also studies showed that the population of several groups of bacteria are important to scab suppression. Results also highlighted the usefulness of both cultivar resistance and soil suppressiveness traits in understanding and managing disease control of crops.

Finally, in the last study we found an important role of resistant potato varieties on changing of soil biosphere in favor of pathogen suppressiveness, and that finding can be also used for selection of proper varieties for a particular field. We suggest, that the effect of more micronutrients on CS severity may be studied together with responses of microbial community to treatments of soil. The new approaches can help us to find the comprehensive and effective methods to cover more fields and lasting longer time.

2.8.1. Biological and chemical factors associated with natural soil suppressivity

Bacterial communities in soil are influenced by various environmental factors, which affect their structure and diversity. Microbial diversity of soil and the implication for the soil's

disease suppressiveness is affected by plant species, soil type, and soil management regime. In some conditions the soil, and in others the plant type represent the key factor distinguishing soil microbial diversity. Some soils are inhospitable to plant pathogens, by restraining either the survival or the growth of pathogens. Such soils are known as pathogen or disease suppressive and are observed all over the world (Garbeva *et al*., 2004, Kopecky *et al*., 2011).

Potato has the highest rank of consumption among vegetables in the US (Brown *et al.,* 2010), however, it can be affected by several diseases. Common scab of potatoes is an important soil-borne disease with worldwide occurrence and it has been rated among the top five diseases of potatoes by seed producers in the USA (Dees and Wanner, 2012). Common scab of potatoes is a disease, which is difficult to manage due to complex interactions of the pathogenic bacteria with soil, microbial community and potato plants. (Garbeva *et al*., 2004, Neeno-Eckwall *et al*., 2001). It is described by deep or shallow-pitted lesions on potato tubers (Bouchek-Mechiche *et al.,* 1998). The infection is caused by actinobacteria from the genus *Streptomyces* that possess a large pathogenicity island in their genomes. The most important pathogenicity determinant is a phytotoxin thaxtomin, coded by *txtAB* genes. These genes are used for determination and quantification of pathogens responsible for the disease (Kers *et al.,* 2005, Hiltunen *et al.,* 2006, Lazarovits *et al.,* 2007). The distribution, severity and incidence of common scab have been widely studied in relationship to physico-chemical and microbial soil characteristics but the disease is still difficult to control. The previous studies demonstrated that the common scab causing organisms are saprophytic living on vegetable remains in soil. They stay until their natural food supply is depleted and only under the stress of hunger switch to their parasitic life style.

Availability of various nutrients may be related to disease suppressivity (Sarikhani *et al*., 2017, Kyselkova and Moenne-Loccoz, 2012). However, the effects are difficult to demonstrate because conditions present during the infection period may not be reflected by concentrations of mineral elements (Ca, P, K, Mg, AI, Fe, Mn, Cu and Zn) measured at harvest in tuber skin, and consequently may not be related to CS occurrence or severity (Kristufek *et al*., 2000). In a large monitoring in Canada, CS disease intensity was anticipated by soil factors such as organic matter, pH, Al, %Ca, %Mg, and %K for PEI but not for Ontario soils. The data indicate that the correlation between scab severity and soil chemical components is complex and potentially soil specific (Lazarovits *et al*., 2007).

Nitrogen levels do not seem to directly impact common scab but many nitrogenous fertilizers have an indirect effect and acidify soil (Stead and Wale, 2004). Generally, high

levels of nitrogen and carbon may be favorable for the pathogen, which is adapted to high nutrient demand and suppress the oligotrophic antagonistic community (NeenoEckwall et al. 2001, Sagova-Mareckova et al. 2015).

Calcium and other cations were also examined for CS control. Strong relation was found between soil pH and exchangeable cations, particularly calcium, and a correlation of calcium with CS development was suggested but without the mechanism specification. CS was not detected on potatoes grown in soil with composed exchangeable Ca, Mg and K at 12 cmolc/kg or less. A strong relation was found between soil pH and these exchangeable cations, particularly calcium (Lacey and Wilson, 2001). Similarly, CS intensity was positively associated to calcium and negatively to phosphorus in skin in another study (Kristufek *et al.,* 2015). The Ca^{2+} influx impelled by thaxtomin A is essential in order to achieve the cell death (Errakhi *et al*., 2008).

The availability of nutrients to plants will be maximized by maintaining a soil pH between 6.3 and 6.8. Low soil pH decreases the availability of phosphorus and enhances the availability of toxic elements like aluminum. To control common scab, soil pH should be maintained within a relatively narrow range (5.0 to 5.2) (Kirkwyland and Thomas, 2013).

Sulphur, magnesium and micronutrients boron, zinc, manganese and copper reduced the tuber infection level and intensity of *Streptomyces scabiei* (Klikocka, 2009). P content was previously often associated with low disease severity (Davis *et al*., 1976, Kristufek *et al*., 2001).

Probability of CS occurrence is decreased at low pH (Lacey and Wilson, 2001, White *et al.*, 2011). The studies demonstrated the effect of low soil pH in decreasing CS disease caused by *S. scabiei*. Generally, it was regarded as being CS suppressive with soil pH below 5.2 (Waksman, 1921; Powelson *et al.*, 1993) and that may be related to availability of P, Zn, Fe, Mn, Cu and B, which is very low in alkaline soils (White *et al.*, 2011). Thaxtomin production is reduced at pH 7.6 and enhanced at 15°C in *S. scabiei* and at 30mM phosphate in *S. acidiscabies* (Natsume *et al.*, 2001). Disease occurrence and variety differ in places and years; this is partially due to diversity in the environment, genetic variability in potato varieties but also due to specific local microbial communities (Wanner *et al*. 2006). Finally, organic matter can enhance disease-suppressive activities of soil microbial communities (Bailey, 2003, Noble and Coventry, 2005).

Several studies demonstrated that the disease can be regulated by the use of resistant varieties, some concluded that they are the best available control (Kotiaho *et al*., 2007, Navarro *et al*., 2013). However currently there are no available commercial potato varieties

showed to be completely resistant to CS (Dees and Wanner, 2012, (Eckwall and Carl, 2000). Some varieties that stimulate disease suppression can increase populations of specific bacteria with antagonistic activity in their rhizosphere toward their pathogens (Mazzola, 2002).

Actinomycete population densities on the tuber surfaces were clearly reflecting differences between the susceptible and resistant potato varieties (Keinath and Loria, 1989). The correlation of disease with soil populations early in the growing season could be useful in a predictive model for scab occurrence (Keinath and Loria, 1989). Nonpathogenic *Actinobacteria* (e.g., *S. diastatochromogenes* and *S. albogriseolus*) generate compositions prohibitory against *S. scabiei* and contribute to disease suppression (Rosenzweig *et al*., 2012). Streptomycetes play various roles in plant-associated microbial communities. Some act as biocontrol agents, prohibiting plant interplays with pathogenic organisms. Owing to the antagonistic characteristics of streptomycetes, they apply a selective pressure on soil microbes, which may not always be for the benefit of plant. Others encourage the formation of symbioses among plant roots and microbes, and this is in part because of their direct positive impact on the symbiotic partner, expressed as, e.g., promotion of hyphal elongation of symbiotic fungi. Lately, streptomycetes have been defined as modulators of plant defense. They facilitate root colonization with pathogenic fungi by suppressing plant responses to pathogens (Schrey and Tarkka, 2008). Modification in the farming practices such as adding organic matter or micronutrient can change the combination of the *Streptomyces* soil sub community and increase biocontrol potential of preventive indigenous strains (Kyselkova and Moenne, 2012). The correlation between greater amounts of nonpathogenic *Streptomyces* and less intense common scab proposes that the interplays among plant genotype and *Streptomyces* microbial community is essential in defining the intensity of common scab on potato, and emphasizes the role of complex interplays among plants and microbial populations on and near plant roots in plant disease outcomes (Neeno-Eckwall *et al*., 2001).

Regarding to the total bacterial community, suppressiveness is very likely to be associated with microbial community structure based on antagonistic functions (Sessitsch *et al*., 2004). Different microbial taxa were found to participate in suppression of diseases either directly or indirectly by enhancing plant nutrition by acquiring limiting nutrients or producing beneficial enzymes (Kyselkova and Moenne-Loccoz, 2012, Tokala *et al.*, 2002) consequently supporting the plant self-protection capabilities. The suppressive character of soils was often associated with multiple species (Rosenzweig *et al.*, 2012, Meng *et al.,*

2012, Kyselkova *et al.,* 2014). The population of several groups of bacteria was showed to be important to scab suppression, e.g. *Acidobacteria, Verrucomicrobia, Bacteroidetes* and *Gemmatimonadetes* (Sarikhani et al 2017). In many examples, the microbial community structure of soil has the ability of suppressiveness of plant pathogens. However, we are far away from comprehending the exact mechanisms in the complex microbial communities of soil that often underlie the increased disease suppression (Garbeva and Veen 2004).

Thaxtomin, which is the most known pathogenicity determinant of common scab, is a phytotoxic secondary metabolite that suppresses cellulose synthesis in potato (Johnson *et al.*, 2007 and 2009). Statistically *txtB* copy numbers in soil could be a good predictive factor for disease suppression (Sagova-Mareckova et al. 2015). The goal of this study was to explore the mutual interaction between soil chemical conditions and microbial community in the development or suppression of CS, and to evaluate the interaction of the soil environmental factors with potato variety in shaping the community inhabiting potato rhizosphere.

2.8.2. Increasing the availability of iron

Common scab of potatoes (CS) is a disease that drastically affects tuber quality due to superficial and pitted lesions that form around the site of infection on the potato periderm. The microorganisms responsible for the infection are plant-pathogenic *Streptomyces* spp., which possess a large pathogenicity island. The most known pathogenicity determinant is thaxtomin, a phytotoxic secondary metabolite that inhibits cellulose synthesis in expanding plant tissues (Johnson *et al*., 2007). The compound is required for plant infection and a positive correlation between thaxtomin production and pathogenicity was found (King *et al*., 1991). Traditional control strategies for CS include the development of resistant cultivars, specific fertilization, increasing soil moisture, chemical treatments and decreasing soil pH. However, control is strongly dependent on local conditions and therefore the results are not clearly predictable (Dees and Wanner 2012; Sagova-Mareckova *et al*., 2015). Many agricultural soils lack a sufficient amount of one or more nutrients so that plant growth is suboptimal (Glick 2012). Despite the fact that iron is the fourth most abundant element on earth, in aerobic soils, iron is not readily assimilated by either bacteria or plants. That is because ferric ion, which is the predominant form in nature, is only sparingly soluble so the amount of iron available for assimilation by living organisms

can be extremely low (Glick 2012). The scarcity of bioavailable iron in soil habitats foments a furious competition (Compant *et al.,* 2010) and plant iron nutrition can affect the structure of bacterial communities in the rhizosphere (Glick 2012).

Application of organic amendments, such as animal and green manure, organic wastes, composts and peats, has been proposed as a strategy for the management of diseases caused by soil borne pathogens (Bonanomi *et al*., 2010). Peat is a natural product derived from the progressive accumulation of plant and moss residues decomposed under waterlogged conditions. During decomposition, peat generally loses its ability to suppress various diseases. This result has been attributed to the progressive reduction of cellulose, carbohydrates and easily degradable organic compounds (Hoitink and Boehm 1999). These chemical changes may drive a progressive shift in the microbial community composition from bacteria, which have antagonistic ability, to those which are less able to antagonize soil-borne pathogens (Bonanomi *et al.,* 2010). Also, the composition of the *Streptomyces* soil sub-community can be modified according to the type of organic soil amendment (Mishra and Srivastava 2004). Above that, soil organic matter is an important factor affecting iron solubility (Carlgren and Mattsson 2001), so its supplementation may change the relationships of iron competition among bacteria. Finally, the CS causing streptomycetes are naturally saprophytic in soil, but, as pathogens, they may take advantage in nutrient utilization from plant tissue (Dees and Wanner 2012). Therefore, the addition of particular organic compounds may change the relationship among the pathogenic and nonpathogenic bacterial communities and consequently influence the disease development. Previously, the use of micronutrients to control CS has been investigated, but most of the effects that were observed could be accounted by changes in soil pH. Reduction of soil pH below 5.2 or an increase above 8.5 appeared to inhibit CS although not kill the pathogen S. scabiei (Neeno-Eckwall 2000; Waterer 2002). Different soil pH also changes the bacterial community composition, which may support either the pathogen or the antagonists. Specifically, changes in the actinobacterial community were correlated not only with soil pH but also with soil nutrients such as carbon and nitrogen showing that pathogen development may be supported in eutrophic conditions (Sagova-Mareckova *et al*., 2015). In the present study, peat and iron in biologically available form were supplemented to soil known for having a high severity of CS; these treatments were compared to naturally

suppressive soil with low CS severity occurring in a nearby field. The study aimed to identify an approach which would decrease the disease severity. In addition, we analyzed the bacterial communities to determine which might be responsible for the observed effects. Bacteria, Actinobacteria and potential thaxtomin producers were quantified by quantitative PCR and community structures were analyzed by Illumina sequencing of the 16S rRNA genes. The study showed that indeed the most successful treatment induced changes in the bacterial community, whose structure became similar to that of the suppressive soil.

2.8.3. Microbial communities associated with disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab

Suppressive soils are described as soils in which disease severity remains low, in spite of the presence of a pathogen, a susceptible host, and climatic conditions favorable for disease development (Baker and Cook, 1974, Janvier *et al.,* 2007) Relatively few soils with suppressive character have been described in the world to date (Kyselková *et al.,* 2012); although it is of prime interest to understand and conserve their functioning because they may help us to learn how to establish suppressive character of soils at other sites (Kinkel *et al.,* 2011). Common scab (CS) of potatoes is a soil-borne disease caused by *Streptomyces* spp. that produce thaxtomin phytotoxins, and for which suppressive soils were reported mostly in the USA (Lorang *et al.,* 1995, Meng *et al.*, 2012). In these systems, disease control is largely attributed to biological interactions (mostly competition and antagonism) between plant-beneficial microbiota and pathogens mediated via antibiotic production or enzymatic activities (Kinkel *et al.,* 2011, Rosenzweig *et al.,* 2012). In particular, nonpathogenic *Streptomyces* spp. were correlated with CS suppressiveness (Meng *et al.*, 2012, Rosenzweig *et al.,* 2012) and it was also hypothesized that other actinobacteria may be involved in this disease suppression (Kinkel *et al.,* 2011).

High levels of resistance to common scab are not found in most commercially significant cultivars of potato (Braun *et al.,* 2017). The resistance to CS is manifested by different quantities of pathogenic streptomycetes in their tubers but not in roots or rhizosphere (Kobayashi *et al.,* 2015). Yet, potato cultivars differing in resistance to common scab also have different ecophysiologies as they differ in chemical composition of the potato periderm and preferences in nutrient utilization (Kristufek *et al.*, 2015). Since various bacterial communities are associated with either resistant or

susceptible cultivars (Kobayashi *et al.,* 2015), interactions between potato plants with different genotypes and associated microbial communities may further influence the disease development under specific soil conditions.

In our previous investigations, CS suppressiveness was studied in two areas (Vyklantice and Zdirec) from the Czech Republic, in field trials (Sagova-Mareckova *et al.,* 2015, Sagova-Mareckova *et al.,* 2017) and pot experiments (Sarikhani *et al.,* 2017).We found that the suppressive character of the fields differed between the two locations, because it was attributed to soil chemical characteristics in the Zdirec area, versus microbial community interactions in the Vyklantice area (Sagova-Mareckova *et al.,* 2015); Therefore, this work we aimed at disentangling the relative effects of soil suppressiveness and potato resistance on the structure of microbial communities in the soil in contact with potato tubers (i.e. tuberosphere, also termed geocauloshere), using suppressive and conducive soils from the Vyklantice area since biotic interactions were determined as responsible for soil supressiveness there. Often, the focus in suppressive soil assessment has been put on bacteria (Rosenzweig *et al.,* 2012, Shi *et al.,* 2019).

Yet, fungi can be also important for crop protection (Kyselkova *et al.,* 2012) and the role of micro-eukaryotes and their participation in top-down control has been typically neglected (Gao *et al.*, 2019); although many of them can be relevant to soil suppressiveness because microfauna and mesofauna members may consume pathogens, increase nutrient turnover or maintain specific diversity by feeding on the dominant bacterial taxa (Zahn *et al,* 2016, Mendes *et al*., 2013).

Archaea also are part of the rhizosphere microbiome, and whether they can participate in biocontrol interactions remains unknown (Taffner *et al*., 2018, Mendes *et al.*, 2011), hence the importance of including them in microbial assessments. Finally, CS-susceptible and resistant potato cultivars have not been compared yet in terms of their respective interactions with the soil microbial community in CS suppressive soils. Our objective was to test whether both suppressive soil and resistant cultivar represent significant ecological factors shaping microbial communities of the potato tuberosphere. To this end, we used a field experiment that included a combination of (i) disease suppressive vs conducive soils, and (ii) resistant vs susceptible cultivars. The study compared spatial compartments of tuberosphere, potato periderm and bulk soil because it was determined that only in tuberosphere

differences between factors influencing CS severity occur (Shi *et al.,* 2019, Kristufek *et al.*, 2000).

Bacterial, archaeal and micro-eukaryote communities in soil and potato tuberosphere were assessed by Illumina sequencing. Above that 16S rRNA taxonomic microarray was used for its semi-quantitative approach in bacterial community assessment (Edgar, 2013, Paliy and Agans, 2012) and also because our taxonomic microarray focuses on bacterial taxa possessing plant growth-promoting and antagonistic traits in soil environments (Kyselková *et al.,* 2009, Kyselková *et al.,* 2014).

For this study, our microarray was extended with probes focusing on CS pathogens. The results were considered in relation to CS severity observed on tuber surface, quantity of thaxtomin biosynthetic genes *txtB*, quantities of total bacteria and more specifically of actinobacteria, but also against chemical characteristics of soil and potato periderm. That was done in order to identify interactions between potato plants, microbial community and soil characteristics in common scab manifestation.

2.8.4. Interaction between bacteria in potato tuberosphere

Actinobacteria are one of the dominating groups in soil, metabolically highly active, efficient in degrading complex biopolymers. They interact with soil chemical environment and with other groups of bacteria using secondary metabolites in signal transmission and/or defense processes. Actinobacteria have the greatest known morphological differentiation based on filamentous elements or hyphae (Kalakoutski and Agre, 1975). Actinobacteria are known as a good sources of natural products. Approximately two thirds of natural antibiotics originate from actinobacteria (Okami and Hotta, 1988). The genus *Streptomyces* has expanded ability to produce different groups of secondary metabolites exhibiting different biological activities (Nakashima *et al*., 1999).

Nonpathogenic *Actinobacteria* (e.g., *S. diastatochromogenes* and *S. albogriseolus*) produce compounds inhibitory against *S. scabiei* and contribute to disease suppression (Bowers *et al*., 1996 and Lorang et al., 1995). Nonpathogenic *Streptomyces* spp. are believed to play a major role in disease suppressiveness and they also produce a range of antibiotics that may contribute to disease suppression. Moreover, the frequency of antagonistic bacteria such as pseudomonads and

streptomycetes were significantly higher in suppression soil (Meng *et al*., 2012, Loria *et al.*, 2006). In several cases, suppressive soils have higher populations of nonpathogenic *Streptomyces* spp., that are associated with less severe common scab, showing an interaction between plant and *Streptomyces* microbial communities that affects disease severity of common scab (Rosenzweig *et al*., 2012).

Assuming the antibiotic production is important in biological control, the use of strain combinations in practical biological control will reduce the chances of selecting pathogenic strains resistant to antibiotics produced by suppressive strains (Liu *et al*., 1996). Previously, biocontrol nonpathogenic suppressive strains of streptomycetes that exhibit antibiotic activity against pathogenic *S. scabiei,* were isolated from the lenticels of tubers in field-pot trials. These isolates were added to conducive soil and significantly decreased common scab (CS) occurrence (Wanner *et al*., 1999, Liu *et al.,* 1995, 1996). In a study in 2001, NeenoEckwall, showed that when the pathogens were inoculated into soil alone, a positive correlation was seen between population density and disease severity, but when the pathogens were inoculated with suppressive strains, higher total streptomycete population densities were correlated with lower amounts of disease (NeenoEckwall *et al.*, 2001).

Both antibiosis and competition contributed to detention of pathogenic strains, however the combination of the *Streptomyces* soil subcommunity can be changed considering the type of organic soil amendment, proposing that the biocontrol potential of preventive indigenous strains could be increased via appropriate selection of farming practices (Kyselkova *et al*. 2012).

Determination of both the growth rate of strains and the antibiotic activity of strains in order to inoculate them in soil against *S. scabiei* is important. The organisms that are operative in pathogen suppression do so via different mechanisms including competition for nutrients, antibiosis and induction of host resistance. Some varieties that stimulate disease suppression can increase populations of specific bacteria with antagonistic activity in their rhizosphere towards their pathogens. Methods that change resident microbial communities in a manner which induce natural soil suppressiveness have potential as parts of environmentally sustainable systems for controlling soilborne plant pathogens (Mazzola, 2002). The growth rate is an important issue with two aspects: the first is competition between pathogen and suppressive bacteria to obtain the nutrient and keep the abundance in habitat, and the second is the application of biocontrol species that has an effect on overall plant growth in favour of enhancing

the plant defense (Arseneaul *et al.*, 2013). In a study, biocontrol bacteria showed to be easy to grow, they can also easily colonize the rhizosphere surrounding the roots of numerous plant species, adapt to a wide variety of environmental conditions and produce numerous bioactive metabolites (Chin-A-Woeng *et al*., 2003; Weller, 2007).

In this study, the 16S rRNA was used to measure quantity of actinobacteria and *txtB* gene to measure quanitity of *S. scabiei*, because it is a specific determinant of pathogenicity in *S. scabiei.* In the mutants altered in the production of thaxtomin the pathogenicity was decreased or mutants were nonpathogenic (Goyer *et al*. 1998). There are more copies of 16S rRNA gene in each genome but only 1 copy of *txtB* gene. An operon encodes for *txtA* and *txtB*, a monooxygenase, a nitric oxide synthase and a regulator (Tapia, 2010). The copies of 16S rRNA gene in actinobacteria were determined: 3.1±1.7, in genome size: 5.03±2.53 (Mb) (Vetrovsky and Baldrian, 2013). Wencong showed that in the fields with low and high severity, the proportion of 16S/*txtB* gene copies respectively was around 10^6 and 10^5.

Vermiculite is a mineral composed mainly of clay and mica. It is very light, airy and has good water retention. The strains were grown on oatmeal–vermiculite to prepare inoculum for the pathogenicity and suppression assays. The suppressive strains were grown on a vermiculite-oatmeal broth base and mixed with scab-conducive soil. Effects of vermiculite dilution and/or oatmeal broth nutrients on scab reduction were significant. Using a vermiculite system improved nutrient uptake efficiency and the acquisition of both nitrogen (N) and phosphorus (P) from the environment in crop breeding (Neeno – Eckwall *et al*., 2001, Liu *et al.*, 2017).

In this study, actinobacterial strains isolated from CS suppressive soils and selected for antibiotic activity against *S. scabiei* were tested for suitability in the pathogen suppression. Their grow rate and ability to produce secondary metabolites with antibiotic activities were evaluated in two different liquid and solid media and also in an enriched soil free vermiculite. The growth and activity zone of suppressive bacteria and *Streptomyces scabiei* against *Kocuria rhizophila* was done in parallel. *Kocuria* rhizophila was used as a sensitive Gram positive actinobacteria in sensitivity test (Savini *et al*., 2010).

3. Materials and methods

3.1. Experimental sites

In the Czech Republic, 32 sites located in different climatic areas were selected for monitoring of common scab (CS) disease severity. Seventeen potato varieties differing in susceptibility to CS were grown in soils contrasted by pH, organic matter content and physicochemical characteristics. Three plants were sampled at each site, where no experiment was carried out. At all sites, quantities of total bacteria, actinobacteria and the gene *txtB* were analyzed by real-time PCR. Soil and potato skin were characterized by contents of carbon, nitrogen, phosphorus, sulphur, calcium, magnesium and iron. Other parameters as pH and the soil texture based on particle size were measured.

Vyklantice is a site where fields suppressive (49.5630N, 15.0575E; L for low disease severity) and conducive (49.5614N, 15.0546E; H for high disease severity) to potato CS occur at about 100 m distance. The two fields differ in common scab severity by observations over 30 years, while their geological context, soil type, climate and management are similar. The fields were regularly planted under a four-year crop rotation system including rapeseed, clover, potatoes, and grains (wheat or oats) in the past two decades (Sagova-Mareckova, *et al.,* 2015).

3.2. Pot experiment

Iron supplementation was selected based on significant differences in available iron content determined previously in the studied suppressive and conducive soils. The suppressive soil had a three times higher iron content determined in ammonium acetate extract than the conducive soil, while iron content determined in EDTA extract and total iron content were similar in the two soils (unpublished data). The pot experiment was conducted in 2012. A potato cultivar Agria, susceptible to potato common scab, was planted in 12 L pots with control conducive soil VH, suppressive soil VL, and the VH soil supplemented with 2.5 L peat / 10 L soil (VHP treatment), 815 mg DTPA chelated iron / 10 L soil (VHFe treatment), and a combination of both (VHPFe treatment). The treatments were performed in five replicates (Table 1). The pots were kept submerged in the field soil without irrigation and received regular pesticide treatment throughout the growing season.

Table 1. Design of the pot experiment

Sample	Description
VH-1 to	high scab severity - conducive soil
VHP-1	high scab severity – peat treatment
VHFe-1	high scab severity - DTPA chelated iron
VHPFe-	high scab severity - a combination of both
VL-1 to	low scab severity- suppressive soil

3.3. Field experiment

Potatoes were planted in the beginning of May and sampled on July 16. Samples of bulk soil, tuberosphere soil and potatoes were collected. A CS susceptible cultivar Agria (Agrico Bohemia, Tabor, Czech Republic) and a resistant cultivar Kariera (Sativa Kerkov, Pribyslav, Czech Republic) were used. Potatoes were all certified seed tubers (common scab below 5% of surface). Four plots of each cultivar were planted at each field and the plots were arranged in a Latin square design. Each plot was planted with 3 rows of 12 potato plants (36 plants) separated by 50 cm of bare soil. Fields were fertilized with 100 kg N/ha (ammonium sulfate, 21% N), 35 kg P/ha (monocalcium phosphate, 35% P_2O_5), and 60 kg K/ha (potassium salt, 50% K_2O). Potatoes were treated with pesticides, once with Nurelle D (EC) (chlorpyrifos, cypermethrin) 62 days after planting at 0.6 l/ha to prevent Colorado potato beetle (*Leptinotarsa decemlineata*), and twice with Acrobat MZ (dimethomorph, mancozeb), 48 and 62 days after planting at 2 kg/ha against the potato blight. Fungicides were not used.

3.4. Sampling

For the field sampling, three potato plants and three samples of bulk soils (without influence of the plant) were randomly sampled from each site. Tuberosphere (rhizosphere) soil samples were collected no further than 3 mm from a potato tuber. A tuber was located by careful uncovering the top soil surrounding the plant, slightly pressed to the remaining soil and taken out. The socket remaining in soil after potato

extraction was carefully scratched by a sharp spoon to collect a thin layer of soil. Soil was also collected from the tuber itself if any soil remained attached on the tuber in a thin layer. Bulk soil was collected at a distance of 30 cm from the closest plant within each plot using a small sterile spade. Potatoes from the sampled plant were collected and washed in distilled water. All potatoes were carefully peeled with a potato peeler taking approximately 1 mm thick skin samples, the peels were homogenized and mixed and subsamples were taken for further analyses. Common scab severity was evaluated on 20 potatoes per plot using a 9 degree scale 1: no scab; 2: 0.1–0.8 %; 3: 0.9–2.8 %; 4: 2.9–7.9 %; 5: 8.0–18.0 %; 6: 18.1–34.0 %; 7: 34.1–55.0 %; 8: 55.1–77.0 %; 9: 77.1–100 % (Wenzl and Demel, 1967). Potatoes used for evaluation were those of the collected plant and several more plants from each plot to achieve at least 20 measurements per plot.

The samples from the pot experiment were collected 89 days after planting. All tubers from each pot were collected and treated in the same way as described for the field sampling.

For the field experiment, one potato plant growing in the center of each plot was sampled, stored in a cooler and processed upon the arrival to the laboratory in the same way as described for the field sampling. Potatoes used for the CS severity evaluation were those of the collected plant and several more plants from each plot to achieve at least 20 measurements per plot.

3.5. Soil and potato skin analyses

To determine total soil C, N and S content, 2-gram aliquots of homogenized soil samples from both bulk and tuberosphere (rhizosphere) were dried, milled, and analyzed using Vario MAX CNS analyzer (Elementar Analysensysteme, Hanau, Germany). To determine all other soil elements soil subsamples were leached with boiling nitro-hydrochloric acid (aqua regia) and assessed by optical emission spectroscopy with inductively coupled plasma (ICP-OES) in Aquatest company (Prague, Czech Republic). To determine the concentrations of available iron at planting and harvest, soil subsamples were extracted with 1 M ammonium acetate (20 g in 100 mL) and assessed by optical emission spectroscopy with inductively coupled plasma (ICP-OES) by Aquatest (Prague, Czech Republic).

The analyses of potato skin were performed by the service laboratory of the Institute of Botany (Trebon, Czech Republic). For total nitrogen analysis, 1–3 mg dried skin was mineralized by modified Kjeldahl method in H_2SO_4 with catalyzer at 360° C. For total phosphorus analysis, 20 mg dried skin was sequentially decomposed by HNO_3 and $HClO_4$ (Kopacek and Hejzlar, 1995) In the mineralized samples, both N and P were determined by flow injection analysis with spectrophotometric detection using FIA Lachat QC 8500 analyzer (Lachat Instruments, Hach Company, Loveland, CO). Cation contents in skin were determined by atomic absorption spectrometry using AAS spectrometer ContrAA 700 (Analytik Jena, Jena, Germany) after mineralization with nitro-hydrochloric acid. A composite sample of the bulk soil was assayed for P, K, Mg, and Ca by extraction in Mehlich III solution, for N by mineralization in sulphuric acid, the numbers represent the total contents determined after mineralization (for C,N,S by burning, for other elements by decomposition in aqua regia). The sieve test was performed to measure soil fractions according to particle size of dry weight. A coarse grained soil includes sand (0.06-2 mm) and gravel (2-20 mm) and a fine-grained soil includes clay (< 0.002 mm) and silt (0.002-0.06mm). Soil pH was measured in a water extract after overnight incubation of 5 g soil in 20 mL deionized water.

3.6. Cultivation of actinobacterial strains

3.6.1. Submerged culture experiment

Twenty one strains of actinobacteria isolated from suppressive soils (Vyklantice and Zdirec) and with antibiotic activity against *S. scabiei* and type strain of *S. scabiei* DSM 41658 were cultured on plates and slant tubes of medium supplement BG (GYM: Glucose Yeast Malt extract), which is used as a basic medium for actinobacteria. Fresh colonies with no sporulation after 2 or 3 days of cultivation were used.

The amount of colonies that were carried on the plate or in the flasks (YME, A and G media) was adjust in nutrient medium A, to turbidity of 1 McFarland,an approach to determine microorganism concentration by standard solution of barium chloride and sulfuric acid with a density corresponding to approximately 3 x 10^ 8 bacteria/ml of suspension (McFarland, 1907).

3.6.2. Cultivation media:

A: 10 g starch, 4 g yeast extract, 2 g pepton, pH: 6.5 (close to the soil pH); total volume by distilled water to 1 litre.

Gauze (G): 0.5 g NaCl, 20 g starch, 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 10 mg $FeSO_4$, Total volume: 1 litre, pH=6.

YME: 4 g yeast extract, 10 g malt extract, 4 g glucose, total volume: 1 litre, pH=7.2.

B1 agar: 10 g Beef extract, 10 g pepton, 5 g NaCl, 20 g agar, total volume: 1 litre, pH=7.2.

GYM (BG) agar: 4.0 g glucose, 4.0 g yeast extract, 10.0 g malt extract, 2.0 g $CaCO_3$, 12.0 g agar, total volume: 1 litre, pH=7.2.

The strains 1 to 21 and *S. scabiei* DSM 41658 were grown in 5 flask of submerged culture in A medium for 24, 48, 72, 120 and 240 h.

The 100 ml Erlenmeyer flasks were incubated in the shaker incubator with 180 rpm speed and 28°C. 2 ml of the nutrient medium A inoculated with *S. scabiei* was added to 10 ml of medium YME prepared in sterile 100 ml Erlenmeyer flasks. Harvesting the cultures was done on the 1st, 2nd, 3rd, 5th and 10th day after 24, 48, 72, 120 and 240 hours, by filtration through filter papers (dried in the 105°C oven for 2 h), the papers were weighted before and after filtration, dried in the oven at 105°C to constant weight and weighted to determine the dry weight of cultured bacteria. Dry weight of filter papers was measured after 2 h keeping in the 105°C oven. The spent media were collected to 15 ml falcon tubes and transferred to -20°C to freeze before the sensitivity testing. The submerged culture experiment, was also performed with Gauze medium in the same way for all strains and *S. scabiei*.

3.6.3. Sensitivity test:

The filtered submerged media (A and G) of day 5 (120 h) were selected for the sensitivity test (time point: 120 h, based on maximum growth rate). It was performed on B1 agar on the 9 cm plates. Paper disks with diameter of 5 mm were prepared and sterilized. 25 µl of filtered spent media (kept in -20°C) was transfered on the disk and allowed to dry in a sterile plate at room temperature. An overnight culture of *Kocuria rizophila* was diluted in 3 ml of sterile water to earn a turbidity of 1 McFarland. One ml of this suspension was spread on B1 agar by gentle shaking and the extra liquid was removed by a sterile pipette. Finally, the dried disks were placed on the plate (5

for each plate) and were incubated in 30°C for 24 h, afterwards, the sizes of inhibition zones around the disks were measured.

3.6.4. Vermiculite medium experiment:

The vermiculite medium is an enriched and soil free culture to evaluate the interaction and competition between suppressive actinobacterial species and *S. scabiei* as pathogen, without impacts of soil factors. Combination of suppressive actinobacteria and *S. scabiei* in vermiculite medium can give a good profile of growth and antagonistic activity between them.

The experiment was started for *S.scabiei* to test if the quantitative PCR result for copy numbers of the *txtB* and 16S rRNA genes will correspond. The time point was selected based on the maximum growth rate related to dry weight from the submerged culture experiment. The highest average growth rate was obtained in the nutrient medium after 5 days or 120 h of incubation. The cultivation was done at first in 50 ml YME in 250 ml flasks, shaking for 2 days at 28°C and 180 rpm then, they were centrifuged in 50 ml sterile falcon tubes at 2000g, 4°C for 15 min. The supernatant was discarded and the culture was suspended in 10 ml sterile distilled water in the same falcon tubes. Inoculation was done from 1ml of suspended pellet with addition of 8ml Ac (2 times concentrated A) to Erlenmeyer flasks with sterile vermiculite, and incubated at 28°C, in a stable incubator, mixed once a day by hand. For combined flasks, 0.5 ml of actinobacteria enriched in YME and 0.5 ml of enriched *S.scabiei* in the same media was inoculated (with 8 ml of 2 times concentrated of A or Ac).

3.6.5. Vermiculite media

50 ml vermiculite and 8ml distilled water were sterilized for 90 min, left 1 day at room temperature, and the sterilization was repeated again for 90 min.

Harvesting of vermiculite medium was done after 1, 2, 3, 5, 7, 10, 14, 16 and 20 days. The vermiculite medium was crashed in a washed and dried mortar, homogenized 0.3 g was transferred to 2ml tubes for DNA extraction, the remaining part was kept in the dark vials in 80% methanol, 19%water and 1% absolute acetic acid for HPLC and antibiogram.

3.7. DNA extraction

Subsamples of 0.5 g tuberosphere and bulk soil or 0.3 g vermiculite culture were used for DNA extraction by method SK described by Sagova-Mareckova et al. (2008). Briefly, the method is based on bead-beating and phenol/chloroform extraction followed by purification with $CaCl_2$ and GeneClean Turbo kit (MP Biomedicals, Santa Ana, CA). For DNA extraction from potato periderm, 3 g of periderm samples were fine cut in sterile Petri dish, homogenized, and a 0.3 g subsample was processed in the same way as soil samples to obtain total periderm DNA. DNA quantity and quality were evaluated using agarose gel and UV-absorption spectrometry with Nanophotometer (Implen, Munich, Germany).

3.8. Quantitative real-time PCR

Quantifications were performed with primers eub338f (5'-ACTCCTACGGGAGGCAGCAG-3') (Lane, 1991) and eub518r (5'-ATTACCGCGGCTGCTGG-3') (Muyzer *et al.,* 1993) amplifying a 197 bp fragment of the 16S rRNA gene from Bacteria, act235f (5'-CGCGGCCTATCAGCTTGTTG-3') (Stach *et al.,* 2003) and eu518r yielding a 280 bp product for Actinobacteria, and StrepF (5'-GCAGGACGCTCACCAGGTAGT- 3') and StrepR (5'-ACTTCGACACCGTTGTCCTCAA-3') yielding a 72 bp amplicon of the thaxtomin biosynthetic gene *txtB* (Qu *et al.*, 2008), respectively. The analyses were done on a StepOne Plus Real- Time PCR System (Applied Biosystems, Foster City, CA) using 96-well plates with GoTaq qPCR Master Mix (Promega, Madison, WI) containing SYBR Green as a double-stranded DNA binding dye. The reaction mixture contained in a total volume of 15 ml: 1× GoTaq qPCRMasterMix, 0.2 mM primers, and 0.2–2 ng diluted DNA sample. For all of the mentioned targets the PCR cycling protocol consisted of initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. Melting curves were recorded to ensure qPCR specificity. Baseline and threshold calculations were performed with the StepOne v. 2.2.2 software. The inhibition was tested by serial DNA dilution from each site. All qPCR measurements were done in duplicate. The qPCR standards were prepared by cloning the fragments of the target genes in pGEM-T Easy vector system (Promega). After PCR verification and isolation of the cloned constructs by Pure Yield Plasmid

Miniprep System (Promega), a linear standard was prepared by cleaving with SalI enzyme (New England Biolabs, UK) in a 200 ml reaction mixture containing 1× reaction buffer, 2 mg circular plasmid, and 20 U restriction endonuclease for 2h in 37°C. The linearized plasmid DNA was purified by phenol-chloroform extraction. The aliquots of linearized and purified standard diluted to 20 ng/ml were stored in-70°C.

3.9. Illumina MiSeq sequencing and analysis.

From the DNA samples, fragments of the 16S rRNA gene including the variable region V4 were PCR amplified using universal primers with 5'linkers CS1_515F/CS2_806R (Caporaso *et al.,* 2011) for bacteria, and CS1_ARC344F/CS2_Arch806R (Takahashi *et al.,* 2014), for archaea (Table 2). PCRs were performed in 25 µl reaction volumes using the *Ex Taq* HS DNA Polymerase (Takara, Kusatsu, Japan), and the PCR conditions were as follows: 5 min initial denaturation at 95 °C, followed by 28 cycles of: 30 s denaturation at 95 °C, 45 s annealing at 55 °C for *Bacteria* or 50 °C for *Archaea*, and 30 s extension at 72 °C. Fragments of the eukaryotic18S rRNA gene including the variable region V9 were amplified using primers CS1_Euk1391F/CS2_EukBr (Amaral-Zettler *et al.,* 2009) (Table 2). PCR conditions were according to the standard protocol of the Earth microbiome project (http://www.earthmicrobiome.org): 3 min initial denaturation at 94 °C, followed by 28 cycles of: 45 s denaturation at 94 °C, 60 s annealing at 57 °C, and 90 s extension at 72 °C. Construction of amplicon libraries including the second PCR and sequencing using MiSeq sequencer (Illumina, San Diego, CA) were done at the DNA Services Facility, Research Resources Center, University of Illinois (Chicago, IL). Resulting paired sequence reads were merged, filtered, aligned using reference alignment from the Silva database[54], and chimera checked using integrated Vsearch tool[61] according to the MiSeq standard operation procedure (Miseq SOP, February 2018) (Kozich *et al.,* 2013), in Mothur v. 1.39.5 software (Schloss *et al.,* 2009). A taxonomical assignment of sequence libraries was performed in Mothur using the Silva Small Subunit rRNA Database, release 128 (Yilmaz *et al.,* 2014), adapted for use in Mothur (https://mothur.org/w/images/b/b4/Silva.nr_v128.tgz) as the reference database. Sequences of plastids, mitochondria, and those not classified in the domain Bacteria were discarded. The sequence library was clustered into OTUs using

the Uparse pipeline in Usearch v10.0.240 softwar (Edgar, 2013) and the OTU table was further processed using tools implemented in the Mothur software. Distance matrices describing the differences in community composition between individual samples were calculated using the Yue-Clayton theta calculator (Yue and Clayton, 2005) Analysis of molecular variance (AMOVA) (Martin, 2002) was based on a matrix of Yue-Clayton theta distances. Metastats analysis (White *et al.*, 2009) was used to detect differentially represented OTUs.

Table 2. Primers used in qPCR and amplicon preparation for microarray and Illumina sequencing analyses

Primer	Sequence (5'-3')[a]	Sense	Target
T7-pA	TAATACGACTCACTATAG-AGAGTTTGATCCTGGCTCAG	forward	16S rRNA gene, bacteria
pH	AAGGAGGTGATCCAGCCGCA	reverse	
CS1_515F	ACACTGACGACATGGTTCTACA-GTGCCAGCMGCCGCGGTAA	forward	16S rRNA gene, bacteria
CS2_806R	TACGGTAGCAGAGACTTGGTCT-GGACTACHVGGGTWTCTAAT	reverse	
CS1_ARC344F	ACACTGACGACATGGTTCTACA-AC-GGGGYGCAGCAGGCGCGA	forward	16S rRNA gene, archaea
CS2_Arch806R	TACGGTAGCAGAGACTTGGTCT-GG-ACTACVSGGGTATCTAAT	reverse	
CS1_Euk1391F	ACACTGACGACATGGTTCTACA -CG - GTACACACCGCCCGTC	forward	18S rRNA gene, eukaryotes
CS2_EukBr	TACGGTAGCAGAGACTTGGTCT-CA-TGATCCTTCTGCAGGTTCACCTAC	Reverse	

[a] The sequences aligning to the target are underlined for the primers with 5' overhang parts.

3.10. 16S rRNA gene-based taxonomic microarray.

A taxonomic microarray based on DNA probes targeting 16S rRNA genes representing 19 bacterial phyla at different taxonomic levels (Kyselkova *et al.,* 2009)

was used to assess soil samples from potato fields. This microarray was validated previously (Kyselkova *et al.,* 2009, Bouffaud *et al.,* 2012). Twelve probes targeting the genus *Streptomyces*, as well as *S. scabies* and relatives (Table 1) were added to the previous probe set (1033 probes) in this study. The probe KO 08 (Franke-Whittle *et al*., 2005), for genus *Streptomyces* was obtained via the *probeBase* server (Greuter *et al*., 2016) (http://probebase.csb.univie.ac.at). The other 11 probes (20-mers) were designed in this study using ARB sofware (Ludwig *et al.,* 2004) (http://www.arb-home.de) and the parameters of the Probe Design function chosen by Sanguin et al.(Ludwig *et al.,* 2004, Sanguin *et al.,* 2006). Probe specificity was tested with the Probe Match function in ARB against the reference Silva-104 and with the TestProbe online tool against Silva 126 database (Quast *et al.,* 2013) (http://www.arb-silva.de), at the weighted mismatch value of 1.5 (Kyselkova *et al.,* 2009). Hybridization properties of probes (e.g. melting temperature, potential formation of secondary structures and 3'dimers) were further tested *in silico*, according to Sanguin et al. (Sanguin *et al.,* 2006, Sanguin *et al.,* 2006).

Universal bacterial primers T7-pA/pH (Table 2) were used to amplify 16S rRNA genes from total DNA extracts (Bruce *et al.,* 1992). Primer T7-pA includes at the 5' end the sequence of T7 promoter, which enabled T7 RNA polymerase-mediated *in vitro* transcription using purified PCR products as templates. PCR reactions were carried out using Taq Expand High Fidelity (Roche Applied Science, Meylan, France) and cycling conditions described in Kyselkova et al. (2009). Purified PCR products (50 ng/µl) were fluorescently labelled (Cy3) by *in vitro* transcription, according to Stralis-Pavese et al. (2004).

Purified RNA was fragmented by incubation with $ZnSO_4$, as described (Stralis-Pavese *et al.,* 2004) and 400 ng subjected to hybridization on the microarray. Each probe was present in four copies per slide, and two slides were hybridized per sample. Hybridization was carried out according to Sanguin et al. (Sanguin *et al.,* 2006). Slides were scanned at 532 nm, images were analyzed with GenePix Pro 7 (Molecular Devices, Sunnyvale, CA), and spot quality was checked visually, as described previously (Sanguin *et al.,* 2006). Data filtration was conducted using R 3.3.0 (Core Team, 2018) (http://www.r-project.org). Hybridization of a given spot was considered positive when 80% of the spot pixels had intensity higher than the median local background pixel intensity plus twice the standard deviation of the local background. Intensity signals (median of signal minus background) were replaced by their square

root value and intensity of each spot was then expressed as a fraction of the total intensity signal of the basic pattern it belongs to (Sanguin *et al.,* 2006); Finally, a given feature probe was considered as truly hybridized when (i) hybridization signals were superior to the mean signal of the negative controls and (ii) at least 3 of 4 replicate spots displayed positive hybridization (Kyselkova *et al.,* 2009).

3.11. Statistical analysis

The differences between samples (suppressive and conducive fields, soils and cultivars, for soil chemical parameters and log copy numbers of bacterial and actinobacterial 16S rRNA genes, and *txtB* genes in soil and periderm samples) were tested by ANOVA and Welch's two sample t-test (allowing differences between variability of variables), which aims at detection of differences in mean values or by ANOVA and Fisher LSD tests. All variables were log-transformed to make their distribution more similar to a normal distribution. P-values for the pairwise comparison were adjusted for multiple comparison problems with the help of the Max-abs-t-distribution method (Hothorn et al. 2011). AMOVA was used to test differences between distance matrices (Yue-Clayton theta) between Illumina samples. The distance matrices were plotted by non-metric multidimensional scaling (Venables and Ripley, 2002) using Mass package, and vectors of environmental variables were fitted to the ordination using Vegan package in the R software environment (R Core Team, 2018).

Two tests based on distance matrices were used. While PERMANOVA (McArdle and Anderson, 2001) tests how much the within group distances are on average shorter than the between group distances (aiming at detection of different mean profiles), dispersion test (denoted later as 'disp') introduced by Gijbels and Omelka (2013) tests how much the average within group distances differs among the groups (aiming at detecting of different dispersions of the samples). The correlation coefficients at different fields were compared through the permutation test introduced in Omelka and Pauly (2012). All statistical calculations were done in the R computing environment (R Core Team, 2018).

3. Results

3.1. Determination of biological and chemical factors associated with natural soil suppressivity to potato common scab at contrasting sites.

The sites differed in nutrient contents and biological properties in soil and skin.(Table 3A, Fig. 1-6). There was a significant (p value ≤ 0.001) difference between location and chemical variables: total contents of N [%], C [%], S [ppm], P [mg/kg], Mg [mg/kg], Ca [mg/kg], and Fe [mg/kg] in soil, and N [%], P [mg/kg], Mg [mg/kg], Ca [mg/kg], and Fe [mg/kg] in skin, showed that the nutrient properties of the fields are locally specific (Table 3B).

Similarly, severity of CS varied strongly between sites. The highest severity, around 6 on the 9 point scale was observed at the high-severity field in Zdirec (Zdirec cesta, Zc). A significant difference was determined between the values of CS severity in summer and autumn (ANOVA, p< 0.001) (Fig. 24, 25, Table 3B).

The maximum of severity in summer was seen in variety Valfi and Rumba. The minimum was seen in variety Carrera, Marabella and Kariera. The maximum of severity in autumn was seen in variety Valfi, Agria and David. The minimum was seen in variety Volex (Fig. 26, 27).

A positive correlation was determined between CS severity and N in soil and skin, Fe in soil and skin, and Mg in soil and skin, while for *txtB* gene in soil it was a negative correlation with N in soil and skin, Fe in soil and skin, Mg in soil, S in soil, P in soil, Mg in skin, positive with Ca in soil and skin.

The *txtB* gene in skin was positively correlated with C in soil, N in soil but not skin, Fe in soil and skin and a negatively correlated with Ca in soil. The correlations were also specific for summer and autumn sampling. Important differences in nutrient contents occurred also between the bulk and rhizosphere. There was a significant difference between bulk and rhizosphere in the nutrients: N, S and Fe in soil (Methodology: Welch version of the two-sample t-test and Two-sample problem with random effects.

Vyklantice uvoz (Vu) was one of the field with maximum severity in summer, the severity of scab was also was high in autumn (Fig. 24, 25). Vyklantice uvoz (Vu) is close to another field (Vyklantice kostel (Vk) which was one of the field with minimum severity in summer and also a low severity in autumn. Vyklantice uvoz (Vu) and Vyklantice kostel (Vk) had a high content of S, Mg and Fe in soil and the copy numbers of bacteria and actinobacteria in soil and *txtB* in skin in both fields was high. In V. uvoz the content of Fe,

Mg and copy numbers of total bacteria in rhizosphere was higher than bulk (r>b) while in V. kostel, the content of Fe, Mg and copy numbers of total bacteia was higher in bulk soil (b>r). In both fields, copy numbers of *txtB* and 16S rRNA gene from actinobacteria were higher in bulk soil; V. uvoz (Vu) also had more copy numbers of *txtB* gene and 16 S rRNA from actinobacteria in skin and a high content of N in skin, C in soil (r=b), and Ca in skin.

Compared to Zdirec les (Zl), Zdirec cesta (Zc) had higher copy numbers of *txtB* gene, total bacteria and actinobacteria in bulk and rhizosphere (r>b) and higher content of N, C, S, Mg, Ca and Fe in rhizosphere and bulk (rhizosphere more than bulk, except Ca) and also higher content of N and Ca in skin. Both fields had the maximum of proportion of *txtB* in skin/*txtB* in soil, maximum of actinobacterial 16S rRNA gene in skin and minimum of *txtB* in bulk between 32 fields. The *txtB* in rhizosphere of Zdirec les was minimum between all fields (Fig. 6-9).

Hostice (Hs) was another field with higher severity in summer. It was one of the fields with minimum of actinobacteria and total bacterial copy numbers in rhizosphere and bulk and *txtB* gene in skin. In opposite of poor biological factors, Hostice (Hs) had a higher content of P and Mg in bulk and rhizosphere, the contents in bulk were higher than in rhizosphere (b>r).

Slavkov-Vinohrady (Sl) is the last field with maximum severity in summer, described to make better comparison and understanding of the fields with high severity. Slavkov-Vinohrady (Sl) had a high content of N (r=b), Ca (r>b), C (the same in rhizosphere and bulk), pH (the same in rhizosphere and bulk), and the high content of P in skin. The copy numbers of *txtB* gene in skin were low compared to other fields.

The minimum severity in summer was seen in Polepy uvoz (Pu), Ruzyne (Ru), Stankov (St), Sutom (Su), Vysoke u Pribrame (VP) and Vyklantice kostel (Vk) and in autumn was seen in Ruzyne (Ru), Stankov (St), Malonty (Ma) and Horazdovice (Hr).

Vyklantice kostel (Vk) with low severity had high content of S, Mg and Fe in soil, the copy numbers of 16S rRNA from bacteria and actinobacteria in soil and *txtB* gene in skin. In V. kostel (Vk), the content of Fe and Mg and copy numbers of total bacteria were higher in bulk soil.

Polepy uvoz (Pu) had the minimum of severity in summer with the low content of N, P, Fe and Mg (bulk more than rhizosphere except P) (Fig. 10, 11, 16, 17, 22, 23, 24). Polepy uvoz (Pu) had the minimum of *txtB* gene in skin and the maximum of *txtB* gene in soil (bulk more than rhizosphere), and also the maximum of sand was seen in Polepy uvoz (Pu) (Table 3A).

Ruzyne (Ru) had a low severity in summer and autumn. Ruzyne (Ru) had a high pH and high content of C, N, Ca and S (rhizosphere more than bulk, except Ca), and low content of Mg (r>b), N in skin, *txtB* in skin and H+.

Stankov (St) had a low severity in summer and autumn with low content of actinobacteria and total bacteria (bulk more than rhizosphere) and *txtB* in skin.

Sutom (Su) had a low severity in summer, with high content of N, C, S, pH (rhizosphere more than bulk), P, Ca, Fe (bulk more than rhizosphere) and N in skin and low numbers of bacteria and actinobacteria in soil (rhizosphere more than bulk) and low numbers of *txtB* gene in skin. The maximum of clay was seen in Sutom (Su) (Table 3A).

Vysoke u Pribrame (VP) had a low severity in summer and low content of Mg and Ca (b>r). Horazdovice (Hr) had a low severity in autumn and low content of Ca (r>b), P in skin, *txtB* gene in skin and high content of Mg (r>b). Malonty (Ma) had a high severity in summer and a low severity in autumn, a high range of N, C, Fe, Ca, S and P in rhizosphere and bulk (in rhizosphere more than bulk, except S), and also a low content of Ca in skin and soil.

Table 3A. The average of chemical parameters and biological variables, measured in the soil samples (included rhizosphere and bulk) and skin of potatoes in 32 sites.

	Location	N(tot)[%] soil	Ct [%] soil	St[ppm] soil	P(tot)[mg/kg] soil	Mg [mg/kg] soil	Ca [mg/kg] soil	Fe [mg/kg] soil	N(tot) [g/kg] skin	P(tot)[mg/kg] skin	Ca [g/kg] skin	Mg [g/kg] skin	Fe [g/kg] skin	pH	actino 16S copies/g skin	txt copies/g skin	bacteria 16S copies/g soil	actino 16S copies/g soil	txt copies/g soil	scab severity in summer	scab severity in autumn	clay[l%]	silt [%]	sand[%]	gravel [%]
1.	Čechtice (Če)	0.17442	1.5	283	820	7281	3288	29942	21.6	1695	1.02	0.9	0.77	6.1	1E+09	9E+06	4E+09	1E+09	1E+06	2.68	3.1	3	32	45	20
2.	České Budějovice (CB)	0.13156	1.3	199	713	1379	2046	11208	17.2	3104	0.77	1.02	0.69	6.1	7E+08	2E+06	5E+09	2E+09	9E+05	1.025	1.2	5	27	65	3
3.	Havlíčkova Borová (HB)	0.16367	1.5	293	916	6007	1938	24467	21.2	2058	0.68	0.75	0.47	5.2	1E+09	1E+06	2E+09	6E+08	6E+05	1.92	3.2	2	41	47	10
4.	Horažďovice (Hr)	0.18158	1.7	368	1044	9540	3208	30133	20.1	1718	0.85	0.77	0.3	5.7	6E+08	7E+05	4E+09	1E+09	9E+05	1.33	1	2	33	51	14
5.	Hoštice (Hs)	0.1425	1.3	243	1675	16517	5410	35500	19.4	3063	0.85	1.26	0.78	6	1E+09	3E+06	1E+09	2E+08	8E+05	3.36	1.7	2	21	64	13
6.	Krásné Údolí (KU)	0.18533	1.8	278	1195	6118	6937	32283	20.1	2505	0.84	0.73	0.22	5.6	1E+09	2E+06	4E+09	1E+09	8E+05	1.35	1	2	22	56	20
7.	Kujavy (Ku)	0.15583	1.4	229	874	2485	2733	15300	15	3416	0.97	0.79	0.3	6.2	2E+09	3E+06	1E+09	3E+08	1E+06	3.3	1.7	7	78	15	0
8.	Lípa u Havl. Brodu (Li)	0.1955	1.9	313	892	3010	3065	21717	18.7	2131	0.54	0.63	0.04	6.1	1E+08	2E+06	5E+09	1E+07	8E+05	1.31	1	9	41	46	4
9.	Lukavec (Lu)	0.18192	1.7	303	788	6897	1905	27067	15.2	2740	1.3	0.75	0.08	5.9	7E+07	2E+06	2E+09	7E+08	8E+05	1.21		5	30	55	10
10.	Malonty (Ma)	0.27033	2.7	445	950	8970	3433	34233	16	3107	0.78	1.01	0.96	6.1	3E+08	1E+06	2E+09	3E+08	8E+05	4.06	1	5	40	47	8
11.	Milotičky (Mi)	0.177	1.7	386	898	8583	1795	30600	17.2	2159	0.48	1.05	0.57	5.9	5E+08	1E+06	2E+09	5E+08	8E+05	1.18	1.1	1	26	42	31
12.	Mostiště (Mo)	0.17633	1.6	292	1066	9632	3840	37167	16.9	3786	0.92	0.85	0.09	6.3	5E+07	2E+06	4E+09	1E+09	9E+05		1.1	4	41	55	0
13.	Netřebice (Ne)	0.194	1.8	320	806	7453	2495	26050	25.2	2104	0.73	1.47	0.86	5.7	8E+08	1E+06	2E+09	7E+08	9E+05	1.25	1.2	4	37	54	5
14.	Polepy-cesta (Pc)	0.14454	1.8	333	959	1721	3539	11850	26.7	2847	1.07	0.93	0.16	6.3	6E+08	1E+06	6E+09	3E+09	1E+06	1.475		10	20	71	0
15.	Polepy-drahota (Pd)	0.17208	1.6	306	740	3450	8840	18517	25.3	2255	2.07	0.85	0.21	7.1	1E+09	2E+06	6E+09	3E+09	1E+06	1.21		20	41	39	0
16.	Polepy-úvoz (Pu)	0.10008	1.3	241	632	1163	2648	8563	22.1	3092	1.07	0.95	0.2	6.4	3E+08	1E+06	3E+09	1E+09	1E+06	1.045		7	15	78	0
17.	Radostín nad Oslavou (Ra)	0.15558	1.4	287	786	4100	2093	18750	25.1	1802	0.85	0.94	0.14	5.8	1E+09	1E+07	2E+09	6E+08	1E+06	3.45	2.8	5	47	47	1
18.	Ruzyně (Ru)	0.21008	2.5	376	1032	3002	9647	24017	16.4	2480	0.96	0.72	0.03	7.2	1E+08	1E+06	4E+09	1E+09	1E+06	1	1	19	64	11	6
19.	Slavkov-Vinohrady (Sl)	0.28933	3.6	467	1250	6578	27767	21817	15.1	3522	1.05	0.84	0.25	7.3	2E+08	7E+05	1E+09	6E+08	1E+06	1.28		12	63	23	2
20.	Staňkov (St)	0.13775	1.4	298	669	3267	2675	17533	16.7	2170	0.92	0.73	0.05	6	8E+07	2E+06	1E+09	5E+08	9E+05	1	1	12	52	36	0
21.	Starý Pelhřimov (SP)	0.1505	1.5	228	694	4958	2028	24400	20.7	2400	1.07	0.69	0.03	6	4E+07	2E+06	2E+09	8E+08	6E+05	1.55	2	3	32	61	4
22.	Sutom (Su)	0.36733	4.5	604	1387	8393	47233	38533	26.3	2356	1.02	1.03	0.09	7.3	3E+08	1E+06	1E+09	8E+08	6E+05	1		27	46	12	15
23.	Trutnov (Tr)	0.12225	1.2	208	852	2510	1977	14050	22.2	2767	0.79	0.98	0.12	6	4E+08	6E+06	2E+09	2E+09	6E+05	1.47	2.2	5	54	40	1
24.	Vodňany Svobodné Hory (V o)	0.14733	1.4	276	1517	8887	4433	17583	20.2	3596	0.8	1.58	1.78	5.7	6E+08	1E+06	1E+09	6E+08	7E+05	1.51		2	23	55	20
25.	Vyklantice-kostel (Vk)	0.15457	1.4	745	964	12073	2110	41025	22.1	2339	0.77	0.94	0.27	5.4	9E+07	1E+06	1E+10	3E+09	3E+05	1.857	1.4	3	16	72	9
26.	Vyklantice-úvoz (Vu)	0.21873	2.1	673	1169	11518	3339	44936	21.6	2610	1.08	0.92	0.35	6	1E+09	3E+07	1E+10	4E+09	3E+05	3.011	2.6	0	30	62	8
27.	Vyšní Lhoty (VL)	0.18158	1.8	269	885	2898	2838	25017	15.9	2258	0.69	0.83	0.27	6.1	1E+09	1E+07	4E+09	2E+09	7E+05	1.54	1.1	8	60	30	2
28.	Vysoké u Příbrami (VP)	0.16783	1.7	308	724	3683	3100	21133	20.1	2466	0.79	0.74	0.22	5.6	8E+08	5E+06	2E+09	1E+09	1E+06	1.04		6	50	24	20
29.	Zderaz (Zd)	0.20358	2	315	636	2003	2368	9312	22	2640	0.93	0.69	0.16	5.6	1E+09	7E+06	5E+09	2E+09	1E+06	1.82	1.6	4	32	39	25
30.	Ždírec-cesta (Zc)	0.22144	2.3	305	722	4559	4153	27075	23.5	3178	1	1.07	0.71	6.4	3E+09	5E+07	2E+10	6E+09	7E+05	4.448	6.2	6	56	35	3
31.	Ždírec-les (Zl)	0.17772	1.7	244	858	3777	2338	22513	23.3	2713	0.68	1.12	0.7	6.4	4E+09	4E+07	1E+10	4E+09	2E+05	6.685	3.9	12	56	29	3
32.	Židovice (Zi)	0.16403	2	315	962	5605	14550	27013	14.3	3063	0.9	0.61	0.02	6.8	7E+07	4E+06	4E+09	2E+09	8E+05	1.19	1.1	10	42	42	5.6

Table 3B. Statistical significance between variables.

Nutrients and bacterias vs. Severity		Nutrients vs. *txtB* soil		Nutrients vs. *txtB* skin		Rhizospeheres vs. bulk	
value	p-value	value	p-value	value	p-value	value	p-value
txt.skin actino.skin bacteria.soil actino.soil Fe.skin N.skin Fe.skin Mg.skin Fe.skin N.skin N.skin Fe.skin Mg.skin N.soil Fe.soil Fe.soil N.soil txt.soil	<0.001	Fe.soil Fe.soil Mg.soil Mg.soil St.soil bacteria.soi St.soil bacteria.soi Ca.skin P.soil Ca.skin P.soil actino.soil actino.soil	<0.001	actino.skin actino.skin actino.soil actino.soil bacteria.soil bacteria.soil	<0.001	N.soil Ct.soil	<0.001
Ca.soil Ct.soil Mg.soil	0.005 0.009 0.12	Mg.skin pH Mg.skin N.soil N.skin P.skin N.skin N.soil	0.011 0.001 0.014 0.013 0.081 0.087 0.097 0.33	Fe.skin Fe.skin N.soil Mg.skin Mg.skin Ca.skin Ca.skin N.soil Ct.soil	<0.005	St.soil Ca.soil actino.soil txt.soil	<0.05

				P.skin			
P.soil Ct.soil P.soil Ca.soil Ca.skin Mg.soil Ca.skin pH P.skin St.soil P.skin St.soil	>0.1	Fe.skin txt.skin Ca.soil P.skin actino.skin txt.skin actino.skin Ca.soil Fe.skin Ct.soil Ct.soil	>0.1	N.skin P.skin Fe.soil Fe.soil N.skin Ct.soil txt.soil pH Ca.soil Mg.soil txt.soil P.soil P.soil Ca.soil Mg.soil St.soil St.soil	>0.05	P.soil Mg.soil Fe.soil pH bacteria.soil	>0.05

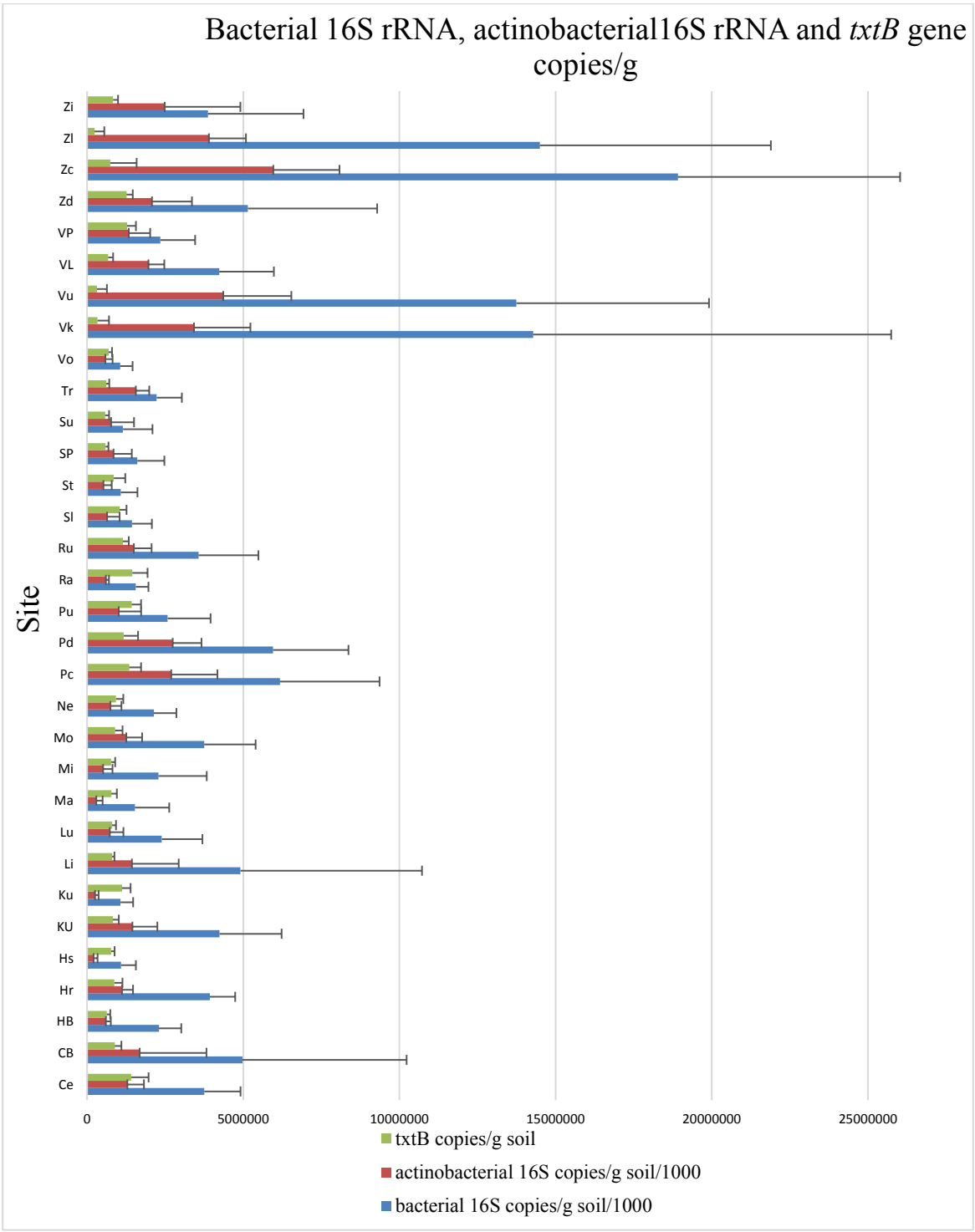

Fig. 1. Comparison of copy numbers of total bacterial 16S rRNA, actinobacterial 16S rRNA and *txtB* gene in soil samples. To make the data possible to show the copy numbers of 16S rRNA, divided to 1000. (means ± standard deviations, n=24 for CB, n=12 for Pc and Pu and Ce, n=28 for Vk, Vu, n=16 for Zc and Zl, n=15 for Zi, n=6 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne.

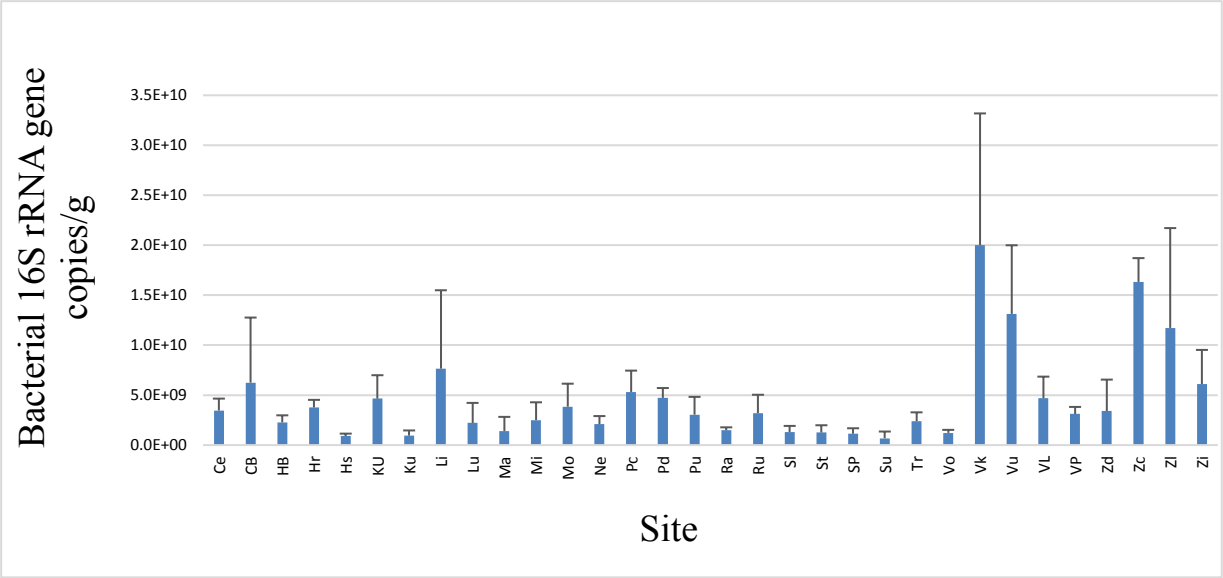

Fig. 2. The copy numbers of total bacterial 16S rRNA gene in bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

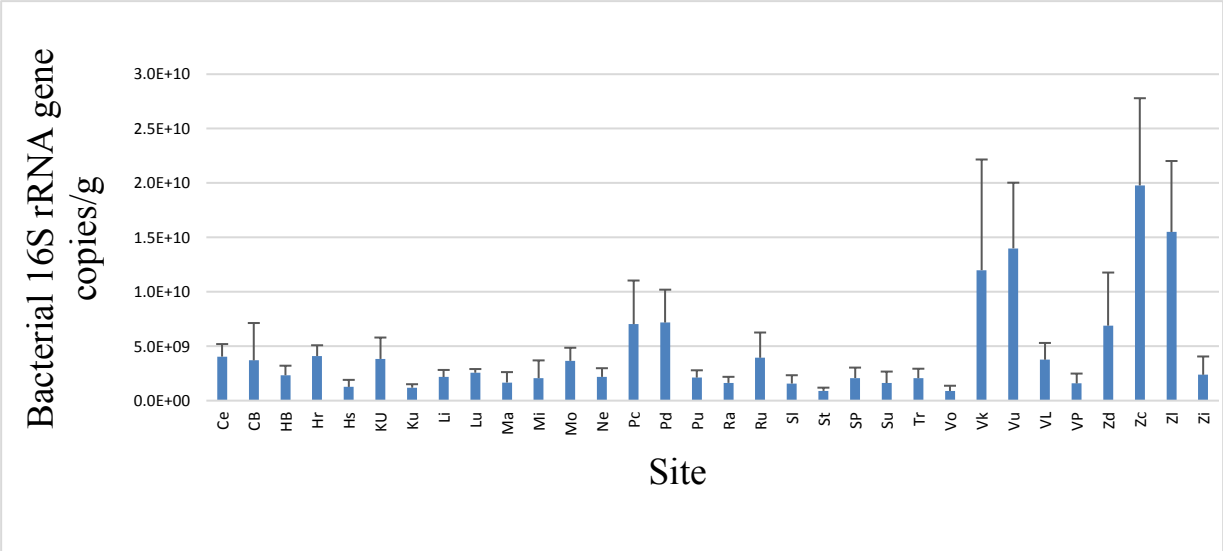

Fig. 3. The copy numbers of total bacterial 16S rRNA gene, in rhizosphere samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

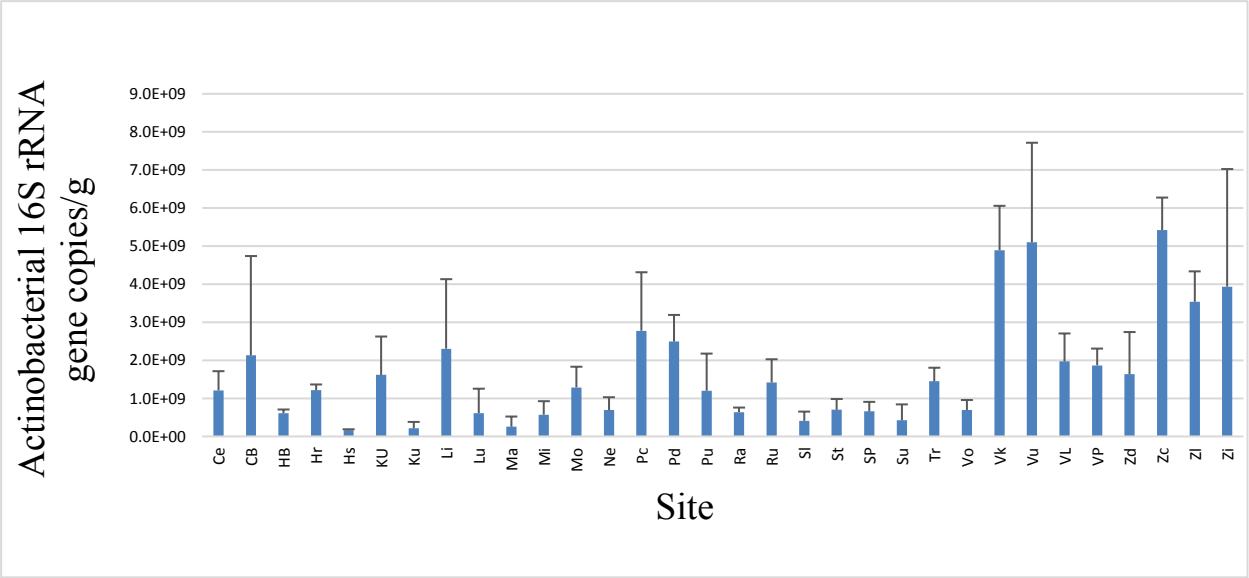

Fig. 4. The copy numbers of total actinobacterial 16S rRNA gene, in bulk samples. (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

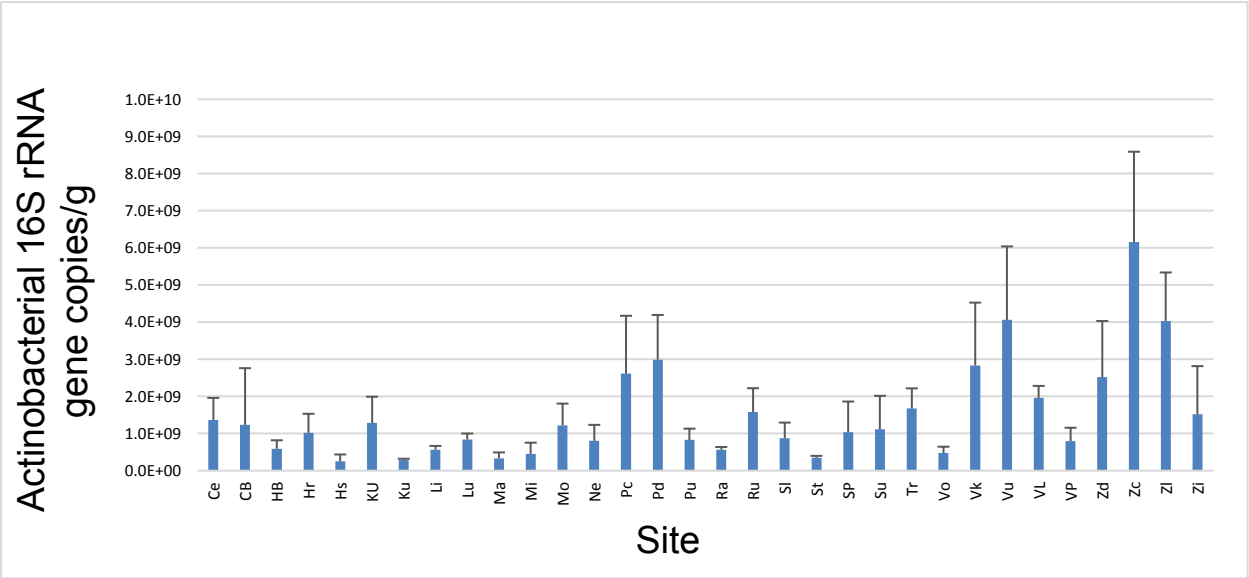

Fig. 5. The copy numers of total actinobacterial 16S rRNA gene in rhizosphere samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

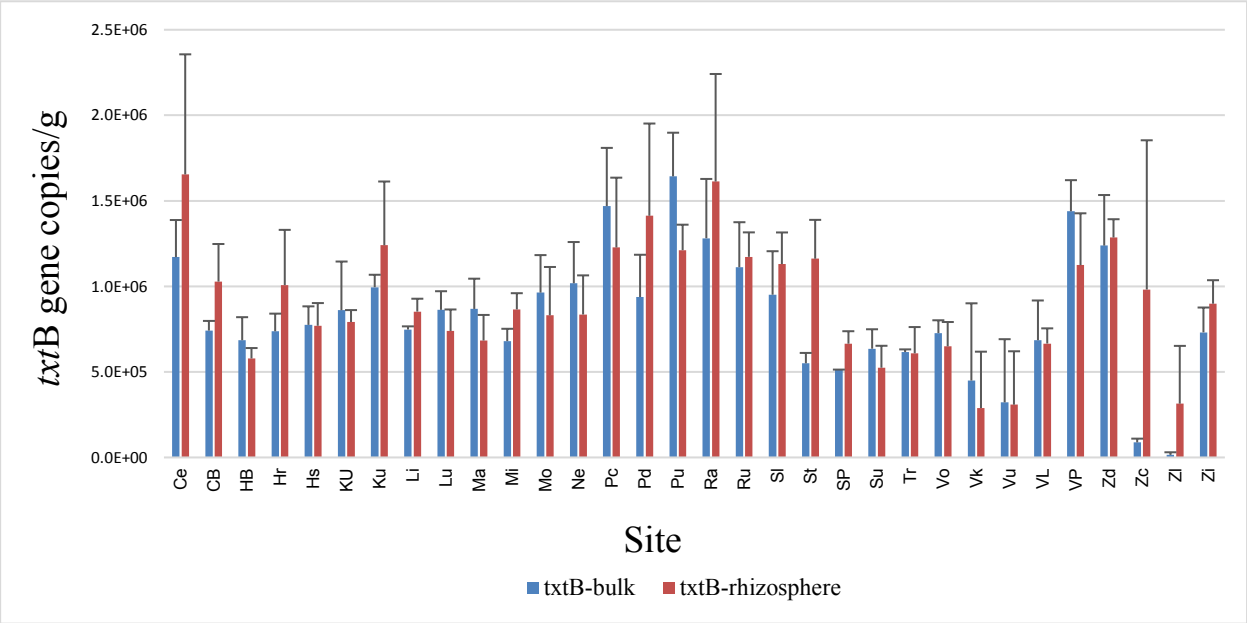

Fig. 6. The comparison of copy numbers of *txtB* in bulk and rhizosphere. For bulk samples n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl) and for Rhizosphere samples n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

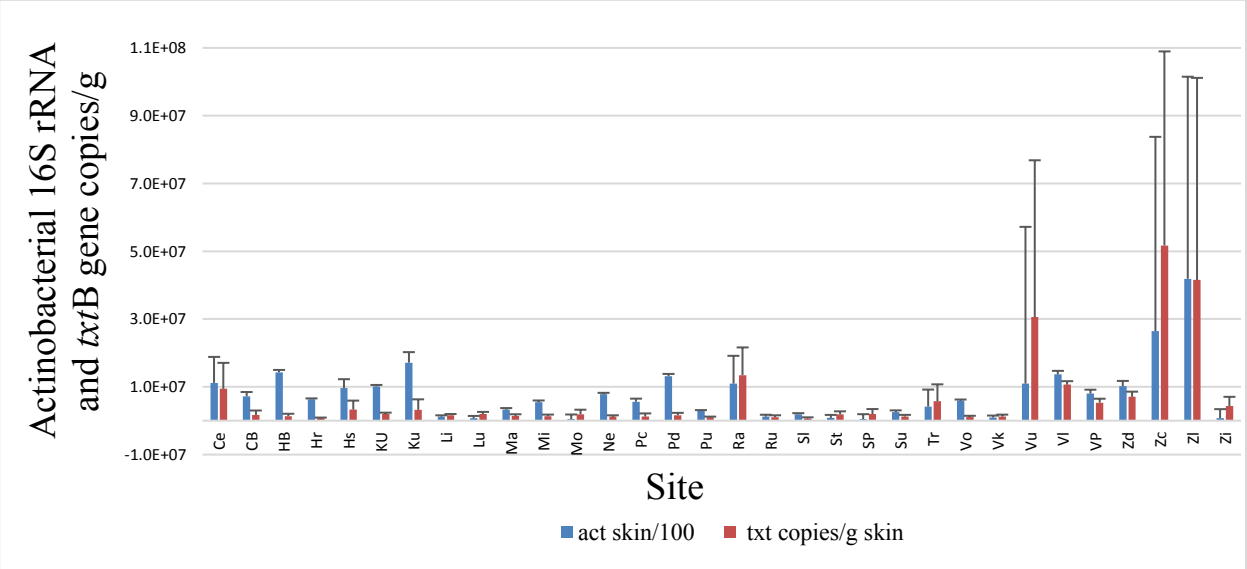

Fig. 7. The comparison of copies of two genes in the skin samples: 16S rRNA gene from actinobacteria and *txtB*. (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi).

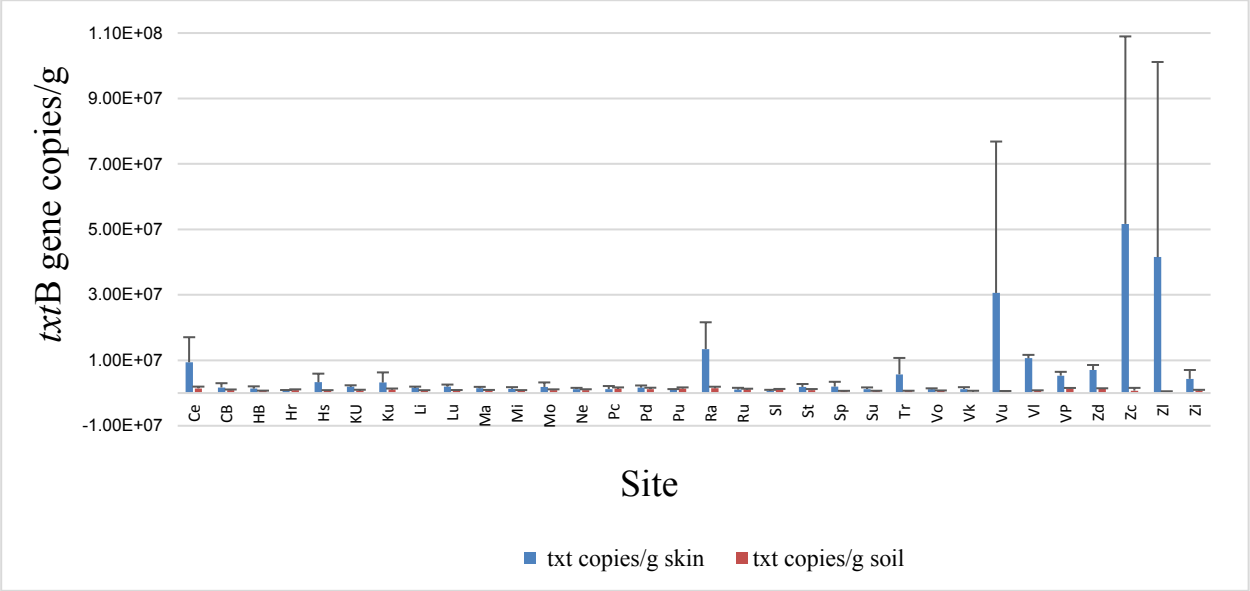

Fig. 8. Comparison of copy numbers *txtB* gene, in skin and soil. (means ± standard deviations, for skin: n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi for soil n=24 for CB, n=12 for Pc and Pu and Ce, n=28 for Vk, Vu, n=16 for Zc and Zl, n=15 for Zi, n=6 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne.

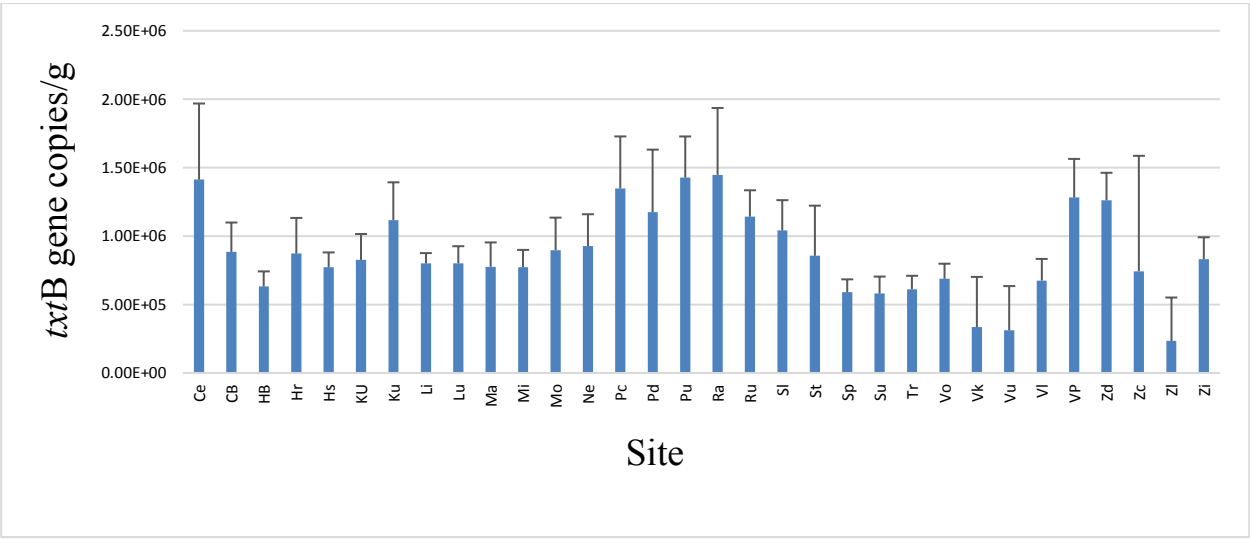

Fig. 9. The copy numbers of *txtB* in soil (means ± standard deviations, n=24 for CB, n=12 for Pc and Pu and Ce, n=28 for Vk, Vu, n=16 for Zc and Zl, n=15 for Zi, n=6 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne.

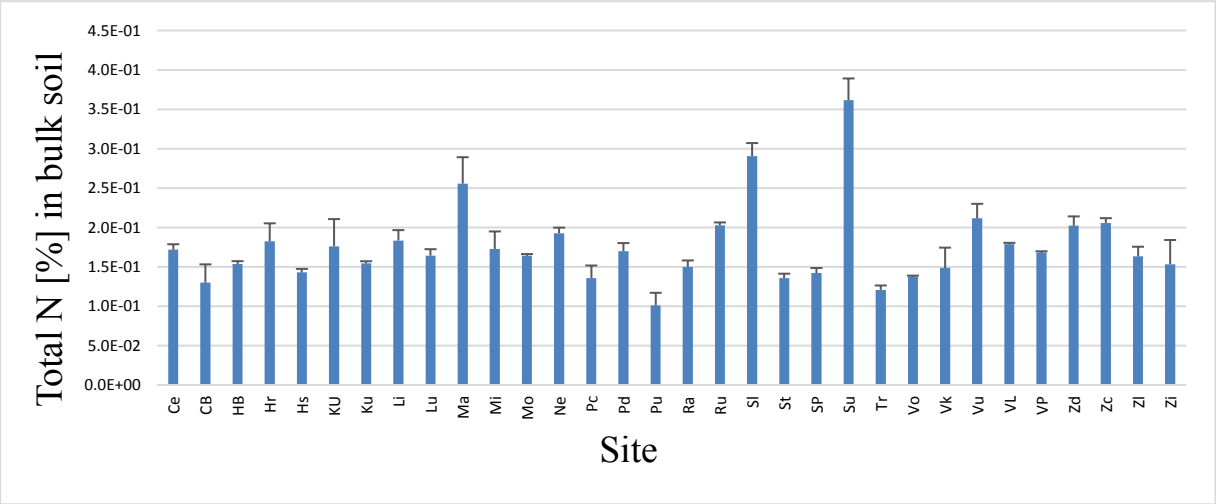

Fig. 10. The average of N [%], in bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

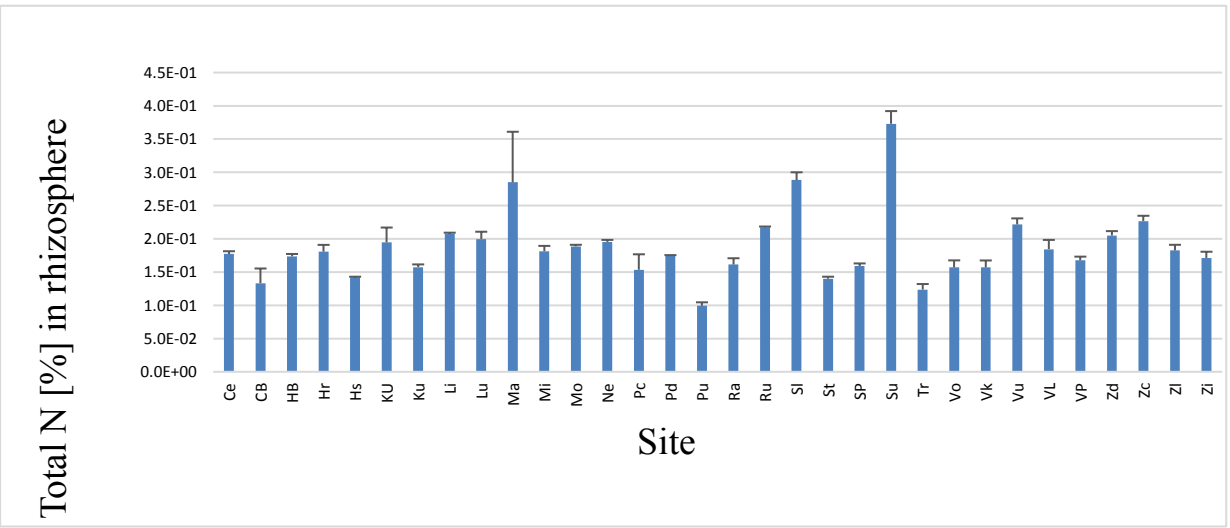

Fig. 11. The average of N [%], in the rhizosphere (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

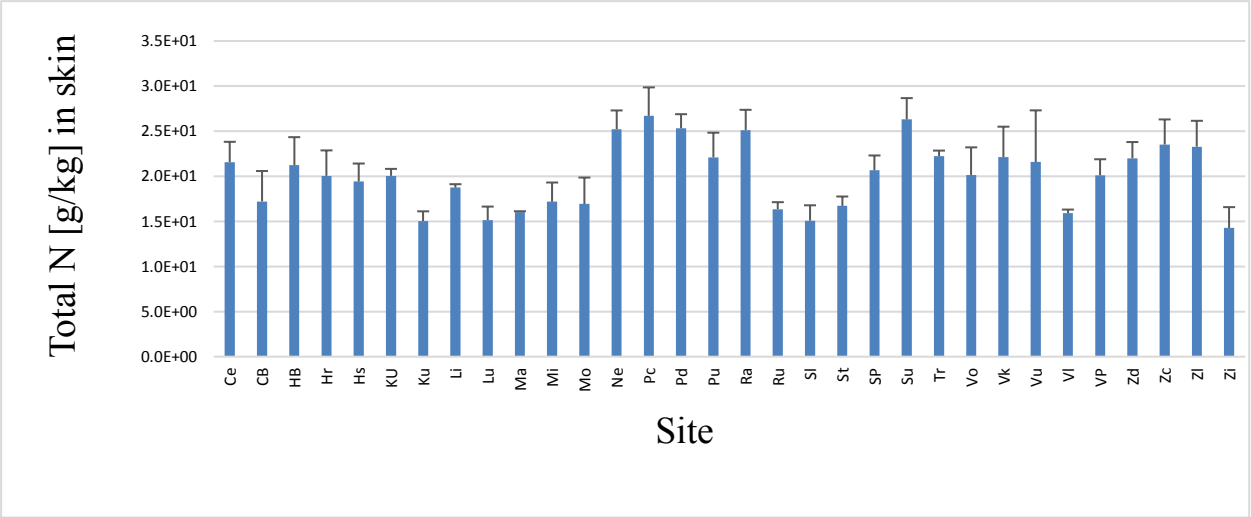

Fig. 12. The average of N(tot) in the skin samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi).

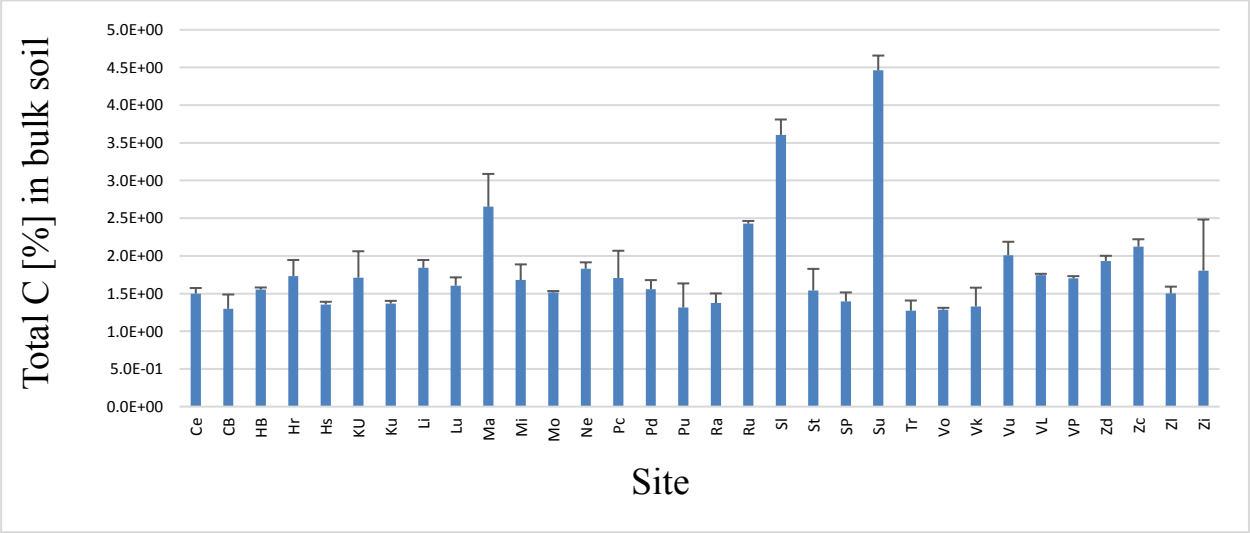

Fig. 13. The average of Ct (%) in the bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

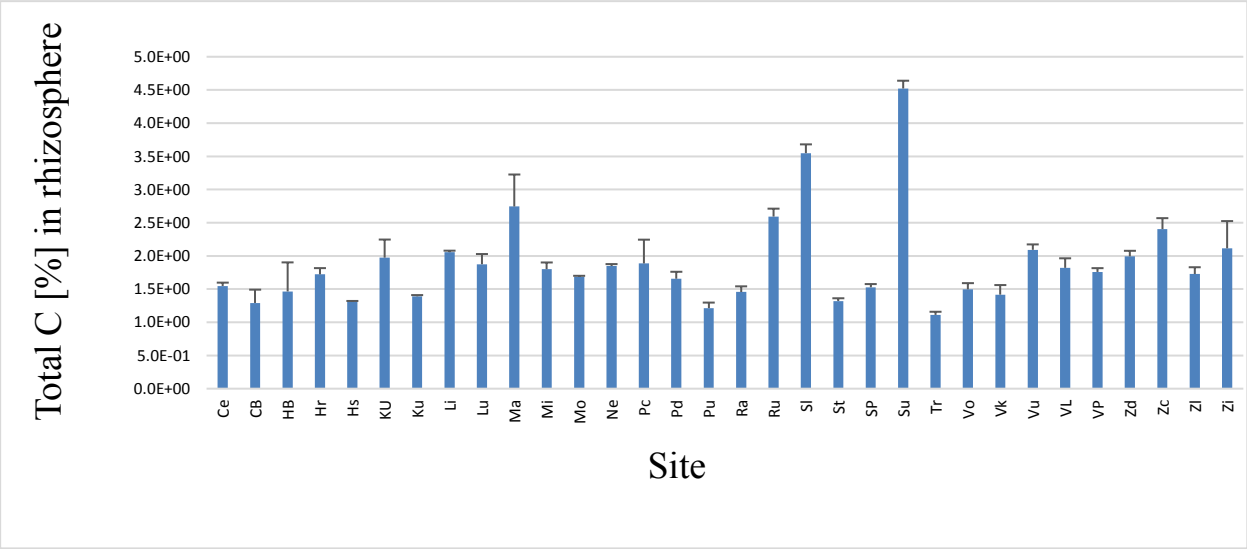

Fig. 14. The average of Ct (%) in rhizosphere (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

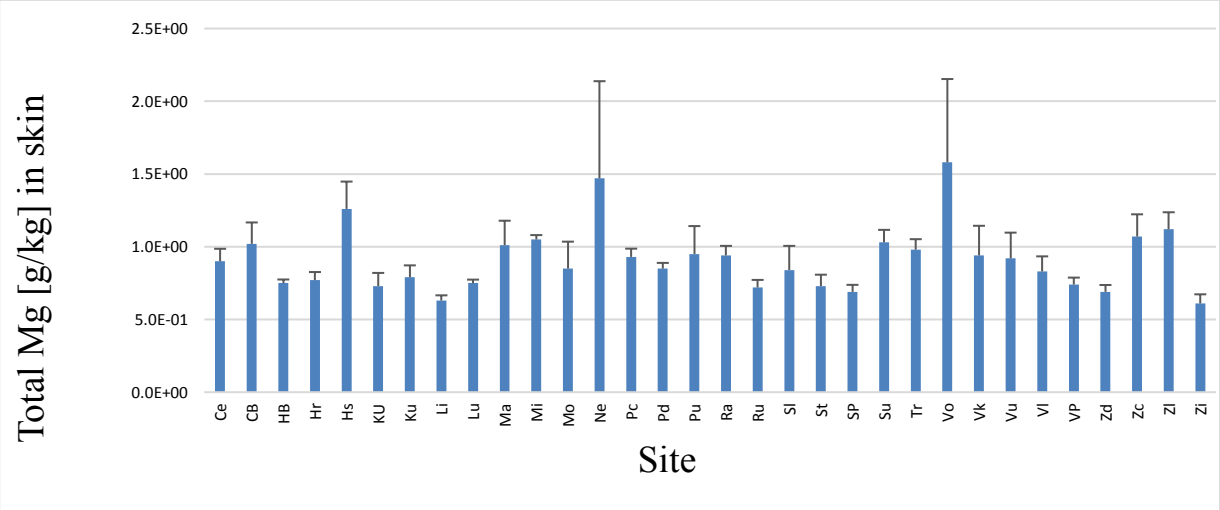

Fig. 15. The average of Mg in skin samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi).

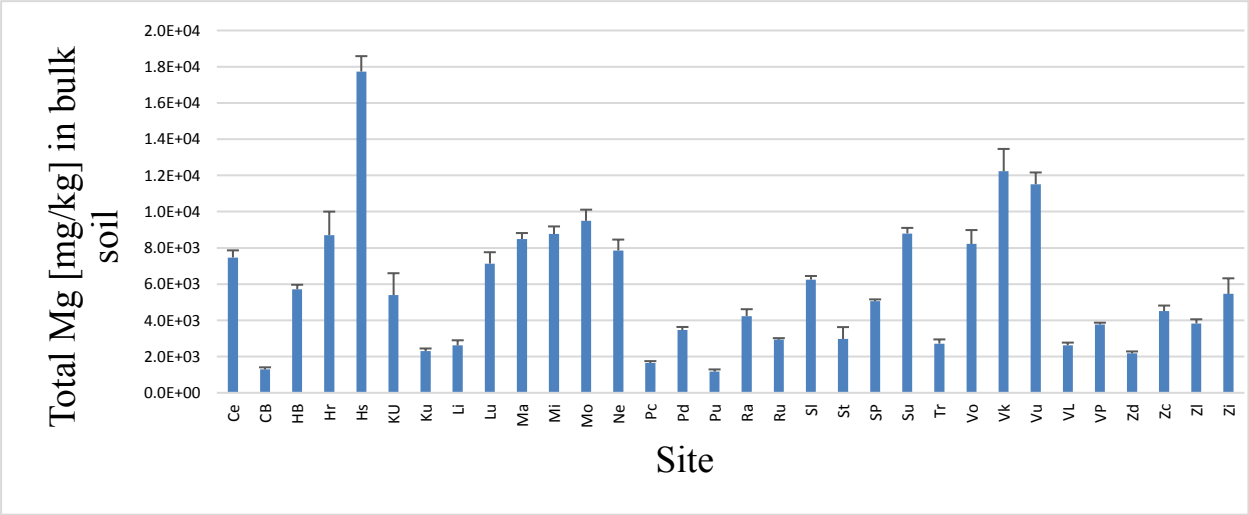

Fig. 16. The average of Mg in bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

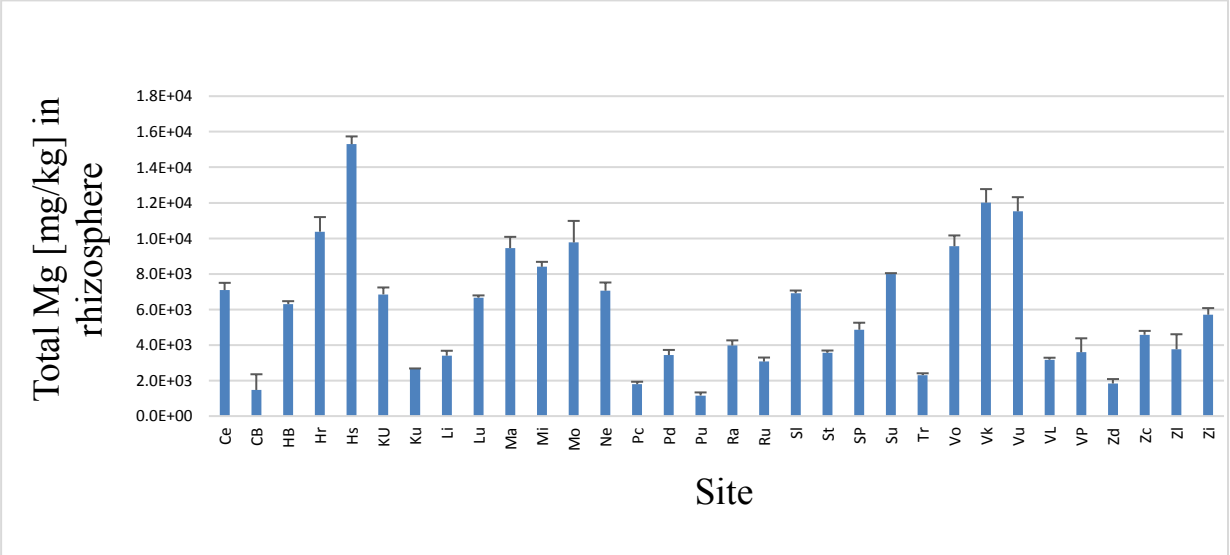

Fig. 17. The average of Mg in rhizosphere (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

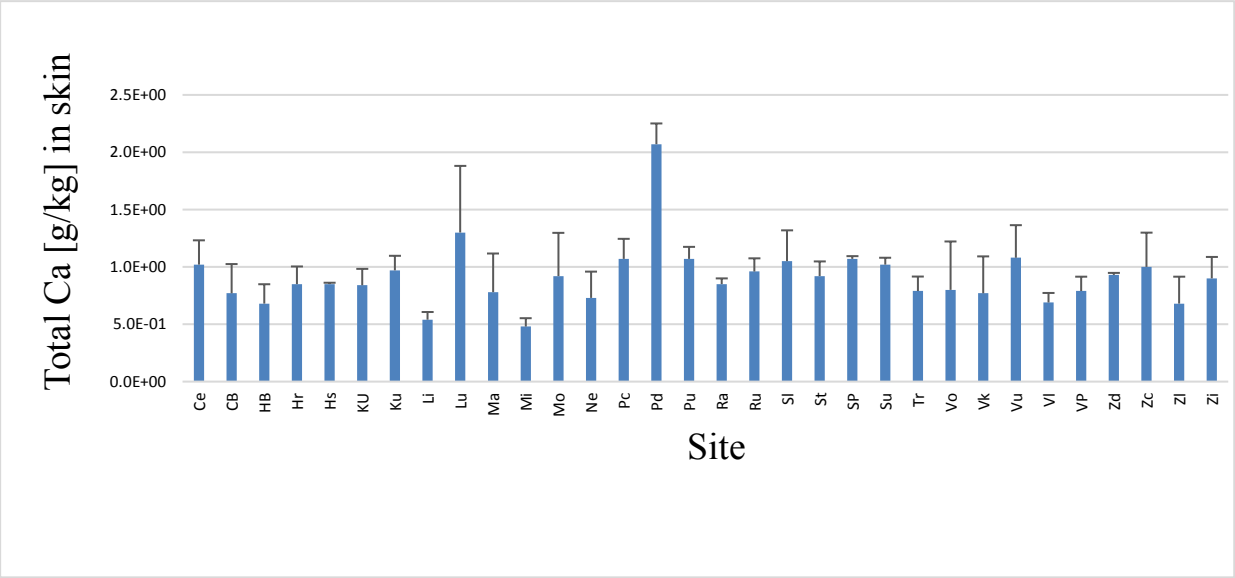

Fig. 18. The average of Ca in skin samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi).

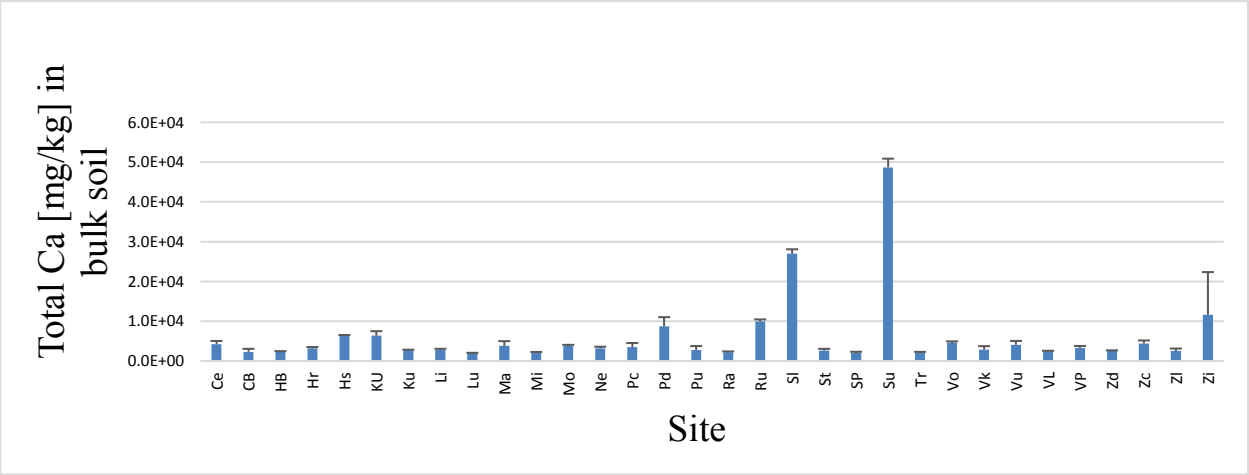

Fig. 19. The average of Ca in bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

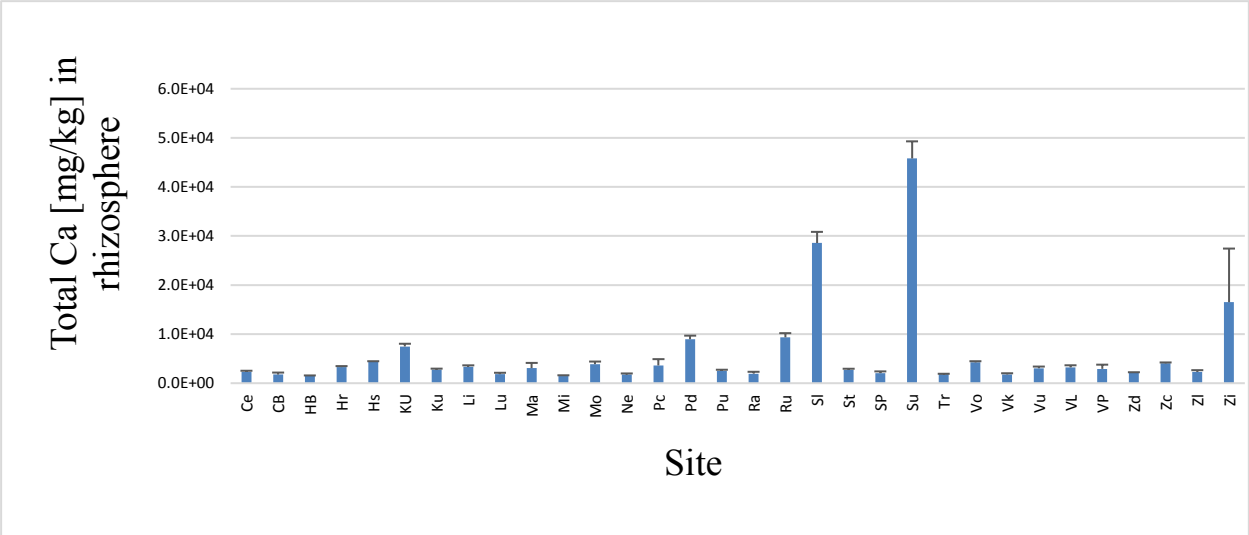

Fig. 20. The average of Ca in rhizosphere (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

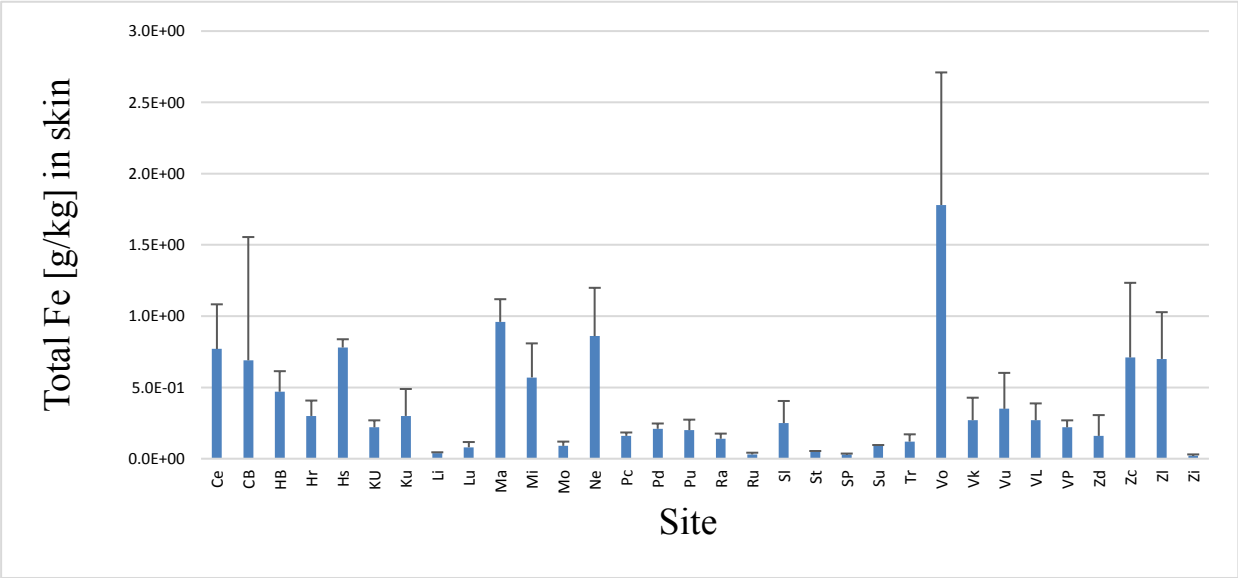

Fig. 21. The average of Fe in the skin samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk and Vu and n=9 for Zi).

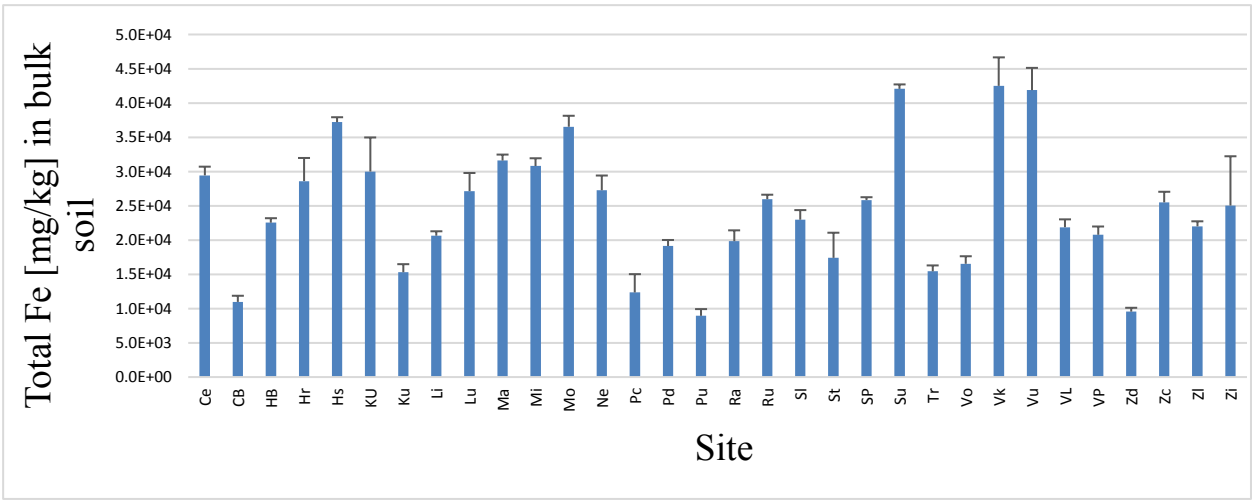

Fig. 22. The average of Fe in the bulk samples (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc, Zi and Pu, n=12 for CB, n=8 for Vk, Vu and n=4 for Zc, Zl).

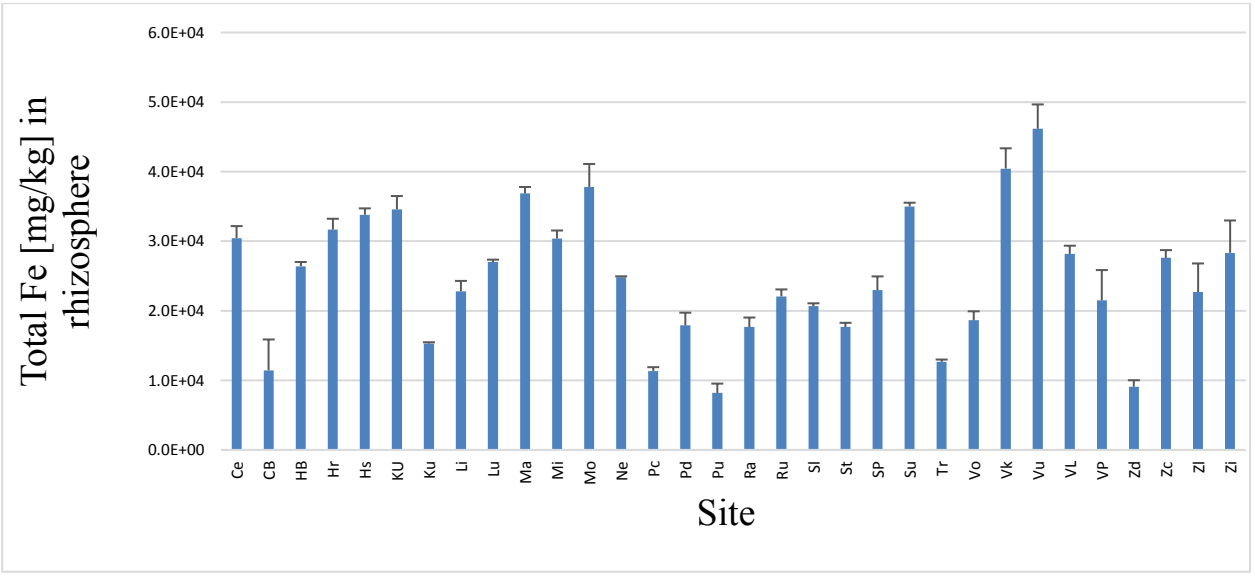

Fig. 23. The average of Fe in rhizosphere (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu n=9 for and Zi).

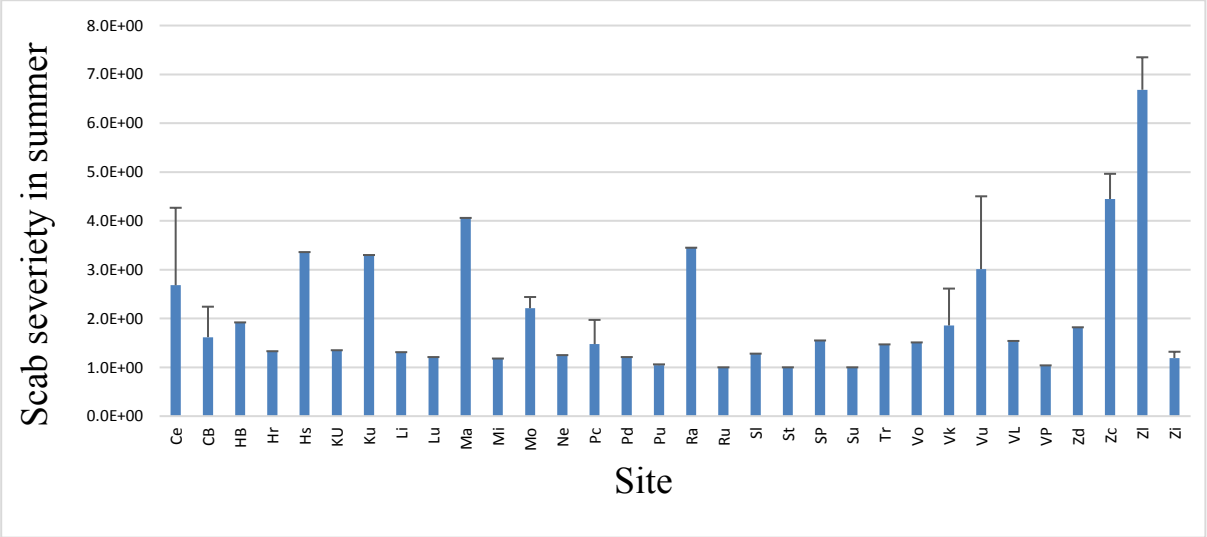

Fig. 24. Severity of common scab in summer (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu, n=9 for and Zi).

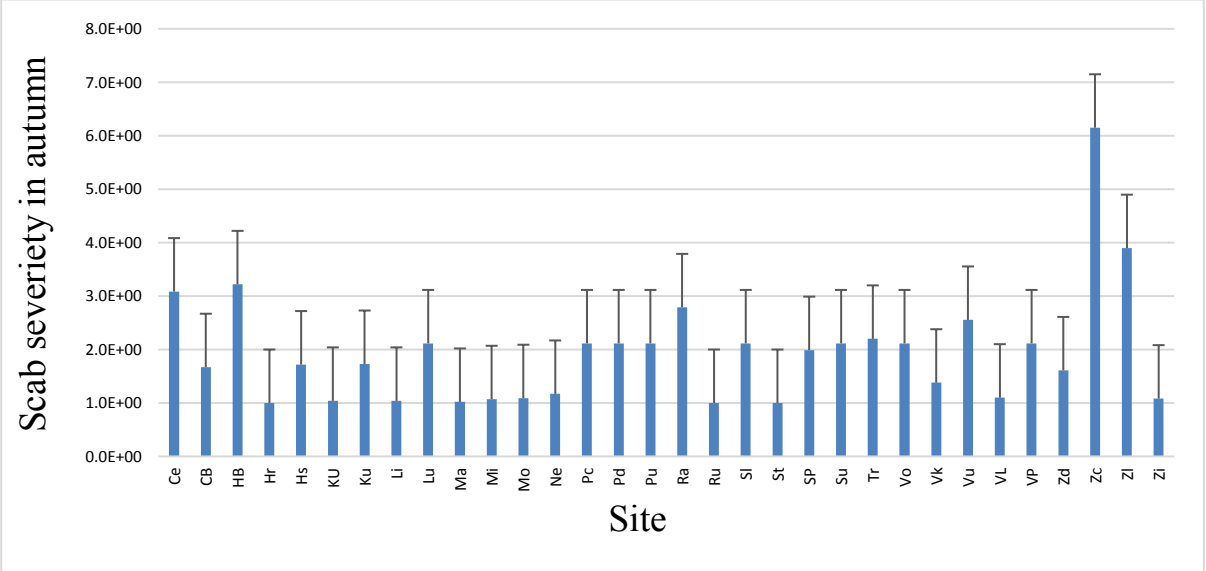

Fig. 25. Severity of common scan in autumn (means ± standard deviations, n=3 for Ra, Ru, Sl, St, SP, Su, HB, Tr, Vo, VL, VP, Zd, Pd, Hr, Hs, Ku, KU, Li, Lu, Ma, Mi, Mo, Ne, n=6 for Ce, Pc and Pu, n=12 for CB, Zc and Zl, n=20 for Vk, Vu, n=9 for and Zi).

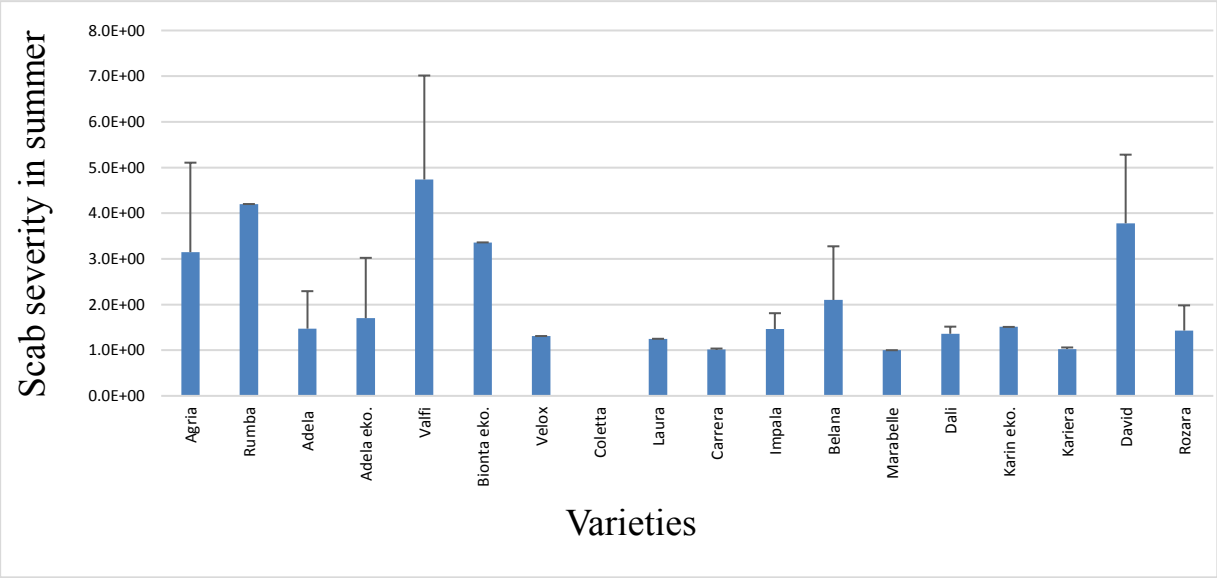

Fig. 26. Severity of common scab in different varieties (cultivars) in summer (means ± standard deviations, n=30 for Agria, n=21 for Adela, n=16 for Valfi and David, n=15 for Adela eko., n=12 for impala, n=9 for Belana, n=8 for Kariera, n=6 for Rozara, Carrera and Dali, n=3 for Rumba, Bionta eko., Velox, Laura, Marabelle and Karin eko. And n=0 for Coletta).

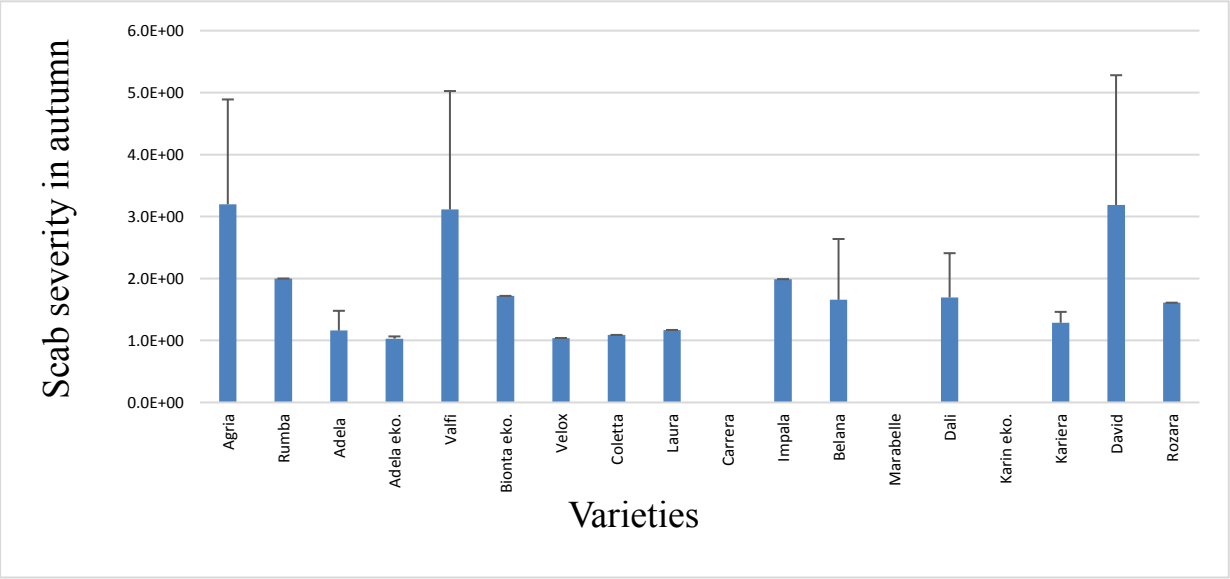

Fig. 27. Severity of common scab in different varieties (cultivars) in autumn (means ± standard deviations, n=33 for Agria, n=15 for Adela, n=19 for Valfi, n=16 for David, n=9 for Adela eko. and Belana, n=8 for Kariera, n=6 for Dali, n=3 for Rumba, Bionta eko., Velox, Coletta, Laura, Impala and Rozára and n=0 for Carrera, Marabelle and Karin eko).

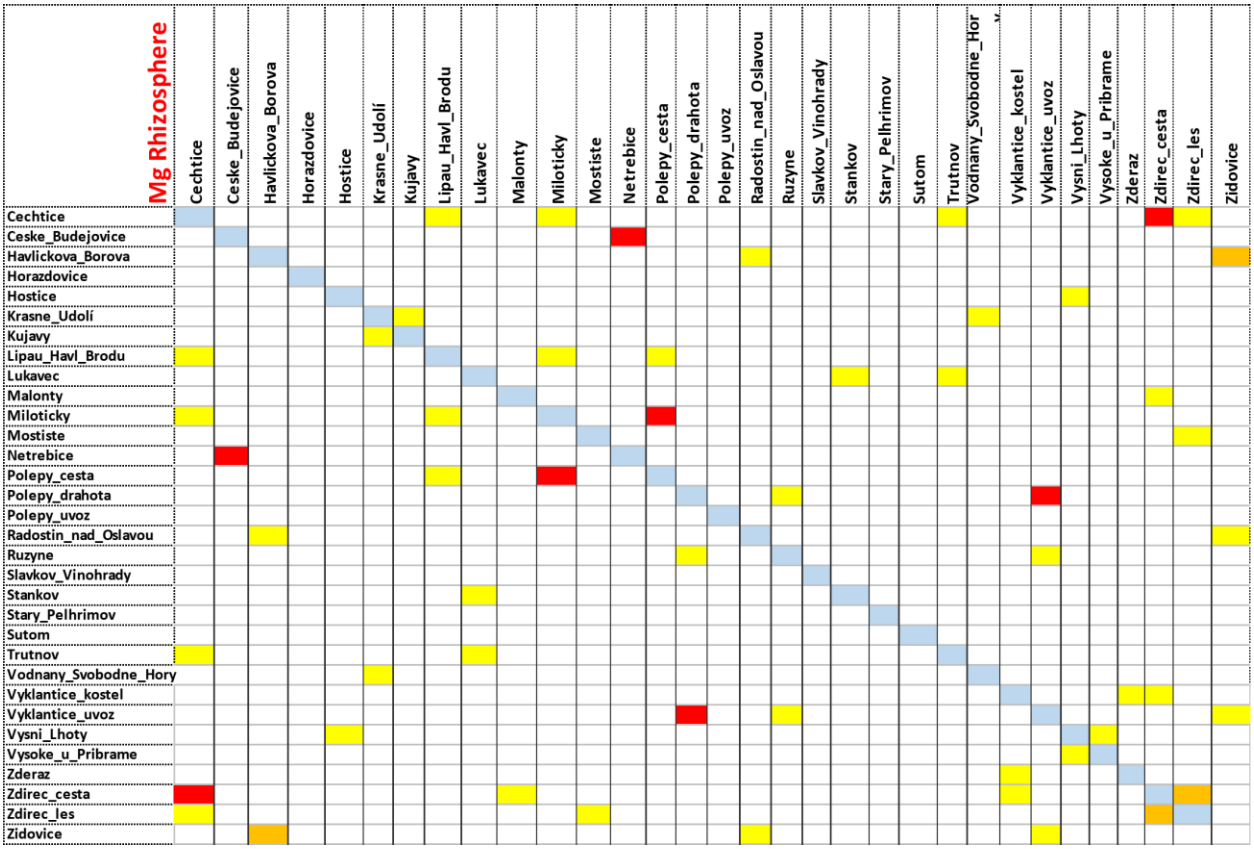

Fig. 28. Correlation between sites and nutrients (Mg [mg/kg] of rhizosphere samples). P value ≤ 0.05 showed by yellow, P value ≤ 0.01 showed by orange and P value ≤ 0.001 showed by red.

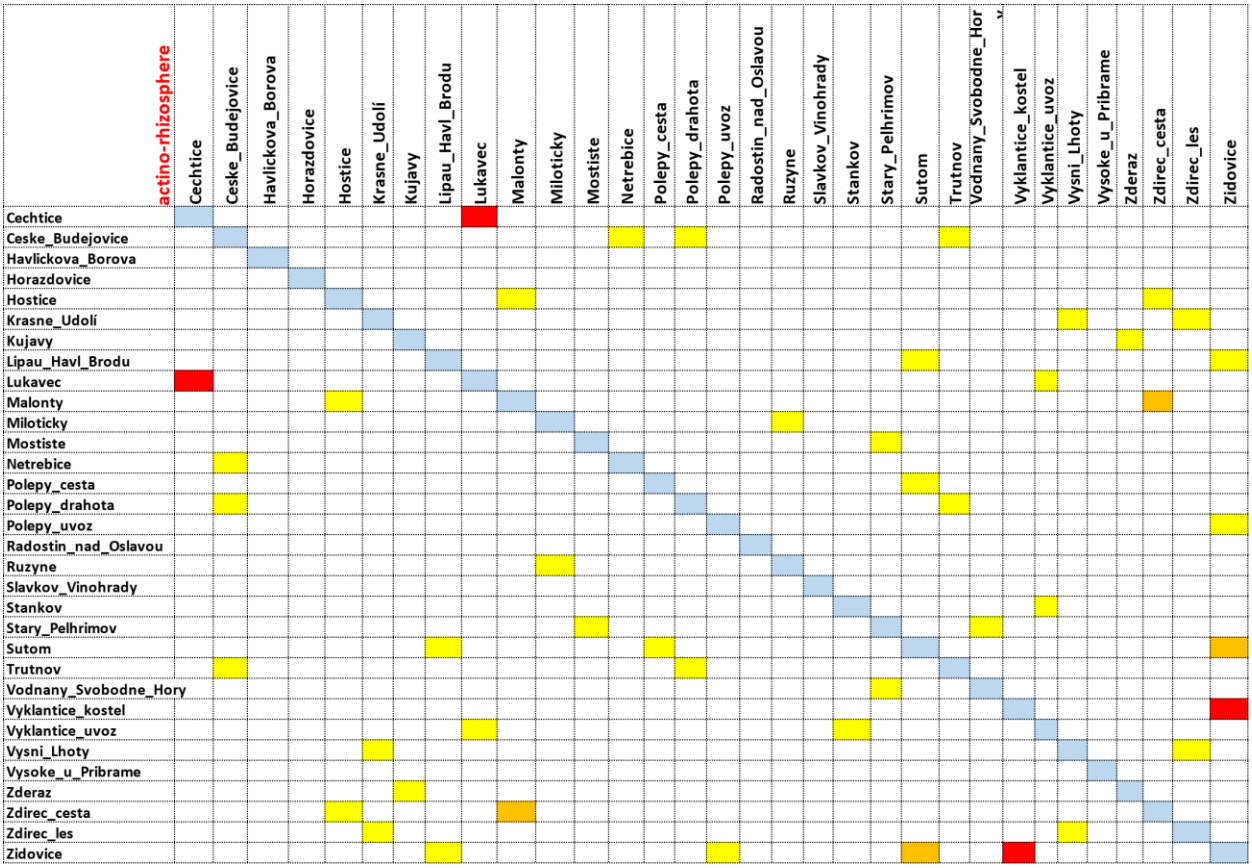

Fig. 29. An instance of the statistical correlation between the sites and biological variables (copy numbers of 16S rRNA gene from actinobacteria of rhizosphere samples). P value ≤ 0.05 showed by yellow, P value ≤ 0.01 showed by orange and P value ≤ 0.001 showed by red.

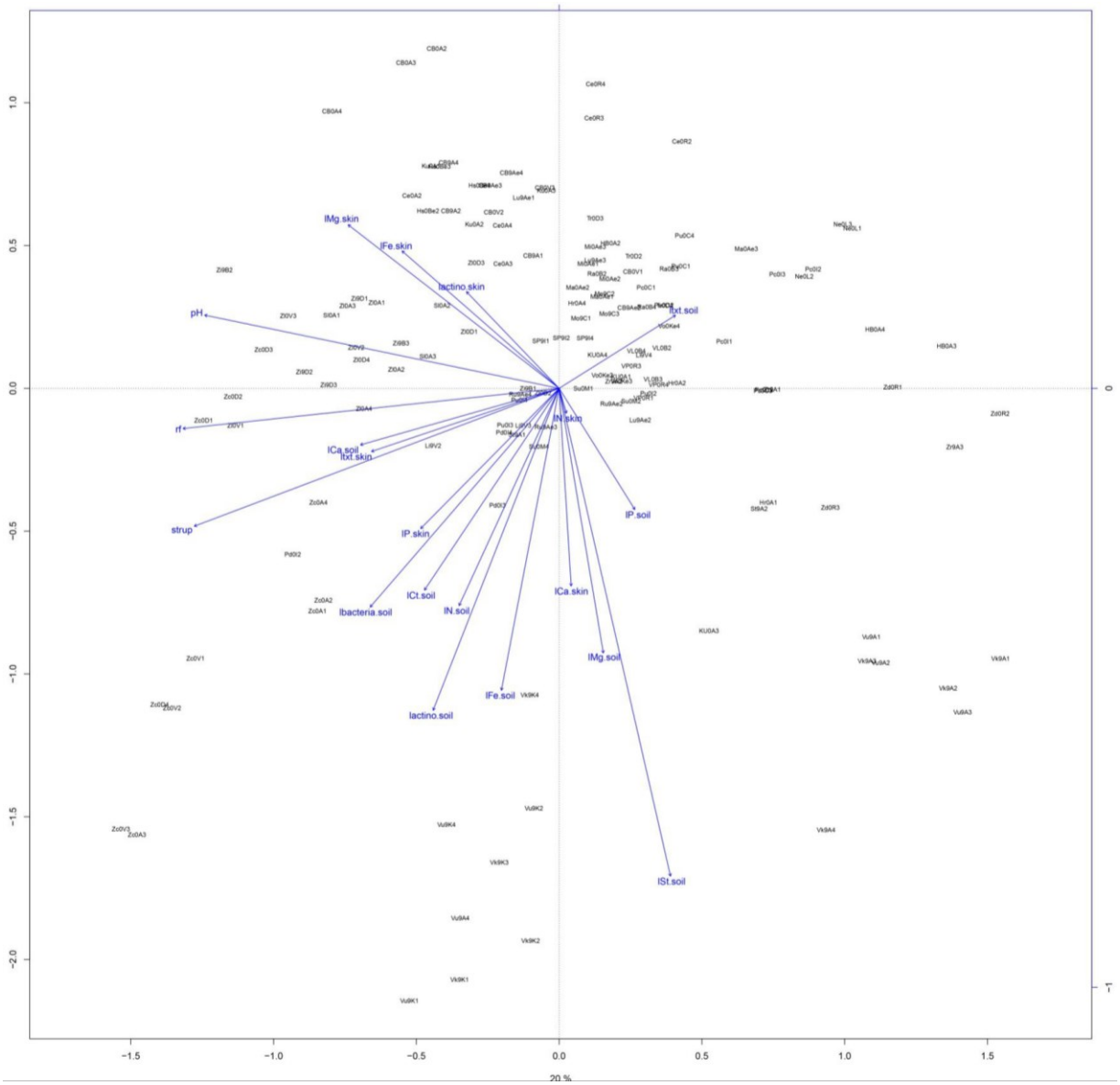

Fig. 30. The thetaYC-based distances calculated for PCA analysis, showing ordination based on bacterial community composition in the rhizosphere soil with vectors of chemical and biological variables.

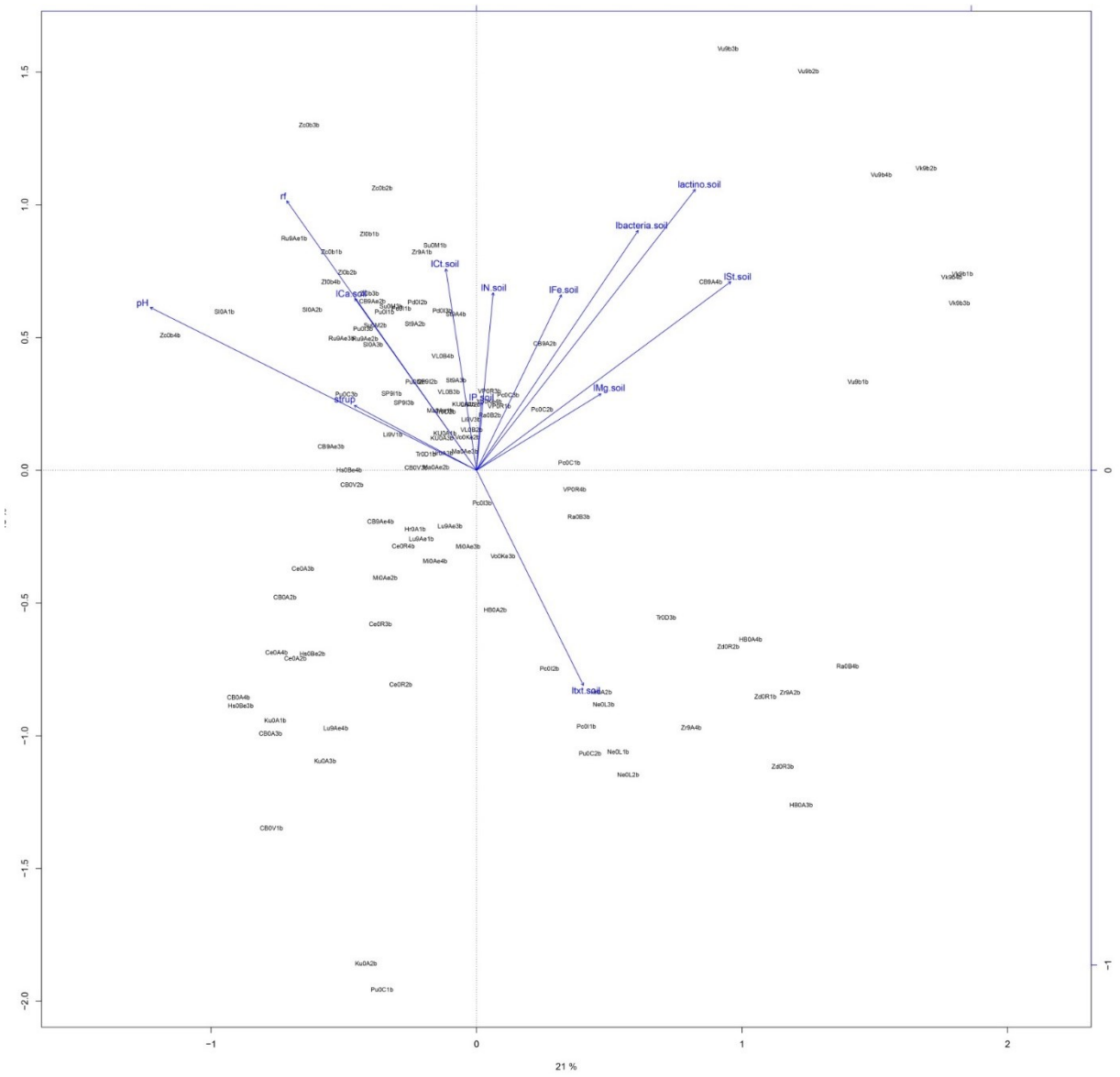

Fig. 31. The thetaYC-based distances calculated for PCA analysis, showing ordination based on bacterial community composition in the bulk soil with vectors of chemical and biological variables.

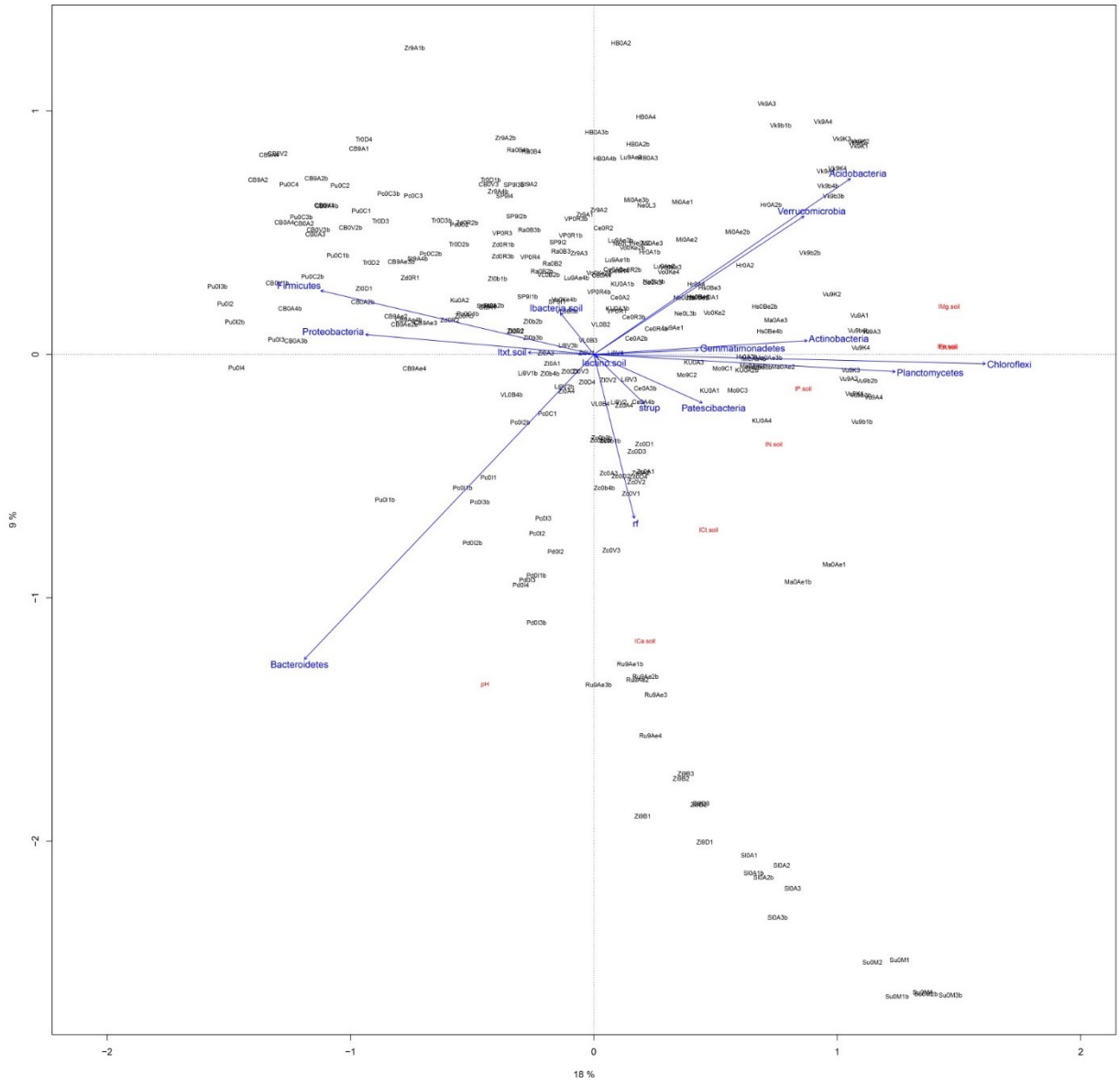

Fig. 32. The distances between chemical properties of the soil samples with the vectors showing properties of the bacterial communities – proportions of individual phyla and diversity (rf).

3.2. The effect of peat and iron supplements on the severity of potato common scab and bacterial community in tuberosphere soil

The highest CS severity was observed in the control of the conducive soil VH, while the lowest was in the suppressive soil (VL) and treatments VHP (peat added) and VHPFe (peat and iron added). Significant differences in scab severity were found between the control VH and treatments VHP, VHPFe ($p< 0.001$), and VHFe ($p < 0.05$) including the suppressive soil VL ($p< 0.001$) (Fig. 33A). Soil pH was the highest in VH and the lowest in VL and VHPFe. The treatments differed significantly by pH ($p < 0.001$, ANOVA, Supplementary Table S1) and the differences were significant between the control VH and both treatment VHPFe and suppressive soil VL ($p < 0.001$) (Fig. 33B). The initial amount of iron added was the same for the two Fe treatments. At the end of the experiment, the treatments were significantly different in the content of extractable iron ($p < 0.001$, ANOVA, Supplementary Table S1). The lowest content of extractable iron was in VHP. The content of extractable iron in both iron supplemented treatments VHFe and VHPFe significantly differed from the control VH ($p < 0.01$ and $p < 0.001$ resp.) (Fig. 33C). The treatments did not affect soil bacteria, *Actinobacteria* and *txtB* gene quantities (Supplementary Table S1). However, the numbers in periderm *Actinobacteria* 16S rRNA and *txtB* gene copies were significantly different between the treatments ($p=0.006$ and $p < 0.001$ resp., ANOVA, Supplementary Table S2). In particular, *Actinobacteria* numbers were significantly lower in the treatment VHP than in the control VH ($p < 0.05$) and *txtB* gene copies were significantly lower in VHP and VL than in the control VH (both $p < 0.05$) (Supplementary Table S1).

A total of 1 321 716 sequences were mapped to 10 515 OTUs defined at a 97% similarity level. Conducive soil VH had a higher number of OTUs than the suppressive soil VL and the treatments VHFe and VHPFe, while the highest number of OTUs was found in treatment VHP. In the whole bacterial community, the relative abundance of *Proteobacteria* and *Bacteroidetes* increased, while *Actinobacteria* and *Firmicutes* decreased in the VHP and VHPFe treatments. *Gemmatimonadetes* *i*ncreased in the VHFe and VHPFe treatments. *Chloroflexi* was high in the suppressive soil VL but decreased in VHPFe (Supplementary Fig. S1, Supplementary Fig. S2). In a plot of bacterial communities done by non-metric multidimensional scaling,

replicates of individual treatments were significantly closer to each other than to those from the other treatments (PERMANOVA, $p<0.001$). Bacterial communities of both the VHP and VHFe treatments were significantly closer to the community of conducive soil VH than to the other communities ($p<0.012$), while the communities of the treatment VHPFe and the suppressive soil VL were significantly closer to each other than to the other treatments (PERMANOVA, VHPFe : VL, $p<0.03$, VL < VHPFe, $p<0.005$, Fig. 34). Clustering of the bacterial communities (represented by sequence libraries) of the treatments to metacommunities based on Dirichlet multinomial mixtures showed the best fit for a model of two metacommunities (community types). The first one included the conducive control soil VH and the treatments VHP and VHFe, while the second included the treatment VHPFe and the suppressive soil VL (Supplementary Table S3). There were 155 dominating OTUs, which represented 50% of the community. Out of those, 5 OTUs (14, 34, 53, 18, 100) changed only in the treatment VHFe and 10 OTUs (19, 37, 44, 55, 75, 79, 81, 95, 144, 218) changed in both iron treatments; 21 OTUs (2, 4, 3, 6, 10, 15, 16, 29, 47, 58, 66, 68, 102, 274, 291, 1048, 1247, 1848, 2597, 9319, 9821) changed in both peat treatments, and 34 changed in the treatment VHPFe towards the percentage characteristic for the suppressive soil VL. In particular for the VHPFe treatment, the increasing OTUs belonging to *Alphaproteobacteria* were OTUs 1, 16, 17, 355; *Betaproteobacteria* OTUs 27, 102; *Gammaproteobacteria* OTUs 30, 47; *Actinobacteria* OTUs 126, 813; *Verrucomicrobia* OTUs 88, 70; *Acidobacteria* OTUs 40, 67 and *Bacteroidetes* OTU 29. The decreasing OTUs belonged to *Acidobacteria* OTUs 5, 72, 106; *Actinobacteria* OTUs 2, 6, 8, 21, 32, 31, 77, 1247; *Alphaproteobacteria* 7, 314; *Betaproteobacteria* 35, 37, 44, 57, 144 and *Chloroflexi* OTU 61 (Supplementary Table S4).

The conducive and suppressive soils VH and VL differed by 1453 OTUs (Metastats, $p<0.05$); out of those the proportional abundance of 1133 changed in the treatment VHPFe in the direction towards VL, i.e. 349 OTUs increased and 784 OTUs decreased. OTUs of *Acidobacteria* and *Actinobacteria* decreased, while the OTUs of *Alphaproteobacteria* mostly increased after the iron and peat treatment, becoming similar in percentage to VL. OTUs of *Firmicutes* and *Chloroflexi* mostly did not respond to the treatment (Fig. 35). In the phylum *Proteobacteria*, the relative abundance of *Methylophilaceae* ($R=0.54$; $n=14$) was positively correlated with CS

severity, while *Xanthobacteraceae, Bradyrhizobiaceae, Sphingomonadaceae, Burkholderiaceae* decreased with increasing CS severity. The relative abundance of *Acetobacteraceae* was high in VL. (Supplementary Fig. S3). In the phylum *Actinobacteria,* the relative abundances of families *Solirubrobacteraceae* (R=0.86; n=14), *Micrococcaceae* (R=0.76; n=14) and *Nocardioidaceae* (R=0.59; n=14) were positively correlated with CS severity, while *Acidimicrobiaceae* decreased with increasing CS severity. *Thermomonosporaceae* and *Micromonosporaceae* were high in the suppressive soil VL, and *Gaiellales* and *Conexibacteraceae* decreased in the peat treatment (Supplementary Fig. S4).

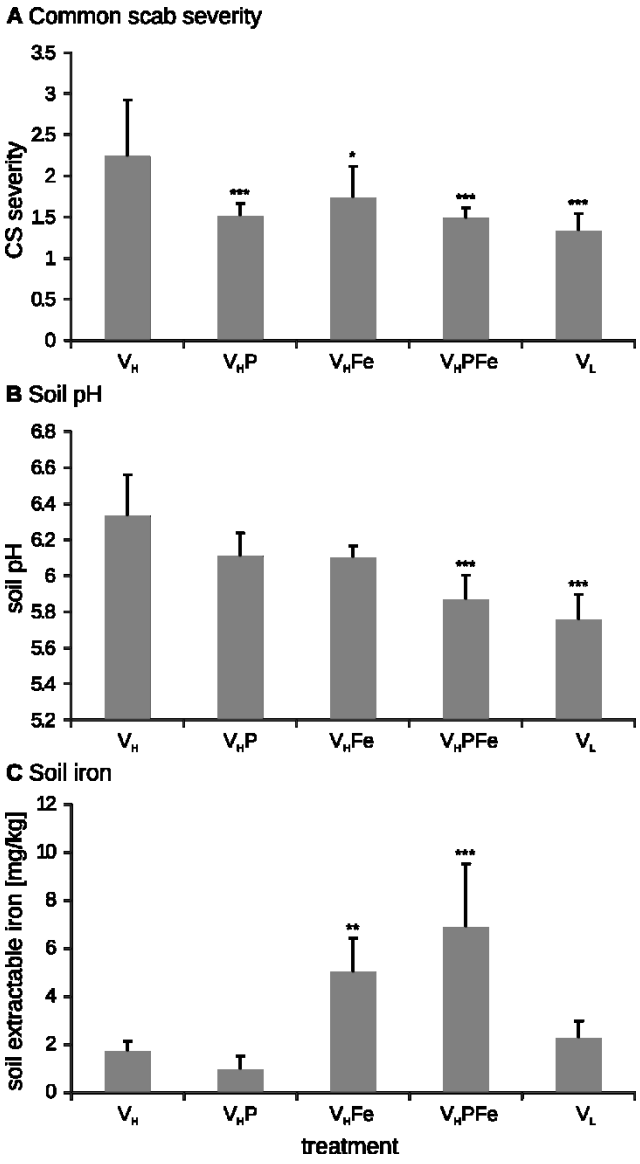

Fig. 33. Severity of the potato common scab (A), tuberosphere soil pH (B), and the contents of available iron extracted with ammonium acetate (C) at the end of cultivation. The potato cultivar Agria was grown in common-scab conducive soil - VH, the same soil amended with peat - VHP, DTPA-chelated iron - VHFe, or both iron and peat - VHPFe, and common-scab suppressive soil - VL. The bars represent averages of five replicates with standard deviations. Significant differences (ANOVA) in comparison to the conducive soil VH are marked with asterisks (P < 0. 05 - *, P < 0.01 - **, P < 0.001 - ***).

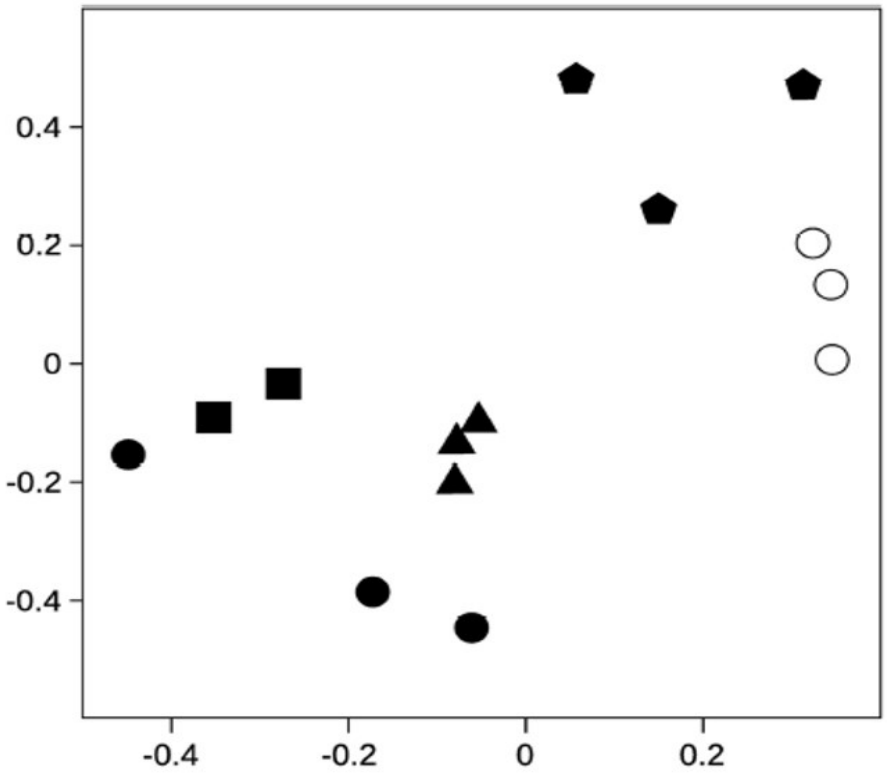

Fig. 34. An ordination showing distances between the bacterial communities represented by amplicon sequence libraries in the treatments. Non-metric multidimensional scaling was based on a matrix of Yue-Clayton theta distances. The sequenced communities were from potato tuberosphere in common-scab conducive soil VH (closed circles), the same soil amended with peat VHP (squares), DTPA-chelated iron VHFe (triangles), both iron and peat VHPFe (pentagons), and common-scab suppressive soil VL (open circles).

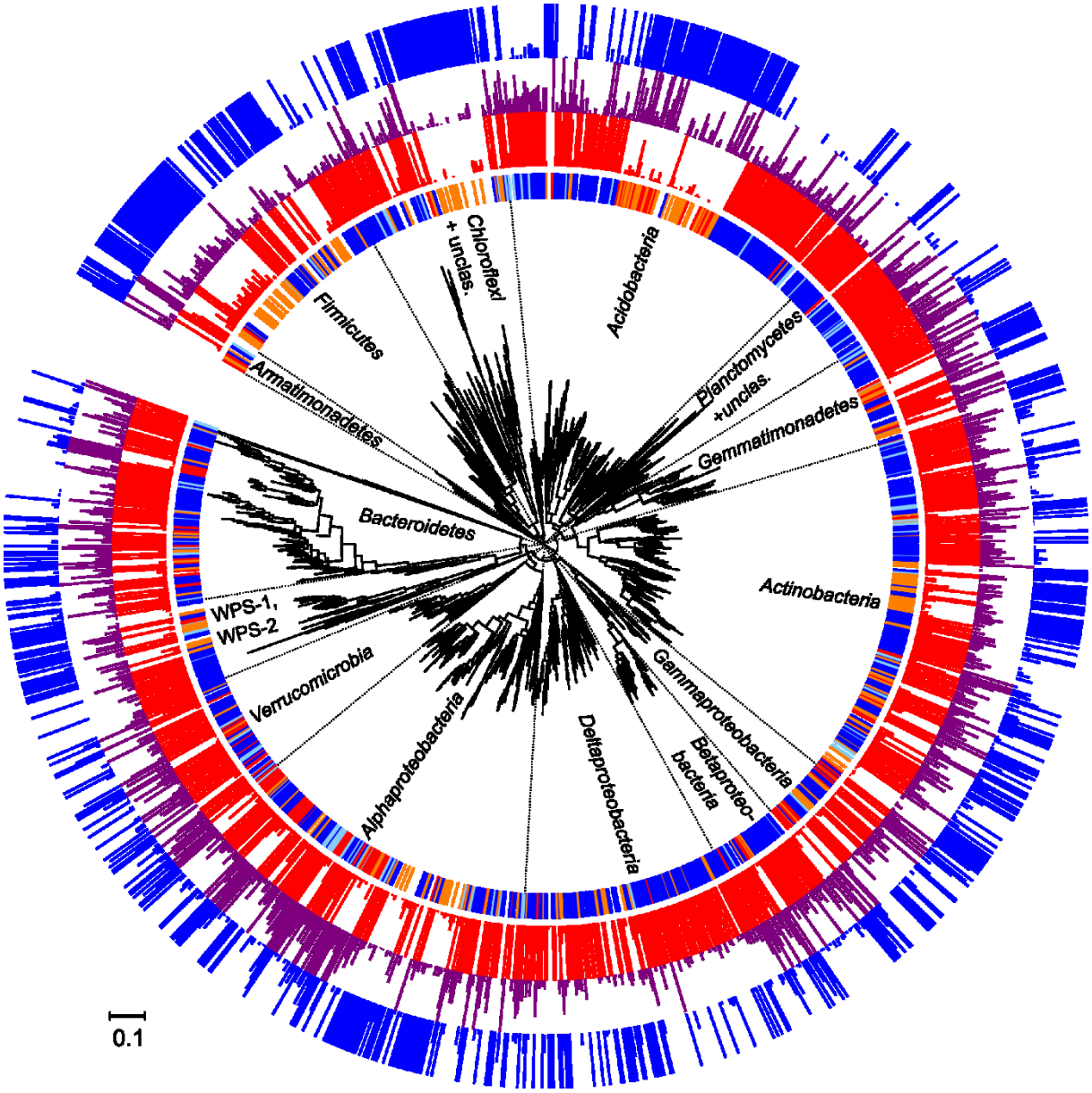

Fig. 35. Phylogenetic tree (constructed by maximum likelihood of representative sequences of each OTU) of OTUs which significantly differ between the conducive and suppressive soil in relative abundance. Red circle - conducive soil VH, blue - suppressive soil VL, purple - iron and peat treatment VHPFe. The inner most circle shows how the proportion of individual OTUs changed from VH to VL in the treatment VHPFe. Dark blue - strong decrease, light blue - weak decrease, dark red - strong increase, light red - weak decrease, white - no change.

3.3. Bacterial, archaeal and micro-eukaryotic communities characterize a disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab.

3.3.1. Common scab severity and quantities of thaxtomin biosynthetic genes.

In conducive soil H, severity of CS (resulting from natural field infestation) was significantly higher in susceptible cultivar Agria than resistant cultivar Kariera (Fig. 36; ANOVA, $p<0.001$). In suppressive soil L, CS severity did not differ between the cultivars, and was as low as for the resistant cultivar in conducive soil. The number of *txtB* gene copies was similar in both soils (Tables S1A and S3A), while in periderm it was significantly higher ($p=0.006$) in conducive than suppressive soil (Tables S1B and S3B). The two cultivars grown in the same soil had comparable quantities of *txtB* gene copies in their periderm. In summary, CS control required resistant cultivar (independently of the soil) or suppressive soil (for susceptible cultivar).

3.3.2. Chemical composition of tuberosphere soil and periderm.

In tuberosphere, contents of N, C, P, Ca, and soil pH were significantly higher in conducive than suppressive soil (ANOVA; all $p<0.001$), while S content was significantly higher in suppressive soil (ANOVA; $p<0.001$). Ca content was significantly higher in bulk soil than in tuberosphere of both soils (ANOVA; $p<0.001$; Tables S2A and S3A). In periderm, N content was significantly higher in both cultivars from suppressive soil (ANOVA; $p<0.001$), Ca content was significantly higher in susceptible cultivar Agria in both soils (ANOVA; $p=0.011$), and Mg content was significantly higher in resistant cultivar Kariera in both soils (ANOVA; $p<0.001$). Fe content was significantly higher in tuberospheres of both cultivars in conducive soil (Supplementary Table S2A,) but it was highest in periderm of the resistant cultivar in suppressive soil (Supplementary Tables S2B). Fe content was affected by both field and field x cultivar interaction in both soil and periderm (ANOVA; $p=0.035$; ANOVA; $p=0.006$ resp. Supplementary Tables S3 A and B) In summary, (i) lower content of N, C, P, and Ca and higher content of S were found in the suppressive soil (ii) higher content of Mg, P or Fe were found in the resistant cultivar. In addition, S and Fe contents were significantly higher in tuberosphere for the combination of suppressive soil × resistant cultivar, showing an interaction effect.

3.3.3. Quantities of total bacteria and actinobacteria.

In tuberosphere, the quantities of bacteria (ANOVA; $p<0.001$) and more specifically of actinobacteria ($p=0.006$) were higher in conducive than in suppressive soil. In suppressive soil, the quantity of both bacteria ($p=0.011$) and actinobacteria ($p=0.019$) was significantly lower in plant tuberosphere compared to bulk soil (Tables S1A and S3A). In periderm, the quantity of actinobacteria (ANOVA; $p=0.021$) was significantly higher in conducive than in suppressive soil, and was also significantly higher in susceptible cultivar Agria than in Kariera in conducive soil (Tables S1B and S3B). In summary, quantities of total bacteria and actinobacteria depended on soil (suppressive vs conducive) × cultivar (resistant vs susceptible) × compartment (periderm vs tuberosphere vs bulk soil) combination, with a trend for lower number (s) in suppressive soil and resistant cultivar.

3.3.4. Bacterial community composition in bulk soil and tuberosphere by microarray analysis.

The 16S rRNA taxonomic microarray previously validated for bacterial community analysis of rhizosphere soil samples[23,25] was expanded for coverage of the genus *Streptomyces*, including pathogen species *S. scabies* and relatives (Table 4).

Non-metric multidimensional scaling (NMDS) plot of sample distances calculated from microarray data demonstrated that bacterial communities in conducive and suppressive soils were distinct, and in tuberosphere they were also influenced by cultivar (Fig. 37A). According to PERMANOVA, cultivar explained 42% variability and field site 13% variability. In particular, bacterial community in tuberosphere of the susceptible cultivar was separated from those of the resistant cultivar and bulk soil. Bacterial communities were significantly closer to each other within conducive or suppressive soil when compared to all samples (PERMANOVA; $p=0.003$), and samples of bacterial communities were significantly closer within each cultivar (PERMANOVA; $p<0.001$) but not within each bulk soil. In tuberosphere, bacterial communities of resistant cultivar Kariera differed between the soils (PERMANOVA; $p<0.001$), while bacterial communities of susceptible cultivar Agria did not differ significantly between the two soils but differed from those of resistant cultivar Kariera in each soil (PERMANOVA; $p=0.029$). The permutation test identified significant

relations of bacterial communities with *txtB* gene copies and Mg soil content, which were significantly higher in suppressive soil, and soil pH, C, N and diversity of bacteria, which were significantly higher in conducive soil. Diversity of micro-eukaryotes pointed to the susceptible cultivar Agria in both soils (Fig. 37A, Table S4).

In summary, analysis of bacterial community by taxonomic microarray revealed differences between both suppressive vs conducive soil and resistant vs susceptible cultivar. This was indicated by two biotic and four abiotic factors, which separated the two soils, and one biotic factor, which separated the susceptible cultivar.

3.3.4.1. Discriminant 16S microarray probes according to soil and potato cultivar.

The Metastats analysis revealed the probes distinguishing the individual treatments in pairwise comparisons (Fig. 38A). The most pronounced differences were found between tuberospheres of the two cultivars, which were separated by signal intensities of 34 and 38 probes in suppressive and conducive soil, respectively. Only two probes discriminated between the two soils when assessing bulk soil samples, while 13 probes separated tuberospheres of the resistant cultivar Kariera, and 26 probes those of the susceptible cultivar Agria (Fig. 38A).

Considering the entire dataset including both tuberospheres of the cultivars and bulk soil, the samples from suppressive and conducive soils were significantly distinguished by the signals of 22 probes (Metastats $p<0.05$). Among 13 of them with higher hybridization signals in suppressive soil, the most significantly contributing probes were Aceto3A, Acdp821, Aci1 (targeting the family *Acetobacteraceae*), PalgiG3 (*Paenibacillaceae*), Pseu33 (*Pseudomonadaceae*), Strepto5 (*Streptomycetaceae*), and Brady4 (*Bradyrhizobiaceae*). Nine of the probes were significantly higher in conducive soil, and probes Janaga 2 and 3 (*Oxalobacteraceae*) contributed most significantly to the separation of the two soils (Table S5A).

Tuberosphere samples of the cultivars were distinguished by 65 probes, 13 with higher signal in the resistant cultivar Kariera and 52 in the susceptible cultivar Agria. The probes most significantly contributing to separation of the cultivars were Strepto1, 2, and 3 (targeting the family *Streptomycetaceae*), Rzbc1247 (*Rhizobiales*), BET940 (Betaproteobacteria), and Azo5 (*Rhodospirillaceae*) with a higher signal in Kariera, and a diverse set of probes targeting *Proteobacteria* (15 probes), *Firmicutes*

(2), *Planctomycetes* (2), *Actinobacteria* (2), *Bacteroidetes* (1) and *Acidobacteria* (1) with higher signal in Agria (Table S5B).

In summary, 22 probes targeting various bacterial taxa discriminated between suppressive and conducive soils, and 65 probes did between resistant and susceptible cultivars. Signals of probes targeting the CS pathogen were detected in the tuberosphere of the susceptible cultivar grown in conducive soil only.

3.3.5. Bacterial community composition in bulk soil and tuberosphere by Illumina sequencing.

A total of 1,213,004 16S rRNA gene sequences were obtained, out of which 944,597 (i.e. 78%) were mapped to 4001 OTUs. On a NMDS plot, bacterial communities of resistant cultivar Kariera, susceptible cultivar Agria, and bulk soil were separated from one another within each field (Fig. 37B). The bacterial communities differed according to treatments (AMOVA, $p<0.001$), with a significant difference between cultivars (AMOVA, $p<0.001$) but not between suppressive and conducive soil (except when only bulk soils were compared). The permutation test identified significant influence of bacterial diversity, which pointed to the resistant cultivar Kariera (Fig. 37B, Table S4).

Both fields and cultivars were compared using significantly different OTUs (Metastats $p<0.05$). The number of discriminating OTUs (Fig. 38B) was only 85 between both fields for resistant cultivar Kariera, 382 between bulk soil and resistant cultivar Kariera in conducive soil, and 316 between bulk soil and susceptible cultivar Agria in suppressive soil, whereas the other pairwise differences between treatments implicated 954-1676 discriminating OTUs.

The relative proportion of bacterial phyla did not differ between bulk soils, except that *Actinobacteria* were higher and *Acidobacteria* lower in suppressive than in conducive soil (Fig. 39A). Based on comparison with bulk soil, the tuberosphere communities implicated (i) an increase in relative proportion of *Chloroflexi* and decrease in that of *Verrucomicrobia*, *Gemmatimonadetes*, *Planctomycetes* and *Proteobacteria* in both cultivars (in the two fields; Fig. 39A), (ii) an increase in relative proportion of *Bacteroidetes* (particularly the family *Sphingobacteraceae*) in resistant cultivar Kariera (in the two fields; Fig. S1C in the supplemental material),

and (iii) an increase in relative proportion of *Firmicutes* (especially the family *Paenibacillaceae*) and *Actinobacteria* (especially the orders *Gaiellales*, *Micrococcales*, *Frankiales* and *Streptomycetales*) in susceptible cultivar Agria (in the two fields; Fig. S1A, B). This increase in *Streptomycetales* was contributed by OTU 176, to which also the CS pathogen belongs. However, other members of this OTU contributed more significantly because this OTU was defined by centroid sequence, which was at 2.1-2.7% distance from the pathogen (Table S6B).

Rarefaction curves for bacterial communities showed that diversity did not differ between suppressive and conducive soils but differed between cultivar tuberospheres and bulk soil (two-way ANOVA, $p<0.001$). The diversity was lowest in susceptible cultivar Agria, followed by bulk soil and resistant cultivar Kariera in both soils (Fig. S2A; Table S7).

In summary, bacteria community by Illumina sequencing revealed differences between resistant and susceptible cultivars, the latter displaying lower bacterial diversity. Differences were also found between suppressive and conducive soils, but only for bulk soil samples.

3.3.5.1. Discriminant bacterial OTUs according to soil and potato cultivar.

When considering bulk as well as tuberosphere soil samples, based on the discriminating OTUs (Metastats, $p<0.05$; Fig. 39A) suppressive soil was enriched in *Plantomycetes* (OTUs 385, 2780) and *Bacteroidetes* (OTUs 1402, 1154, 1408) and conducive soil in *Actinobacteria* (OTUs 355, 1230, 886) and *Chloroflexi* (OTU 1478). Different OTUs separating the two soils were found within *Proteobacteria* (with OTUs 92, 253, 68, 592, 835 enriched in suppressive soil vs OTUs 369, 899, 2391, 1832, 2001 in conducive soil) and *Firmicutes* (OTU 3391 enriched in suppressive soil vs OTUs 2120, 2105, 1772 in conducive soil) (Table S6A).

The tuberosphere of Agria was enriched in taxa from actinobacterial orders *Frankiales* (*Frankiaceae*, *Acidothermaceae*, *Geodermatophilaceae*; OTUs 63, 20, 54, 117) and *Micrococcales* (*Intrasporangiaceae*; OTUs 13, 10) (Table S6B, Fig. S1A) and phylum *Gemmatimonadetes* (*Gemmatimonadaceae*; OTU 36), while tuberosphere of Kariera displayed significant enrichment in taxa from phylum *Acidobacteria* (OTUs 51, 275, 143, 138, 76; Table S6B, Fig. 39A). Tuberosphere communities of both

cultivars were also separated by different OTUs belonging to the same taxonomic groups. These discriminating taxa included (i) *Betaproteobacteria, Burkholderiales* (OTU 38 in Agria vs OTU 199 in Kariera), (ii) *Alphaproteobacteria, Sphingomonadales* (OTU 1 in Agria vs OTUs 48, 696, 282 in Kariera), (iii) *Actinobacteria, Propionibacteriales* (OTU 138 in Agria vs OTU 6 in Kariera), *Gaiellales* (OTUs 21, 140, 41, 114, 104, 19, 8, 309, 213, 110, 23, 946 in Agria vs OTUs 16, 12, 30, 46, 107, 105, 24 in Kariera) and *Solirubrobacterales* (OTU 31 in Agria vs OTU 69 in Kariera), and (iv) *Chloroflexi* (OTUs 18, 26, 77, 137 in Agria vs OTUs 4, 55, 164, 29, 123, 284, 74 in Kariera) (Table S6B).

In summary, suppressive soil was enriched in *Plantomycetes* and *Bacteroidetes* and conducive soil in *Actinobacteria* and *Chloroflexi*, and soils also differed in their *Proteobacteria* and *Firmicutes* profiles. Resistant and susceptible cultivars differed based on 1 *Gemmatimonadetes*, 5 *Acidobacteria*, 6 *Proteobacteria*, 29 *Actinobacteria* and 11 *Chloroflexi* discriminant OTUs.

3.3.6. Archaeal community composition in bulk soil and tuberosphere by Illumina sequencing, and discriminant OTUs.

A total of 987,680 archaeal 16S rRNA gene sequences were obtained, out of which 545,211 (i.e. 55.2%) were mapped to 112 OTUs. On a NMDS plot, archaeal communities were primarily separated according to conducive vs suppressive soil, though samples were more variable in conducive than suppressive soil (Fig. 37C). The archaeal communities differed overall from each other (AMOVA, $p=0.017$) but while the bulk soils were significantly different (AMOVA, $p=0.006$), the cultivars were not. The permutation test identified significant relations of the archaeal community composition with soil bacteria and actinobacteria quantities, diversity of micro-eukaryotes and archaea, soil pH and soil contents of C, N, P, Ca and Fe in conducive soil, while content of S was important in suppressive soil (Fig. 37C, Table S4).

The same pattern was obtained when considering discriminant archaeal OTUs (Metastats $p<0.05$), as the two soils differed for 30 OTUs in bulk soil, 20 OTUs in cultivar Kariera and 27 OTUs in cultivar Agria, while within a same soil only a few OTUs separated one cultivar from the other, and from bulk soil (Fig. 38C).

The relative proportion of archaeal phyla did not differ between bulk soils and

resistant cultivar Kariera. Yet, it differed between soils for susceptible cultivar Agria, which had about 60% of *Thaumarchaeota* and 40% of *Euryarchaeota* in conducive soil, versus 48% of *Thaumarchaeota* and 52% of *Euryarchaeota* in suppressive soil (Fig. 39B). Within these archaeal phyla, the same pattern was found for respectively the *Methanosarcinales* and *Nitrososphaerales* orders, and there was also an increase of *Nitrosotaleales* and *Methanomicrobiales* in suppressive soil (Fig. S3). Rarefaction curves showed that higher archaeal diversity occurred in conducive soil than in suppressive soil (two-way ANOVA; $p<0.001$) and in the treatments the lowest diversity was in both cultivars in suppressive soil (two-way ANOVA; $p<0.05$) (Fig. S2B, Table S7). When considering discriminant OTUs, differences were found mostly between the two soils, especially for *Thaumarchaeota* and *Euryarchaeota* OTUs. Suppressive soil was particularly enriched in 7 OTUs and conducive soil in 20 OTUs (Table S8).

In summary, Illumina sequencing of archaeal 16S rRNA genes showed major differences between conducive and suppressive soil, regardless of whether bulk soil, susceptible cultivar Agria or resistant cultivar Kariera were considered. The difference between cultivars was also significant but to a lesser extent. Four biotic and six abiotic factors increased with respect to archaea community in conducive soil, one increased in suppressive soil.

3.3.7. Micro-eukaryotic community composition in bulk soil and tuberosphere by Illumina sequencing.

A total of 1,244,356 18S rRNA gene sequences were obtained, out of which 896,483 (i.e. 72%) were mapped to 3,754 OTUs. On a NMDS plot, samples from suppressive soil were relatively close to each other, whereas samples from conducive soil were more dispersed (Fig. 37D). In suppressive soil, there was relatively good separation of bulk soil, susceptible cultivar Agria and resistant cultivar Kariera, whereas treatments did not differ in conducive soil. The micro-eukaryotic communities differed overall (AMOVA, $p=0.006$) and suppressive soil samples tended to differ from conducive soil samples, but this was significant only for bulk soils (AMOVA, $p <0.001$). The permutation test identified significant relation between the micro-eukaryotic community and quantities of soil total bacteria and actinobacteria, diversity of archaea,

soil pH and contents of C, N, P, Ca and Fe in conducive soil, and S content in suppressive soil. Above that, the diversity of micro-eukaryotes was higher in conducive bulk soil (Fig. 37D, Table S4).

Significantly different OTUs (Metastats $p<0.05$) showed major differences between suppressive and conducive soils, with 258 discriminant OTUs for bulk soils, 327 for resistant cultivar Kariera, and 522 for susceptible cultivar Agria. Micro-eukaryotic communities differed least between bulk soil and cultivar Kariera in conducive soil (Fig. 38D).

There was a higher proportion of Ascomycota (Fig. 39C), in classes Pezizomycetes, Leotiomycetes, Eurotiomycetes, and particularly in Eurotiomycetes' Chaetothyriales order (Fig. S4A) and Basidiomycota in suppressive bulk soil and a higher proportion of Chlorophyta, Ciliophora in classes Spirotrichea, Litostomatea and superclade CONThreeP (Fig. S4B), Myxogastria and Apicomplexa in conducive bulk soil. Compared with bulk soil, Chlorophyta and Cercozoa were in lower proportion with resistant cultivar (in conducive soils) and in similar proportion in both cultivars in suppressive soil. Basidiomycota were in higher proportion with both cultivars (in conducive soil), and the macro-eukaryotic phylum Arthropoda with cultivars Kariera (in conducive soil) and Agria (in suppressive soil) (Fig. 39C).

Rarefaction curves showed a slightly higher eukaryotic diversity in conducive soil than in suppressive soil overall, and diversity was generally lower in resistant cultivar than in susceptible cultivar, yet none of the differences was statistically significant (Fig. S2C; Table S7).

In summary, Illumina sequencing of eukaryotic 18S rRNA genes showed differences between conducive and suppressive soil but to some extent also between the cultivars, particularly for ciliates and fungi in conducive soil.

3.3.7.1. Discriminant eukaryotic OTUs according to soil and potato cultivar.

According to the differences observed at the level of eukaryotic phyla, OTUs from Ascomycota, Basidiomycota and Cercozoa were enriched in suppressive soil, while OTUs from Chlorophyta and Ciliophora were enriched in conducive soil. Specific OTUs of Ascomycota, Basidiomycota and Cercozoa were also prevalent in susceptible

cultivar Agria in suppressive soil, while particularly OTUs of Chlorophyta and Ochrophyta were prevalent in cultivar Agria in conducive soil. Eukaryotic communities of resistant cultivar Kariera were separated in the two soils by OTUs of various phyla (Table S9).

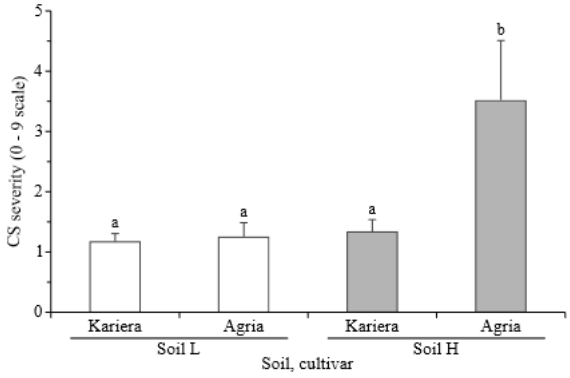

Fig. 36. Severity of common scab of susceptible cultivar Agria and resistant cultivar Kariera in suppressive (L, low severity) and conducive (H, high severity) soils (means ± standard deviations, n = 4). Statistical significance between treatments (ANOVA) are shown with letters a and b.

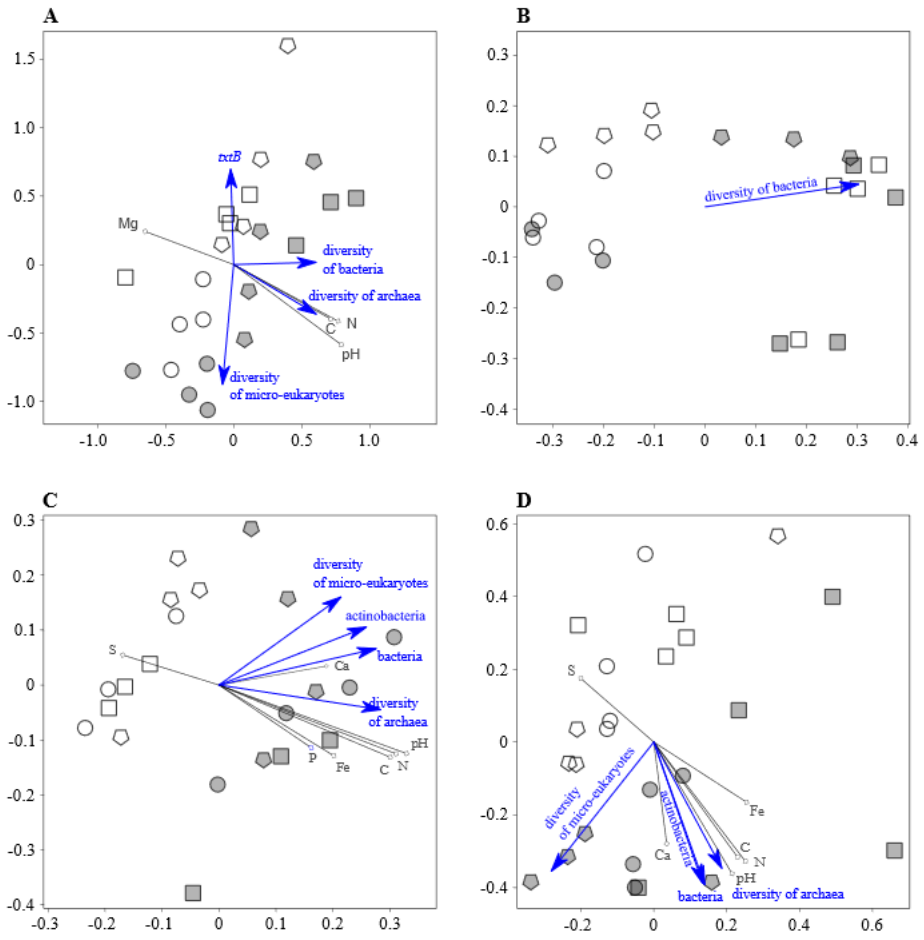

Fig. 37. Differences in soil communities of bacteria (A - assayed by 16S microarray hybridization, and B - by 16S rRNA gene Illumina MiSeq amplicon sequencing), archaea (C - 16S Illumina), and eukaryotes (D - 18S Illumina), and the relationships to other biological and chemical characteristics of tuberosphere soil. Samples of tuberosphere (circles - susceptible potato cultivar Agria; squares - resistant cultivar Kariera), and bulk soil (pentagons) were from the fields suppressive (open symbols) and conducive (grey symbols) to the potato common scab.

Non-metric multidimensional scaling of distance matrices was based on Bray-Curtis calculator with fitted vectors of environmental variables. The vector length shows the relative strengths of contributions/responses. Vectors are pointing to the same direction for positively correlated variables, and to the opposite direction for negatively correlated ones; perpendicular vectors indicate no mutual relationship.

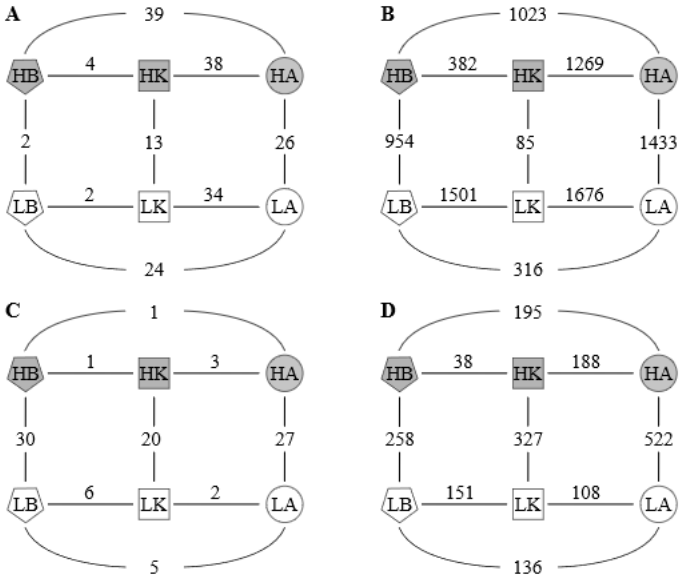

Fig. 38. Pairwise comparisons of soil communities of bacteria (A - assayed by 16S microarray hybridization, and B - by 16S rRNA gene Illumina MiSeq amplicon sequencing), archaea (C - 16S Illumina), and eukaryotes (D - 18S Illumina) in tuberosphere of susceptible cultivar Agria (circles) and resistant cultivar Kariera (squares), and bulk soil (pentagons) from suppressive (L, open symbols) and conducive fields (H, grey symbols). Numbers indicate the probes (A) and OTUs (B, C, D) significantly contributing to the difference between samples in pairwise comparisons (Metastats, $p < 0.05$).

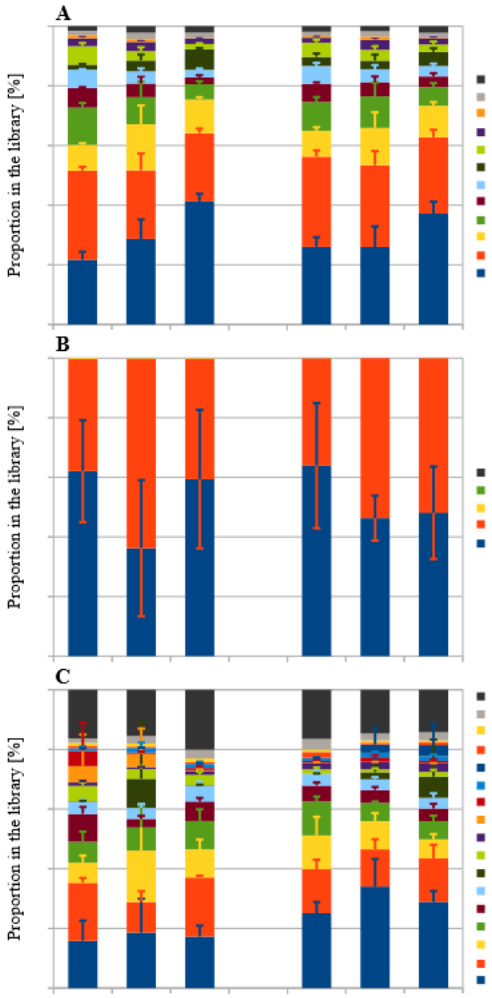

Fig. 39. Proportions of phyla (means ± standard deviations, n = 4) in the sequence libraries of ribosomal small subunit genes from bacteria (A), archaea (B), and eukaryotes (C). (Taxa for organisms generally larger than the sample size (*Arthropoda*, *Annelida*) were included to display the whole community although based only on shaded cells and products. Samples of tuberosphere of susceptible potato cultivar Agria (HA, LA) and resistant cultivar Kariera (HK, LK), and bulk soil (HB, LB) were from the fields suppressive (L) and conducive (H) to the potato common scab. Illumina MiSeq sequencing of amplicons were prepared with domain-specific primers.

Table 4. Coverage of the probes added to the 16S rRNA microarray.

Probe	Probe sequence (5'-3')	Coverage of *Streptomyces*		Ref.
		genus [%]	species	
KO 08	ACGGCTTCGCAGCTCATTGTA	28.0	-	Franke-Whittle et al. 2005[0]
Strepto1	CACGTGTGCAGCCCAAGACA	98.1	-	this work
Strepto2	ACGTGTGCAGCCCAAGACAT	98.1	-	this work
Strepto3	TTAGACCCCGTTTCCAGGGC	95.2	-	this work
Strepto5	GTATTAGACCCCGTTTCCAG	95.2	-	this work
Scab1	CCACACTCATCGGATGCCCG	1.7	*S. scabiei, stelliscabiei, europaeiscabiei,*	this work
Scab5	TCCACACTCATCGGATGCCC	1.7	*bottropensis, variabilis,* "	this work
Scab6	TCATCGGATGCCCGAGAGTG	2.6	as "Scab1" + *S. variabilis, ipomoeae, neyagawaensis, torulosus*	this work
Scab7	ATGCCCGAGAGTGTCGTATC	1.5	*S. scabiei, stelliscabiei, variabilis, ossamyceticus,*	this work
Scab8	GATGCCCGAGAGTGTCGTAT	2.2	*ipomoeae, neyagawaensis,* as "Scab6"	this work
Scab9	GCTTTCCACACTCATCGGAT	1.7	as "Scab1"	this work
Scab11	GAGCTTTCCACACTCATCGG	1.7	as "Scab1"	this work

3.4. Interaction between isolated actinobacteria from suppressive soil and *Streptomyces scabiei* in vitro.

3.4.1. Liquid culture experiments

The 21 strains of actinobacteria were cultured in liquid A and G media in different time points of 24, 48, 72, 120 and 240 h. The highest average biomass was obtained at 120h of growth. Consequently, this point of maximum biomass was also selected for the sensitivity test (Fig. 40).

The maximum biomass on A medium was obtain for strains 09VU19, 09VK39, 09VK12, 09VK62, 09VK70 and the maximum biomass on G medium was obtained for strains 09VK12 and 09VU16 (Fig. 41). The cultivation of *Streptomyces scabiei* was done in parallel with the selected actinobacteria strains. Its maximum biomass was reached between 48 and 120 h of growth and differences between cultivation media were not significant. The maximum of dry weight of *S. scabiei* was similar to the highest biomass of the actinobacteria strains. The biomass accumulated differently on the two media (Fig. 44).

Both spent media were used for filter paper disk sensitivity test. The resulting antibiotic activity against the Gram positive Kocuria rhizophila was for most strains higher on A medium but for a few on G medium. The largest inhibition zone was observed for strain 09VK70 on A medium. Large zones were observed also for strains 09ZI22 and 09ZI7 (Fig. 42). The average antibiotic activity was not dependent on the strain biomass (or growth) because some strains had relatively large zones and small biomass and vice versa (Fig. 43). *S. scabiei* is also antibiotically active against *K. rhizophila* and the average inhibition zone size was 10 mm on both media.

3.4.2. Vermiculite experiment.

The copy numbers of 16S rRNA gene and *txtB* gene of *S. scabiei* were determined at different time points (Fig. 45). The maximum copy numbers of both 16S rRNA and *txtB* gene for *S. scabiei* were observed at day 3. Quantification of 16S rRNA and *txtB* genes was done for both DNA extracted directly from colony that inoculate and shook for 24 h in YME and DNA extracted from vermiculite culture. The result showed that the proportion of 16S rRNA of actinobacteria to *txtB* copies average in vermiculite (n=3 replicates) was 5.89E+03 and in pure colony (n=3 replicates) 2.64E+6, therefore

in *S.scabiei* the ratio of 16S rRNA gene from actinobacteria/*txtB* gene of pure colony was higher than those in vermiculite.

Based on the results for *S. scabiei,* the biomass and antibiotic activity in vermiculite were determined for all strains after 3 and 14 days of cultivation. The copy numbers of 16S rRNA gene of the 21 actinobacteria strains were determined after 3 and 14 days of cultivation.

The antibiotic activity against *K. rhizophila* determined for the 21 strains was always higher after 14 days than after # days of cultivation. The largest zones from the cultivation on vermiculate were observed for strains 09ZI22, 09VK26, 09VK70, 09VK39 (Fig. 46).

The combined cultivation of actinobacteria strains and *S. scabiei* showed that the strains were able to grow together in vermiculite (Fig. 49, 52). The copy numbers of 16S rRNA gene from actinobacteria of combined actinonacteria isolates and *S.scabiei* in vermiculite differed between days 3 and 14. In some situations the numbers were lower, in some higher with respect to the length of co-culture period. More specifically, for 10 strains the copy number was higher on day 14 showing the overgrowth of the antagonistic strain (Fig. 47).

The copy numbers of *txtB* gene from the combined culture were different between days 3 and 14 for most strains in vermiculite. More specifically, it was higher for 8 strains on day 14 than day 3 showing the overgrowth of the pathogenic strain (Fig. 48).

The proportion of copy numbers of the *txtB* and 16S rRNA genes varied between the strains after co-culture with *S.scabiei* at both day 3 and 14 (Fig. 49 and 52).

16S rRNA gene copy numbers are divided by 100 to be comparable in the Fig. 49 and 52. Although the proportion is similar between the two days of culture, in some strains the dominance of antagonistic strain was more pronounced on day 14.

Total numbers of actinobacteria at day 3 were almost the same in both individual and combined samples. For combined samples, actinobacteria and *txtB* decreased after 14 days compared with individual ones. Generally, the pathogen which possess the *txtB* gene, was suppressed in the combined cultures (Fig. 49, 50). The proportion was highest for the strain 09ZI13 after 3 days of cultivation and for the strains 09ZI22 and 09VK44 after 14 days of cultivations. In contrast, the proportion

was lowest for the strain 09VU19 for both periods. The co-culture with this strain was the only, in which the pathogen largely overgrew the antagonistic strain (Fig. 49, 50).

The inhibition zones against *Kocuria rizophila*, measured for average of both submerged culture (21 isolates and *S. scabiei*) and vermiculite culture (21 individual, 21 combined and 9 individual cultures of *S.scabiei*). For vermiculite culture of *S.scabiei,* the largest inhibition zone was seen on day 3 and the largest inhibition zone in 21 isolates whether in submerged, individual and combined culture belonged to 09ZL22, 09Vk26 and 09Vk70. The inhibition zone of the co-culture of actinobacteria and *S. scabiei* against *K. rhizophila* was generally higher than when the strains were cultured individually or in the submerged culture. The inhibition zones were larger after 14 days of cultivation. The largest inhibition zones were observed in 09ZI22, 09VK26, 09VK44, 09VK70 and 09VU19 (Fig. 54). Several species with higher antibiotic activity suppressed *S. scabiei* more effectively. The high ratio of 16S rRNA/*txtB* gene copy numbers on day 14 was in strains 09ZI22, 09VK11 and 09VK44, which also had large inhibition zone in the same harvest time. In contrast, 16S rRNA/*txtB* gene copy numbers ratio for strains 09VU19, 09VK70 and 09VK26 were not at the maximum range, however, the inhibition zone was high (Fig. 51, 53).

Finally, the comparison of copy numbers of 16S rRNA gene from actinobacteria, copy numbers of *txtB* gene and inhibition zone on day 14 showed that the largest inhibition zone was observed in 09ZI22, 09ZI7, 09VK11, 09VK26, 09VK44, 09VK70 and 09VU19, the copy numbers of 16S rRNA gene decreased from day 3 to day 14 in: 09VK26, 09VK70, 09ZI7 and increased in 09ZI22, 09VK11, 09VK44 and 09VU19. The copy numbers of *txtB* increased in 09VK11 (slightly) and 09VU19, and decreased in 09ZI22, 09ZI7, 09VK26, 09VK44 and 09VK70.

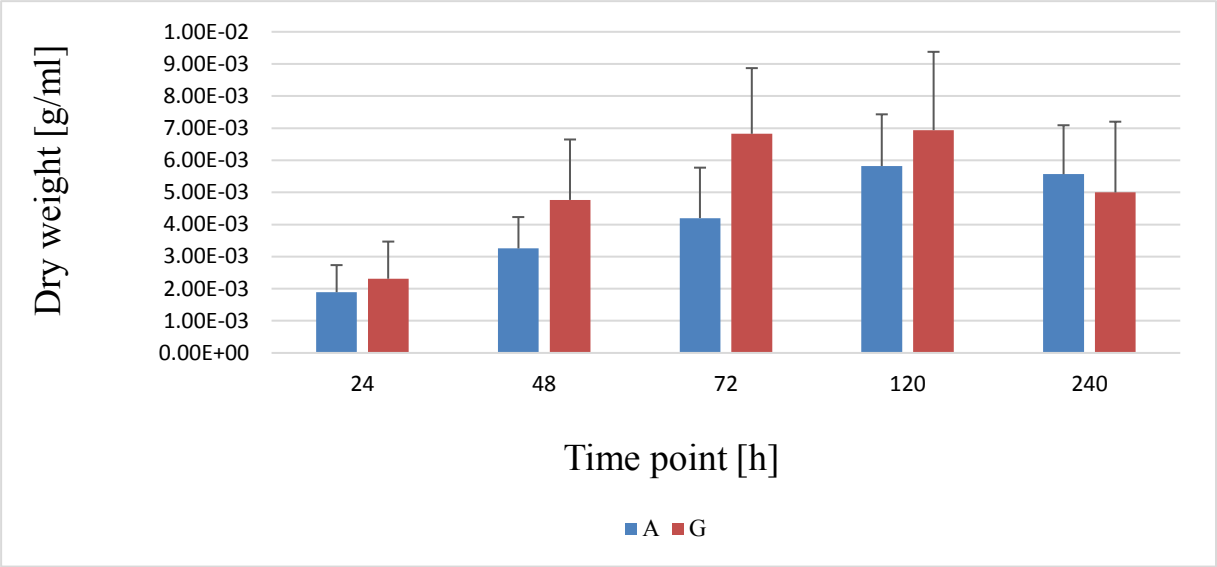

Fig. 40. The average dry weights of 21 actinobacterial strains (g/ml), harvested from A and G medium at different time points (means ± standard deviations, n=21).

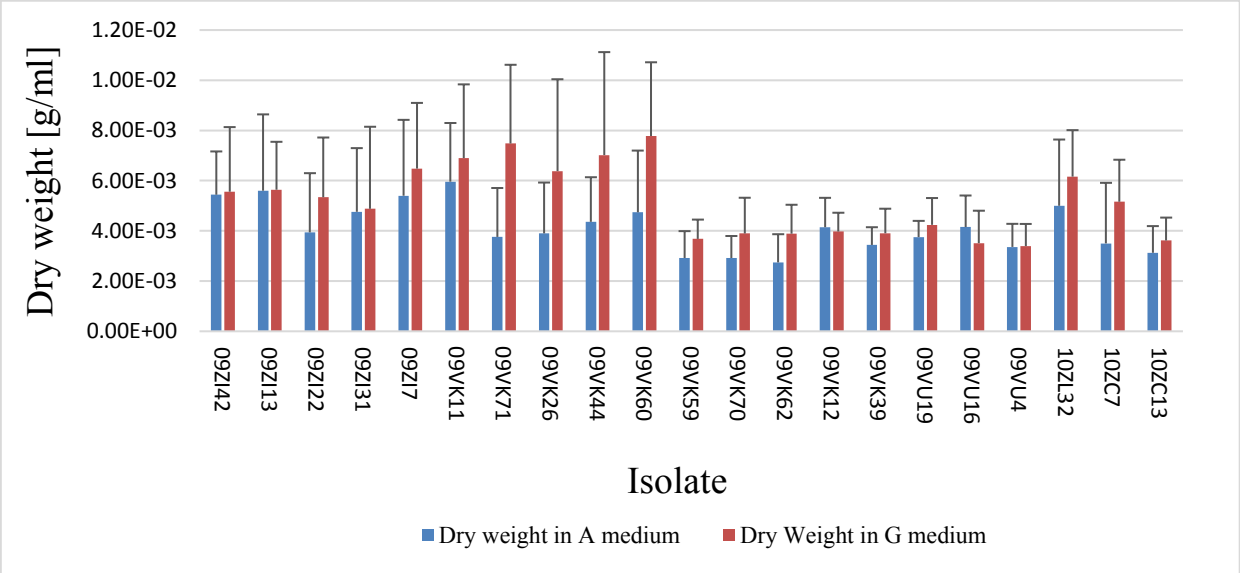

Fig. 41. The comparison of average growth (g/ml) in G and A medium at 5 time points (means ± standard deviations, n=5).

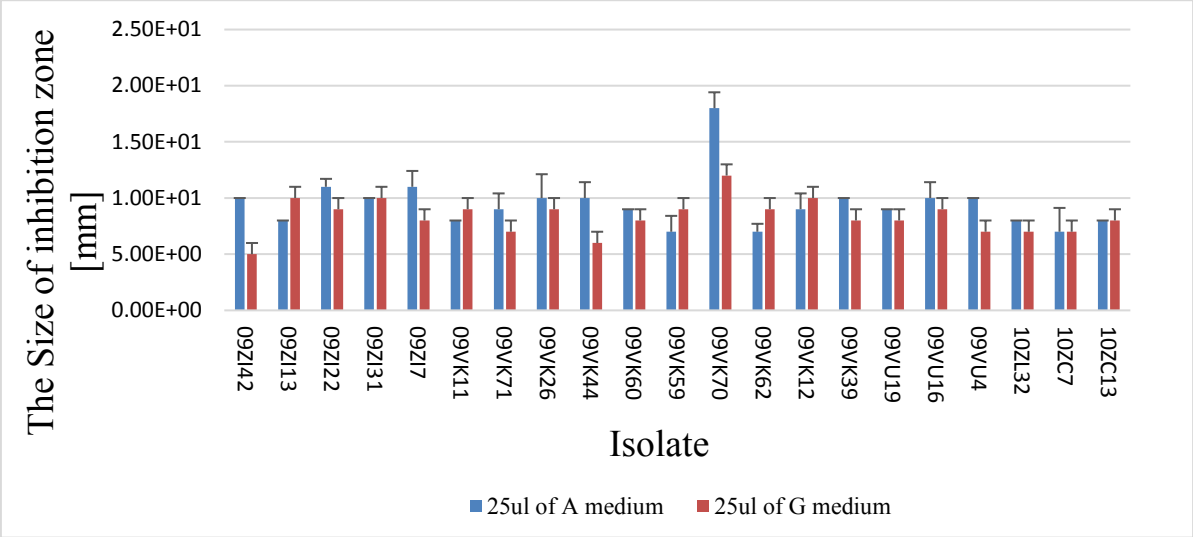

Fig. 42. The Sizes of average inhibition zones (mm)on agar plates inoculated with *Kocuria rhizophila* around discs with 25 µl of spent cultivation media A and G of the tested actinobacteria strains (means ± standard deviations, n=2).

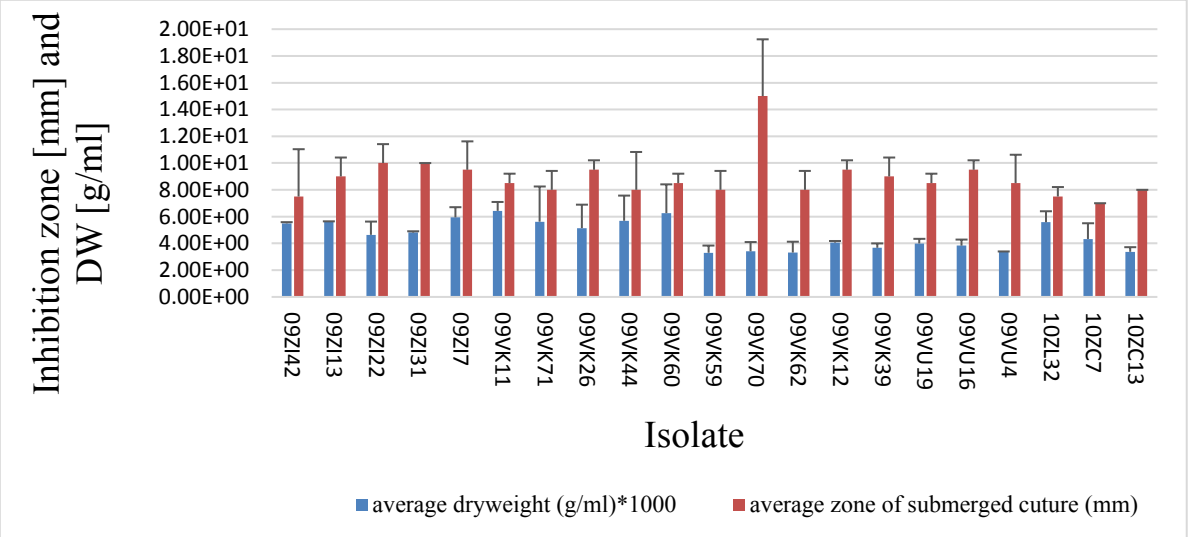

Fig. 43. The average of inhibition zone against *Kocuria* (mm) in submerged culture and average dry weight of actinobacteria (g/ml) in A and G medium (means ± standard deviations, n=2).

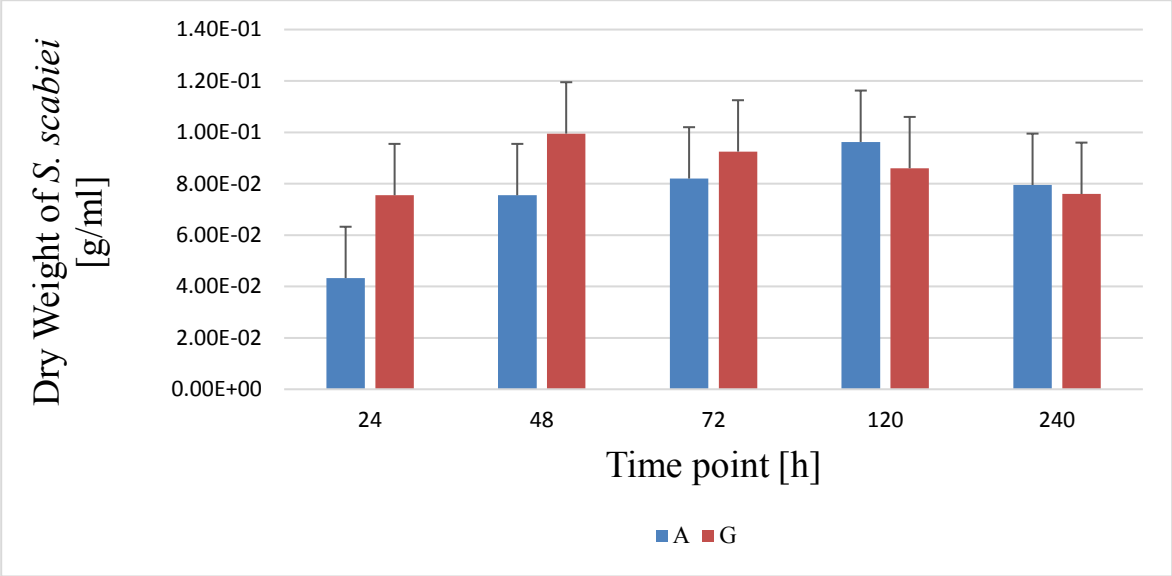

Fig. 44. The dry weight of *S. scabiei* (g/ml) at different time points of A and G media (means ± standard deviations, n=2).

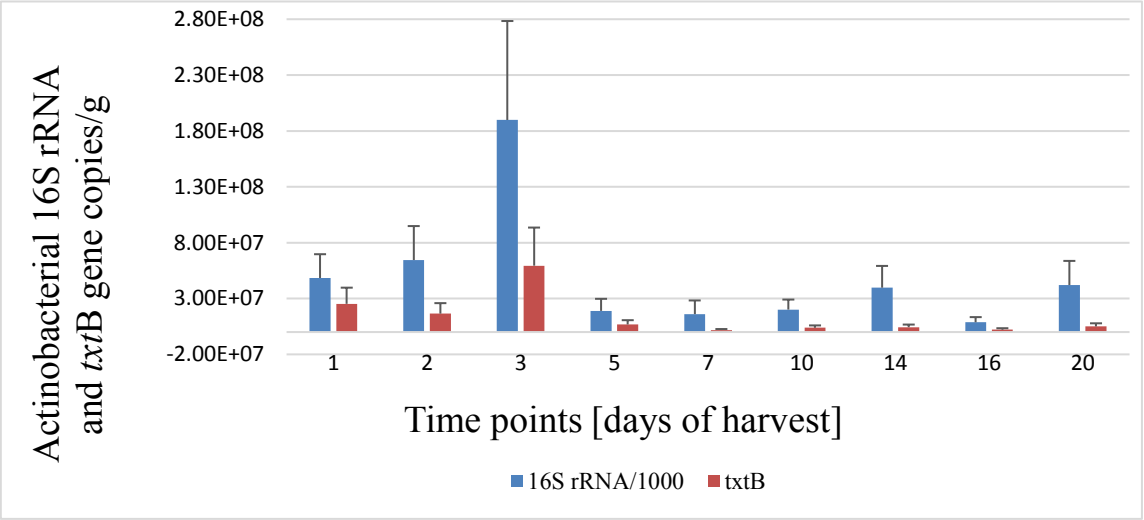

Fig. 45. The copy numbers of 16S rRNA gene from actinobacteria and *txtB* gene of *S. scabiei* /g vermiculite (means ± standard deviations, n=6, 2 technical and 3 experimental replicates).

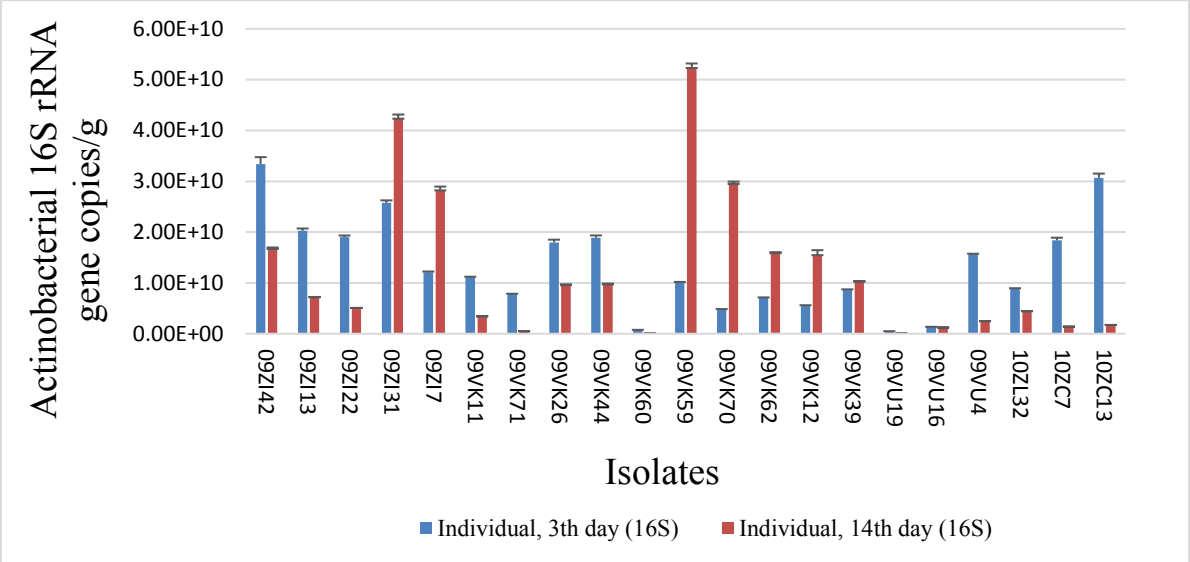

Fig. 46. The inhibition zone of 21 isolates of individual actinobacteria against *Kocuria,* at days 3 and 14 of cultivation /g vermiculite (means ± standard deviations, n=2).

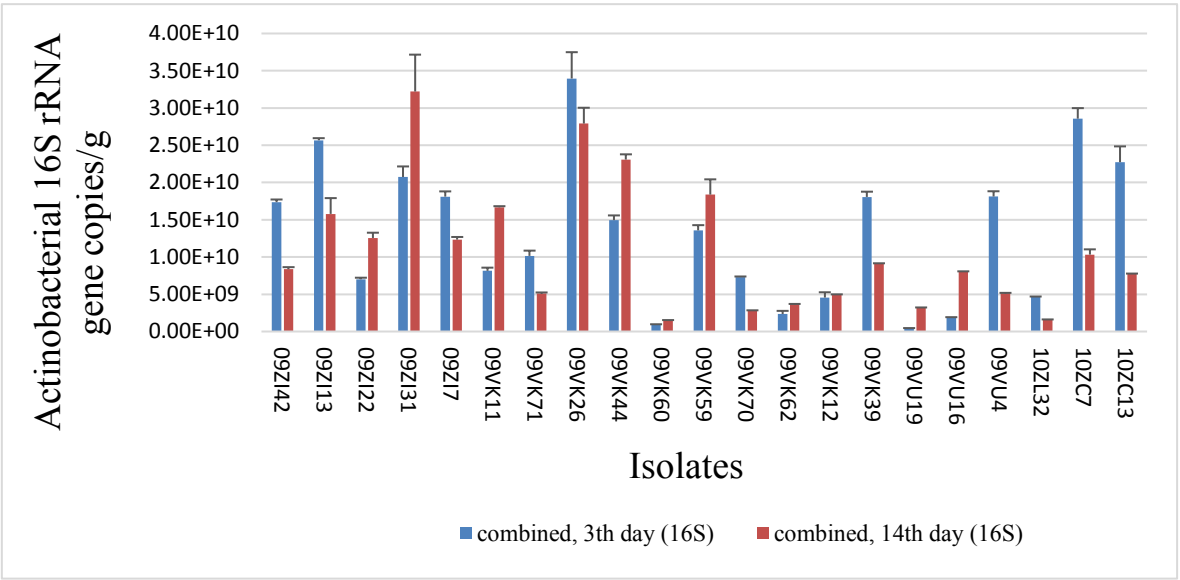

Fig. 47. The copy numbers of 16S rRNA gene from actinobacteria of combined 21 actinonacteria isolates and *S. scabiei* /g vermiculite at to different points at days 3 and 14 of incubation (means ± standard deviations, n=2).

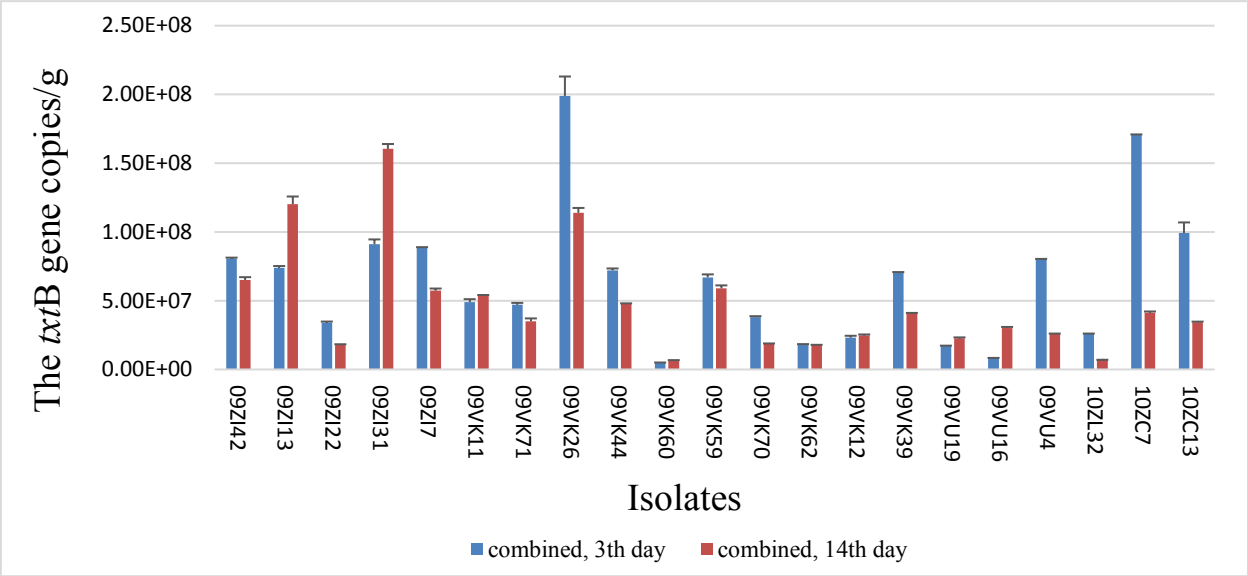

Fig. 48. The copy numbers of *txtB* gene of combined 21 actinonacteria isolates and *S.scabiei* /g vermiculite at to different points at days 3 and 14 of incubation (means ± standard deviations, n=2).

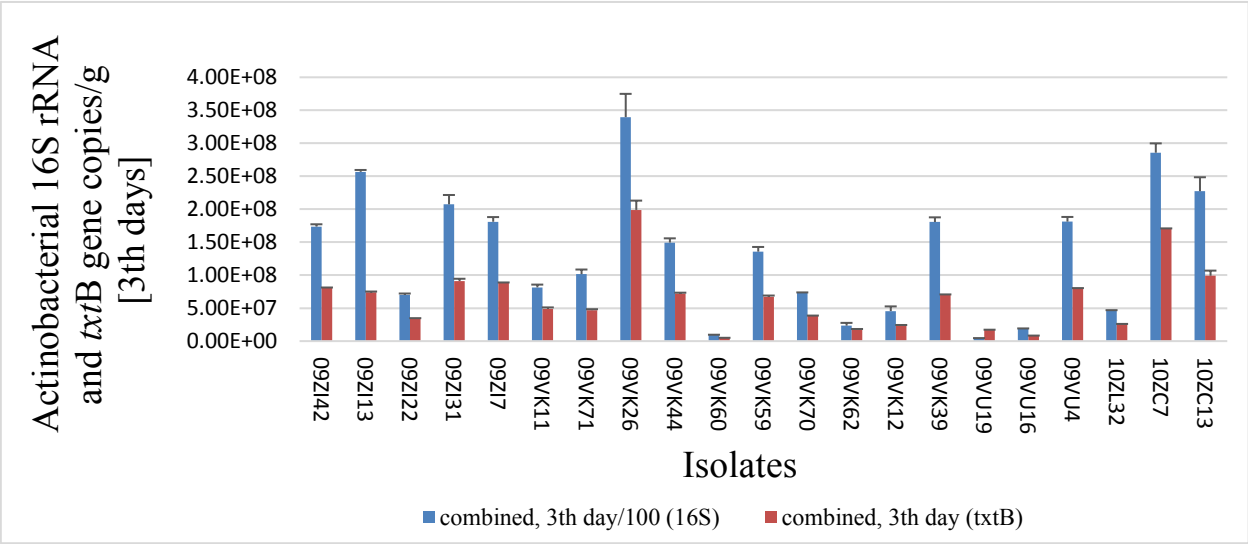

Fig. 49. The copy numbers of *txtB* and 16S rRNA gene from actinobacteria (divided to 100 to make it comparable in the chart) of combined 21 actinonacteria isolates and *S. scabiei* /g vermiculite at days 3 and 14 of incubation (means ± standard deviations, n=2).

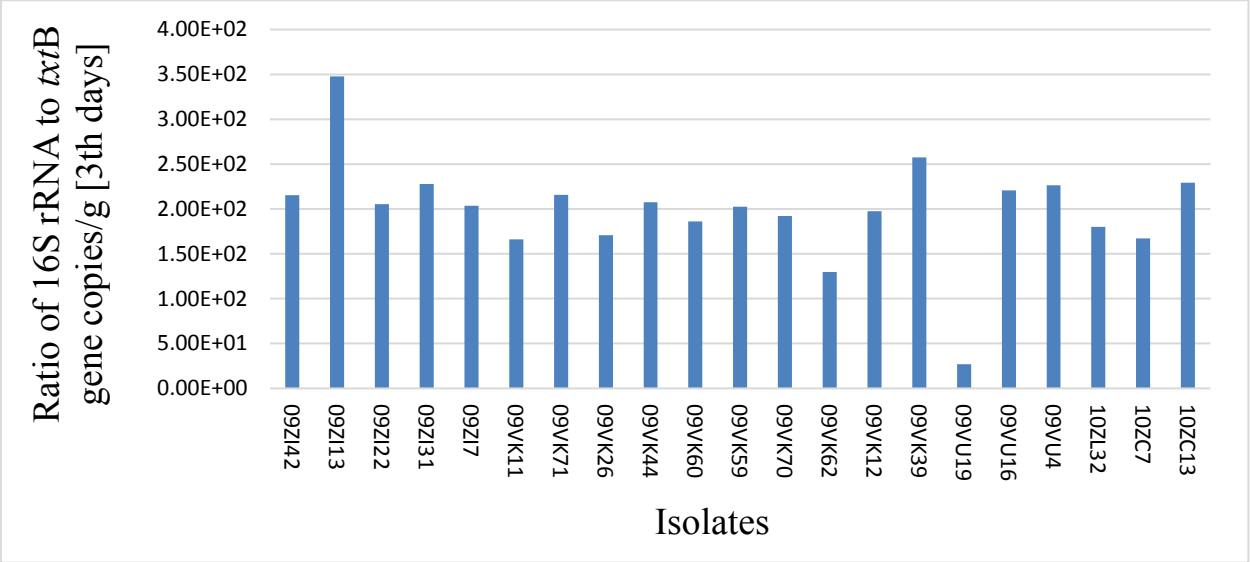

Fig. 50. The ratio of 16S rRNA gene from actinobacteria to *txtB* copies at day 3 of harvest

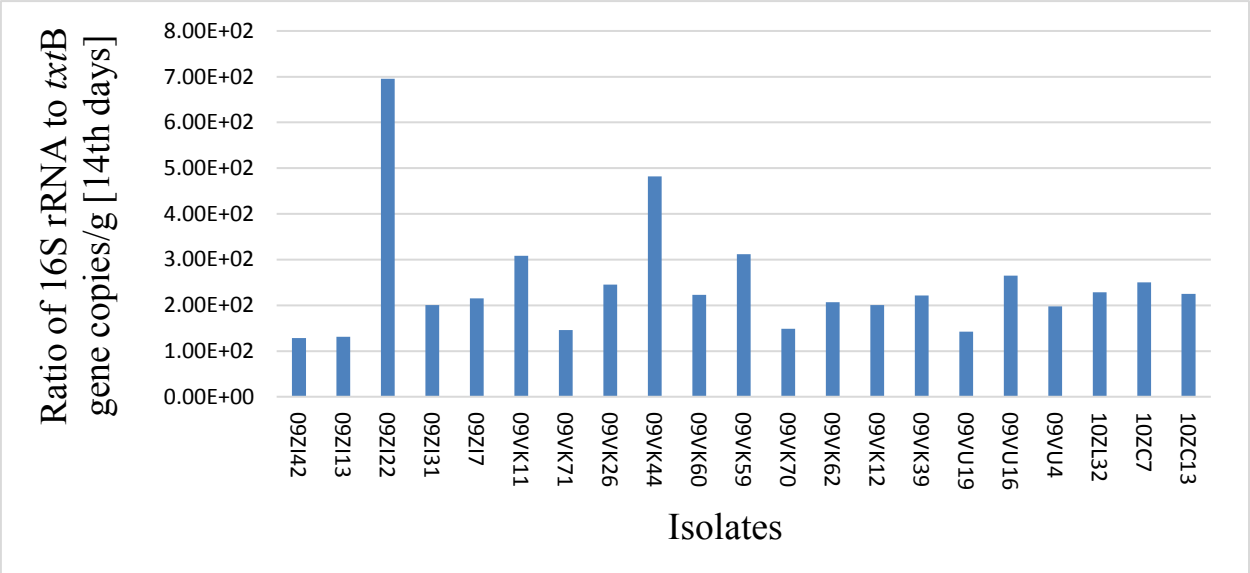

Fig. 51. The ratio of 16S rRNA gene from actinobacteria to *txtB* copies at day 14 of harvest.

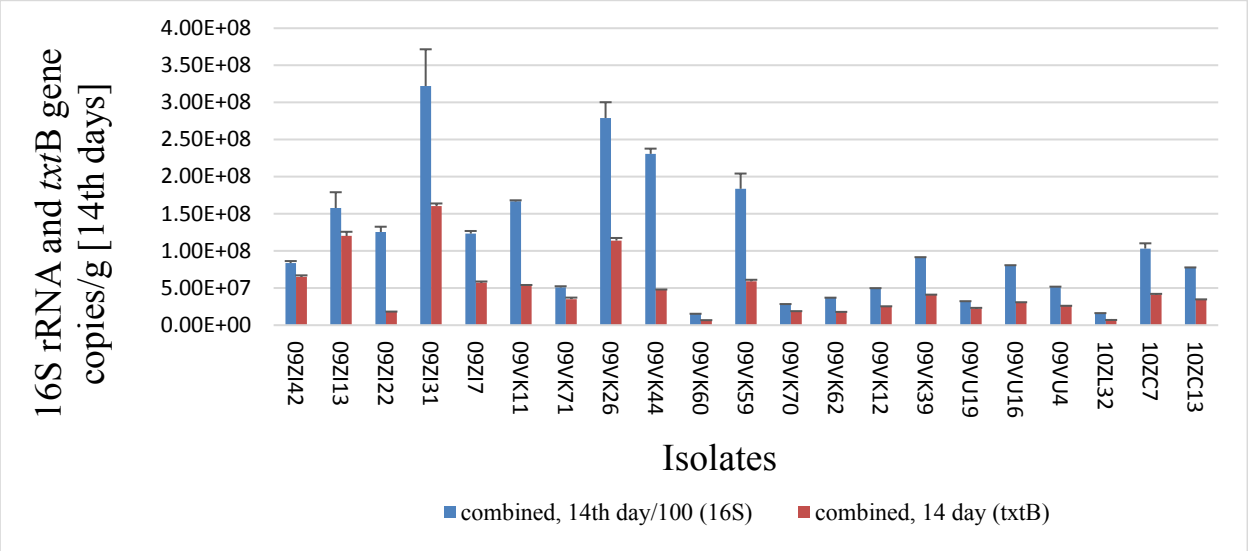

Fig. 52. The copy numbers of *txtB* and 16S rRNA gene from actinobacteria (divided to 100 to make it comparable in the chart) of combined 21 actinonacteria isolates and *S. scabiei* /g ver miculite at day 14 of incubation (means ± standard deviations, n=2).

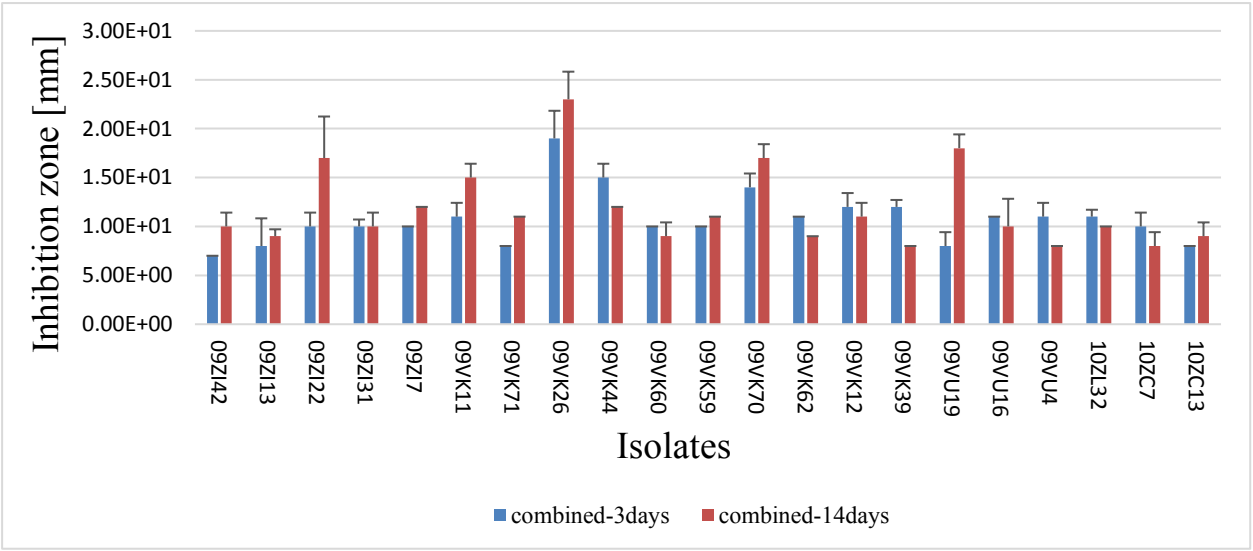

Fig. 53. The average of inhibition zone (mm) of combined cultures of actinobacteria and *S. scabiei* against *Kocuria* at days 3 and 14 of culture (means ± standard deviations, n=2).

4. Discussion

4.1. Biological and chemical factors associated with natural soil suppressivity to potato common scab.

In a total, 32 fields with different CS severity were selected for the study. The biological parameters focused on total bacteria and actinobacteria in soil and potato skin. The chemical parameters focused on determining the concentration of macroelements C, N, S, P, Mg, Ca and Fe in relationship to the biological parameters.

The previous studies demonstrated that various factors affect CS severity but the site can be determinative and change the predicted roles of other factors (Schlatter *et al.,* 2013; Sagova-Mareckova *et al.*, 2015). In this study, the comparisons between the sites showed a low level of correlation (Fig. 28, 29). The differences between biological parameters in the different sites were significant, which means the sites were clearly distinguished.

Some studies showed that the fields with low distance from each other showed similar relationships of nutrients and CS severity (Lazarovits *et al*., 2007). However, other studies demonstrated that neighbor fields may show drastically different associations between soil properties and CS severity (Rosenzweig *et al*., 2012; Sagova-Mareckova *et al*., 2015; Lazarovits *et al*., 2007). In this study, a significant correlation occurred between the site and CS severity.

Several field sites had special properties compared to the others. The maximum severity occurred in two neighboring fields: Zl and Zc. Both of them had a high content of C in the rhizosphere and a rich content of nutrients in skin (N, Mg, Fe, P). In these two fields the skin rich in nutrients provided a niche of high nutrients for pathogens (Fig. 12, 14, 15, 21)

In previous studies, the role of nutrient-rich soil to enhance or suppress the pathogen was showed. A positive correlation was reported between CS and Ca, PO_4, Mn and Fe contents of tuber peelings (Davis *et al,* 1976). Also, Mg, K, Mn and Cu positively correlated with common scab severity (Sheikh, 2010), however, other studies showed a different outcome of high P and Mg in different fields (Sagova-Mareckova *et al*., 2015). In this study, nutrient-rich soil enhanced the pathogen was seen in Hostice, where high CS severity can be possibly attributed to high content of P and Mg in the rhizosphere (in opposite of low values of biological factors: minimum of actinobacterial and bacterial 16S rRNA gene in soil and *txtB* gene in skin). In

Slavkov-Vinohrady (Sl), the effect of high nutrients (N, Ca, C in rhizosphere and P in skin) on CS severity was more noticeable because the copy number of pathogenic agent (*txtB* gene) in skin was low compared to the other fields. The role of rhizosphere environment can be explained also for other two nearby fields, Vu (with high severity) and Vk (with low severity), where both had a high content of S, Mg and Fe in soil. The content of Fe and Mg in the rhizosphere was higher than in bulk, while in Vk it was higher in bulk. Also, Vu had more content of N and Ca in skin. So, there was a different selection for nutrients by plants, microbes but most likely their interaction.

The low CS severity in this study was not related to soil pH. It is noticeable in the fields with minimum CS (Ruzyne, Stankov, Sutom, Horazdovice, Polepy uvoz) with an exception of Vk (low pH and low severity). Statistically, severity showed to be related to Fe, Mg, N (positively) and Ca in skin and soil (negatively). Polepy uvoz (low content of N, P, Fe, Mg soil) and Vysoke u Pribrame (low content of Mg) were a good instance. There was also an exception field with a high severity in summer and a low severity in autumn, with a high range of N, C, Fe, Ca, S and P in rhizosphere and a low content of Ca in skin and soil (Malonty). Similar results with locally specific correlation between CS severity and soil chemical factors (pH, Al, Ca, Mg, and K) and organic matter were observed by Lazarovits *et al*. (2007).

Researches showed different effects of organic matter and nutrients on the scab severity (Lazarovits *et al*., 2007; Lambert *et al*., 2005; Davis *et al*., 1976). However, the effects of nutrient additions differed by locations and situations (Bailey *et al*., 2003; Lazarovits *et al*., 1999; Lazarovits *et al*., 2010; Soltani *et al*., 2010), so again the conclusions were that even after application of amendments the reduction of CS is soil-specific and varies from year to year (Abbasi *et al*., 2010). The reason for that is mostly that the relationships between scab severity and soil chemical components is very complex (Lazarovits *et al*., 2007; Conn and Lazarovits, 2013).

A positive correlation was observed between CS and the Ca, PO_4, Mn and Fe content of potato skin, a positive correlation of number of *txtB* gene copies in potato skin and the content of Mg, P and Fe in skin (Sagova-Mareckova *et al.*, 2017; Lacey and Wilson, 2001; Horsfall *et al.,* 1954; Davies *et al.,* 1976 and Davis *et al,* 1976), and a positive correlation between scab severity and Ca, Fe, Mg, K, Mn, C, N and Cu of soil (Sagova-Mareckova *et al.*, 2015; Sheikh, 2010). Other researches showed a negative correlation between CS and Mg, N, P, Zn, K, Fe, Ca and S in soil (Horsfall *et al.,* 1954; Davies *et al.,* 1976; Sheikh, 2010; Lacey and Wilson, 2001; Sagova-

Mareckova *et al.*, 2015; Kyselkova and Moenne, 2012; Sarikhani, *et al*., 2017; Kristufek *et al*., 2015). The resistant variety had a higher Mg content in skin compared to sensitive varieties (Kopecky *et al*., 2018; Bailey and Lazarovits, 2003; Lazarovits *et al*., 1999; Klikocka, 2009 and Davies *et al.,* 1974).

In this study, the results support the complex character of CS severity determinants because of contrasting examples and leads to a conclusion that CS relationships are completely site-specific. Moreover, the nutrient-rich environment of rhizosphere may play two contradictory roles. On the one hand, it can make a rich environment for pathogen causing a high severity, and on the other hand, it can support and enhance the antagonistic population that may lead to decrease of CS severity.

The differences between rhizosphere and bulk soils were shown. There was a significant difference between bulk and rhizosphere in several nutrients (totally N, C, S of rhizosphere was more than bulk and Ca of bulk was more than rhizosphere). However, the interpretation of interaction between chemical and biological characteristics might be complicated but in our study, the content of nutrients such as Fe, Mg, N and C in soil and skin had predictive value. The negative correlation between CS severity and Ca in soil and skin can be a good indicator for management of CS.

Previous studies showed the importance of resistant potato varieties to decrease the disease severity and even more to change the microbial interactions in the rhizosphere in favor of scab suppression (Weinert *et al*., 2010; Dees and Wanner 2012; Eckwall and Carl, 2000 and Kopecky *et al*., 2019). In this study, a significant correlation between the variety and CS severity demonstrated the effectiveness of potato variety selection in CS severity control.

Several field sites had special properties compared to others that may help to get a better understanding of CS profile. The maximum severity occurred in two neighboring fields: Zl and Zc. Both of them had the maximum of *txtB* gene copies in skin/soil, maximum of actinobacteria numbers (in skin) and minimum of *txtB* in bulk among the 32 fields. The *txtB* in the rhizosphere in Zl was the minimum among all fields. So, it seems that accumulation of pathogens and respective genes in the skin is the main reason of CS severity. In addition, in these two fields the skin rich in nutrients provided a niche of high nutrients for pathogens (Fig. 12, 15, 18, 21).

The role of rhizosphere environment can be explained also for other two closed fields, Vu (with high severity) and Vk (with low severity), where both had a high copy numbers of bacteria and actinobacteria in soil and *txtB* in skin. In both fields, *txtB* gene and actinobacteria was higher in bulk soil. In Vu, the copy numbers of total bacteria in the rhizosphere was higher than in bulk, while in Vk it was higher in bulk. Also, Vu had more copy numbers of *txtB* gene and actinobacteria in skin.

The low CS severity in this study can be attributed to low copy numbers of *txtB* and actinobacteria in skin. Researches showed that the plant roots are colonized by living bacteria added to the starter fertilizer, which feed the bacteria with carbon-rich root exudates: in turn, the microbes enhance nutrient uptake, making fertilizers more efficient, and produce metabolites that stimulate healthy growth and suppress disease as a side effect (Abram, 2009). In this study, the differences between rhizosphere and bulk soils were showed. There was a significant difference between bulk and rhizosphere in biological variables (actinobacteria in rhizosphere and *txtB* in bulk soil showed to have larger quantities).

The Zl and Zc had higher severity and maximum copy numbers of 16S rRNA gene from actinobacteria, total bacteria and *txtB* gene in skin among the 32 fields. The previous studies proved the relation between bacterial population and CS disease (Rosenzweig *et al.,* 2012; Han *et al*., 2005; St-Onge *et al*., 2010). Interestingly, Zl had the maximum of severity in summer and minimum of *txtB* gene copy number in soil. That is explained by the accumulation of pathogen in the potato skin (Sagova-Mareckova et al. 2015).

In spite of correlation between CS severity and several chemical and biological characteristics, the relationships did not follow a unique rule. That may be because it was a vast monitoring with varied factors and those might have been affected by interactions with other factors not comprised in this research. This study proved the previous founds that management of nutrients can change the CS severity, e.g. by making the change in availability of essential nutrients for plant and antagonistic bacteria. Nutrients can also affect *txtB* gene production or density of pathogen on the plant skin or change the bacterial population in favor of increasing the biocontrol species of bacteria with antagonistic activity against pathogen. Nutrients can also enhance the plant defence or even enrich a pathogen as a saprophyte bacteria that is not dependent on plant skin to live (Lazarovits *et al.,* 2007; Millard, 1923; Han *et al*., 2005; Larkin and Tavantzis, 2013; Abram, 2009).

However, the interpretation of interaction between chemical and biological characteristics might be complicated but *txtB* copy numbers in soil and skin could be a good predictive factor for CS disease occurrence and at some sites the high amount of *txtB* in soil will define a low accumulation of *txtB* and pathogen on skin.

In this study, CS severity had a positive correlation with 16S rRNA gene from actinobacteria and 16S rRNA gene from total bacteria copy numbers in soil and 16S rRNA gene from actinobacteria and *txtB* copy numbers in skin, however, it cannot be generalized to all fields. There are also different results about the role of actinobacteria and bacterial community in CS disease progress. In a greenhouse study, the treatment with the lowest CS occurrence also had the lowest density of actinomycetes in the rhizosphere (Keinath and Loria, 1989). Nonpathogenic actinobacteria produce metabolites against *S. scabiei* and contribute to the disease suppression (Rosenzweig *et al*., 2012). Regarding to the total bacterial community, suppressiveness is very likely to be associated with microbial community structure based on antagonistic functions (Sessitsch *et al*., 2004).

The RDA plot showed that *txtB* abundance in soil has a different direction compared to other variables, particularly *txtB* in skin (as Zl and Zc), coordinated to the low scab severity points such as CB, Mo, KU, VP, Vc and Vl. It demonstrated that the copy numbers of *txtB* in the soil and skin are two factors that predict the scab suppressiveness or conductivity, respectively. In the same plot, a different situation can be seen (points towards 4-5 and 6-7 o'clock), those points are relatively far apart of other fields (Vk and Vu). Interestingly however, the severity of Vk and Vu are in the opposite way but the properties of fields seems to be in the same position (actinobacterial 16S rRNA in soil). It was demonstrated that at Vyklantice site suppressivity (Vk) was associated with low *txtB* gene copies in the tuberosphere (rhizosphere) and discussed with respect to the interaction between potato plant and soil microbial community. As previous studies showed, compared to the high scab severity group of soil, the low severity group exhibited a lower *txtAB* gene copy number, lower bacterial 16S copy number and higher diversity (Wencong *et al*., 2019). A study of the two nearby fields demonstrated that the suppressivity was associated with low *txtB* gene copies in bulk soil, while at other site it was associated with low *txtB* gene copies in the tuberosphere (Sagova-Mareckova *et al*., 2015).

In the plot of bulk samples (Fig. 31), again the abundance of *txtB* in soil has a different direction compared to other variables, and the same coordination and

overlapping to the low severity points: Pu, Ne, Zr, Hb and Pc, confirm that *txtB* gene in soil can show the disease profile component points of bulk samples in the plot showed to be scattered, moreover according to statistical analysis, the average of N, C, S, Fe and actinobacterial copy numbers in the rhizosphere was significantly more than bulk soils, it seems that the profile of bulk soils cannot be sufficiently determinative to predict the disease.

Acidobacteria and *Verrucomicrobia* were on the direction from Mi and Hr with a low CS severity towards Vk (Fig. 32), identified previously as CS suppressive soil (Sarikhani *et al.,* 2017; Rosenzweig *et al.,* 2012; Kopecky *et al.* 2019; Wencong *et al*., 2019). *Proteobacteria* and *Firmicutes* showed a trend to Pu, CB and Zd with low CS severity and Zl with high CS severity. A recent study showed that the taxa enriched in high scab severity levels were mainly found in *Proteobacteria* and *Bacteroidetes*, and the taxa enriched in low scab severity levels mainly belonged to *Acidobacteria*, *Actinobacteria* and *Firmicutes* (Wencong *et al*., 2019). *Bacteroidetes* had a direction to Pu, Pc and Pd with a low severity in summer. *Gemmatimonadetes* pointed to the direction of Li with low severity, *Planctomycetes* and *Chloroflexi* and *Actinobacteria* pointed to the direction towards Vu a conductive soil (Kopecky *et al*., 2019). *Patescibacteria* was showed to be correlated with KU (low severity) and N (in soil). There was not any report of *Patescibacteria* and *Planctomycetes* in relation to common scab in the previous studies. It seems that the CS conductivity in Zc is not related to a special group of bacteria, however, it was showed that it correlated with actinobacteria (in soil). The results are comparable with the results of previous studies, which demonstrated that soil treatments can change the bacterial community in favor of scab suppression. The treatment can increase the population of *Acidobacteria, Verrucomicrobia, Gemmatimonadetes*, *Proteobacteria*, and several genera of *Actinobacteria* and *Bacteroidetes* in the direction towards the suppression (Sarikhani *et al.,* 2017). In the same study *Chlorofelexi* had naturally a higher number of OTUs in CS suppressive soil. Moreover, *Firmicutes* and several genus of *Actinobacteria* decreased in the treated suppressive soils. Common phyla based on relative sequence abundance were *Acidobacteria, Proteobacteria* and *Firmicutes*. Sequences of *Lysobacter* were found in significantly higher numbers in the disease-suppressive soil (Rosenzweig *et al.,* 2012). Meng et al. (2012) showed four groups of potential antagonists (general bacteria, streptomycetes, fluorescent pseudomonads and bacilli) pairing in culture with *S. scabiei.*

In conclusion, the most important factors predicting the CS severity were nutrients Fe, Mg, N, C, Ca and P in soil. Also, *txtB* copy numbers in soil could be a good predictive factor for disease suppression. The disease severity was positively correlated with numbers of 16S rRNA gene from actinobacteria and 16S rRNA gene from total bacteria in soil and also 16S rRNA gene from actinobacteria and *txtB* copy numbers in skin, however, this cannot be generalized to all fields. The population of several groups of bacteria was showed to be important to scab suppression (*Acidobacteria, Verrucomicrobia, Bacteroidetes* and *Gemmatimonadetes).*

4.2. The effect of iron availability on potato common scab and tuberosphere bacterial community

The soil conducive for common scab (CS) used in this study was previously characterized by high carbon, nitrogen, and soil pH. Also, the soil had the same quantity of *txtB* gene copies (representing the pathogen) and low available iron in comparison to the nearby occurring soil suppressive for CS (Sagova-Mareckova *et al*., 2015). Supplementation of the soil by iron and peat significantly decreased the CS severity to the level of the suppressive soil but the addition of iron alone was less effective than the addition of peat and the combination of both.

The disease was suppressed in the treatments but the quantity of *Actinobacteria* and *txtB* genes was not affected in either soil or periderm. So, it seemed that in our study the iron supplement supported plant defense but did not change pathogen colonization. Indeed, plant iron can influence host-pathogen relationships by affecting the pathogen's growth or virulence as well as the host's defense (Expert *et al*., 2012). For example, iron starved maize plants were more susceptible to pathogen infection, while adequate Fe nutrition conferred a more resistant state (Aznar *et al*., 2015). Similarly, in *A. thaliana*, Fe starved plants were unable to produce reactive oxygen species (ROS) in response to pathogen infection (Phuong Kieu *et al*., 2012). Possibly, iron was important for plant defense in our experiment also because Agria (the potato cultivar used in this study) requires more iron than other cultivars (Ozturk *et al*., 2011). Therefore, Fe deficiency may favor disease susceptibility of this cultivar in particular.

In the peat treatment, both *Actinobacteria* and potential thaxtomin producers decreased significantly compared to the conducive soil control. It seemed that peat had a direct effect on the pathogen or improved its suppression by antagonists. In other studies, peat and other organic materials were used to reduce soil pH (Carlgren and Mattsson, 2001) but in our study the pH in the peat treatment remained the same possibly because of soil buffering capacity. Therefore, the decrease of CS severity was not due to a decrease in soil pH but possibly due to changes in organic matter quality supporting antagonistic microorganisms. A similar observation was demonstrated previously because peat and other organic matter supplements increased the biocontrol potential of suppressive strains (Bonanomi *et al*., 2010; Kyselkova *et al*., 2012). In particular, application of organic matter amendments altered rhizobacterial communities, which correlated with a varying degree of antibiosis against S. scabiei (Driscoll 2007; Junaid *et al*., 2013).

In the treatment combining iron and peat, CS severity was significantly suppressed, while extractable iron increased and soil pH decreased. Similarly to the peat treatment, the numbers of *txtB* gene copies decreased in the periderm so it seemed that the pathogen was suppressed at the very surface of the tubers. At first, the decrease of soil pH seemed to be the most important factor because it was significantly reduced to the level of the suppressive soil only in this treatment. The effect of soil pH on CS was well documented previously although CS severity decreased due to both low and high pH in several studies (Lacey and Wilson 2001; Waterer 2002). Soil pH is connected also to the availability of iron and other nutrients because they are more soluble in acidic soils (Schulte, 2004). However, in this treatment, the most interesting was the change in the bacterial community, which became similar to that of the suppressive soil. It seems that although iron and peat alone supported some plant-growth promoting bacteria, the combination of both supplements was successful in simulating the most favorable community composition either due to decreasing soil pH or due to the addition of iron and organic matter supplements together. Finally, according to the preferential food hypothesis, the pathogenic *Streptomyces* has a saprophytic nature in soil, until their natural food is available and their parasitic stage will start only under hunger stress (Millard 1923). Therefore, supplements of peat as an organic matter and iron as an important micronutrient may lead to keeping the pathogen on the surface of tubers only as saprophytic bacteria without proceeding to the virulence stage.

Generally, both biotic and abiotic elements of the soil environment contribute to suppressiveness, however, in most defined systems biological elements were identified as primary factors in disease suppression (Mendes *et al*., 2011; Kyselkova *et al*., 2012). In our study, the iron treatment alone decreased CS severity but not soil pH and only a few OTUs, mostly from the orders of *Bacillales* and *Gaiellales* changed in the direction towards the suppressive soil. The peat treatment alone decreased CS severity but again not soil pH but the number of OTUs changed was higher possibly because many saprotrophic bacterial taxa respond to addition of organic matter (Bonanomi *et al*., 2010). The OTUs responding to peat addition were mostly from the orders of *Rhizobiales, Burkholderiales, Xanthomonadales* and *Bacillales* some of which were influenced also by soil supplement with small organic molecules (Eilers *et al*., 2010). The suppression of CS by peat may be also due to some organic compounds, namely lignin which can induce production of secondary metabolites (Schlatter *et al*., 2009). It seemed that the combination of iron and peat supplement had a more pronounced effect on CS severity possibly due to decrease in soil pH as it is well established that soil pH has a strong effect on bacterial community structure (Fierer *et al*., 2009).

In our study, the number of bacterial OTUs was lower in all treatments but peat compared to the conducive soil. This result was obtained for OTUs defined at both the 0.03 and 0.10 levels (data not showed) which is in contrast to the observation of Rosenzweig *et al*. (2012) who found a higher number of OTUs in suppressive than conducive soil. Therefore, diversity alone was not responsible for disease suppression, but rather the dominance of particular taxonomic groups, which possibly have some promoting traits was important.

With respect to that, *Proteobacteria* and *Bacteroidetes* increased at the proportional expense of *Actinobacteria* in the suppressive soil and also in the most successful treatment with peat and iron supplement. Mendes *et al*. (2011) identified *Betaproteobacteria* and *Gammaproteobacteria* (*Pseudomonadaceae*, *Burkholderiaceae, Xanthomonadales*) as the most dynamic taxa dominating suppressive soils and similarly in our study, OTUs from *proteobacterial* orders of *Burkholderiales*, *Xanthomonadales* but also *Sphingomonadales*, and *actinobacterial* order of *Gaiellales* belonged to the most responsive taxonomic groups. For example, *Sphingomonas* sp. with 100% identity of OTU1 was regularly isolated and cloned from agricultural soil (GenBank acc. no. LN876437), *Burkholderia* sp. with 100%

identity of OTU 27 was isolated from rotten wood (GenBank Acc. No. KX822674) or uncultured *Gammaproteobacterium* represented by OTU 47 from soil of *Solanum muricatum* (GenBank acc. no. KT785778). Some taxa coming from those groups or their relatives were previously associated with disease suppression. For example, bacteria affiliated to *Sphingomonadaceae* (*Alphaproteobacteria*) were prevalent in the tobacco rhizosphere in black root rot suppressive soil compare to conducive soil (Kyselkova *et al*., 2009). *Pseudomonas* sp. LBUM223 (*Gammaproteobacteria*) exhibited antagonistic properties against *Streptomyces scabiei* (Larkin and Stellos, 2013; Marschner, 1995), and also other *Gammaproteobacteria* had a biocontrol role against *S. scabiei* (Sessitsch *et al*., 2004). Finally, *Xanthomonadaceae* (*Gammaproteobacteria*), which was enriched in our study, was found to be important in Japan, where they were enriched in cultivars resistant to CS (Kobayashi *et al*., 2015). This was also found in soil suppressive to CS in Michigan, in which *Lysobacter* (*Xanthomonadaceae, Gammaproteobacteria*) was also significantly elevated (Rosenzweig *et al*., 2012). In contrast, *Acetobacteraceae* were elevated in disease suppressive soil in the study of Rosenzweig *et al*. (2012) but diminished in our study.

Although the relative abundance of total *Actinobacteria* decreased in the suppressive soil, and some OTUs of *Actinobacteria* were found more abundant in conducive, others were more abundant in suppressive soils similarly as in other studies (Mendes *et al*., 2011; Kyselkova *et al*., 2009). Here, OTUs of the families *Micromonosporaceae* and *Thermomonosporaceae* and were elevated in the suppressive soil and in the peat and iron treatment. *Thermomonosporaceae* is found in the rhizosphere microbial community of certain plants and some colonize root tissues as plant growth promoting endophytes supporting N-fixation and plant growth (Szoboszlay *et al*., 2016). In other patho-systems, *Thermomonospora* sp. and *Bacillus subtilis* intensely reduced bacterial wilt (Reddy 2014). In contrast, *Nocardioidaceae* were elevated in disease suppressive soil in the study of Rosenzweig *et al*. (2012) but diminished in our study.

In this study, the effect of peat addition was more important than that of iron for suppression of common scab but the most effective was the combination of both treatments. Although some abiotic factors i.e. soil pH were changed by the treatments it seemed that the manipulation was most important in stimulating the beneficial bacterial community. Possibly some of the OTUs enriched in suppressive soil as well as in both the peat and iron and peat treatments might produce antibiotic compounds

or were successfully competing with the pathogen. Alternately, the bacterial community might promote plant defense, which seemed to be the case with supplementation of iron. It seemed that, although the relationships are very complex, members of some taxa, namely *Solirubrobacteraceae, Xanthomonadaceae* or *Sphingomonadaceae,* were associated with disease suppression not only in our work but also in other studies.

4.3. Bacterial, archaeal and micro-eukaryotic communities in potato tuberosphere

Disease suppressiveness of Vyklantice soil L was shown in previous studies (Sagova-Mareckova et al. 2015; 2017). In the current field experiment, previous work on common scab severity was extended by including two potato cultivars susceptible or resistant to CS. According to expectation, the CS severity of the susceptible cultivar grown in the suppressive soil was as low as for (i) the resistant cultivar in the same soil, and (ii) the resistant cultivar in the conducive soil. These results proved that the experimental set up was relevant and evidenced a similar potential of both types of CS control mechanisms. Suppressive soil was differentiated from conducive soil by (i) lower diversity and quantity of bacteria, and specifically of actinobacteria, (ii) lower N, C, P, Ca, Fe contents and pH, and higher S content. Above that, in suppressive soil, higher Mg, P, Fe contents were found in the periderm of the resistant cultivar.

The quantity of pathogenic streptomycetes (based on numbers of *txtB* genes) did not change with soil suppressiveness status or cultivar in tuberosphere and bulk soil, but in suppressive soil the number of pathogens decreased in potato periderm, possibly due to both microbial interactions and soil chemical conditions depending on location (Kobayashi *et al.,* 2015; Sagova-Mareckova *et al.,* 2015).

Yet, the increased numbers of actinobacteria in periderm of susceptible cultivar did not correspond to pathogenic streptomycetes, so perhaps an antagonistic community of actinobacteria developed there as a response to pathogen infection, similarly as in Rosenzweig et al. (2012) or Tomihama et al. (2016).

Bacterial community structure has been identified as a major factor in CS control (Rosenzweig *et al.,* 2012; Shi *et al.,* 2019). In this work, microarray analysis evidenced mainly effects of suppressive vs conducive soil, with higher signals in suppressive soil for *Streptomyces* (*Actinobacteria*), *Bradyrhizobium*, *Burkholderia*

(*Proteobacteria*), known to include plant-beneficial species and strains (Kyselková *et al.,* 2012; Brader *et al.*, 2014), and *Nitrospira* (*Nitrospirae*), known for participation in nitrite oxidation (Uroz *et al.*, 2016).

In conducive soil, higher signals were detected for *Acidobacteria*, *Pseudomonas*, *Agrobacterium* and *Janithobacterium* (*Proteobacteria*), some of them also known for plant protection and antibiotic activities against fungi (Ditt *et al.,* 2002; Loudon *et al.,* 2014).

To some extent, Illumina sequencing discriminated between the two soils similarly to what microarray did, with a prevalence of *Bradyrhizobiaceae* (and other *Proteobacteria*), *Bacteroidetes* and *Firmicutes* in suppressive soil, and lower levels for different families of *Proteobacteria*, *Actinobacteria* and *Firmicutes* in conducive soil. However, Illumina sequencing showed that major effects were due to resistant vs susceptible cultivars. *Chloroflexi* and *Gaiellales* (*Actinobacteria*) were enriched in resistant cultivar Kariera, and *Burkholderia*, *Sphinomonas* (*Proteobacteria*) and *Actinobacteria* in susceptible cultivar Agria. Highest bacterial diversity was primarily associated with the resistant cultivar Kariera, which is reminiscent of the general importance of diversity in disease control (Shi *et al.,* 2019).

Overall, (Various taxa were associated with low CS (i.e., suppressive soil or resistant cultivar), which is consistent with previous studies which, however, provided results only for suppressive soils or only for resistant cultivars (Shi *et al.,* 2019; Tomihama *et al.*, 2016; Cha *et al.,* 2016).

Here, differences were obtained by the two methodologies, as microarray pointed to general differences in soil community profiles, whereas Illumina sequencing enabled more detailed identification of bacterial OTUs, which highlighted lower taxonomic level differences between cultivars (Edgar, 2013; Kyselkova *et al.,* 2014; Sanguin *et al.,* 2006; Donn *et al.,* 2014).

Mainly soil effects were evidenced with the archaeal community. *Methanosarcinales* (*Euryarchaeota*), implicated in methylotrophic methanogenesis, were prevalent in both cultivars in suppressive soil and resistant cultivar Kariera in conducive soil, while *Nitrososphaerales,* (*Thaumarchaeota*), implicated in ammonia oxidation, were prevalent in both bulk soils and susceptible cultivar Agria in conducive soil. This might indicate changes in oxygen availability, which have been associated with *Nitrososphaerales* to *Methanosarcinales* ratio in situations of water level manipulation. Due to specific functions of the two archaeal groups, this may

have further consequences for C and N cycling (Breidenbach *et al.*, 2016).

The Micro-eukaryotic community (especially fungi, parasitic Apicomplexa, Cercozoa, with weaker contributions from various bacterivores and autotrophs) differed between soils, as found with archaea and bacteria (especially with the microarray approach). Chlorophyta together with Myxogastria, Apicomplexa and Ciliophora were enriched in conducive soil, which consequently displayed increased micro-eukaryotic diversity. Enrichment of Chlorophyta suggests higher water content of that soil, which is compatible with a lower slope position (Seppey *et al.*, 2017).

Cercozoa and *Acanthamoeba* graze on bacteria[36]. Also, most Ciliophora are bacterivorous, but some species consume the content of fungal hyphae (Jousset, 2017). Certain Myxogastria species are fungivores, so they probably feed of the relatively abundant fungi in conducive soil and they might also affect bacterial-fungal dynamics (Fiore-Donno *et al*., 2016).

Since feeding preferences may be reflected in both diversity and quantity of prey, (Gao *et al.*, 2019; Mendes *et al*., 2013) and in our study, differences were found in both, this raises the possibility of complex food-web interactions, potentially specific to soil and cultivar conditions, and suggests that top-down control of rhizosphere microbiome might be important to consider (Jousset, 2017; Bonanomi *et al*., 2016). Trophic interactions between the domains can modify nutrient cycling and plant nutrition (Gao *et al.*, 2019; Abdallah *et al*., 2019; Jousset, 2017) which can be relevant for soil suppressiveness (Sarikhani *et al.,* 2017; Kristufek *et al.*, 2000) with potential feedback effects of microbial communities themselves. In our study, content of P and Fe increased particularly by resistant cultivar accumulation so possibly, distinct microbial interactions occur also in various cultivars. As suppressive soil had lower N contents, this agrees also with the dominance of ammonia oxidizing archaea and suggests that N was recycled more intensively in suppressive than conducive soil (Gao *et al.*, 2019) Contents in Mg, S and P may influence the composition and functioning of microbial communities in potato rhizosphere (Barnett *et al*., 2015; Inceoglu *et al*., 2012) and here we found differences in those nutrients between suppressive and conducive soils. A negative relationship between Mg periderm concentration and disease severity was found previously by Lazarovits et al. (2007) and similarly Lacey and Wilson (2001) found that CS disease severity was related to contents in exchangeable Ca, Mg, and K cations. Mg may by associated to phosphorus, which also agrees with increased P periderm concentration in healthy

potatoes (Kristufek *et al.*, 2015).

In conclusion, microbiome features differed when comparing suppressive vs conducive soil as well as resistant vs susceptible cultivar (Sagova-Mareckova, *et al.,* 2015; Shi *et al.,* 2019); but the relative importance of soil suppressiveness and cultivar resistance depended on the microbial community considered. Results suggest that the possible role of archaea and protists in suppressivity mechanisms deserves further attention. They also suggest that potential interactions between the three microbial domains would need to be considered for a comprehensive understanding of tuberosphere functioning and microbial CS control taking place in suppressive soils.

4.4. Strains antagonistic to the CS pathogen

Using Gauze medium helped to isolate the members of phylum Actinobacteria as source of bioactive compounds, notably antibiotics (Rangseekaew *et al*., 2019; Nimaichand *et al*., 2015). In antimicrobial screening test, several culture media were evaluated, and GAUZE's medium was the best one for the majority of actinobacteria isolates. The extracts from GAUZE's medium showed no obvious antimicrobial difference from other media, but all the tested strains could be cultured on the GAUZE's medium (Wei *et al*., 2018).The actinomycete isolate showed moderate growth on nutrient agar, relatively good growth on modified Gauze's (Ma *et al*., 2017)

In a previous study, International *Streptomyces* project media (ISP-1, Tryptone glucose yeast extract) and ISP-2 (Yeast extract-malt extract-dextrose) supported high antimicrobial potential after 5–6 days of growth (Kavitha and Savithri, 2017). A medium is constructed of yeast extract and pepton, The A medium can be used selectively for evaluation of production of secondary metabolites. The impact of yeast extract and pepton on induction of secondary metabolite production has been demonstrated before. (Sorenson, 2013; Filterborg, 1990; Naik *et al*., 2015 and Oskay *et al*., 2004).

The growth rate based on dry weight in our study was comparable with growth curve of bacteria based on colony forming units (typically for G medium, Fig. 40). The stationary culture conditions provide five phases of bacterial growth: (1) lag phase – bacteria adapt themselves metabolically to the new conditions of growth (before time pint 24 h), (2) log or exponential phase – bacteria undergo rapid reproduction (time

pint 24-72 h), (3) declining phase because of depletion of nutrients and accumulation of waste products, the bacterial reproduction slows down (time point 72 h), (4) stationary phase – the number of alive bacteria is constant and death rate equals the growth rate (time pint 72-120 h), (5) death phase – when the death rate is greater than the growth rate and all nutrients are exhausted (time pint 120-240 h) (Paulton, 1991). For A medium, the stationary phase seems to be later, in 120-240 time point. Totally the dry weight and growth rate of actinobacteria in G medium was larger and faster (Fig. 40, 41).

Measurements of actinobacteria growth in stationary liquid cultures and observation of colony growth of actinobacteria were done in previous studies. Strains of *Streptomyces lividans* were grown on MS agar medium at 30°C to prepare spore solutions, seed cultures were inoculated with 10^6 spores ml^{-1} in TSBS liquid media in a rotating incubator. 10 ml of culture broth was used for dry weight measurements which were performed with the freeze-dried mycelium. Seed cultures were grown for 24, 48 and 72 h, and filtered through 100, 40, 5 and 0.22 micrometer filters. The cultures were then grown for another 8 h, after which they were again filtered through a 100 micrometer filter. 1 ml of the diluted filtrate of liquid-grown cultures was evenly distributed on the surface of MS agar plates by gently swirling the plates, after which these were allowed to dry in the fume hood for 30 min. Colony forming units were counted after 48 h of growth at 30°C (Zacchetti *et al*., 2018). Growth rates of streptomycetes were also measured by comparison of turbidimetric and gravimetric techniques. A linear relationship between turbidity and dry weight was obtained for all of the strains (Flowers and Williams, 1976).

Previous studies also evaluated the growth rate of *S. scabiei*. The *S. scabiei* RB2 was grown for 72 h in oatmeal broth. Growth was measured as OD_{650}. No yellow-pigmented thaxtomin compounds were detected in culture filtrates of exponentially growing cells. However, when the culture reached late exponential to early stationary phases of growth (24 to 33 h), five yellow compounds were visible on the TLC plate, this results are comparable with our results in submerged culture (Fig. 44) and maximum production at day 3 of harvest in vermiculite experiment (Fig. 45) (Babcock *et al*., 1993).

Previous studies showed that thaxthomin production does not require induction by living host tissue or enzymatic modification by the host and it can happen *in vitro* (Loria, 1995). The study was performed with the expectation that the maximum

expression of *txtB* gene and consequently the thaxtomine molecule will coincide with the production of other secondary metabolites in the co-cultivation of *S. scabiei* and antagonistic actinobacteria strains. It was suggested to culture *Actinobacteria in vitro* at least for 3 days to observe any secondary metabolite production (Brook, 2012). Other studies suggested that actinobacteria enter the stationary phase after 14 days, and that the microbes produce the antibacterial compounds (secondary metabolite) in that stage to prevent the competition of nutrition and space (Rante *et al*, 2017). Consequently, we carried the experiment at both days, which may be most important for the interaction between the pathogens and antagonists.

Since it was observed that secondary metabolites are synthesized only in 'stationary phase' or in cultures that have low growth rates (Demain, 1982; Demain *et al.,* 1979), we may assume that different strains reached that growth phase at different time points because the maximum antibacterial activity determined as the size of inhibition zone against *Kocuria* was seen on day 3 and 14 depending on the strain. However, different situation may occur in vermiculite because neither day 3 nor day 14 can be distinguished as stationary phase because the conformity of antibiotic activity with growth in vermiculite cannot be clearly determined due to its very complex structure; nevertheless between isolates with higher antagonistic activity (09ZI22, 09VK11, 09VK26, 09VK44, 09VK70, 09VK62, 09VU19, 10ZC7), 75 % had more activity in day 14 of individual culture than day 3 and 63 % had more antagonistic activity in day 14 of combined cultures than day 3, it can be understood that stationary phase of vermiculite may be in 14 days of culture. The antagonistic activity of actinobacteria was seen even in absence of pathogens but was increased in the presence of pathogen. Above that, the size of inhibition zones in cultures grown in vermiculite were larger than those in submerged cultivation. That demonstrated that vermiculite supplemented with a liquid medium can play a beneficial role in induction of secondary metabolite production.

The cultivations of antagonistic strains on vermiculite were done earlier. It was demonstrated that vermiculite enriched with medium can support growth of both the *S. scabiei* pathogen and antagonistic actinobacteria strains; however the disease suppression occur more than when the pathogen strain is alone (Neeno-Eckwall *et al.*, 2001). In our study, the ratio of actinobacterial 16S to *txtB* gene was higher in day14 than day 3 of harvest in 62% of total samples and 88% of isolates with higher

antagonistic activity (09ZI22, 09VK11, 09VK26, 09VK44, 09VK70, 09VK62, 09VU19, 10ZC7) (Fig. 50, 51).

The competition between coexisting streptomycetes is strongly dependent on neighboring bacteria. *Streptomyces* strongly regulate their secretions based on the presence of other strains, in two pathways: one way is to sense specific compounds released by others; in this way, a bacterium can sense a variety of competitors and respond deferentially to them (Abrudan *et al*., 2015; Jauri *et al*., 2014). Another strategy is for a bacterium to use its own physiological state (such harm caused by other cells or stress condition) to sense competition directly and thereby respond physiologically to other strains. *Streptomyces* species have several established examples, with antibiotic production, up-regulated by starvation, envelope damage and, potentially, DNA damage (Hesketh *et al*., 2007; Cornforth, 2013). In this study, generally, the co-culture of actinobacteria with pathogen increased the ratio of copy numbers of 16S rRNA from actinobacteria to *txtB* gene, and antagonistic activity after 14 days. It showed that the pathogen which possess the *txtB* gene, was suppressed in combined cultures.

The 80.95 % of disk inhibition zones in vermiculite and submerged were determined with an enhanced inhibition zone immediately behind the sensitivity inhibition zone. It demonstrated that actinobacteria species can produce two groups of secondary metabolites: the first ones were antibiotics against *Kocuria* that can make an inhibition zone around the disk, and the second ones were the favorable secondary metabolites that can enhance and induce *Kocuria* to accumulate immediately after inhibition zone. It may compare with flattening of zone of inhibition which is caused by antagonism between two antibiotics (Laishram et al., 2017) or may be similar to D-effect, can be seen in disk diffusion method between different antibiotics placed in close proximity which inhibits the sensitivity zone of another antibiotic in its proximity (Bhardwaj *et al.* 2016), however the disks in our study did not cover a unique compound and may had several antagonistic or synergistic metabolites. The most numbers of enhanced inhibition zone around the disks were seen in vermiculite combine culture showed the induction of secondary metabolites by pathogen and the effectiveness of vermiculite medium.

Finally, it must be considered that in the soil conditions the antagonistic activities against *S. scabiei* will be further modified. The most important factor seems

to be the local microbial community, which will further modify both the antagonistic functioning and growth rate of the supplemented strain (Garbeva *et al*., 2004).

In conclusion, the actinobacterial strains isolated from suppressive soils showed different growth and antagonistic activity in the experiments. The 71.4% of actinobacteria strains suppressed the growth of *S. scabiei* (decreased copy numbers of *txtB* gene). The copy numbers of *txtB*, decreased most in 09ZI22, 09ZI7, 09VK26, 09VK44 and 09VK70. The largest inhibition zone was seen after 14 days of cultivation in 09ZI22, 09ZI7, 09VK11, 09VK26, 09VK44, 09VK70 and 09VU19. The growth rate (based on dry weight of culture) and antibiotic production (based on inhibition zone) of actinobacterial isolates, in G media were higher than A media. The growth curve of actinobacteria in several time points, based on dry weight was matched to the growth curve based on plating and counting of colony forming units. The vermiculite medium is suitable to evaluate the interaction and competition between antagonistic actinobacterial strains and *S. scabiei* as pathogen.

4.5. Conclusions

The control of common scab (CS) of potatoes includes resistant cultivars, specific fertilization, increase of soil moisture, and chemical treatments. Yet, these management practices do not have common or reproducible results at differing sites (Potatoes production guideline, 2013; Dees and Wanner, 2012; Kirkwyland *et al.,* 2013; Dees *et al.,* 2012). The relation between biological parameters such presence of antagonistic bacteria, total bacterial community, copy numbers of *txtB* gene (Larkin, 2008; Meng *et al.,* 2012), and chemical parameters such various micronutrients in soil and periderm, evaluated in the previous studies (Lazarovits *et al*., 2001; 2003; 2007; Stead and Wale, 2004; Bailey *et al.,* 2003; Kristofek *et al*., 2000). The results showed that the parameters may affect scab severity but it also showed that the results may change by locations and years (Rosenzweig *et al*., 2012; Sagova-Mareckova *et al*., 2015; Lazarovits *et al*., 2007). An extensive monitoring study was done at 32 field sites to evaluate the relation between potato common scab and biological/chemical parameters. The content of nutrients such as Fe, Mg, N, C, Ca and P in soil and periderm had a predictive value for potato scab disease. In this study disease severity had a positive correlation with the copy numbers of 16S rRNA gene from

actinobacteria and total bacteria in soil and 16S rRNA gene from actinobacteria and *txtB* gene copy numbers in potato skin. The population of several groups of bacteria was showed to be important to scab suppression (*Acidobacteria, Verrucomicrobia, Bacteroidetes* and *Gemmatimonadetes*). Modifying pH and using Iron and natural amendment separately showed to be effective to scab control (Kyselkova and Moenne-Loccoz, 2012; Glick, 2012; Expert *et al*., 2012; Carlgren and Mattsson, 2001). Consequently, in the second study we aimed to determine the effects of soil organic matter, available iron and pH on CS development. Peat and DTPA chelated iron were supplemented to pots filled with soil conducive for CS. All results were compared with the same data obtained for a suppressive soil, which has naturally low severity of CS and occurs nearby. Both peat and iron supplements controlled potato scab and the combination of the two supplements reduced CS most effectively. The bacterial community composition was modified by all treatments but the change was most profound after the combined peat and iron treatment, when the community changed towards the composition in the suppressive soil. It seemed that iron supplement supported plant defense while both iron and peat additions changed the bacterial community in favor of CS suppression. In this study, the effect of peat addition was more important than that of iron for suppression of common scab but the most effective was the combination of both treatments. Although some abiotic factors i.e. soil pH were changed by the treatments it seemed that the most important effect of the manipulation was in stimulating the beneficial bacterial community. Possibly some of the OTUs (*Proteobacteria*, *Bacteroidetes and Gemmatimonadetes*) enriched in suppressive soil as well as in both the peat and iron and peat treatments might produce antibiotic compounds or otherwise successfully compete with the pathogen. Alternatively, the bacterial community might promote plant defense, which seemed to be the case with supplementation of iron. It seemed that, although the relationships are very complex, members of some taxa, were associated with disease suppression not only in our work but also in other studies (Sessitsch *et al*., 2004; Mendes *et al*., 2011; Larkin and Stellos, 2013; Marschner, 1995; Kobayashi *et al*., 2015). Connections between the structure of bacterial communities in suppressive soils and potato resistance to common scab (CS) are not yet well understood (Merete *et al.*, 2012; Agrios, 2005; Hosaka *et al.,* 2000; Powelson *et al.,* 1993); In the third study, one resistant and one susceptible cultivar were grown in a conducive and suppressive field to assess cultivar resistance × soil suppressiveness interactions. The resistant cultivar

had a higher Mg content in periderm than the susceptible cultivar, while suppressive soil had lower pH (5.3 vs 5.9), N, C, P, and Ca contents but higher Fe and S contents compared with the conducive soil. Bacteria and actinobacteria 16S rRNA gene copy numbers were higher in the conducive soil. Copy numbers of *txtB* gene were similar in both soils but were higher in periderm samples of the susceptible cultivar from the conducive soil. Taxonomic microarray analysis and Illumina sequencing of 16S rRNA genes amplicon showed that bacterial community differed between resistant and susceptible cultivar and between suppressive and conducive soil. We demonstrated the cultivar-specific community selection related to the susceptibility or resistance to CS and above that we compared this trait in suppressive and conducive soils. We showed that CS can be controlled either with resistant cultivar or with suppressive soil, with no additive effect between them. Out of the two factors, potato cultivar had a higher effect on tuberosphere bacterial community composition than soil in our experiment. The results highlighted the usefulness of both cultivar resistance and soil suppressiveness traits in understanding and managing crop disease control. The previous studies showed that members of some species of *Actinobacteria* were associated with disease suppression by production of secondary metabolites (Kyselkova and Moenne-Loccoz, 2012). In the final experiment the growth rate and antibiotic production of actinobacteria isolated from CS suppressive soils was studied. The actinobacterial strains were cultured in liquid media to evaluate the growth rate and to extract the secondary metabolites. The isolates also were cultivated in a soil free media (vermiculite), individually and combined with the pathogen (*S. scabiei*) to study the interaction between them. The results showed different growth and antagonistic activity against *Kocuria* (a control as a gram positive and sensitive bacteria) in the experiment, however several species that produced antagonistic metabolites in vermiculite and submerged cultures and had also higher antibacterial activity, could suppress the *S. scabiei* and causal pathogenic gene (*txtB*) in vermiculite media, more effectively. The 71.4% of actinobacteria isolates with higher antagonistic activity suppressed the *S. scabiei, i.e.* decreased copy numbers of *txtB* gene, *in vitro*.

4.6. The main outcomes

- The content of nutrients such Fe, Mg, N, C, Ca, P, in soil and/or periderm, and the copy numbers of actionobacteria, total bacteria in soil and the *txtB* copy numbers in periderm were in correlation with potato common scab and determinative to severity of the disease.
- Although some abiotic factors i.e. soil pH were changed by the treatments, the manipulation was most important in stimulating the beneficial bacterial community. Iron supplement supported plant defense while both iron and peat additions changed the bacterial community in favor of CS suppression. The proportions of several groups of bacteria (such *Proteobacteria*, *Bacteroidetes, Acidobacteria, Verrucomicrobia, Firmicues* and *Gemmatimonadetes*) are important to scab suppression and had more presence in enriched fields (traditionally enriched by micronutrients) and treated fields (manually treated by peat and iron).
- The CS can be controlled either with resistant cultivar or with suppressive soil, with no additive effect between them. Out of the two factors, potato cultivar had a higher effect on tuberosphere bacterial community composition. Some differences seen between susceptible and resistant cultivars: The resistant cultivar had a higher Mg content in periderm compare to susceptible cultivar. Moreover bacterial community differed between resistant vs susceptible cultivar and between suppressive vs conducive soil. Results highlighted the usefulness of both cultivar resistance and soil suppressiveness traits in understanding and managing disease control of crops. The results showed that archaea and micro-eukaryotes differed between resistant and susceptible cultivar and between suppressive and conducive soil.
- The vermiculite medium is an enriched and soil free culture can be selected to evaluate the interaction and competition between suppressive actinobacterial isolates and *S. scabiei* as pathogen, without impacts of soil factors. Combination of actinobacteria isolated from suppressive soil and *S. scabiei* in vermiculite showed a good antagonistic activity between them. Several species of actinobacteria isolated from suppressive soils, with higher antibacterial activity against *Kocuria* could suppress the *S. scabiei* in combined culture effectively.

5. References

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Appendix

The author declares that all work on the thesis was done by herself except the following: Ing. Jan Kopecký, Ph.D. – bioinformatics (alignments, phylogenetic tree constructions, indices computation), RNDr. Markéta Marečková, Ph.D. – soil properties measurements, help to writing the paper and thesis, Assoc. Prof. Ing. Marek Omelka, Ph.D. – all of the statistical computations. The author is grateful for their contribution and help.

Author's publication activity

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Supplementary material

Supplementary material to the chapter

3.2. The effect of peat and iron supplements on the severity of potato common scab and bacterial community in tuberosphere soil

Supplementary Table S1. Quantities of total *Bacteria*, *Actinobacteria* and *txtB* gene [copies g^{-1}] in tuberosphere soil and potato periderm. Average of five replicates with standard deviations.

Treatment	Soil						Periderm			
	Bacteria		Actinobacteria		*txtB*		Actinobacteria		*txtB*	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
V_H	4.66E+09	4.02E+09	6.41E+08	4.64E+08	5.53E+05	2.00E+04	5.26E+08	2.97E+08	7.31E+06	6.02E+06
V_HP	4.77E+09	1.68E+09	8.45E+08	3.65E+08	5.75E+05	9.88E+04	*1.05E+08**	*8.16E+07*	*1.02E+06**	*8.76E+05*
V_HFe	2.11E+09	9.00E+08	4.95E+08	2.51E+08	7.64E+05	1.94E+05	3.25E+08	2.05E+08	1.09E+06	5.72E+05
V_HPFe	2.25E+09	2.29E+08	4.55E+08	2.37E+08	6.43E+05	6.82E+04	7.32E+08	4.15E+08	1.28E+07	7.67E+06
V_HFe5	2.02E+09	4.95E+08	3.26E+08	1.14E+08	7.35E+05	1.20E+05	8.94E+08	5.74E+08	2.70E+07	2.91E+07
V_HPFe5	2.61E+09	1.15E+09	3.88E+08	2.48E+08	6.04E+05	1.25E+05	1.05E+09	6.99E+08	4.05E+07	3.73E+07
V_L	2.37E+09	7.75E+08	4.89E+08	3.11E+08	7.09E+05	1.09E+05	2.95E+08	2.07E+08	*8.97E+05**	*4.51E+05*

Supplementary Table S2. Comparison of variables between the treatments

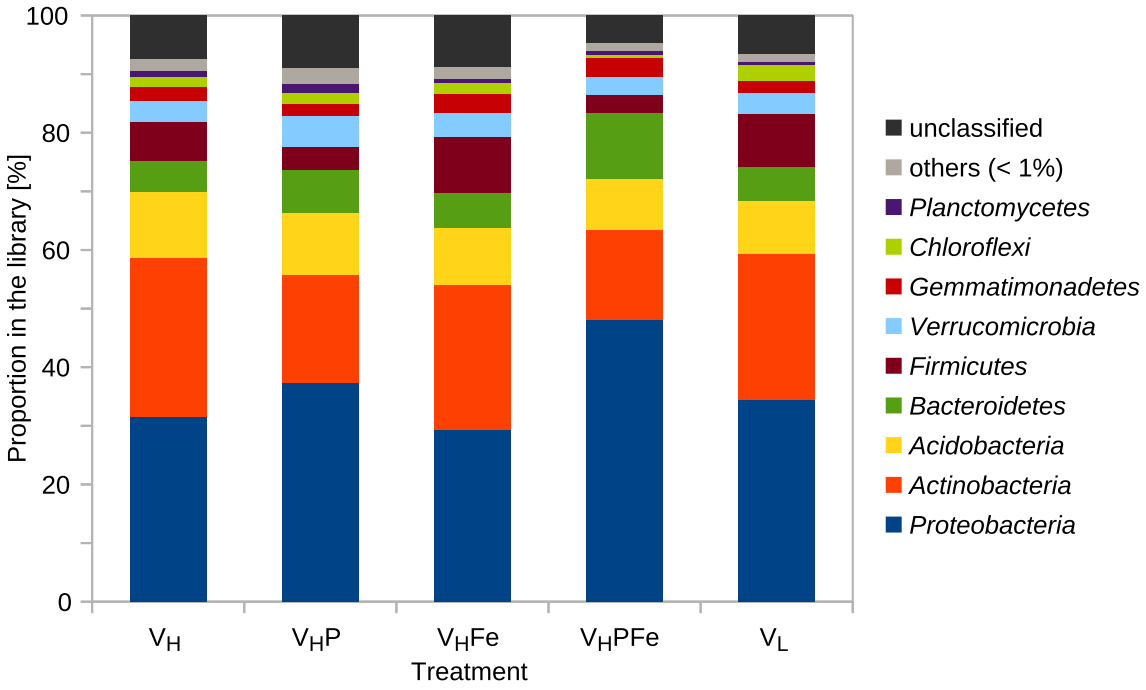
Actinobacteria 16S rRNA gene copies in potato periderm			
	Df	F	P
treatment	6	3.975	0.006

txtB gene copies in potato periderm			
	Df	F	P
treatment	6	7.302	<0.001

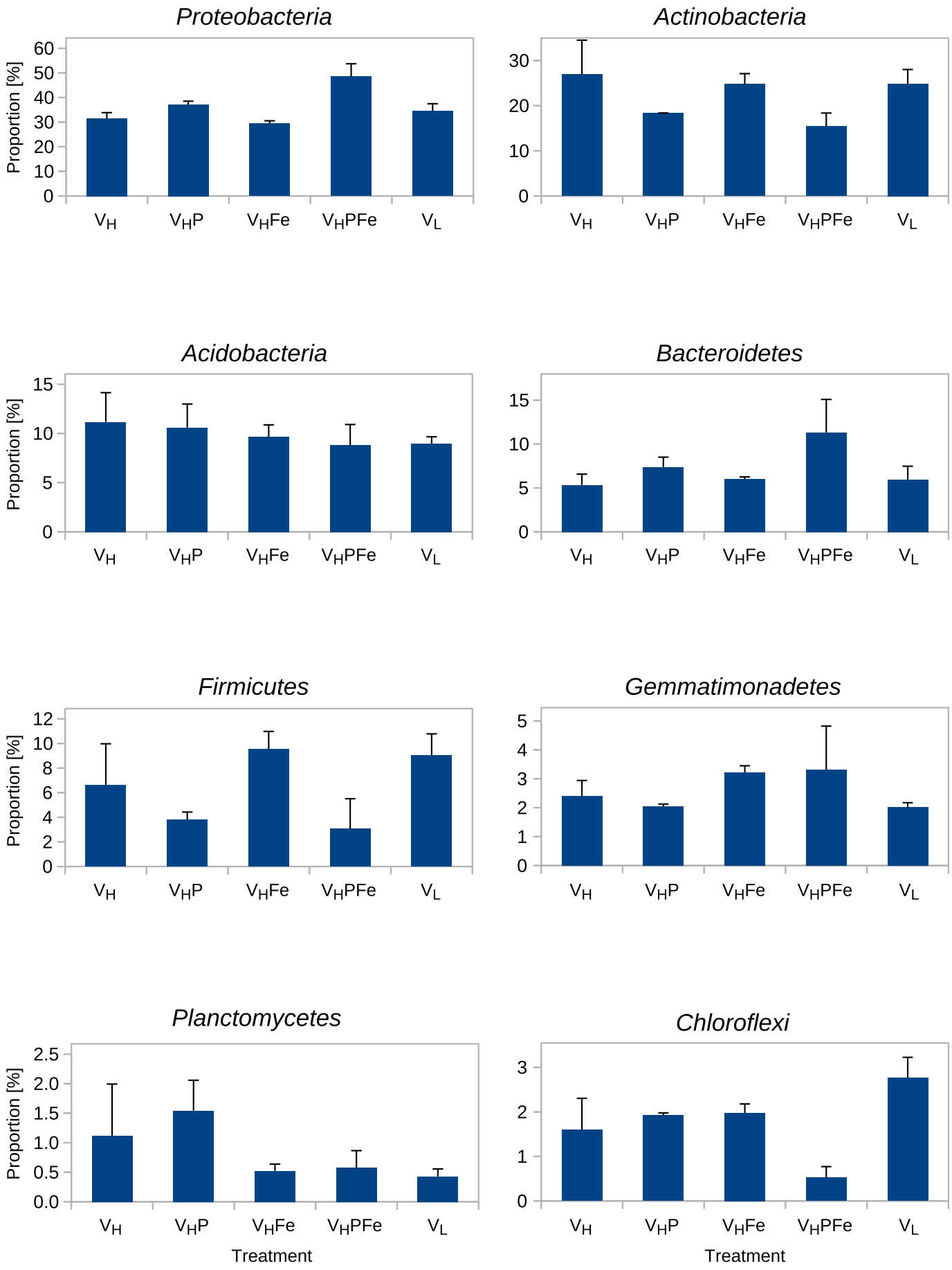
soil pH			
	Df	F	P
treatment	6	8.669	<0.001

soil Fe at the end of the growth period			
	Df	F	P
treatment	6	12.89	<0.001

Supplementary Figure S1. Relative proportions of bacterial phyla in Illumina MiSeq 16S rRNA gene amplicon sequence libraries. Samples representing the bacterial communities in scab conducive soil - V_H , the same soil amended with peat - V_HP, DTPA-chelated iron - V_HFe, or both iron and peat - V_HPFe, and common-scab suppressive soil - V_L.



Supplementary Figure S2. Relative proportions of bacterial phyla in the Illumina MiSeq sequence libraries from the individual treatments. The averages of three replicates with standard deviations.



Supplementary Table S3. Clustering the sequence libraries of the treatments to metacommunities based on Dirichlet multinomial mixtures

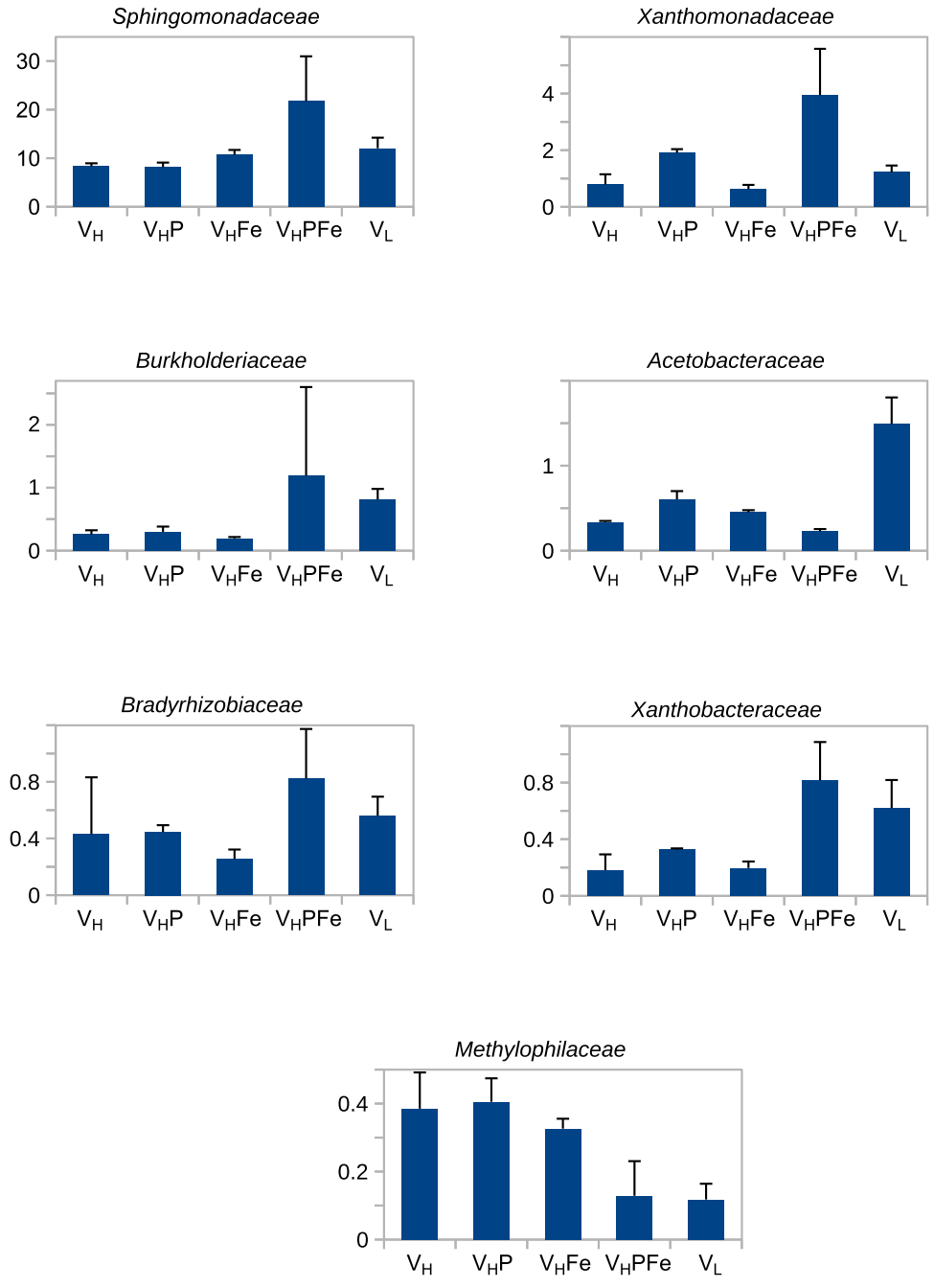
A. Laplace approximation for increasing number of Dirichlet mixture components (K)

K	Laplace
1	187697.3
2	180922.93
3	188048.92
4	193340.4
5	201021.21

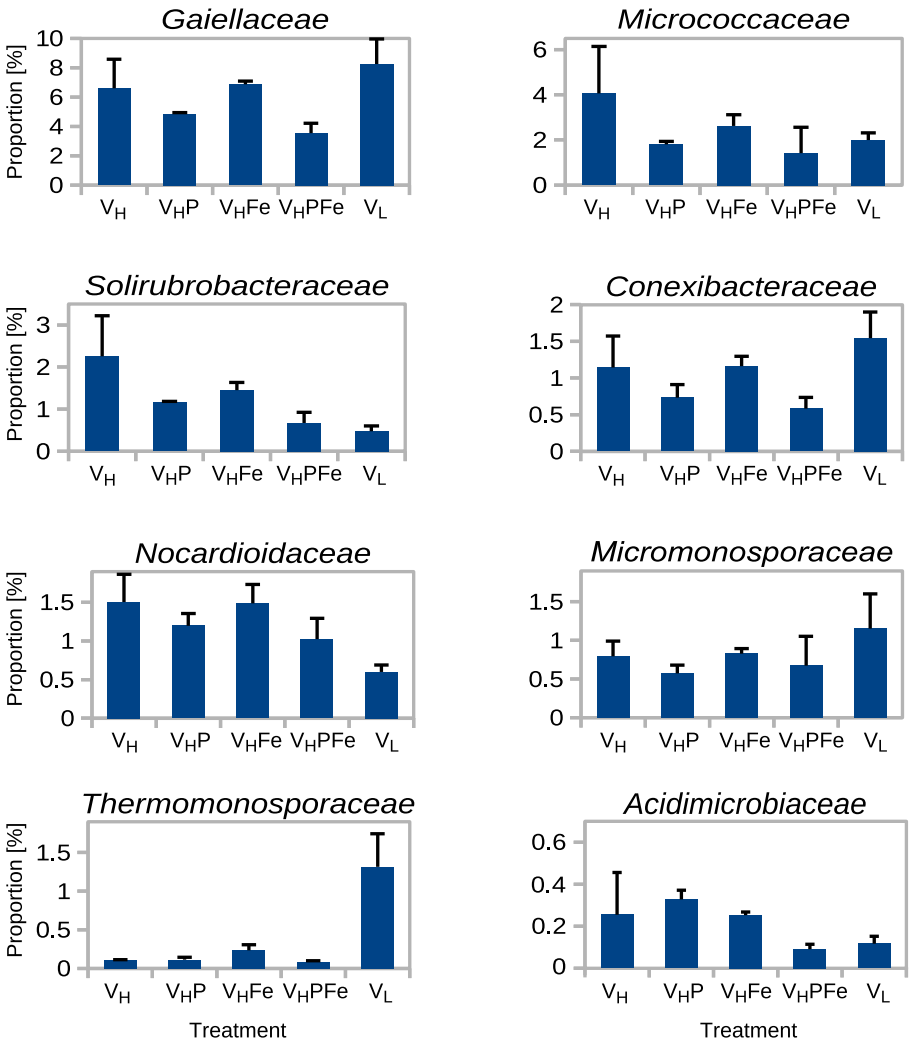
B. Assignment of the treatments to the two determined community types

Treatment	Community type
V_H1	Partition_1
V_H2	Partition_1
V_H3	Partition_1
V_HP1	Partition_1
V_HP2	Partition_1
V_HFe1	Partition_1
V_HFe2	Partition_1
V_HFe3	Partition_1
V_HPFe1	Partition_2
V_HPFe2	Partition_2
V_HPFe3	Partition_2
V_L1	Partition_2
V_L2	Partition_2
V_L3	Partition_2

Supplementary Figure S3. Relative proportions of selected families within the phylum *Proteobacteria* in the Illumina MiSeq sequence libraries from the individual treatments. The averages of three replicates with standard deviations.



Supplementary Figure S4. Relative proportions of selected families within the phylum *Actinobacteria* differentially represented in the Illumina MiSeq sequence libraries from the individual treatments. The averages of three replicates with standard deviations.



Supplementary Table S4. List of the most abundant OTUs constituting 50% of the total number of sequences in the library. The OTUs are ordered by the difference between their proportions in the suppressive soil V_L and the conducive soil V_H.

OTU	V_H	V_HP	V_HFe	V_HPFe	V_L	Class	Order	Family
OTU1	465	753	1286	3992	2696	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU292	12	3	15	3	593	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
OTU230	13	24	36	21	412	Rubrobacteria	Gaiellales	Gaiellaceae
OTU50	2	4	2	3	332	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae
OTU38	94	93	120	92	403	Rubrobacteria	Gaiellales	Gaiellaceae
OTU45	14	15	18	10	281	Actinobacteria	Streptosporangiales	Thermomonosporaceae
OTU153	6	9	11	9	262	Rubrobacteria	Gaiellales	Gaiellaceae
OTU16	83	170	79	248	322	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
OTU117	2	3	9	1	227	Bacilli	Bacillales	Paenibacillaceae 1
OTU355	85	114	231	533	302	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU22	28	34	48	73	234	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae
OTU132	7	5	6	5	202	Thermoleophilia	Solirubrobacterales	Conexibacteraceae
OTU27	18	43	12	294	197	Betaproteobacteria	Burkholderiales	Burkholderiaceae
OTU126	26	31	39	101	198	Actinobacteria	Micromonosporales	Micromonosporaceae
OTU4	6	178	25	708	140	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU88	8	29	42	99	132	Verrucomicrobia Subdivision3	Subdivision3 gen. inc. sedis	
OTU14	89	74	160	89	205	Rubrobacteria	Gaiellales	Gaiellaceae
OTU813	7	16	28	56	110	Rubrobacteria	Gaiellales	Gaiellaceae
OTU43	0	12	29	110	102	Holophagae	Holophagales	Holophagaceae
OTU71	15	22	29	33	113	Actinobacteria	Propionibacteriales	Nocardioidaceae
OTU29	4	56	24	398	96	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU102	15	71	20	114	106	Betaproteobacteria	Burkholderiales	Comamonadaceae
OTU17	13	38	100	210	103	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae
OTU289	35	50	36	20	125	Verrucomicrobia Subdivision3	Subdivision3 gen. inc. sedis	
OTU30	2	57	11	518	85	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
OTU158	33	51	44	26	113	Verrucomicrobia Subdivision3	Subdivision3 gen. inc. sedis	
OTU70	1	26	12	96	76	Verrucomicrobia Subdivision3	Subdivision3 gen. inc. sedis	
OTU34	48	48	115	24	120	Bacilli	Bacillales	Paenibacillaceae 1
OTU67	15	36	25	52	85	Acidobacteria Gp1	Gp1	
OTU23	76	58	77	57	145	Actinobacteria	Micrococcales	Intrasporangiaceae
OTU47	0	36	9	149	64	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
OTU11	136	139	154	449	195	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU28	104	105	57	140	160	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae
OTU53	43	36	88	46	96	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae
OTU99	43	43	45	60	94	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae
OTU49	73	73	69	31	123	Actinobacteria	Nakamurellales	Nakamurellaceae
OTU52	86	36	59	23	136	Actinobacteria	Micrococcales	Micrococcaceae
OTU25	2	6	3	227	49	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU2597	5	49	20	138	44	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU107	0	7	5	119	38	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
OTU171	13	38	15	79	51	Alphaproteobacteria	Alphaproteobacteria inc. sedi	Rhizomicrobium
OTU15	67	112	46	153	104	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae
OTU66	5	68	5	126	41	Alphaproteobacteria	Alphaproteobacteria inc. sedi	Rhizomicrobium
OTU101	105	54	84	102	138	Actinobacteria	Micrococcales	Microbacteriaceae
OTU74	0	5	1	233	31	Acidobacteria Gp1	Terriglobus	
OTU26	138	74	63	45	167	Actinobacteria	Micrococcales	Intrasporangiaceae
OTU18	86	56	105	34	109	Bacilli	Bacillales	Bacillaceae 1
OTU79	23	13	47	67	44	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU100	56	66	71	46	71	Rubrobacteria	Gaiellales	Gaiellaceae
OTU42	0	253	4	165	14	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
OTU36	51	85	54	156	64	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
OTU86	59	83	40	68	72	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
OTU179	44	69	49	100	56	Actinobacteria	Geodermatophilales	Geodermatophilaceae
OTU223	48	52	23	30	60	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
OTU2165	28	39	55	38	41	Actinobacteria	Streptomycetales	Streptomycetaceae
OTU130	0	55	1	194	12	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae
OTU46	83	68	39	80	93	Spartobacteria	Spartobacteria gen. inc. sedis	
OTU9	0	14	1	355	8	Alphaproteobacteria	Alphaproteobacteria inc. sedi	Rhizomicrobium
OTU3670	52	5	8	146	57	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU94	22	22	38	73	26	Thermoleophilia	Solirubrobacterales	Conexibacteraceae
OTU1131	45	35	123	58	46	Bacilli	Bacillales	Bacillaceae 1
OTU24	0	15	0	322	0	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
OTU59	35	74	32	25	33	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae
OTU146	70	41	72	50	69	Actinobacteria	Micrococcales	Intrasporangiaceae
OTU180	47	63	39	40	43	Acidobacteria Gp6	Gp6	
OTU104	64	33	85	33	59	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae
OTU257	44	39	47	48	39	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
OTU7667	40	49	28	38	35	Betaproteobacteria	Burkholderiales	Burkholderiales inc. sedis
OTU7133	56	54	122	58	51	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU105	53	72	46	65	47	Alphaproteobacteria	Rhizobiales	Rhizobiaceae

OTU13	99	94	194	282	90	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU103	44	45	40	43	31	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae
OTU7654	66	61	76	67	54	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU2640	48	55	31	28	34	Acidobacteria Gp6	Gp6	
OTU41	30	110	48	31	13	Bacteroidetes inc. sedis	Ohtaekwangia	
OTU12	229	97	249	80	211	Bacilli	Bacillales	Bacillaceae 1
OTU54	38	53	65	72	14	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
OTU85	53	56	27	26	25	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae
OTU154	109	83	103	79	80	Actinobacteria	Corynebacteriales	Mycobacteriaceae
OTU400	56	36	58	23	27	Thermoleophilia	Solirubrobacterales	Conexibacteraceae
OTU3166	50	66	29	41	20	Acidobacteria Gp6	Gp6	
OTU84	49	90	47	16	19	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae
OTU115	34	58	63	50	4	Acidobacteria Gp3	Gp3	
OTU87	34	62	144	58	3	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU80	41	126	35	15	9	Opitutae	Opitutales	Opitutaceae
OTU186	47	62	54	25	14	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae
OTU40	43	69	52	45	9	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae
OTU197	77	57	55	67	43	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
OTU62	46	82	20	41	12	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae
OTU4563	77	46	71	36	42	Acidobacteria Gp16	Gp16	
OTU68	62	28	66	41	24	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU162	43	42	68	46	5	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU1848	164	65	179	56	124	Bacilli	Bacillales	Planococcaceae
OTU213	77	60	45	50	35	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae
OTU121	52	62	78	53	7	Acidobacteria Gp6	Gp6	
OTU8040	100	96	74	100	54	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
OTU288	64	47	66	35	18	Actinobacteria	Streptomycetales	Streptomycetaceae
OTU401	51	52	50	47	4	Acidobacteria_Gp4	Gp4	
OTU291	99	35	32	37	51	Actinobacteria	Micrococcales	Microbacteriaceae
OTU173	50	54	71	15	2	Chloroflexia	Chloroflexales	Oscillochloridaceae
OTU183	80	117	40	19	32	Nitrospira	Nitrospirales	Nitrospiraceae
OTU140	75	54	39	41	26	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU60	51	70	75	90	2	Acidobacteria Gp4	Gp4	
OTU138	54	30	75	23	4	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU33	56	60	71	29	5	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae
OTU1048	98	33	74	30	46	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae
OTU76	60	75	65	100	7	Actinobacteria	Propionibacteriales	Nocardioidaceae
OTU89	68	62	65	44	14	Spartobacteria	Spartobacteria gen. inc. sedis	
OTU203	78	32	45	15	24	Deltaproteobacteria	Myxococcales	Cystobacteraceae
OTU90	64	51	45	38	10	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU20	63	58	102	49	3	Acidobacteria Gp16	Gp16	
OTU274	70	40	49	25	9	Thermoleophilia	Solirubrobacterales	Conexibacteraceae
OTU81	136	118	80	85	74	Betaproteobacteria	Burkholderiales	Burkholderiales inc. sedis
OTU95	75	55	31	37	14	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae
OTU57	67	74	111	24	5	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae
OTU96	68	66	77	36	2	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU65	67	48	117	36	0	Bacilli	Bacillales	Bacillaceae 1
OTU218	72	55	30	26	5	Acidobacteria Gp4	Gp4	
OTU55	90	141	69	54	23	Acidobacteria Gp4	Gp4	
OTU106	90	66	95	33	22	Acidobacteria Gp16	Gp16	
OTU9319	154	81	203	55	85	Bacilli	Bacillales	Bacillaceae 1
OTU56	72	45	52	32	3	Rubrobacteria	Gaiellales	Gaiellaceae
OTU9821	82	36	63	46	10	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU278	73	56	73	51	0	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU19	157	123	112	111	84	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
OTU149	75	39	84	16	1	WPS-2 gen. inc. sedis		
OTU77	76	106	50	17	0	Actinobacteria	Streptomycetales	Streptomycetaceae
OTU3793	183	111	136	72	101	Actinobacteria	Micrococcales	Intrasporangiaceae
OTU51	112	91	75	43	24	Rubrobacteria	Gaiellales	Gaiellaceae
OTU61	91	84	56	16	2	Anaerolineae	Anaerolineales	Anaerolineaceae
OTU75	117	100	46	75	28	Acidobacteria Gp6	Gp6	
OTU144	98	60	49	19	9	Betaproteobacteria	Burkholderiales	Burkholderiaceae
OTU48	107	101	89	72	13	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU1579	114	59	87	55	20	Acidobacteria Gp16	Gp16	
OTU35	137	123	98	39	38	Betaproteobacteria	Methylophilales	Methylophilaceae
OTU63	194	172	109	118	93	Betaproteobacteria	Burkholderiales	Comamonadaceae
OTU10	172	98	190	73	64	Actinobacteria	Geodermatophilales	Geodermatophilaceae
OTU44	109	69	41	8	0	Betaproteobacteria	Burkholderiales	Burkholderiaceae
OTU64	148	128	143	92	34	Actinobacteria	Propionibacteriales	Nocardioidaceae
OTU72	125	90	110	42	8	Acidobacteria Gp16	Gp16	
OTU58	176	87	139	76	56	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae

OTU37	125	65	52	12	5	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae
OTU32	124	83	121	46	0	Rubrobacteria	Gaiellales	Gaiellaceae
OTU83	142	104	127	82	15	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU1247	127	49	73	16	0	Rubrobacteria	Gaiellales	Gaiellaceae
OTU39	147	95	127	159	18	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU21	152	94	159	44	2	Rubrobacteria	Gaiellales	Gaiellaceae
OTU8	235	154	236	109	18	Rubrobacteria	Gaiellales	Gaiellaceae
OTU3	500	183	469	183	250	Bacilli	Bacillales	Bacillaceae 1
OTU314	273	193	206	110	5	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU31	306	129	208	80	2	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae
OTU7	359	307	271	234	4	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
OTU6	465	218	301	114	7	Rubrobacteria	Gaiellales	Gaiellaceae
OTU5	860	602	822	382	154	Acidobacteria Gp16	Gp16	
OTU2	1287	551	765	419	562	Actinobacteria	Micrococcales	Micrococcaceae

Supplementary material to the chapter

3.3. Bacterial, archaeal and micro-eukaryotic communities characterize a disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab.

Supplementary Table S1. Quantitative real-time PCR analyses. Data were assessed by ANOVA and Fisher's LSD tests, and letters indicate significantly different samples ($p < 0.05$). L stands for suppressive and H for conducive Vyklantice soils.

A. Quantitative analyses of bacteria in bulk soil and tuberosphere samples. Data are shown as means with standard deviations (n = 4).

Sample	Cultivar / bulk soil	Bacterial 16S rRNA gene		Actinobacterial 16S rRNA gene		*txtB* gene	
		copies/g	SD	copies/g	SD	copies/g	SD
LB	bulk soil	$9.69 \times 10^{9\ a,b}$	3.43×10^{9}	$4.20 \times 10^{9\ a,b}$	1.02×10^{9}	8.64×10^{5}	1.12×10^{5}
LK	Kariera	$1.38 \times 10^{9\ c}$	4.23×10^{8}	$1.15 \times 10^{9\ c}$	4.65×10^{8}	6.85×10^{5}	5.80×10^{4}
LA	Agria	$1.43 \times 10^{9\ b,c}$	2.37×10^{9}	$2.27 \times 10^{9\ b}$	1.00×10^{9}	6.76×10^{5}	1.30×10^{4}
HB	bulk soil	$1.37 \times 10^{10\ a}$	5.53×10^{9}	$6.69 \times 10^{9\ a}$	2.50×10^{9}	6.34×10^{5}	2.09×10^{5}
HK	Kariera	$1.14 \times 10^{10\ a,b}$	7.07×10^{9}	$4.84 \times 10^{9\ a,b}$	3.03×10^{9}	7.17×10^{5}	1.33×10^{5}
HA	Agria	$1.24 \times 10^{10\ a}$	6.19×10^{9}	$4.85 \times 10^{9\ a,b}$	2.01×10^{9}	5.97×10^{5}	1.23×10^{5}

B. Quantitative analyses of bacteria in periderm samples. Data are shown as means with standard deviations (n = 4).

Sample	Cultivar	Actinobacterial 16S rRNA gene		*txtB* gene	
		copies/g	SD	copies/g	SD
LK	Kariera	$3.31 \times 10^{8\ b}$	8.29×10^{7}	$7.74 \times 10^{5\ b}$	4.84×10^{5}
LA	Agria	$8.66 \times 10^{7\ b}$	5.44×10^{7}	$1.21 \times 10^{6\ b}$	2.43×10^{5}
HK	Kariera	$2.62 \times 10^{8\ a,b}$	1.53×10^{8}	$4.61 \times 10^{6\ a,b}$	1.40×10^{6}
HA	Agria	$1.61 \times 10^{9\ a}$	1.68×10^{9}	$6.01 \times 10^{7\ a}$	6.72×10^{7}

Supplementary Table S2. Chemical analyses. Data were assessed by ANOVA and Fisher's LSD tests, and letters indicate significantly different samples ($p < 0.05$). L stands for suppressive and H for conducive Vyklantice soils.

A. Chemical analyses of bulk soil and tuberosphere samples. Data are shown as means ± standard deviations (n = 4).

Sample	Cultivar / bulk soil	N [g/kg]	C [g/kg]	S [g/kg]	P [g/kg]	Mg [g/kg]	Ca [g/kg]	Fe [g/kg]	pH
LB	bulk soil	1.67 ± 0.16 ^{b}	14.9 ± 2.0 ^{b}	0.642 ± 0.095 ^{b,c}	1.03 ± 0.08 ^{b,c}	11.2 ± 0.7	3.40 ± 0.78 ^{b}	39.5 ± 2.8 ^{b,c}	5.36 ± 0.16 ^{b}
LK	Kariera	1.67 ± 0.10 ^{b}	15.7 ± 0.9 ^{b}	0.855 ± 0.044 ^{a}	1.04 ± 0.06 ^{b,c}	12.0 ± 0.3	1.98 ± 0.10 ^{c}	38.6 ± 1.1 ^{c}	5.37 ± 0.15 ^{b}
LA	Agria	1.64 ± 0.12 ^{b}	15.4 ± 1.3 ^{b}	0.745 ± 0.091 ^{b}	1.00 ± 0.14 ^{c}	11.5 ± 0.5	1.81 ± 0.08 ^{c}	37.0 ± 2.0 ^{c}	5.36 ± 0.06 ^{b}
HB	bulk soil	2.24 ± 0.08 ^{a}	21.2 ± 1.1 ^{a}	0.661 ± 0.043 ^{b,c}	1.14 ± 0.06 ^{a,b}	11.3 ± 0.5	4.77 ± 0.32 ^{a}	39.7 ± 1.6 ^{b,c}	5.86 ± 0.16 ^{a}
HK	Kariera	2.29 ± 0.13 ^{a}	21.2 ± 1.3 ^{a}	0.623 ± 0.005 ^{c}	1.10 ± 0.04 ^{a,b,c}	10.7 ± 0.4	2.85 ± 0.36 ^{b}	42.6 ± 1.8 ^{a,b}	5.98 ± 0.08 ^{a}
HA	Agria	2.24 ± 0.08 ^{a}	20.9 ± 0.9 ^{a}	0.617 ± 0.014 ^{c}	1.21 ± 0.04 ^{a}	11.3 ± 0.5	2.94 ± 0.26 ^{b}	45.0 ± 1.9 ^{a}	5.99 ± 0.12 ^{a}

B. Chemical analyses of periderm samples. Data are shown as means ± standard deviations (n = 4).

Sample	Cultivar	N [g/kg]	P [mg/kg]	Ca [g/kg]	Mg [g/kg]	Fe [g/kg]
LK	Kariera	19.3 ± 1.8 ^{a,b}	2.85 ± 0.47	0.91 ± 0.20 ^{b}	0.793 ± 0.019 ^{a}	0.174 ± 0.049 ^{a}
LA	Agria	21.3 ± 2.0 ^{a}	3.00 ± 0.52	1.21 ± 0.08 ^{a}	0.652 ± 0.057 ^{b}	0.076 ± 0.005 ^{b}
HK	Kariera	14.7 ± 0.9 ^{c}	3.52 ± 0.47	1.04 ± 0.03 ^{a,b}	0.778 ± 0.063 ^{a}	0.091 ± 0.009 ^{b}
HA	Agria	16.0 ± 2.5 ^{b,c}	2.97 ± 0.31	1.24 ± 0.14 ^{a}	0.668 ± 0.046 ^{b}	0.076 ± 0.027 ^{b}

Supplementary Table S3. ANOVA of field (suppressive and conducive) and cultivar (resistant and susceptible) effects on data of soil and periderm chemical and microbial analyses. Levels of significance are indicated by asterisks, $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

A. Soil analyses

	Df	N	C	S	P	Mg	Ca	Fe	pH	*Bacteria*	*Actinobacteria*	*txtB* gene
Field	1	0.021 ***	1.992 ***	77703 ***	101530 **	1475104	7537604 ***	98010417 ***	2.024 ***	6.78 ***	3.88 **	0.144
Cultivar	2	2.65E-05	0.003	15910	2117	58958	7647463 ***	4321667	0.012	2.78 *	1.74 *	0.037
Field × cultivar	2	1.68E-05	0.004	31708 **	10067	1903958	128904	30831667 **	0.011	1.20	0.38	0.076

B. Periderm analyses

	Df	N	P	Ca	Mg	Fe	*Actinobacteria*	*txtB* gene
Field	1	96.91 ***	412015	0.03	0.000184	0.0069 *	31.4 *	42.52 **
Cultivar	1	10.96	158614	0.23 *	0.05946 ***	0.0134 **	10.46	15.47
Field × cultivar	1	0.48	484017	0.01	0.000878	0.0063 *	0.08	0

Supplementary Table S4. Correlation between environmental variables and differences in community composition of bacteria, archaea, and micro-eukaryotes assessed by non-metric multidimensional scaling based on Bray-Curtis distance matrices. Levels of significance are indicated by dots, $p < 0.1$, and asterisks, $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

Environmental variable		Microarray analysis			Illumina amplicon sequencing								
		bacteria			bacteria			archaea			micro-eukaryotes		
		r	p		r	p		r	p		r	p	
N	total content in tuberosphere soil	0.4501	0.006	**	0.1099	0.334		0.8073	0.001	***	0.5999	0.001	***
C		0.3950	0.013	*	0.1274	0.284		0.7728	0.001	***	0.5373	0.001	***
S		0.1533	0.202		0.0777	0.456		0.2259	0.096	.	0.2484	0.040	*
P		0.1001	0.343		0.0118	0.885		0.2818	0.059	.	0.1102	0.294	
Mg		0.2858	0.034	*	0.0109	0.902		0.1314	0.289		0.1495	0.205	
Ca		0.1794	0.139		0.2099	0.102		0.2636	0.071	.	0.2792	0.029	*
Fe		0.1432	0.208		0.0774	0.466		0.4105	0.010	**	0.3240	0.023	*
diversity of bacteria	area under the rarefaction curve	0.2173	0.090	.	0.7478	0.001	***	0.0019	0.973		0.0331	0.703	
diversity of micro-eukaryotes		0.4593	0.002	**	0.1174	0.295		0.5137	0.002	**	0.7220	0.001	***
diversity of archaea		0.2961	0.037	*	0.0121	0.887		0.5925	0.002	**	0.5459	0.001	***
pH		0.5751	0.002	**	0.2048	0.120		0.8889	0.001	***	0.6228	0.001	***
total bacteria	16S rRNA gene copies	0.0887	0.377		0.1490	0.236		0.5786	0.001	***	0.6104	0.001	***
actinobacteria		0.1399	0.208		0.1718	0.180		0.5610	0.002	**	0.5933	0.001	***
txtB	gene copies	0.2887	0.037	*	0.1960	0.128		0.1927	0.150		0.0979	0.307	

Supplementary Table S5. Average signal intensities of probes (n = 4)

A. Probes significantly separating the soils L and H (Metastats, p < 0.05)

Probe	Target group	LB	LK	LA	HB	HK	HA	p-value
Aceto3A	Some Acetobacteraceae	0.010	0.020	0.017	0.007	0.000	0.006	< 0.001
Acdp821	Acidiphilium	0.012	0.022	0.022	0.011	0.000	0.011	< 0.001
PalgiG3	Paenibacillus alginoliticus et rel. cluster	0.013	0.017	0.027	0.003	0.000	0.016	< 0.001
Pseu33	Pseudomonas citronellolis and Pseudomonas nitroreducens	0.016	0.033	0.021	0.022	0.000	0.011	0.001
Aci1	Acidiphillium	0.021	0.018	0.022	0.011	0.000	0.012	0.001
Strepto5	Streptomyces	0.009	0.020	0.012	0.012	0.000	0.014	0.003
Brady4	Bradyrhizobiaceae	0.030	0.034	0.038	0.034	0.000	0.030	0.005
StspSUB1	Streptosporangiales	0.003	0.011	0.015	0.002	0.000	0.013	0.007
Nit1B	Most Nitrosospira	0.000	0.016	0.022	0.008	0.000	0.013	0.013
Frank11	Frankia	0.000	0.005	0.007	0.000	0.000	0.003	0.037
Aceto3B	Acetobacteraceae	0.000	0.004	0.014	0.000	0.000	0.007	0.041
Burkho4B	Some Burkholderia	0.000	0.000	0.014	0.000	0.000	0.003	0.044
Glob2	Rhodopila globiformis	0.000	0.005	0.006	0.000	0.000	0.002	0.049

Probe	Target group	LB	LK	LA	HB	HK	HA	p-value
Janaga2	Janthinobacterium agaricidamnosum	0.000	0.000	0.012	0.024	0.045	0.016	0.005
Janaga3	Janthinobacterium agaricidamnosum	0.008	0.005	0.023	0.052	0.061	0.025	0.008
Acido-c	Uncultured Acidobacteria (Acidobacteria_4 cluster)	0.000	0.000	0.000	0.010	0.000	0.010	0.017
Barto2	Bartonella	0.000	0.000	0.000	0.003	0.006	0.009	0.020
RhizoLCSA2	Maize rhizosphere clones affiliated to Acidobacteria (Acidobacteria_7 cluster)	0.000	0.000	0.000	0.007	0.000	0.009	0.022
B6-603	Agrobacterium (G1, G3, G4, G7), A. rubi, A. larrymoorei, some Rhizobium and some Brevundimonas	0.000	0.000	0.000	0.000	0.000	0.008	0.026
MyxCor1	Myxococcus/Corallococcus	0.000	0.000	0.000	0.000	0.000	0.011	0.027
PseuD	Pseudomonas	0.000	0.000	0.000	0.015	0.000	0.014	0.034
AcidUnc	Uncultured Acidobacteria (Acidobacteria_6 cluster)	0.015	0.000	0.015	0.026	0.008	0.022	0.045

B. Probes significantly separating the two varieties A and K (Metastats, p < 0.05)

Probe	Target group	LB	LK	LA	HB	HK	HA	p-value
Strepto3	Streptomyces	0.022	0.037	0.020	0.021	0.039	0.014	< 0.001
Strepto1	Streptomyces	0.072	0.068	0.045	0.067	0.069	0.029	< 0.001
Strepto2	Streptomyces	0.059	0.052	0.034	0.046	0.055	0.020	< 0.001
Rzbc1247	Rhizobiaceae, Brucellaceae, Bartonella, Phyllobacteriaceae, Blastochloris, Azospirillum irakense and A. amazonense	0.037	0.044	0.033	0.059	0.061	0.027	0.001
BET940	Betaproteobacteria (except Comamonadaceae, Nitrosomonadaceae and Methylophilaceae)	0.060	0.051	0.046	0.054	0.043	0.037	0.001
Azo5	Azospirillum, some Roseomonas, Rhodospirillum, Rhodocista, Skermanella	0.009	0.017	0.017	0.021	0.023	0.016	0.003
Actino1	Streptosporangiales	0.017	0.025	0.012	0.013	0.016	0.007	0.003
Plancto4-mB	Most Planctomycetes	0.054	0.038	0.031	0.039	0.034	0.028	0.005
Rhizo157	Rhizobiaceae (except Agrobacterium), Bradyrhizobiaceae, Brucellaceae and Brevundimonas	0.068	0.064	0.033	0.058	0.024	0.025	0.011
Mycoba2	Mycobacterium	0.025	0.030	0.023	0.022	0.021	0.016	0.014
Mycoba1	Mycobacterium	0.025	0.028	0.021	0.021	0.024	0.018	0.019
CYA664	Most Cyanobacteria and some Chloroplasts	0.009	0.015	0.013	0.011	0.016	0.012	0.030
Kisp9	Kitasatospora griseola	0.012	0.025	0.023	0.028	0.022	0.020	0.032

Probe	Target group	LB	LK	LA	HB	HK	HA	p-value
Gludi	Gluconacetobacter diazotrophicus	0.000	0.000	0.021	0.000	0.000	0.015	< 0.001
TDRNO1030	Thermodesulforhabdus norvegica	0.000	0.000	0.012	0.003	0.000	0.013	< 0.001
Lacto39	Lactobacillus	0.000	0.000	0.006	0.000	0.000	0.007	< 0.001
Xan	Xanthobacter	0.000	0.000	0.018	0.000	0.000	0.021	< 0.001
Hyme3	Hymenobacter	0.000	0.000	0.008	0.000	0.000	0.011	< 0.001
Polycell	Polyangium cellulosum	0.000	0.000	0.009	0.000	0.000	0.011	< 0.001
Rhodobact1B	Rhodobacteraceae (excepted Paracoccus, Amaricoccus, Rhodobacter and Rhodovulum)	0.000	0.000	0.023	0.009	0.000	0.020	< 0.001
XAN818	Xanthomonas	0.000	0.000	0.020	0.000	0.000	0.009	< 0.001
Pho1	Photorhabdus	0.008	0.000	0.015	0.000	0.000	0.005	< 0.001
Acidocella1	Acidocella/Acidiphilium	0.006	0.004	0.020	0.011	0.000	0.022	0.001
Plancto12	Gemmata cluster	0.013	0.004	0.021	0.012	0.000	0.018	0.001
Stsp16	Streptosporangium	0.000	0.000	0.007	0.000	0.000	0.012	0.001
Ehrli1	Ehrlichiaceae (except Ehrlichia risticii and Cowdria)	0.000	0.000	0.006	0.000	0.000	0.012	0.001
Campy	Campylobacter	0.005	0.004	0.019	0.011	0.000	0.019	0.001
Bacpsf1	Bacillus pseudofirmus	0.000	0.000	0.006	0.000	0.000	0.010	0.001
Bkhcari1	Some Burkholderia (B. caribensis and B. hospita)	0.000	0.000	0.012	0.003	0.000	0.010	0.001
Dietz7	Dietzia	0.000	0.000	0.006	0.002	0.000	0.008	0.001
Nit1C	Some Nitrosospira	0.007	0.005	0.027	0.013	0.000	0.019	0.002
Polyang10	Polyangium	0.000	0.000	0.005	0.000	0.000	0.008	0.002
anaermycln	Acidobacteria (Subgroup 11)	0.000	0.000	0.007	0.004	0.000	0.010	0.002
pirelcln	Planctomycetaceae (Pir1 lineage)	0.000	0.000	0.014	0.009	0.000	0.014	0.003
Hypho5	Some Hyphomicrobium	0.000	0.004	0.011	0.000	0.000	0.009	0.005
Sphingo5B	Most Sphingomonadaceae	0.000	0.006	0.013	0.000	0.000	0.013	0.005
Delac3	Delftia acidovorans	0.000	0.000	0.004	0.000	0.000	0.009	0.006
Nso1225b	Betaproteobacteria ammonia oxidizers	0.000	0.000	0.006	0.000	0.000	0.008	0.006
Sacchps10	Saccharopolyspora	0.000	0.000	0.003	0.000	0.000	0.013	0.006
Cow1	Cowdria	0.000	0.000	0.004	0.000	0.000	0.014	0.007
Pagg6	Pantoea agglomerans	0.000	0.000	0.002	0.000	0.000	0.010	0.007
StreptomycesD7	Streptomyces	0.000	0.000	0.005	0.000	0.000	0.009	0.007
OP11-3	Uncultured eubacteria (OP11 division)	0.000	0.000	0.006	0.000	0.000	0.007	0.008
Bkand	Burkholderia andropogonis	0.000	0.000	0.006	0.000	0.000	0.012	0.008
Comtes2	Comamonas (mainly C. testosteroni)	0.000	0.000	0.005	0.000	0.000	0.010	0.008
Nancs7	Nannocystis	0.000	0.000	0.004	0.000	0.000	0.009	0.009
Rhi	Rhizobiaceae (except Agrobacterium)	0.000	0.000	0.002	0.000	0.000	0.014	0.011
Rhizo1B	Some Rhizobium	0.000	0.003	0.009	0.002	0.000	0.012	0.013
Spiro5	Spirosoma	0.000	0.000	0.002	0.000	0.000	0.005	0.024
Phyllobact	Mesorhizobium / Rhizobium	0.000	0.000	0.002	0.000	0.000	0.004	0.024
B6-603	Agrobacterium (G1, G3, G4, G7), A. rubi, A. larrymoorei, some Rhizobium and some Brevundimonas	0.000	0.000	0.000	0.000	0.000	0.008	0.025
Frtul7	Francisella tularensis	0.000	0.000	0.003	0.000	0.000	0.005	0.025
CystbSUB1	Cystobacterineae	0.000	0.000	0.003	0.004	0.000	0.008	0.026
MyxCor1	Myxococcus/Corallococcus	0.000	0.000	0.000	0.000	0.000	0.011	0.026
Plancto10	Some Pirrellula	0.000	0.000	0.002	0.000	0.000	0.008	0.028
Pagg5	Pantoea agglomerans	0.000	0.000	0.002	0.000	0.000	0.007	0.029
PseuD	Pseudomonas	0.000	0.000	0.000	0.015	0.000	0.014	0.033
Burkho4B	Some Burkholderia	0.000	0.000	0.014	0.000	0.000	0.003	0.034
PLA46	Planctomycetes, Lentipshaerae and OP3 candidate phylum	0.000	0.000	0.005	0.000	0.000	0.005	0.037
Nitmob	Nitrosococcus mobilis	0.000	0.000	0.004	0.000	0.000	0.005	0.038
Gloe2	Chlorogloeopsis	0.000	0.000	0.003	0.000	0.000	0.006	0.038
Aqutert	Aquicola tertiaricarbonis	0.000	0.000	0.003	0.004	0.000	0.008	0.039
Paen6	Paenibacillus	0.000	0.000	0.007	0.000	0.000	0.004	0.041
Diali14	Dialister	0.000	0.004	0.005	0.000	0.000	0.016	0.042
Baccir1	Bacillus circulans	0.000	0.000	0.008	0.000	0.000	0.008	0.043

Supplementary Table S6. Average frequencies (n = 4) of selected OTUs significantly contributing to separation of bacterial communities in the individual samples.

A. The most abundant OTUs significantly separating the soils L and H (Metastats, $p < 0.05$)

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu92	126	52	88	60	28	45	0,045	Proteobacteria	Betaproteobacteria	SC-I-84		
Otu253	115	61	104	68	27	55	0,015	Proteobacteria	Betaproteobacteria	SC-I-84		
Otu68	62	68	66	36	31	56	0,020	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia
Otu592	18	5	18	5	0	6	0,038	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Rhodopseudomonas
Otu835	17	33	25	12	18	15	0,023	Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	
Otu385	16	1	10	8	1	2	0,040	Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	
Otu3120	13	7	12	10	3	3	0,043	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys
Otu516	31	15	16	30	8	11	0,022	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dokdonella
Otu1402	1	4	7	0	2	0	0,029	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium
Otu1154	0	5	9	2	2	0	0,012	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium
Otu2920	7	2	2	2	0	0	< 0.001	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Sorangium
Otu1408	6	7	6	7	4	1	0,047	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Flavisolibacter
Otu1343	4	1	4	2	1	0	0,031	FBP				
Otu2780	4	0	2	1	0	0	0,028	Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	
Otu3391	4	0	1	1	0	0	0,033	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu355	16	17	22	21	33	32	0,040	Actinobacteria	MB-A2-108			
Otu369	32	18	28	22	39	37	0,008	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	
Otu1230	6	9	7	8	12	18	0,040	Actinobacteria	Thermoleophilia	Gaiellales		
Otu1478	5	6	5	9	11	9	0,045	Chloroflexi	TK10			
Otu886	5	6	3	7	11	7	0,033	Actinobacteria	Thermoleophilia	Solirubrobacterales		
Otu2120	7	8	10	4	14	17	0,018	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina
Otu899	7	3	4	20	1	1	0,019	Proteobacteria	Betaproteobacteria	SC-I-84		
Otu2391	2	1	1	5	6	1	0,015	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	
Otu2105	4	4	4	2	6	11	0,017	Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Shimazuella
Otu1621	2	3	2	3	6	5	0,012	unclas. Bacteria				
Otu1832	6	1	1	6	4	3	< 0.001	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Dongia
Otu2001	0	0	0	1	3	1	0,031	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio
Otu1772	1	2	2	2	3	5	0,020	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus
Otu820	19	7	8	12	13	13	0,002	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium
Otu3265	1	2	0	2	3	2	0,045	Cyanobacteria	ML635J-21			

B. The most abundant OTUs significantly separating the varieties Kariera (K) and Agria (A) (Metastats, $p < 0.05$)

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu4	347	956	374	576	1177	386	< 0.001	Chloroflexi	KD4-96			
Otu16	13	435	7	198	615	24	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu55	406	766	464	504	1011	450	0,019	Chloroflexi	KD4-96			
Otu48	24	283	9	155	278	15	< 0.001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu12	96	360	101	233	439	165	0,001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu51	77	303	49	269	273	42	< 0.001	Acidobacteria	Subgroup_6			
Otu164	59	249	55	121	328	61	< 0.001	Chloroflexi	KD4-96			
Otu30	6	215	2	127	243	15	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu69	22	195	21	98	238	37	< 0.001	Actinobacteria	Thermoleophilia	Solirubrobacterales	288-2	
Otu46	2	162	3	105	183	5	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu11	281	252	93	450	221	49	0,039	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	
Otu29	17	164	8	75	169	10	< 0.001	Chloroflexi	Gitt-GS-136			
Otu275	149	252	113	290	242	99	0,002	Acidobacteria	Subgroup_6			
Otu107	1	106	0	53	166	1	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu38	153	213	62	295	152	34	0,005	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Piscinibacter
Otu33	41	165	12	129	131	18	< 0.001	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas
Otu105	2	125	2	58	140	5	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu696	6	164	8	94	110	10	< 0.001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu143	42	145	20	162	145	19	< 0.001	Acidobacteria	Subgroup_6			
Otu141	0	110	0	42	142	1	< 0.001	Actinobacteria	MB-A2-108			
Otu123	17	123	11	75	150	17	< 0.001	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Roseiflexus
Otu24	70	167	77	118	234	81	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu64	239	193	67	413	141	31	0,026	Verrucomicrobia	Spartobacteria	Chthoniobacterales	DA101_soil_group	
Otu61	11	101	8	68	142	10	< 0.001	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	
Otu283	60	141	53	102	196	60	0,002	Chloroflexi	KD4-96			
Otu74	2	105	6	42	132	8	< 0.001	Chloroflexi	Thermomicrobia	JG30-KF-CM45		
Otu282	10	122	9	66	117	10	< 0.001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	WW2-159	
Otu73	63	151	36	202	119	32	0,005	Acidobacteria	Subgroup_6			
Otu138	21	131	29	62	127	37	< 0.001	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
Otu76	80	140	29	206	96	19	0,006	Acidobacteria	Blastocatellia	Blastocatellales	Blastocatellaceae_Subgr.4	RB41

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu1	1213	687	1980	746	522	1971	< 0.001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu21	633	145	841	236	169	836	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu6	288	67	558	86	73	652	< 0.001	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
Otu140	178	13	306	24	23	341	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu18	397	50	323	127	46	370	< 0.001	Chloroflexi	JG37-AG-4			
Otu63	176	56	311	56	62	370	< 0.001	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	Jatrophihabitans
Otu41	160	33	263	44	39	342	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu114	221	38	315	85	46	298	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu104	212	35	303	60	30	263	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu19	183	8	257	25	12	247	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu20	147	35	241	69	47	314	< 0.001	Actinobacteria	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus
Otu31	160	16	246	37	26	263	< 0.001	Actinobacteria	Thermoleophilia	Solirubrobacterales	TM146	
Otu54	175	45	248	67	55	267	< 0.001	Actinobacteria	Actinobacteria	Frankiales	uncultured	
Otu84	73	3	191	5	1	228	0,001	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Rhodanobacter
Otu26	138	8	215	15	11	198	< 0.001	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	
Otu8	221	195	387	162	253	439	0,016	Actinobacteria	Thermoleophilia	Gaiellales		
Otu309	109	6	194	14	5	186	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu13	139	93	248	106	97	275	< 0.001	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Oryzihumus
Otu10	181	136	308	127	106	255	< 0.001	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter
Otu137	82	13	166	12	12	180	< 0.001	Chloroflexi	Ktedonobacteria	C0119		
Otu50	89	14	160	22	20	187	< 0.001	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillales Inc. Sedis	Cand. Alysiosphaera
Otu176	63	34	187	44	38	175	< 0.001	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces
Otu199	93	9	160	14	5	140	< 0.001	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	(Para)Burkholderia
Otu36	350	15	197	112	4	100	< 0.001	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	
Otu52	213	22	186	73	22	128	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellales_unclassified	
Otu49	110	17	163	31	21	144	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu77	59	18	135	23	18	160	< 0.001	Chloroflexi	Ktedonobacteria	C0119		
Otu23	204	143	271	196	188	302	0,014	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Otu946	136	52	192	76	69	171	< 0.001	Actinobacteria	Thermoleophilia	Gaiellales		
Otu117	44	8	108	17	11	139	< 0.001	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Modestobacter

Supplementary Table S7. Effects of site, treatment, and their interaction on diversity of microbial communities. Two-way analysis of variance, n=4. Levels of significance are indicated by dots, $p < 0.1$, or asterisks, $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

Bacteria

	Sum Sq	Df	F value	Pr(>F)
site	39544	1	2.6604	0.1224
treatment	496817	2	16.7125	0.0001 ***
site:treatment	95263	2	3.2046	0.0675 .
Residuals	237818	16		

Microeukaryotes

	Sum Sq	Df	F value	Pr(>F)
site	77255	1	1.8266	0.1933
treatment	253156	2	2.9928	0.0755 .
site:treatment	72860	2	0.8613	0.4393
Residuals	761301	18		

Archaea

	Sum Sq	Df	F value	Pr(>F)
site	2660.84	1	47.6212	5.1e-06 ***
treatment	445.64	2	3.9878	0.0409 *
site:treatment	194.94	2	1.7176	0.2130
Residuals	838.13	15		

Supplementary Table S8. Correlations between proportions of individual OTUs in the communities of *Bacteria, Archaea,* and microeukaryotes (Spearman, $|\rho| \geq 0.8$).

Bacteria	**Rhizosphere**		**Bulk**	**L soil**	**H soil**
	Agria	**Kariera**	**soil**		
OTUs (min in two samples)	2942	3335	3416	3682	3778
Number of correlations	181026	332847	588300	415347	508320
Avg number of neighbors	61.5	99.8	172.2	112.8	134.5
Positive correlations	110401	174899	352691	254492	285632
Negative correlations	70625	157948	235609	160855	222688

Archaea	**Rhizosphere**		**Bulk**	**L soil**	**H soil**
	Agria	**Kariera**	**soil**		
OTUs (min in two samples)	87	80	88	95	69
Number of correlations	579	672	370	200	133
Avg number of neighbors	6.7	8.4	4.2	2.1	1.9
Positive correlations	426	453	256	142	116
Negative correlations	153	219	114	58	17

Microeukaryotes	**Rhizosphere**		**Bulk**	**L soil**	**H soil**
	Agria	**Kariera**	**soil**		
OTUs (min in two samples)	2339	1801	2148	2495	2229
Number of correlations	142260	67000	72585	20828	16411
Avg number of neighbors	60.8	37.2	33.8	8.3	7.4
Positive correlations	107366	55606	62538	19561	15286
Negative correlations	34894	11394	10047	1267	1125

Supplementary Table S9. Average frequencies (n = 4) of selected OTUs significantly contributing to separation of archaeal communities in the individual samples.

A. The most abundant OTUs significatly separating the soils L and H (Metastats, $p < 0.05$)

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu1	6626	11846	11496	5229	6200	5634	0.024	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina
Otu3	6028	3736	4132	1784	2311	701	0.009	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu5	869	1033	986	428	292	498	0.001	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus
Otu6	1153	596	707	359	462	238	0.028	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu10	584	311	223	1	12	1	0.001	Thaumarchaeota	Nitrososphaeria	Nitrosotaleales	Nitrosotaleaceae	Cand. Nitrosotalea
Otu17	429	426	503	90	105	36	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu93	97	102	130	53	38	41	0.001	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina
Otu29	11	7	30	1	2	0	0.003	Thaumarchaeota	Group_c			
Otu42	8	3	13	0	0	0	0.001	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	uncultured	
Otu69	1	3	2	0	1	0	0.032	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Cand. Nitrocosmicus

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu38	1	0	6	31	9	32	0.006	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu91	6	1	1	23	15	36	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu36	0	0	0	17	13	41	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu55	17	5	4	46	38	36	0.015	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu96	0	0	0	20	46	23	0.002	Euryarchaeota	Thermoplasmata	uncultured		
Otu22	7	0	0	40	47	20	0.002	Nanoarchaeaeota	Woesearchaeia			
Otu76	0	0	0	18	64	14	0.001	Euryarchaeota	Thermoplasmata	unclass.		
Otu25	0	0	0	27	38	36	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu23	0	0	0	23	40	47	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Cand. Nitrososphaera
Otu87	0	0	0	19	79	19	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu20	2	0	0	33	53	40	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu31	0	0	0	35	71	18	0.002	Euryarchaeota	Thermoplasmata	uncultured		
Otu86	68	18	11	83	62	135	0.016	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu65	0	0	0	42	100	47	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu40	2	0	1	59	124	52	0.001	Euryarchaeota	Thermoplasmata	unclass.		
Otu39	1	0	0	54	115	75	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu14	0	0	0	48	143	53	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu78	12	0	12	91	134	85	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu19	0	0	1	63	168	59	0.001	Euryarchaeota	Thermoplasmata	uncultured		
Otu61	4	0	2	115	87	187	0.002	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu15	71	10	50	164	246	126	0.013	Euryarchaeota	Thermoplasmata	uncultured		
Otu18	5	1	3	112	102	216	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu13	4	0	0	249	164	317	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Cand. Nitrososphaera
Otu70	18	6	2	303	217	480	0.002	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu12	30	1	9	498	1166	493	0.001	Euryarchaeota	Thermoplasmata	uncultured		

Otu64	372	117	250	1149	1289	829	0.006	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina
Otu4	765	296	285	1378	1495	1803	0.002	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu9	5	0	4	1655	779	1598	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	
Otu8	201	47	30	1250	1965	1146	0.001	Euryarchaeota	Thermoplasmata	unclass.		
Otu7	106	22	37	1722	1957	2537	0.001	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	

B. The most abundant OTUs significatly separating the varieties Kariera (K) and Agria (A) (Metastats, $p < 0.05$)

Overall comparison

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu112	0	0	0	4	1	0	0.045	Nanoarchaeaeota	Woesearchaeia	unclass.		
Otu75	0	0	0	0	1	0	0.045	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Otu92	0	0	0	0	0	2	0.034	Euryarchaeota	Thermoplasmata	uncultured		
Otu73	55	13	49	34	9	23	0.009	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus

Comparison of the varieties in conducive soil H

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu64	372	117	250	879	1696	829	0.019	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina
Otu17	429	426	503	170	4	36	0.012	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	unclass.
Otu43	74	40	25	159	7	40	0.039	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	unclass.

Comparison of the varieties in suppressive soil L

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu29	11	7	30	2	0	0	0.05	Thaumarchaeota	Group_c			
Otu73	55	13	49	34	9	23	0.018	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus

Supplementary Table S10. Average frequencies (n = 4) of selected OTUs significantly contributing to separation of eukaryotic communities in the individual samples.

A. The most abundant OTUs significatly separating the soils L and H (Metastats, $p < 0.05$)

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu1	1044	2101	1952	400	500	603	0.001	Ascomycota	Eurotiomycetes	Chaetothyriales	unclass.	
Otu10	581	855	688	358	255	564	0.02	Ascomycota	Leotiomycetes	Incertae_Sedis	Pseudeurotiaceae	Pseudogymnoascus
Otu25	452	488	586	227	134	267	0.001	Schizoplasmodiida	Schizoplasmodiida_cl	Schizoplasmodiida_or	Schizoplasmodiida_fa	Ceratiomyxella
Otu34	306	144	177	57	15	40	0.001	Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	Saitozyma
Otu36	119	234	232	32	9	37	0.001	Cercozoa	unclass.			
Otu47	54	405	65	2	15	5	0.001	Basidiomycota	Tremellomycetes	Tremellales	Rhynchogastremataceae	Papiliotrema
Otu45	60	210	223	0	0	2	0.001	unclass.				
Otu80	91	151	198	9	6	6	0.001	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Knufia
Otu74	84	257	113	7	16	12	0.001	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Melastiza
Otu182	119	220	230	56	30	95	0.001	unclass.				
Otu51	251	73	61	1	0	0	0.002	Nematoda	Chromadorea	unclass.		
Otu59	221	92	66	3	5	12	0.001	Mucoromycota	Incertae_Sedis	Mucorales	Mucoraceae	Mucor
Otu1080	95	169	118	8	12	12	0.001	Ascomycota	Leotiomycetes	unclass.		
Otu69	1	152	169	0	2	1	0.007	Arthropoda	Arachnida	Acari_or	Acari_fa	Acari_ge
Otu68	31	121	168	1	6	1	0.001	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	unclass.
Otu42	128	160	128	46	28	44	0.001	Ochrophyta	unclass.			
Otu41	77	230	150	57	63	48	0.008	unclass.				
Otu117	167	45	45	8	8	2	0.001	Klebsormidiophyceae	Klebsormidiophyceae_cl	Klebsormidiales	Klebsormidiales_fa	Klebsormidium
Otu97	136	172	164	56	80	99	0.006	Discosea	Longamoebia	Centramoebida	Centramoebida_fa	Acanthamoeba
Otu76	28	102	121	5	7	5	0.001	Basidiomycota	Agaricomycetes	Agaricales	uncultured	uncultured_ge
Otu102	97	130	5	0	0	0	0.001	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Trechispora
Otu29	147	247	213	131	65	183	0.044	Ochrophyta	Xanthophyceae	Tribonematales	Tribonematales_fa	Xanthonema
Otu108	136	102	52	10	42	20	0.009	unclass.				
Otu1590	67	81	84	4	8	6	0.001	Ascomycota	Leotiomycetes	Helotiales	uncultured	
Otu2050	39	107	44	5	2	1	0.001	Mucoromycota	Glomeromycetes	Paraglomerales	Paraglomeraceae	Paraglomus
Otu49	73	129	117	82	21	44	0.004	Cercozoa	Glissomonadida	Glissomonadida_or	Glissomonadida_fa	Glissomonadida_ge
Otu115	44	41	86	0	0	0	0.001	Cercozoa	Phytomyxea	Phytomyxea_or	Phytomyxea_fa	Plasmodiophora
Otu103	36	90	71	4	6	20	0.002	Schizoplasmodiida	Schizoplasmodiida_cl	Schizoplasmodiida_or	Schizoplasmodiida_fa	Ceratiomyxella
Otu148	46	60	54	6	3	7	0.001	unclass.				
Otu96	106	56	91	37	28	47	0.01	Cercozoa	unclass.			

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu105	28	5	9	47	41	57	0.002	unclass.				
Otu1934	5	0	1	16	82	17	0.004	Ciliophora	Intramacronucleata	Litostomatea	Haptoria	Arcuospathidium
Otu223	1	1	5	4	56	57	0.005	unclass.				
Otu159	27	5	3	36	34	81	0.005	unclass.				
Otu146	0	0	0	59	43	18	0.001	unclass.				
Otu160	14	4	5	20	108	18	0.046	Ochrophyta	Diatomea	unclass.		
Otu155	12	5	16	42	76	43	0.001	Peronosporomycetes	Peronosporomycetes_cl	Peronosporomycetes_or	Peronosporomycetes_fa	Aplanopsis
Otu124	15	17	19	16	69	96	0.006	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinales_fa	Spumella
Otu1245	15	7	12	79	21	67	0.007	Cercozoa	Thecofilosea	Cryomonadida	Rhizaspididae	Rhogostoma
Otu268	0	0	0	31	55	49	0.001	unclass.				
Otu120	1	6	3	66	27	58	0.003	Mucoromycota	Incertae_Sedis	Mucorales	Rhizopodaceae	Rhizopus
Otu192	3	0	3	27	58	65	0.001	Cercozoa	Cercomonadidae	Cercomonadidae_or	Cercomonadidae_fa	Cercomonas
Otu119	22	10	19	102	35	70	0.002	unclass.				
Otu86	31	14	24	102	43	82	0.001	Ciliophora	Intramacronucleata	Conthreep	unclass.	
Otu81	20	11	11	91	35	80	0.001	Chlorophyta_ph	Chlorophyceae	Chlamydomonadales	Chlamydomonadales_fa	Fasciculochloris
Otu87	65	18	38	161	51	77	0.016	Ochrophyta	Eustigmatophyceae	Eustigmatales	Eustigmatales_fa	Eustigmatos
Otu75	25	8	17	126	28	69	0.003	unclass.				
Otu77	27	14	10	117	81	53	0.004	Ciliophora	Intramacronucleata	Litostomatea	Haptoria	unclass.
Otu46	43	24	52	121	72	149	0.015	Myxogastria	Myxogastria_cl	Myxogastria_or	Myxogastria_fa	Lepidoderma
Otu116	0	0	0	0	231	0	0.001	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	Marasmius
Otu84	47	24	21	189	142	65	0.002	Ciliophora	Intramacronucleata	Conthreep	unclass.	
Otu31	102	40	40	275	57	158	0.011	Chlorophyta_ph	Chlorophyceae	Chlamydomonadales	Chlamydomonadales_fa	Tetracystis
Otu40	66	8	31	122	87	240	0.001	Cercozoa	Phytomyxea	Phytomyxea_or	Phytomyxea_fa	Plasmodiophora
Otu57	3	0	2	100	90	174	0.001	unclass.				
Otu89	28	28	57	319	78	125	0.003	Ciliophora	Intramacronucleata	Conthreep	unclass.	
Otu11	305	130	185	418	322	445	0.02	Mucoromycota	Incertae_Sedis	Mortierellales	Mortierellaceae	Mortierella
Otu27	18	21	20	302	116	441	0.001	Ascomycota	Eurotiomycetes	Eurotiales	uncultured	uncultured_ge
Otu17	15	0	0	10	446	797	0.039	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	Sistotrema
Otu23	312	113	237	863	378	741	0.002	Cercozoa	Thecofilosea	Cryomonadida	Rhizaspididae	Rhogostoma
Otu5	0	0	0	1447	1186	42	0.001	Apicomplexa	Conoidasida	Gregarinasina	Neogregarinorida	Neogregarinorida_ge

B. The most abundant OTUs significatly separating the varieties Kariera (K) and Agria (A) (Metastats, $p < 0.05$)

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu63	1	341	0	1	2	0	0.03	Arthropoda	Arachnida	Acari_or	Acari_fa	Acari_ge
Otu94	0	0	0	0	311	0	0.001	Basidiomycota	Agaricomycetes	Agaricales	unclass.	
Otu116	0	0	0	0	231	0	0.001	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	Marasmius
Otu104	290	54	26	8	203	14	0.023	Basidiomycota	Malasseziomycetes	Malasseziales	Malasseziaceae	Malassezia
Otu178	50	60	15	7	72	23	0.004	Ascomycota	Sordariomycetes	Hypocreales	unclass.	
Otu288	0	0	0	10	76	0	0.001	Blastocladiomycota	Blastocladiomycetes	Blastocladiales	Blastocladiaceae	Allomyces
Otu196	46	61	13	3	50	25	0.005	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Chaetomium
Otu286	4	6	1	5	51	2	0.031	Ascomycota	Pezizomycetes	Pezizales	Ascodesmidaceae	uncultured
Otu400	0	47	0	0	0	0	0.001	Mucoromycota	Glomeromycetes	Diversisporales	unclass.	
Otu578	0	0	0	0	36	0	0.001	Basidiomycota	Agaricomycetes	Agaricales	Agaricales_fa	Agaricales_ge
Otu397	0	26	0	2	8	1	0.015	unclass.				
Otu466	6	19	0	1	4	1	0.018	unclass.				
Otu599	0	22	0	0	0	0	0.001	unclass.				
Otu738	0	0	0	0	15	0	0.001	unclass.				
Otu366	8	14	5	4	10	4	0.046	Chlorophyta_ph	Trebouxiophyceae	Incertae_Sedis	Incertae_Sedis_fa	Coccomyxa
Otu764	2	13	0	0	0	0	0.001	unclass.				
Otu3556	0	12	0	0	0	0	0.001	Arthropoda	unclass.			
Otu953	0	0	0	0	11	0	0.001	unclass.				
Otu779	1	8	1	0	4	0	0.006	Chytridiomycota	Incertae_Sedis	unclass.		
Otu1075	2	4	0	0	7	0	0.001	Peronosporomycetes	Peronosporomycetes_cl	Peronosporomycetes_or	Peronosporomycetes_fa	uncultured
Otu710	0	0	0	0	10	0	0.001	Ciliophora	Intramacronucleata	Spirotrichea	Hypotrichia	unclass.
Otu2032	0	0	0	35	9	0	0.001	Ciliophora	Intramacronucleata	Spirotrichea	Hypotrichia	unclass.
Otu905	0	8	0	0	0	0	0.001	Arthropoda	unclass.			
Otu729	2	9	2	0	1	0	0.048	unclass.				
Otu1051	0	8	0	0	0	0	0.001	Euglenozoa	Kinetoplastea	Metakinetoplastina	Trypanosomatida	unclass.
Otu1780	2	6	2	1	7	4	0.046	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	Clonostachys
Otu1158	0	2	0	0	5	0	0.001	unclass.				
Otu1303	2	4	0	0	2	0	0.001	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	unclass.
Otu1044	0	6	0	0	0	0	0.001	unclass.				
Otu2975	0	0	0	0	5	0	0.001	unclass.				

Group	LB	LK	LA	HB	HK	HA	p-value	Phylum	Class	Order	Family	Genus
Otu167	19	15	25	19	3	15	0.045	Chlorophyta_ph	Trebouxiophyceae	unclass.		
Otu277	19	4	25	1	5	5	0.036	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	Sclerotinia
Otu241	19	11	21	7	0	11	0.022	unclass.				
Otu202	12	14	15	13	2	23	0.028	Chlorophyta_ph	Chlorophyceae	Chlorophyceae_or	Chlorophyceae_fa	Chlorophyceae_ge
Otu355	17	7	9	26	2	22	0.027	Cercozoa	Glissomonadida	Glissomonadida_or	Glissomonadida_fa	Glissomonadida_ge
Otu181	19	14	15	37	2	24	0.05	Cercozoa	Cercomonadidae	Cercomonadidae_or	Cercomonadidae_fa	Cercomonas
Otu249	19	7	21	6	3	11	0.019	unclass.				
Otu306	6	0	14	4	4	14	0.003	Cercozoa	unclass.			
Otu404	11	7	12	13	1	22	0.001	unclass.				
Otu429	0	0	0	0	0	27	0.001	Nematoda	Enoplea	Dorylaimia_or	Dorylaimida_fa	Dorylaimida_ge
Otu323	9	2	10	14	8	28	0.05	Schizoplasmodiida	Schizoplasmodiida_cl	Schizoplasmodiida_or	Schizoplasmodiida_fa	Ceratiomyxella
Otu3439	5	0	28	0	0	0	0.001	Ascomycota	Leotiomycetes	unclass.		
Otu248	20	0	7	7	4	25	0.023	Ciliophora	Intramacronucleata	Spirotrichea	Hypotrichia	uncultured
Otu163	12	9	28	34	15	26	0.041	Cercozoa	unclass.			
Otu216	30	11	22	33	6	26	0.009	Chlorophyta_ph	Trebouxiophyceae	Incertae_Sedis		
Otu166	22	4	14	32	5	26	0.031	Cercozoa	Cercomonadidae	Cercomonadidae_or	Cercomonadidae_fa	Cercomonas
Otu2943	28	8	20	17	4	22	0.036	Chlorophyta_ph	Chlorophyceae	Chlorophyceae_or	Chlorophyceae_fa	Chlorophyceae_ge
Otu588	8	0	10	1	0	22	0.003	Zoopagomycota	Basidiobolomycetes	Basidiobolales	Basidiobolaceae	Basidiobolus
Otu141	20	21	40	4	17	34	0.014	Cercozoa	Glissomonadida	Glissomonadida_or	Glissomonadida_fa	Glissomonadida_ge
Otu118	88	35	40	108	17	49	0.048	Chlorophyta_ph	Chlorophyceae	Chlorophyceae_or	Chlorophyceae_fa	Chlorophyceae_ge
Otu191	2	16	38	6	3	24	0.007	unclass.				
Otu90	63	26	47	61	32	61	0.04	unclass.				
Otu168	59	23	40	39	8	42	0.043	Cercozoa	unclass.			
Otu158	74	9	19	31	19	60	0.035	Cercozoa	Cercomonadidae	Cercomonadidae_or	Cercomonadidae_fa	Cercomonas
Otu61	76	71	93	87	42	90	0.026	Ascomycota	Dothideomycetes	Capnodiales	Extremaceae	Extremus
Otu78	38	34	56	38	39	105	0.005	Cercozoa	Cercomonadidae	Cercomonadidae_or	Cercomonadidae_fa	Paracercomonas
Otu133	0	0	126	0	0	0	0.001	Arthropoda	Ellipura	Collembola	Collembola_fa	Collembola_ge
Otu13	730	401	459	565	364	468	0.029	Basidiomycota	Tremellomycetes	unclass.		
Otu255	26	32	171	19	15	50	0.007	Cercozoa	Glissomonadida	Glissomonadida_or	Glissomonadida_fa	Glissomonadida_ge
Otu21	243	136	226	168	123	215	0.001	Ascomycota	Leotiomycetes	Helotiales	Incertae_Sedis	Tetracladium

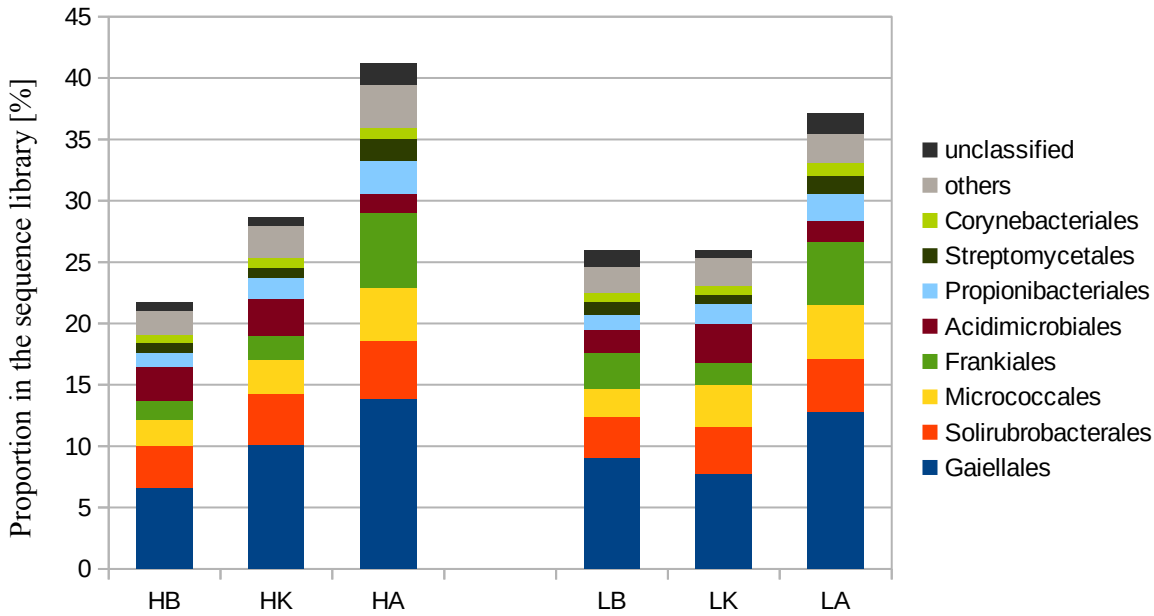
Supplementary Table S11. Comparison of studies related to the topic.

Publication	Rozenzweig et al. 2012	Kobayashi et al. 2015	Sagova-Mareckova et al. 2015	Tomihava et al. 2017	Shi et al. 2019	Kopecky et al. 2019
System description: treatments, sampling	2 fields: induced suppressive and conducive soil, 1 cultivar, 1 compartment	1 field: infested artificially infested, 8 cultivars, 3 compartments	4 fields: 2 naturally suppressive and 2 conducive soils, 3 cultivars	1 field: 1 cultivar and 3 treatments, 3 compartments	1 field: soil patchiness and potato cultivar genotypes (no treatments)	2 fields: naturally suppressive and conducive soil
Actinobacteria* / *Streptomycetaceae	Similar proportion in CS/SS soils		*Actinobacteria* quantity same or high in SS	High actinobacteria in low scab, antagonistic isolates	No differences in Streptomyces	*Actinobacteria* quantity low in SS, *Streptomyces* proportion high in SS
Pathogen / *txt* genes	cultivation/leisons, higher percentage in conducive soil	severity/qPCR txtAB, differences between cultivars in tubers, not rhizosphere	severity/qPCR txtB, differences between tuberosheres (both sites), bulk and periderm (one site)	severity/qPCR txtAB, differences between treatments in tubers	severity/qPCR txtAB, low s in low scab in geocaulosphere	severity / qPCR *txtB,* differences between cultivars in tuberosphere
Suppressive soil / low scab	Bacteria diversity high *Acidobacteria, Nocardioidaceae, Pseudomonadaceae, Lysobacter, Rhizobium*	NA	Bacteria quantity similar	Actinobacteria, Streptomyces	Bacteria diversity high, quantity low, network interactions high	Bacteria quantity low. *Acetobacteraceae, Paenibacillaceae, Pseudomonadaceae, Bradyrhizobiaceae*
Conducive soil / high scab	Bacteria diversity low *Deinococcus-Thermus, Firmicutes, Acetobacteraceae, Bacillaceae, Trupera*	NA	NA			
Cultivars	Snowden - moderately resistant	resistant: Yukirasha, 02005-10, moderately resistant: Snow March, Star Ruby, Snowden, susceptible: Irish Cobbler, Toyoshiro, Piruka	susceptible: Agria, David, Valfi	Nishiyutaka	Favorita - susceptible	Kariera - resistant; Agria - susceptible

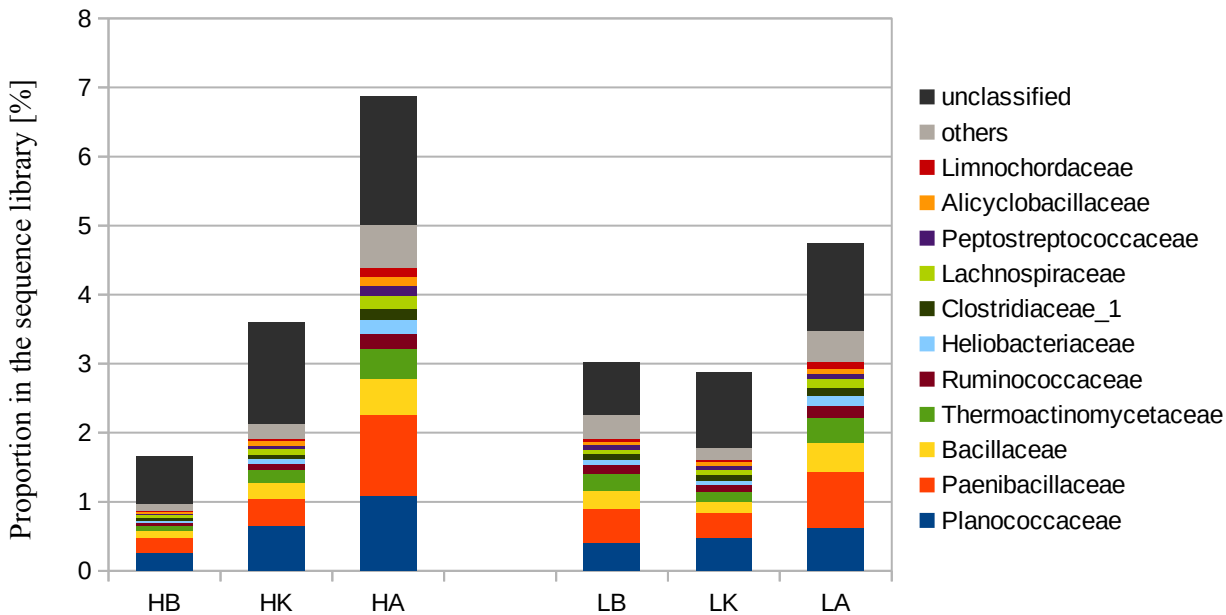
Bacteria: cultivar - resistant / low scab	NA	*Gammaproteobacteria Aquicella siphonis*	NA	NA	*Acidobacteria, Actinobacteria, Firmicutes, Geodermatophilaceae, Nocarioides, Curtobacterium*	Bacterial diversity high, network interactions high. *Streptomycetaceae, Rhizobiales, Sphingobacteraceae*
Bacteria: cultivar - susceptible / high scab	NA	*Rhodococcus, Streptomyces*	NA	NA	*Proteobacteria, Bacteroidetes, Stenotrophomonas, Variovorax, Sphingobium, Agrobacterium*	low bacterial but high archaeal and microeukaryot diversity. *Gaiellales, Micrococcales, Frankiales and Streptomycetales*
Soil compartment effect	NA	rhizosphere	tuberosphere, periderm	rhizoshere, tuber	geocaulosphere	tuberosphere, bulk
Nutrients - low scab	NA	NA	high Mg	OM	low N-NH_4, TC, OM	soil S, periderm Mg, Fe
Nutrients - high scab	NA	NA	high pH, C, N, Ca, Fe (total)			soil N, C, P, Ca
Functions	antibiosis of isolates (Meng et al. 2012)			antibiosis of isolates	high nitrogen metabolism, drug metabolism in high scab, high carbohydrate metabolism, energy metabolism, antibiotic pathways, more complex networks in low scab	

Supplementary Figure S1. Taxonomic composition of tuberosphere and bulk soil bacterial communities.

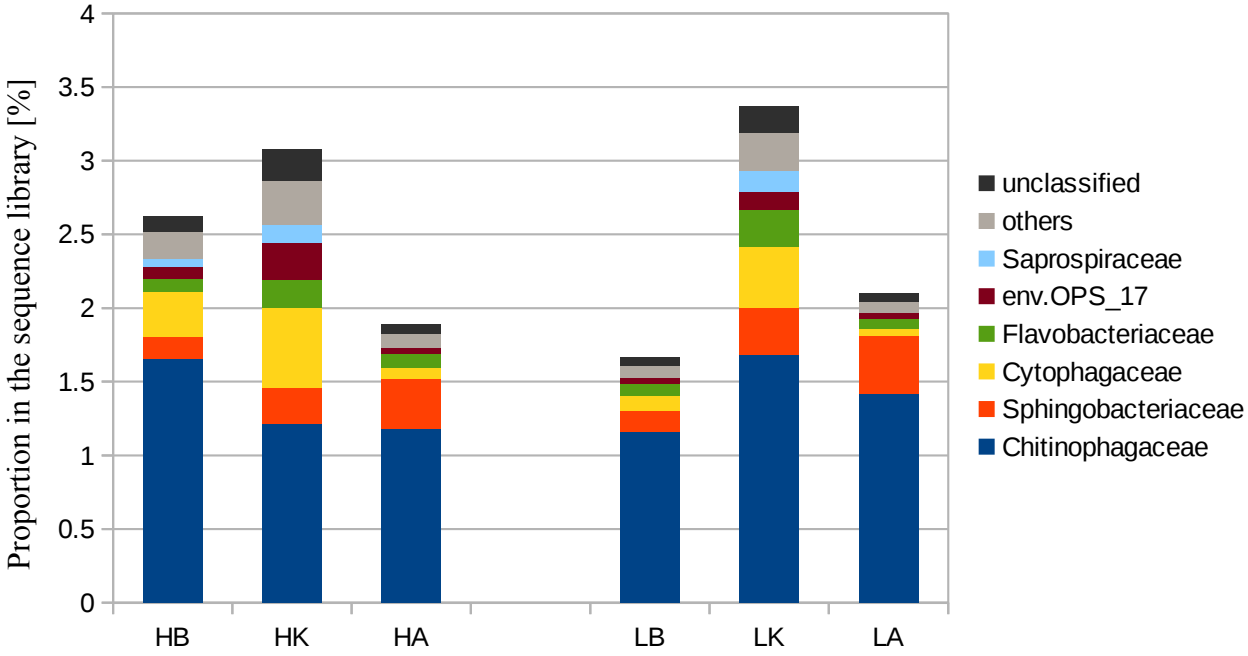
A. Relative proportions of orders within the phylum *Actinobacteria* [%].



B. Relative proportions of families within the phylum *Firmicutes* [%].

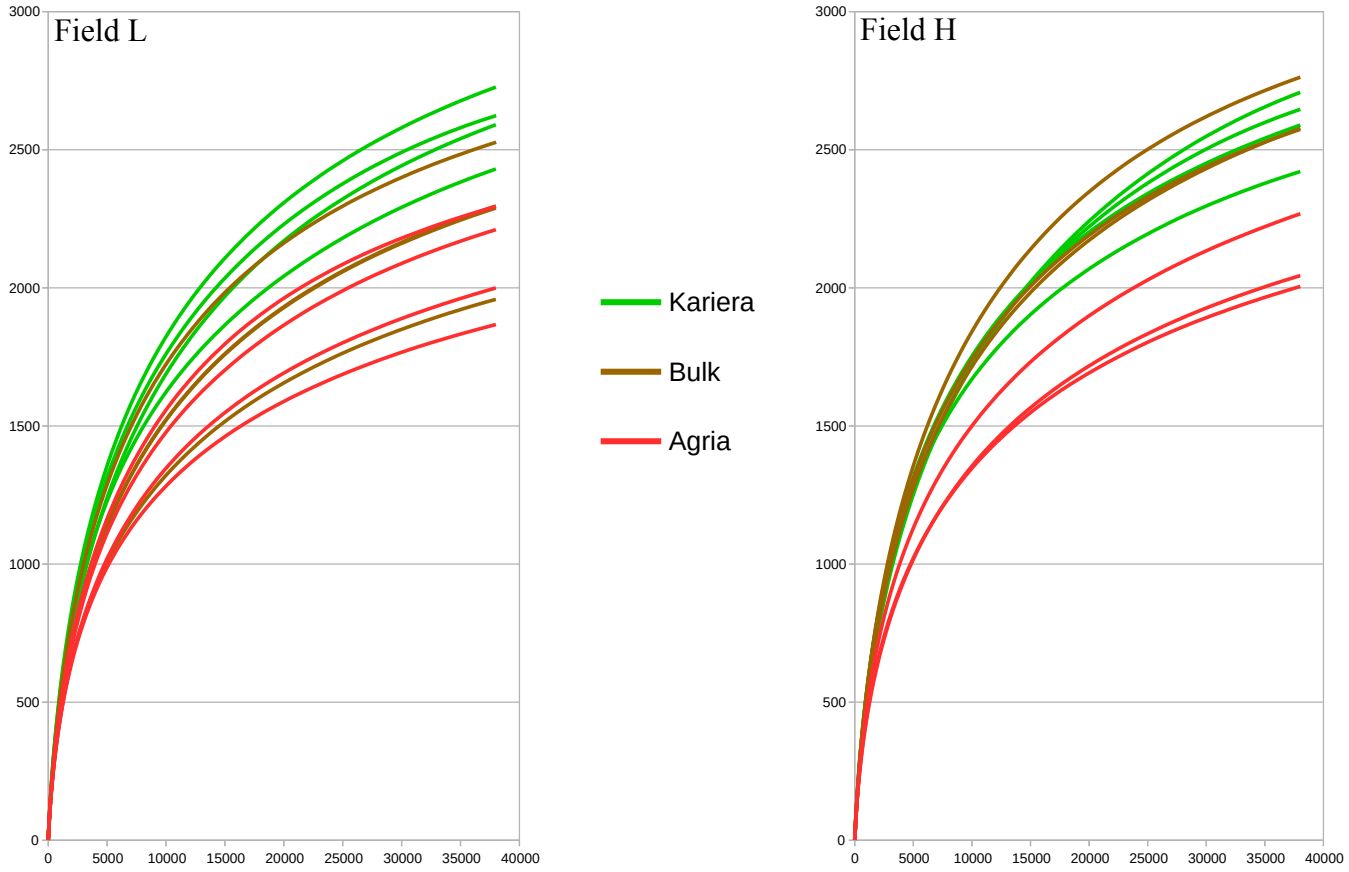


C. Relative proportions of families within the phylum *Bacteroidetes* [%].

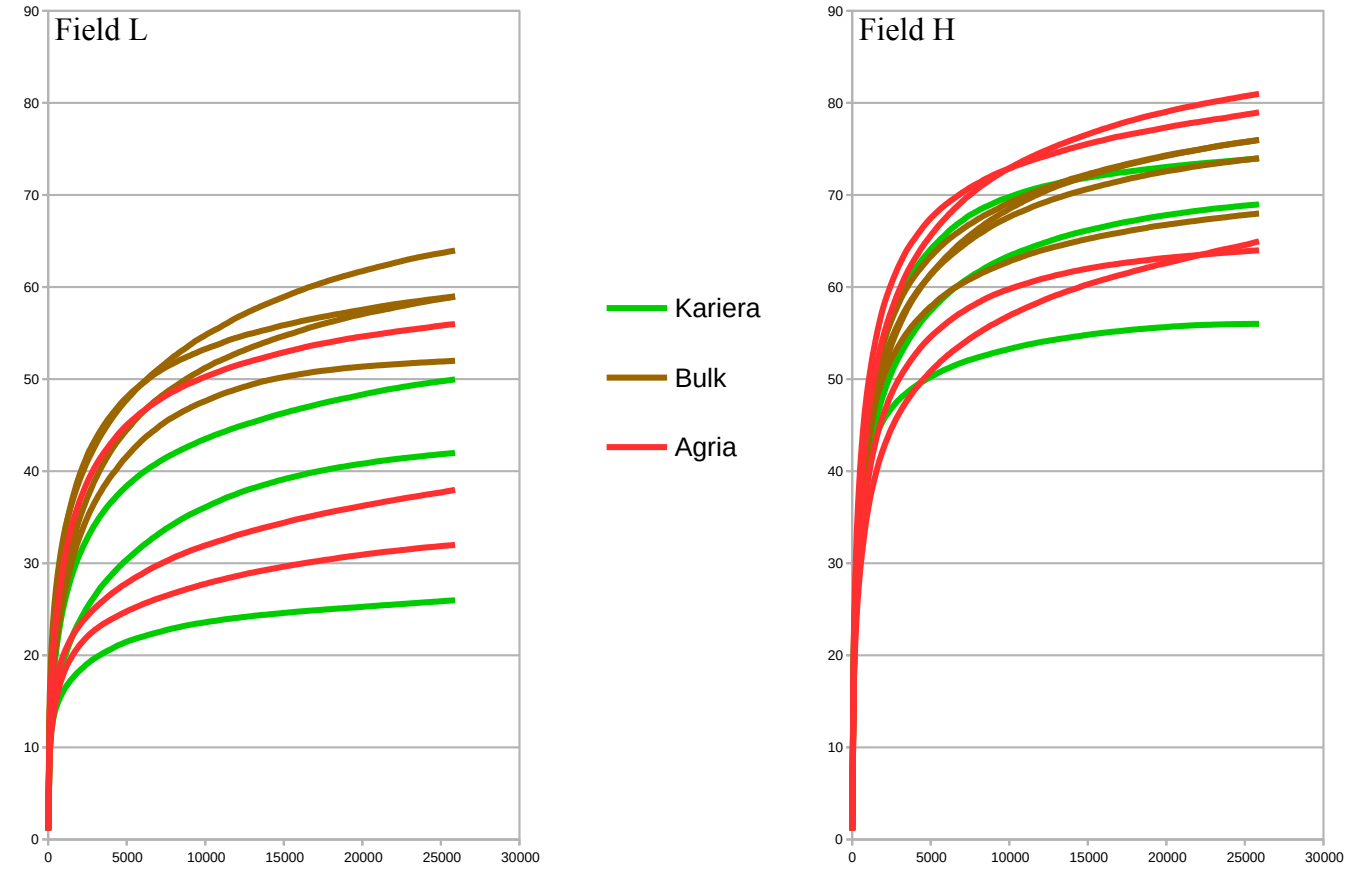


Supplementary Figure S2. Rarefaction analysis of sequence libraries of bacteria (A), archaea (B), and eukaryotes (C) from bulk (brown) and tuberosphere soil of varieties Kariera (green) and Agria (red) from suppressive field L (left) and conducive field H (right).

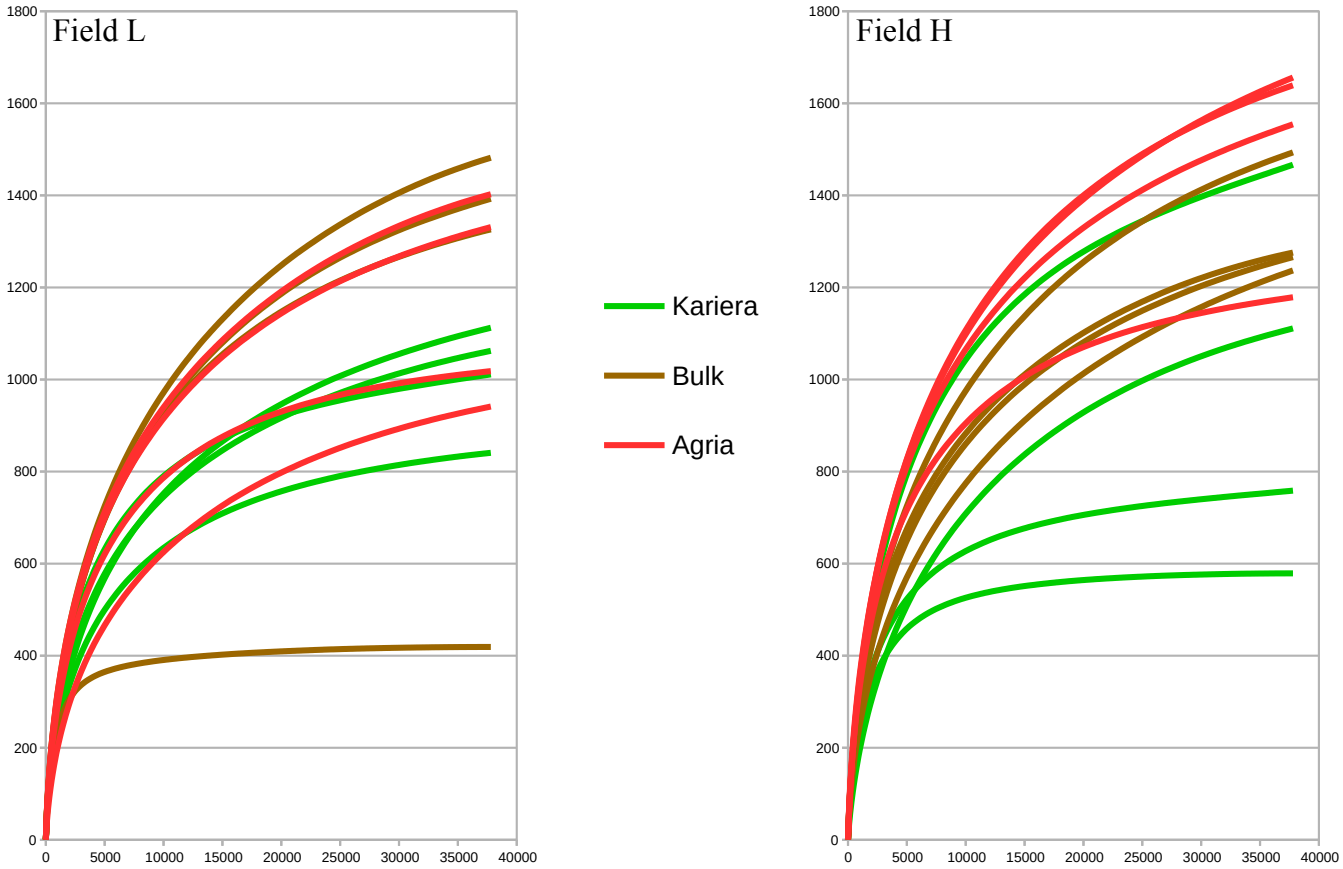
A. Bacteria



B. Archaea

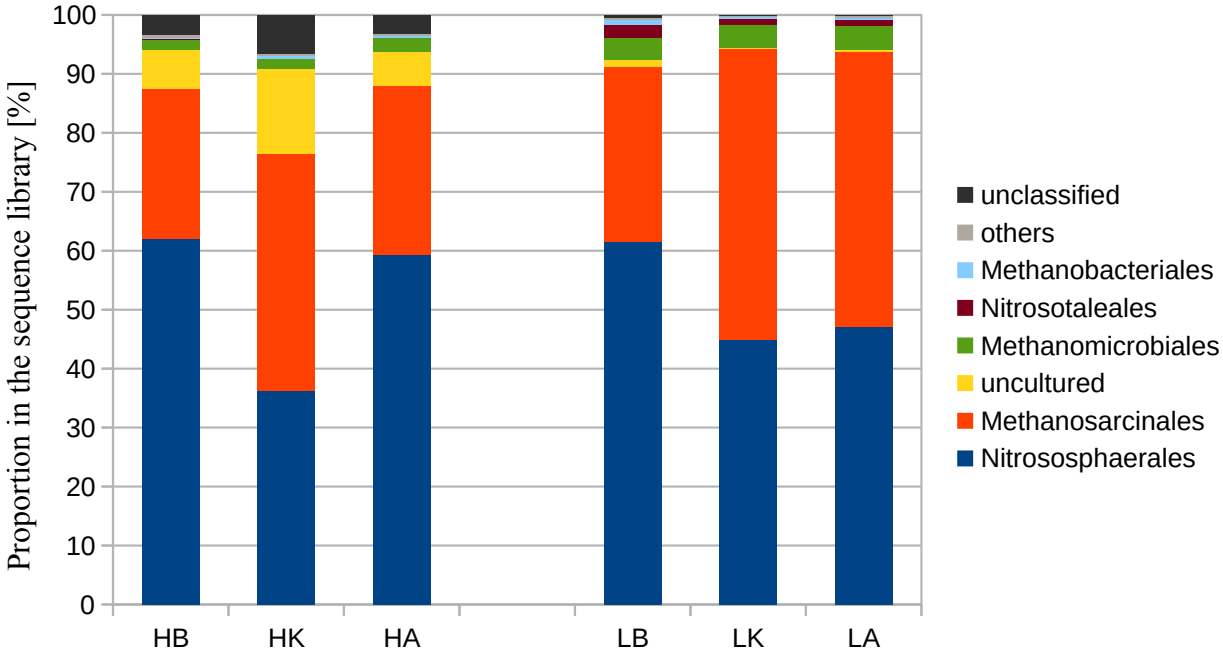


C. Eukaryotes



Supplementary Figure S3. Taxonomic composition of tuberosphere and bulk soil archaeal communities.

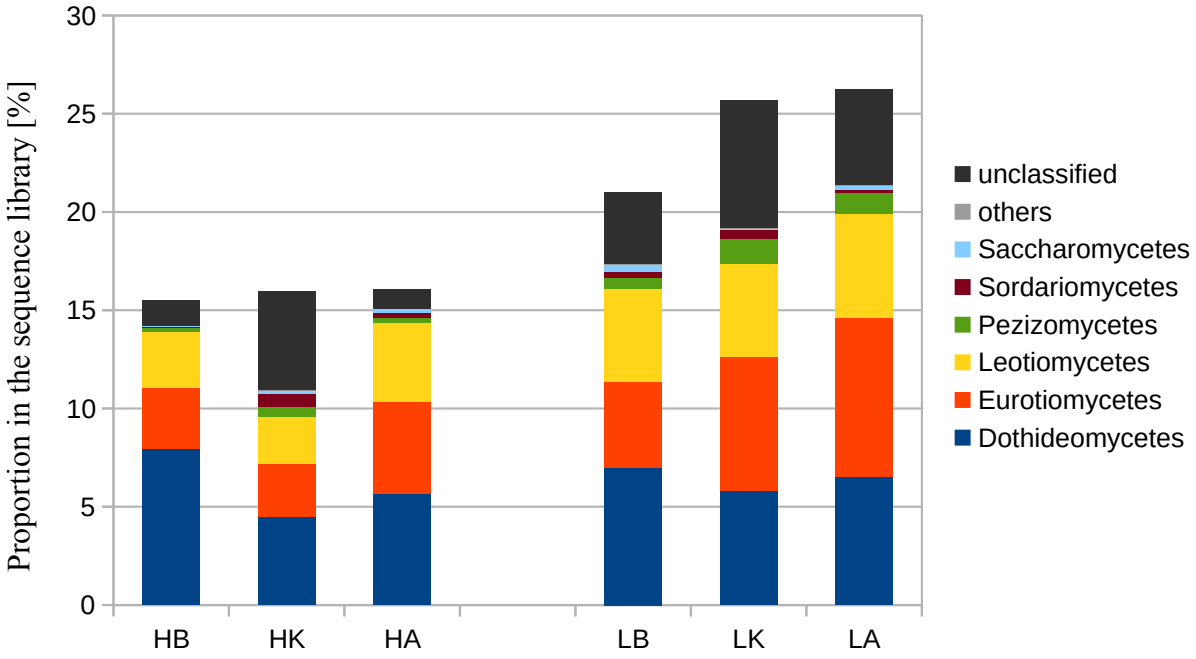
Proportions of orders within the domain *Archaea*.



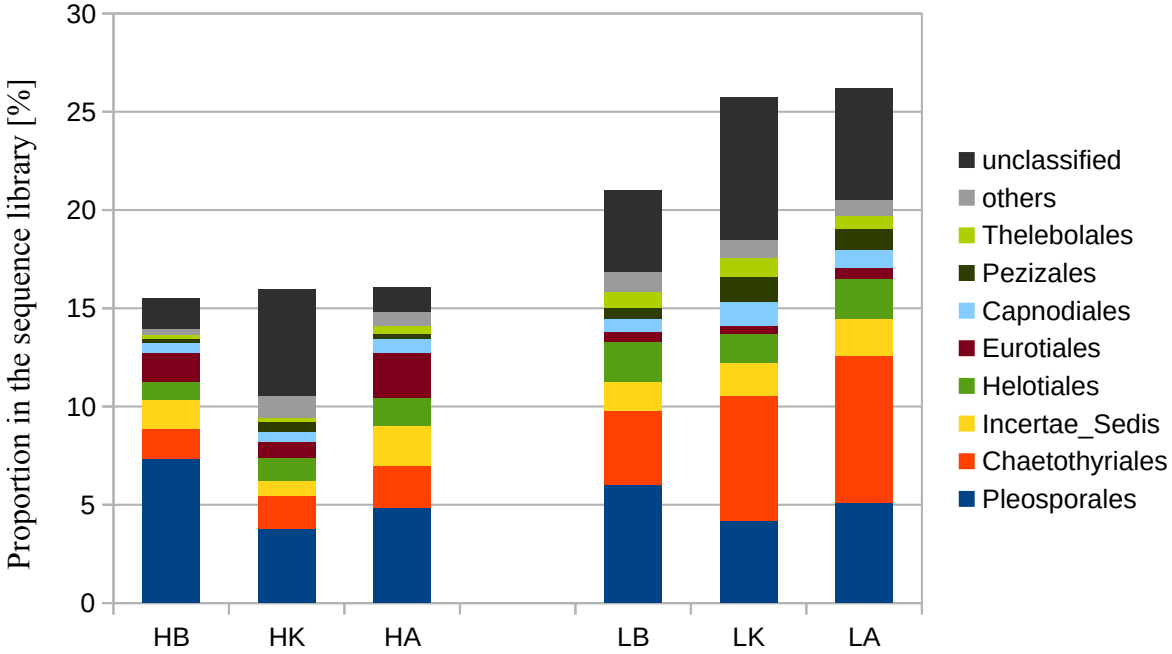
Supplementary Figure S4. Taxonomic composition of tuberosphere and bulk soil communities of micro-eukaryotes.

A. Taxonomic composition of the community of Ascomycota.

Proportions of classes within the phylum Ascomycota.



Proportions of orders within the phylum Ascomycota.



B. Proportions of taxonomic groups (classes and above) within the community of Ciliophora