The effect of susceptible and resistant potato cultivars on bacterial communities in the tuberosphere of potato in soil suppressive or conducive to common scab disease

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Running Head: Resistant potato cultivar and suppressive soil

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ABSTRACT Connections between the structure of bacterial communities in suppressive soils and potato resistance to common scab (CS) are not well understood. In this 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 compare to susceptible cultivar, while suppressive soil had lower pH (5.3 vs 5.9), N, C, P, Ca contents but higher Fe and S compared with the conducive soil. Bacteria and actinobacteria numbers were higher in the conducive soil. Copy numbers of *txtB* gene (coding for a pathogenicity determinant) were similar in both soils but were higher in the conducive soil (for periderm samples) and in the susceptible cultivar (for conducive soil samples). Taxonomic microarray analysis and Illumina sequencing of 16S rRNA genes amplicon showed that bacterial community differed between resistant vs susceptible cultivar and to a lesser extend between suppressive vs conducive soil. Bacteria participating in soil suppression belonged to *Pseudomonadaceae*, *Bradyrhizobiaceae*, *Acetobacteraceae* and *Paenibacillaceae*, while resistant cultivars selected a bacterial community resembling that of the suppressive soil, which was enriched in *Nitrospirae* and *Acidobacteria.* Thus, the analysis of soil suppressiveness×cultivar resistance interactions enabled to gain new insight to CS control in the field.

IMPORTANCE

It was demonstrated that potato cultivars susceptible and resistant to common scab select differing bacterial community and above that this trait is further modified in suppressive and conducive soil. Common scab severity was diminished by either resistant cultivar or suppressive soil but without additive effect between them. Out of the two factors, potato cultivar had a more significant influence on tuberosphere bacterial community composition than soil. Results highlighted the usefulness of both cultivar resistance and soil suppressiveness traits in

48 understanding and managing disease control of crops.

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51 **KEYWORDS** Soil bacterial community, potato rhizosphere, *Actinobacteria*, *Streptomyces*,

52 thaxtomin

Suppressive soils were 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 (1, 2). Relatively few soils with suppressive character have been described in the world to date (3). They represent a unique model for studying plant disease control. It is of prime interest to conserve their functioning, and ultimately, these soils may help us to learn how to establish suppressive character of soil at other sites (4).

It is agreed that soil suppressiveness is related to soil health and crop productivity through physico-chemical conditions or microbial communities (5, 6). Yet, suppressiveness is difficult to understand because in soil, (i) most physico-chemical factors are not independent from one another, (ii) phytopathogenic taxa may display genetic and functional diversity, and (iii) microbial communities are composed of many taxonomic groups with unknown functions and their structure is affected not only by soil but also by the plant host (4). Therefore, the assessment of suppressive soils may necessitate to target potential interactions between all these ecological factors.

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 in the USA (6, 7). 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 (4, 8). In one situation, nonpathogenic *Streptomyces* spp. were correlated with CS suppressiveness (6), and it was also hypothesized that other actinobacteria may be involved in this disease suppression (4).

In our previous investigations, two sites (Vyklantice and Zdirec) from the Czech Republic, where CS suppressive and conducive fields occur next to each other were studied both in field trials (9, 10) and pot experiments (11). The CS suppressive character of the fields differed

77 according to field location, presumably in relationship to local soil chemical properties (9). Potato
78 cultivars susceptible and resistant to CS have different ecophysiologies, and they differ in
79 chemical composition of the potato periderm, which may further influence microbial community
80 structure (12). Therefore, our hypothesis was that pathogen control via plant-enriched taxa
81 occurring in the tuberosphere was a trait associated with both the suppressive soil and cultivar.

82 CS-susceptible and resistant potato cultivars have not been compared yet in terms of their
83 respective interactions with the soil microbial community, so that was the focus of the current
84 work. We wanted to assess the relative importance of suppressive soil and resistant cultivar as
85 ecological factors shaping bacterial community properties in CS disease suppression conditions,
86 and identify the corresponding bacterial taxa. We used a field set-up that included a combination
87 of both disease suppressive vs conducive soils, and resistant vs susceptible cultivars. Bacterial
88 community structure in soil and potato tuberosphere was assessed by 16S rRNA taxonomic
89 microarray and Illumina sequencing, and these results were compared to CS severity observed on
90 tuber surface, quantity of thaxtomin biosynthetic genes *txtB*, quantities of total bacteria and
91 actinobacteria and soil and periderm chemical characteristics.

92 Currently, next generation sequencing is the preferred approach to study bacterial
93 communities. The method enables high throughput and financially-efficient description of
94 bacterial taxa present in a sample. However, the method has its drawbacks. In particular, it is not
95 highly quantitative so only a relative quantity of determined taxa can be assessed, and also it may
96 be difficult to separate sequencing errors from real diversity (13). In comparison, taxonomic
97 microarrays as a method for bacterial community assessments are more laborious (so are slower)
98 and give results only for a selected group of taxa. However, these taxa can be purposefully
99 selected and aimed at determining particular characteristics of bacterial community, and
100 microarrays are semi quantitative (14), which makes microarrays a useful complement to

Illumina sequencing. In this work, we used a validated taxonomic microarray focusing on disease suppressive soils and bacterial taxa possessing plant growth-promoting and antagonistic traits in soil environments (15, 16), which was further extended with probes focusing on CS pathogens. The microarray approach was combined with amplicon Illumina sequencing to obtain deeper insight to relationships of bacterial communities in cultivar resistance and soil suppressiveness.

RESULTS

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. 1; 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 (Supplementary Tables S2A and S4A), while in periderm it was significantly higher ($p=0.006$) in conducive than suppressive soil (Supplementary Tables S2B and S4B). The two cultivars grown in the same soil had comparable quantities of *txtB* gene copies in their periderm, yet, the number of *txtB* gene copies was significantly higher for susceptible cultivar Agria in conducive soil than in suppressive soil. In summary, CS control required resistant cultivar (independently of the soil) or suppressive soil (for susceptible cultivar).

Chemical composition of tuberosphere soil and periderm. In tuberosphere, contents of N, C, P, Ca, Fe 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; $p<0.001$; Supplementary Tables S3A and S4A). 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 conducive soil in both cultivars (ANOVA; p=0.035) and it was higher in the resistant cultivar in both soils (ANOVA; p=0.006; Supplementary Tables S3B and S4B).

In summary, CS control was connected either with lower content of N, C, P, Ca, Fe and lower soil pH in tuberosphere in suppressive soil, or with higher content of Mg in periderm of the resistant cultivar. In addition, S content was significantly higher in tuberosphere for the combination of suppressive soil x resistant cultivar.

Quantities of total bacteria and actinobacteria. In tuberosphere, the quantities of bacteria (ANOVA; p<0.001) and 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 (Supplementary Tables S2A and S4A). In periderm, the quantity of actinobacteria (ANOVA; p=0.021) was significantly higher in conducive than in resistant cultivar Kariera suppressive soil in both cultivars and was also significantly higher in susceptible cultivar Agria than Kariera in conducive soil (Supplementary Tables S2B and S4B). 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 supressive soil and resistant cultivar.

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 (15, 17) was expanded for coverage of the genus *Streptomyces*, including pathogen species *S. scabies* and relatives (Supplementary Table S1).

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. 2A). 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; $p=0.029$).

In summary, our 16S rRNA probe set was expanded to target also the CS pathogens and other *Streptomyces* taxa. The resulting taxonomic microarray evidenced significant differences in bacterial community features when comparing suppressive vs conducive soil and resistant vs susceptible cultivar.

Discriminant microarray probes according to soil and potato cultivar. Microarray probes contributing to separation of suppressive from conducive soil were Brady4 (targeting the family *Bradyrhizobiaceae*), Pseu33 (*Pseudomonadaceae*), Aceto3A (*Acetobacteraceae*), and PalgiG3 (*Paenibacillaceae*), which were significantly higher in suppressive soil, as well as Janaga 2 and 3 (targeting the genus *Janthinobacterium*), which were significantly higher in conducive soil (Supplementary Table S5A). These differences seemed enhanced by tuberosphere of susceptible cultivar Agria, based on lower hybridization of probes targeting the genus *Streptomyces* or families *Rhizobiaceae* or *Bradyrhizobiaceae* but higher signals for probes aiming

at pathogenic *Streptomyces scabiei* group.

Probes contributing by their higher signal intensity to separation of resistant cultivar and bulk soil from the susceptible cultivar Agria were Rhizo157 and Rzbc1247 (both targeting the family *Rhizobiaceae*), Strepto1, 2, and 3 (targeting the genus *Streptomyces*) and Plancto4.mB (targeting the phylum *Planctomycetes*) (Supplementary Table S5B). Relatively small numbers of probes discriminated between the two soils when assessing bulk soil samples (only 2 probes), tuberospheres of the resistant cultivar Kariera (13 probes), and tuberospheres of the susceptible cultivar Agria (26 probes). Differences between samples from a same soil implicated somewhat comparable numbers of discriminating probes (Fig. 3A).

In summary, six probes targeting contrasted bacterial taxa discriminated between suppressive and conducive soils. Higher probe signals for CS pathogen were found with the susceptible cultivar.

Bacterial community composition in bulk soil and tuberosphere by Illumina sequencing. A total of 1,213,004 16S rRNA gene sequence reads 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. 2B). When comparing both fields using significantly different OTUs (Metastats $p<0.05$), bacterial communities of bulk soils were separated, whereas they overlapped when comparisons were made for cultivar Kariera, or for cultivar Agria. The number of discriminating OTUs (Fig. 3B) 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 significantly between bulk soils,

except that *Actinobacteria* were higher and *Acidobacteria* lower in suppressive than in conducive soil (Supplementary Fig. S1A). 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; Supplementary Fig. S1A), (ii) an increase in relative proportion of *Bacteroidetes* (particularly the family *Sphingobacteraceae*) in resistant cultivar Kariera (in the two fields; Supplementary Fig. S1D), 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; Supplementary Fig. S1B,C). 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 (Supplementary Table S6B).

Diversity of bacterial communities based on rarefaction curves showed that in both soils the lowest diversity was in tuberosphere of susceptible cultivar Agria. Above that, the curves were mixed for bulk soil and both cultivars in suppressive soil, while in conducive soil they were mixed only for bulk soil and tuberosphere of resistant cultivar Kariera. Therefore, the diversity in bulk soil was more heterogeneous in suppressive soil and differed more between cultivars in conducive soil (Supplementary Fig. S2).

In summary, Illumina sequencing of 16S rRNA genes evidenced differences between resistant and susceptible cultivars, the latter displaying lower bacterial diversity. Differences were also found and between suppressive and conducive soils, but mainly for bulk soil samples.

Discriminant OTUs according to soil and potato cultivar. When considering bulk as well as tuberosphere soil samples based on the discriminating OTUs (Metastats, $p<0.05$),

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 for *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) (Supplementary Table S6A).

The tuberosphere of Agria was enriched in taxa from orders *Frankiales* (*Frankiaceae*, *Acidothermaceae*, *Geodermatophilaceae*; OTUs 63, 20, 54, 117) and *Micrococcales* (*Intrasporangiaceae*; OTUs 13, 10) (Supplementary Table S6B, Supplementray Fig. S1B) and class *Gemmatimonadetes* (*Gemmatimonadaceae*; OTU 36), while tuberosphere of Kariera displayed significant enrichment in taxa from phylum *Acidobacteria* (OTUs 51, 275, 143, 138, 76; Supplementary Table S6B, Supplementray Fig. S1A). Tuberosphere communities of both cultivars were also separated by different OTUs belonging to the same taxonomic groups. These discriminating taxa included (i) *Betaproteobacteria* i.e. *Burkholderiales* (OTU 38 in Agria vs OTU 199 in Kariera), (ii) *Alphaproteobacteria* i.e. *Sphingomonadales* (OTU 1 in Agria vs OTUs 48, 696, 282 in Kariera), (iii) *Actinobacteria* i.e. *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) (Supplementary 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*

245 discriminant OTUs.

246

247 **DISCUSSION**

248 Disease suppressiveness of Vyklantice soil L was shown in previous studies (9, 11) and
249 since the soil has been regularly interrupted by cropping sequence over long time it can be
250 considered naturally suppressive to the disease (2). In the current experiment, CS disease severity
251 was assessed again in suppressive soil L and conducive soil H, under field conditions, but this
252 time the significance of cultivar status was also investigated, by comparing two potato cultivars
253 susceptible or resistant to CS. Results indicated that CS severity of the susceptible cultivar grown
254 in the suppressive soil was as low as for (i) the resistant cultivar in the same soil, and (ii) the
255 resistant cultivar in the conducive soil. These results showed the effect of soil suppressiveness
256 and cultivar resistance on disease severity, and in particular the similar potential of both types of
257 control mechanisms.

258 In contrast to our previous work, the current set-up enabled to compare soil
259 suppressiveness and host resistance, and we further identified that soil suppressiveness was
260 significantly related to (i) low quantities of bacteria and actinobacteria and (ii) low C, N contents
261 and high S content in the tuberosphere, while cultivar resistance was related to high Mg content
262 in the periderm. In spite of extensive literature on the positive effect of S on CS severity
263 (reviewed in (18)) it does not seem that S was a key factor for CS control in our soils. A
264 combination of both cultivar resistance and soil suppressivness was related to (i) increased Fe
265 content in both tuberosphere and periderm, and (ii) changes in pathogen populations, which is in
266 agreement with our previous work (11), in which additions of available Fe to soil decreased CS
267 severity.

268 Out of the parameters related to CS severity, the quantity of bacteria including

actinobacteria was previously linked with the amount of available carbon, which differed between conducive and suppressive soils (e.g. (19)). Specifically, the quantity and community structure of actinobacteria were connected to pathogen population and its interactions (11, 20). Further to that the quantity of pathogenic streptomycetes (based on numbers of *txtB* genes) did not change with soil suppressiveness 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 (9, 21). In this study, however, 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. (8) or Tomihama et al. (22).

The increase of magnesium in resistant cultivar may be explained by several aspects of CS disease and plant metabolism. Previously, we did not find any correlation between Mg periderm content and CS, probably because the field site effect was more important (12). However, the importance of site Mg concentration was correlated to CS and quantities of thaxtomine gene *txtA* in Lazarovits et al. (23), and similarly Lacey and Wilson (24) found that CS disease severity was related to contents in exchangeable Ca, Mg, and K cations. Generally, Mg has both indirect as well as direct effects on disease (25). Magnesium functions also as a P carrier in plants, and P periderm concentration was negatively correlated with CS in many studies (e.g. (12)). Also, root–microbial activities are key factors that determine plant-available Mg release from soils, and Mg was among nutrients affecting microbial community in potato rhizosphere (26). So, we concluded that Mg connection to resistant cultivar is a result of a combination of several factors, which specifically in our experiment resulted in diminishing of CS severity. Finally, higher Fe content in resistant cultivar than susceptible cultivar regardless of the nutrient

content in soil is probably connected to better acquisition of this element from soil, either by the resistant plant itself or through its selected bacterial community as pointed by Sarikhani et al. (11). This possibility was raised also by Inceoğlu et al. (27), where potato cultivars grown in two soils yielded different contents in C, S and P while selecting microbial communities with different functional capabilities

Bacterial community structure was identified as a major aspect of soil suppressiveness to CS (8). In our microarray study, *Pseudomonadaceae*, *Bradyrhizobiaceae*, *Acetobacteraceae* and *Paenibacillaceae* were associated with disease suppressiveness, and all four include plant-beneficial species and strains (3, 28). This was not expected, as the last three families were more prevalent in CS conducive than suppressive soil in Michigan (8). Our Illumina sequencing indicated that *Rhizobiales* and *Planctomycetales* were enriched in suppressive soil, as in Cha et al. (29), but *Actinobacteria*, *Chloroflexi*, *Bacillaceae,* and *Chitinophagaceae* that increased with CS suppressiveness induced by rice bran (22) were more prevalent in the conducive soil here. Therefore, different biocontrol consortia may be at work in different types of suppressive soils. Several phyla were more prevalent in the susceptible cultivar Agria, whereas others were more prevalent in the resistant cultivar Kariera, including *Nitrospirae* and *Acidobacteria* also enriched in suppressive soil studied by Cha et al. (29).

In this work, Illumina sequencing showed that major effects were due to resistant and susceptible cultivars and microarray analysis also evidenced the effect of suppressive versus conducive soils. The most relevant study to compare is that of Rosenzweig et al. (8) because they also studied CS suppressive soils using next generation sequencing, but they used a single, relatively CS resistant potato variety (Snowden), so they could not demonstrate the effect of cultivar in combination with soil suppressivity. In comparison, Weinert et al. (30) showed the effect of potato cultivar on rhizosphere bacteria selection, while Inceoğlu et al. (27) described

317 more profound influence of soil type over potato cultivar on bacterial rhizosphere communities
318 but studied two soil types not disease suppressive and conducive soil. Therefore, our results
319 brought new insight to bacterial community differences with respect to suppression of CS
320 disease, possibly with a more general implication to other pathogen-plant systems.

321 Finally, the data obtained by Illumina sequencing and taxonomic microarrays are
322 differently biased and to some extend showed different results. Microarray showed more
323 differences between the two soils, while Illumina sequencing stressed differences between
324 cultivars. In particular, microarray demonstrated increase of *Streptomyces* (*Actinobacteria*),
325 *Bradyrhizobium*, *Burkholderia* (*Proteobacteria*) or *Nitrospira* (*Nitrospirae*) in suppressive soil,
326 while increase of *Acidibacteria*, *Pseudomonas*, *Agrobacterium* and *Janithobacterium*
327 (*Proteobacteria*) in conducive soil. *Streptomyces* and *Rhizobiaceae* were associated with resistant
328 cultivar Kariera, while *Burkholderia* and *Stingomonas* (*Proteobacteria*) were associated with
329 susceptible cultivar Agria. Illumina sequencing discriminated the two soils similarly to
330 microarray by increase of *Bradyrhizobiaceae* and other *Proteobacteria*, but also *Bacteroidetes*
331 and *Firmicutes* in suppressive soil, while different families of *Proteobacteria*, *Actinobacteria* and
332 *Firmicutes* increased in conducive soil. For resistant cultivar Kariera, *Chloroflexi*, *Gaiellales*
333 (*Actinobacteria*) were found enriched, while in susceptible cultivar Agria, similarly to microarray
334 results *Burkholderia* and *Sphinomonas* (*Proteobacteria*) and *Actinobacteria* were enriched. We
335 explain the differences by specific characteristics of the two methodological approaches (14–16,
336 31, 32).

337 In conclusion, we demonstrated the cultivar-specific community selection with respect to
338 their susceptibility or resistance to CS and above that we included comparison of this trait in
339 suppressive and conducive soils. We showed that CS can be controlled either with resistant
340 cultivar or with suppressive soil, with no additive effect between them. Out of the two factors,

341 potato cultivar had a higher effect on tuberosphere bacterial community composition than soil in
342 our experiment. Results highlighted the usefulness of both cultivar resistance and soil
343 suppressiveness traits in understanding and managing disease control of crops.
344
345 **MATERIALS AND METHODS**
346 **Sites.** Vyklantice is a site where fields suppressive (L for low disease severity), and
347 conducive (H for high disease severity) to potato CS occur at about 100 m distance. The two
348 fields differ in common scab severity by observations over 30 years, while their geological
349 context, soil type, climate and management are similar. The fields were regularly planted under a
350 four-year crop rotation system including rapeseed, clover, potatoes, and grains (wheat or oats) in
351 the past two decades (9).
352 **Field experiment.** Potatoes were planted in the beginning of May and samples of bulk
353 soil, tuberosphere soil and potatoes were collected after 80 days. One susceptible cultivar (Agria)
354 and another resistant (Kariera) to CS were used. Potatoes were all certified seed tubers (common
355 scab below 5% of surface). Four plots of each cultivar were planted at each field and the plots
356 were arranged in a Latin square design. Each plot was planted with 3 rows of 12 potato plants (36
357 plants) separated by 50 cm of bare soil. Fields were fertilized with 100 kg N/ha (ammonium
358 sulphate, 21% N), 35 kg P/ha (monocalcium phosphate, 35% P_2O_5), and 60 kg K/ha (potassium
359 salt, 50% K_2O). Potatoes were treated with pesticides, once with Nurelle D (EC) (chlorpyrifos,
360 cypermethrin) at 0.6 l/ha to prevent Colorado potato beetle (*Leptinotarsa decemlineata*), and
361 twice with Acrobat MZ (dimethomorph, mancozeb) at 2 kg/ha and Ridomil Gold MZ Pepite
362 (mancozeb, metalaxyl-M) at 2.5 kg/ha against the potato blight. Fungicides were not used.
363 **Sampling.** One potato plant growing in the center of each plot was sampled. Potatoes
364 from this plant were collected and washed in distilled water. All potatoes were carefully pealed

using a sterile potato peeler (taking approximately 1 mm thick periderm samples), peels were homogenized and mixed, and subsamples (1 subsample per plant) were taken for further analyses ('periderm' samples). Tuberosphere soil samples were collected no further than 3 mm from a potato tuber (for details see (9)). Bulk soil was collected at a distance of approximately 30 cm from the closest plant within each plot using a small sterile spade (1 sample per plot). Common scab severity was evaluated on 20 potato tubers per plot using a 9-degree scale (33). 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.

Soil and potato periderm analyses. To determine total soil C, N, and S contents, 2-g samples of homogenized soil from both bulk soil and tuberosphere were dried, milled, and analyzed using Vario MAX CNS analyzer (Elementar Analysensysteme, Hanau, Germany). To determine all other elements, soil subsamples were leached with boiling nitro-hydrochloric acid (aqua regia) and assessed by optical emission spectroscopy with inductively coupled plasma (ICP-OES) by Aquatest Inc. (Prague, Czech Republic). Analyses of potato periderm were performed by service laboratory of the Institute of Botany (Trebon, Czech Republic). For total nitrogen analysis, 1-3 mg dried periderm was mineralized by modified Kjeldahl method in H_2SO_4 with catalyzer at 360°C. For total phosphorus, 20 mg of dried periderm was sequentially decomposed by HNO_3 and $HClO_4$. In 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 periderm were determined by atomic absorption spectrometry using AAS spectrometer ContrAA 700 (Analytik Jena, Jena, Germany) after mineralization with nitro-hydrochloric acid.

Soil DNA extraction. Soil samples from tuberosphere and bulk soil were homogenized and subsamples of 0.5 g were used for DNA extraction by method SK described by Sagova-

389 Mareckova et al. (34). Briefly, the method is based on bead-beating and phenol/chloroform
390 extraction followed by purification with $CaCl_2$ and GeneClean Turbo kit (MP Biomedicals, Santa
391 Ana, CA). For DNA extraction from potato periderm, 3 g of periderm samples were fine cut in
392 sterile Petri dish, homogenized, and a 0.3 g subsample was processed in the same way as soil
393 samples to obtain total periderm DNA.

394 **Real-time PCR (qPCR).** Quantifications were performed with primers eub338f (5'-
395 ACTCCTACGGGAGGCAGCAG-3) (35) and eub518r (5'-ATTACCGCGGCTGCTGG-3') (36)
396 amplifying a 197 bp fragment of the 16S rRNA gene from bacteria, act235f (5'-
397 CGCGGCCTATCAGCTTGTTG-3') (37) and eub518r yielding a 280 bp product for
398 *Actinobacteria*, and StrepF (5'-GCAGGACGCTCACCAGGTAGT-3') and StrepR (5'-
399 ACTTCGACACCGTTGTCCTCAA-3') yielding a 72 bp amplicon of the thaxtomin biosynthetic
400 gene *txtB* (38), respectively. The analyses were done on a StepOne Plus Real-Time PCR System
401 (Applied Biosystems, Foster City, CA) using 96-well plates with GoTaq qPCR Master Mix
402 (Promega) containing SYBR Green as a double-stranded DNA binding dye. The reaction mixture
403 contained in a total volume of 15 µl: 1× GoTaq qPCR Master Mix, 0.2 µM primers, and 0.2-2 ng
404 diluted DNA sample. For all of the mentioned targets the PCR cycling protocol consisted of
405 initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, 60°C for 30 s and
406 72°C for 30 s. Melting curves were recorded to ensure qPCR specificity. Baseline and threshold
407 calculations were performed with the StepOne v. 2.2.2 software. The inhibition was tested by
408 serial DNA dilution from each site, and the dilutions without inhibition of qPCR reactions were
409 used for quantification. All qPCR measurements were done in duplicate. Standards for qPCR
410 were prepared by cloning fragments of target genes from *Streptomyces europaeiscabiei* DSM
411 41802 in pGEM-T Easy vector system (Promega). After PCR verification and isolation of cloned
412 constructs by Pure Yield Plasmid Miniprep System (Promega), a linear standard was prepared by

413 cleaving with SalI enzyme (New England Biolabs, UK) in a 200 µl reaction mixture containing
414 1× reaction buffer, 2 µg circular plasmid, and 20 U restriction endonuclease for 2 h in 37°C. The
415 linearized plasmid DNA was purified by phenol-chloroform extraction. Aliquots of linearized
416 and purified standard diluted to 20 ng /µl were stored in -70°C. Results were expressed per g dry
417 soil. All results (including for *txtB*) were above detection limit.

418 **16S rRNA gene-based taxonomic microarray.** A taxonomic microarray based on DNA
419 probes targeting 16S rRNA genes representing 19 bacterial phyla at different taxonomic levels
420 (15) was used to assess soil samples from potato fields. This microarray was validated previously
421 (15, 17). Twelve probes targeting the genus *Streptomyces*, as well as *S. scabies* and relatives
422 (Supplementary Table S1) were added to the previous probe set (1033 probes) in this study. The
423 probe KO 08 (39) for genus *Streptomyces* was obtained via the *probeBase* server (40)
424 (http://probebase.csb.univie.ac.at). The other 11 probes (20-mers) were designed in this study
425 using ARB sofware (41) (http://www.arb-home.de) and the parameters of the Probe Design
426 function chosen by Sanguin et al. (31, 42). Probe specificity was tested with the Probe Match
427 function in ARB against the reference Silva-104 and with the TestProbe online tool against Silva
428 126 database (43) (http://www.arb-silva.de), at the weighted mismatch value of 1.5 (15).
429 Hybridization properties of probes (e.g. melting temperature, potential formation of secondary
430 structures and 3'dimers) were further tested *in silico*, according to Sanguin et al. (31, 42).

431 Universal bacterial primers T7-pA (forward; TAATACGACTCACTATAG-
432 AGAGTTTGATCCTGGCTCAG) and pH (reverse; AAGGAGGTGATCCAGCCGCA) were
433 used to amplify 16S rRNA genes from total DNA extracts (44). Primer T7-pA includes at the 5'
434 end the sequence of T7 promoter, which enabled T7 RNA polymerase-mediated *in vitro*
435 transcription using purified PCR products as templates. PCR reactions were carried out using Taq
436 Expand High Fidelity (Roche Applied Science, Meylan, France) and cycling conditions described

437 in Kyselková et al. (15). Purified PCR products (50 ng/µl) were fluorescently labelled (Cy3) by
438 *in vitro* transcription, according to Stralis-Pavese et al. (45). Purified RNA was fragmented by
439 incubation with $ZnSO_4$, as described (45), and 400 ng subjected to hybridization on the
440 microarray. Each probe was present in four copies per slide, and two slides were hybridized per
441 sample.

442 Hybridization was carried out according to Sanguin et al. (31). Slides were scanned at 532
443 nm, images were analyzed with GenePix Pro 7 (Molecular Devices, Sunnyvale, CA), and spot
444 quality was checked visually, as described previously (31). Data filtration was conducted using R
445 3.3.0 (46) (http://www.r-project.org). Hybridization of a given spot was considered positive when
446 80% of the spot pixels had intensity higher than the median local background pixel intensity plus
447 twice the standard deviation of the local background. Intensity signals (median of signal minus
448 background) were replaced by their square root value and intensity of each spot was then
449 expressed as a fraction of the total intensity signal of the basic pattern it belongs to (42). Finally,
450 a given feature probe was considered as truly hybridized when (i) hybridization signals were
451 superior to the mean signal of the negative controls and (ii) at least 3 of 4 replicate spots
452 displayed positive hybridization (15).

453 **Illumina MiSeq sequencing and analysis**. From the DNA samples, a fragment of the
454 bacterial 16S rRNA gene including the variable region V4 was amplified by PCR using universal
455 primers with overhang adapters CS1-515F (5'-
456 ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGTAA-3') and CS2-806R 5'-
457 TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT-3') (47). Construction of
458 amplicon libraries and sequencing using MiSeq sequencer (Illumina, San Diego, CA) were done
459 at the DNA Services Facility, Research Resources Center, University of Illinois (Chicago, MI).
460 Resulting paired sequence reads were merged, filtered, aligned using reference alignment from

the Silva database (43), and chimera checked using integrated Vsearch tool (48) according to the MiSeq standard operation procedure (Miseq SOP, February 2018) (49) in Mothur v. 1.39.5 software (50). A taxonomical assignment of sequence libraries was performed in Mothur using the Silva Small Subunit rRNA Database, release 128 (51) 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 software (13), 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 (52). Analysis of molecular variance (AMOVA) (53) was based on a matrix of Yue-Clayton theta distances. Metastats analysis (54) was used to detect differentially represented OTUs.

Statistical analyses. Analysis of variance (ANOVA) and Fisher LSD tests were used to test differences between soils and cultivars for soil chemical parameters and copy numbers of bacterial and actinobacterial 16S rRNA genes, and *txtB* genes in soil and periderm samples. Permutational multivariate analysis of variance (PERMANOVA) was used to compare distance matrices between microarray samples (55). AMOVA was used to test differences between distance matrices (Yue-Clayton Theta) between Illumina samples. The distance matrices were plotted by Sammon's Multidimensional Scaling (56).

Accession number. MiSeq 16S rRNA gene amplicon sequences have been deposited in the NCBI Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) as BioProject PRJNA474544.

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487

488 **References**

489 1. Baker K, Cook RJ. 1974. Biological control of plant pathogens.Biological control of plant
490 pathogens. W.H. Freeman and Co., San Francisco.
491 2. Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Mateille T, Steinberg C. 2007.
492 Soil health through soil disease suppression: Which strategy from descriptors to
493 indicators? Soil Biol Biochem 39:1–23.
494 3. Kyselková M, Moënne-Loccoz Y. 2012. Pseudomonas and other Microbes in Disease-
495 Suppressive Soils, p. 93–140. *In* Lichtfouse E (ed.), Organic Fertilisation, Soil Quality and
496 Human Health, Sustainable Agriculture Reviews 9. Springer Science+Business Media
497 B.V., Dordrecht.
498 4. Kinkel LL, Bakker MG, Schlatter DC. 2011. A Coevolutionary Framework for Managing
499 Disease-Suppressive Soils. Annu Rev Phytopathol 49:47–67.
500 5. Larkin RP. 2008. Relative effects of biological amendments and crop rotations on soil
501 microbial communities and soilborne diseases of potato. Soil Biol Biochem 40:1341–1351.
502 6. Meng Q, Yin J, Rosenzweig N, Douches D, Hao JJ. 2012. Culture-Based Assessment of
503 Microbial Communities in Soil Suppressive to Potato Common Scab. Plant Dis 96:712–
504 717.
505 7. Lorang JM, Liu D, Anderson NA, Schottel JL. 1995. Identification of potato scab inducing
506 and suppressive species of Streptomyces. Phytopathology 85:261–268.
507 8. Rosenzweig N, Tiedje JM, Quensen JF, Meng Q, Hao JJ. 2012. Microbial Communities
508 Associated with Potato Common Scab-Suppressive Soil Determined by Pyrosequencing

509 Analyses. Plant Dis 96:718–725.

510 9. Sagova-Mareckova M, Daniel O, Omelka M, Kristufek V, Divis J, Kopecky J. 2015.
511 Determination of factors associated with natural soil suppressivity to potato common scab.
512 PLoS One 10:1–13.

513 10. Sagova-Mareckova M, Omelka M, Kopecky J. 2017. Sequential analysis of soil factors
514 related to common scab of potatoes. FEMS Microbiol Ecol 93:fiw201.

515 11. Sarikhani E, Sagova-Mareckova M, Omelka M, Kopecky J. 2017. The effect of peat and
516 iron supplements on the severity of potato common scab and bacterial community in
517 tuberosphere soil. FEMS Microbiol Ecol 93:fiw206.

518 12. Krištůfek V, Diviš J, Omelka M, Kopecký J, Sagová-Marečková M. 2015. Site, year and
519 cultivar effects on relationships between periderm nutrient contents and common scab
520 severity. Am J Potato Res 92:473–482.

521 13. Edgar RC. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon
522 reads. Nat Methods 10:996–998.

523 14. Paliy O, Agans R. 2012. Application of phylogenetic microarrays to interrogation of
524 human microbiota. FEMS Microbiol Ecol 79:2–11.

525 15. Kyselková M, Kopecký J, Frapolli M, Défago G, Ságová-Marecková M, Grundmann GL,
526 Moënne-Loccoz Y. 2009. Comparison of rhizobacterial community composition in soil
527 suppressive or conducive to tobacco black root rot disease. ISME J 3:1127–38.

528 16. Kyselková M, Almario J, Kopecký J, Ságová-Marečková M, Haurat J, Muller D,
529 Grundmann GL, Moënne-Loccoz Y. 2014. Evaluation of rhizobacterial indicators of
530 tobacco black root rot suppressiveness in farmers' fields. Environ Microbiol Rep 6:346–
531 53.

532 17. Bouffaud M-L, Kyselková M, Gouesnard B, Grundmann G, Muller D, Moënne-Loccoz Y.

2012. Is diversification history of maize influencing selection of soil bacteria by roots? Mol Ecol 21:195–206.

18. Dees MW, Wanner LA. 2012. In Search of Better Management of Potato Common Scab. Potato Res 55:249–268.

19. McDaniel MD, Tiemann LK, Grandy AS. 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol Appl 24:560–570.

20. Schottel JL, Shimizu K, Kinkel LL. 2001. Relationships of in vitro pathogen inhibition and soil colonization to potato scab biocontrol by antagonistic Streptomyces spp. Biol Control 20:102–112.

21. Kobayashi A, Kobayashi YO, Someya N, Ikeda S. 2015. Community Analysis of Root- and Tuber-Associated Bacteria in Field-Grown Potato Plants Harboring Different Resistance Levels against Common Scab. Microbes Environ 30:301–309.

22. Tomihama T, Nishi Y, Mori K, Shirao T, Iida T, Uzuhashi S, Ohkuma M, Ikeda S. 2016. Rice Bran Amendment Suppresses Potato Common Scab by Increasing Antagonistic Bacterial Community Levels in the Rhizosphere. Phytopathology 106:719–728.

23. Lazarovits G, Hill J, Patterson G, Conn KL, Crump NS. 2007. Edaphic Soil Levels of Mineral Nutrients, pH, Organic Matter, and Cationic Exchange Capacity in the Geocaulosphere Associated with Potato Common Scab. Phytopathology 97:1071–1082.

24. Lacey MJ, Wilson CR. 2001. Relationship of common scab incidence of potatoes grown in Tasmanian ferrosol soils with pH, exchangeable cations and other chemical properties of those soils. J Phytopathol 149:679–683.

25. Huber DM, Jones JB. 2013. The role of magnesium in plant disease. Plant Soil 368:73–85.

26. Barnett BA, Holm DG, Koym JW, Wilson RG, Manter DK. 2015. Site and Clone Effects

on the Potato Root-Associated Core Microbiome and its Relationship to Tuber Yield and Nutrients. Am J Potato Res 92:1–9.

27. Inceoğlu Ö, Salles JF, van Elsas JD. 2012. Soil and Cultivar Type Shape the Bacterial Community in the Potato Rhizosphere. Microb Ecol 63:460–470.

28. Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A. 2014. Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–7.

29. Cha JY, Han S, Hong HJ, Cho H, Kim D, Kwon Y, Kwon SK, Crusemann M, Bok Lee Y, Kim JF, Giaever G, Nislow C, Moore BS, Thomashow LS, Weller DM, Kwak YS. 2016. Microbial and biochemical basis of a Fusarium wilt-suppressive soil. ISME J 10:119–129.

30. Weinert N, Meincke R, Gottwald C, Heuer H, Schloter M, Berg G, Smalla K. 2010. Bacterial diversity on the surface of potato tubers in soil and the influence of the plant genotype. FEMS Microbiol Ecol 74:114–123.

31. Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moënne-Loccoz Y, Grundmann GL. 2006. Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on Agrobacterium spp. and bacterial communities. Appl Environ Microbiol 72:4302–12.

32. Donn S, Almario J, Muller D, Moënne-Loccoz Y, Gupta VVSR, Kirkegaard JA, Richardson AE. 2014. Rhizosphere microbial communities associated with Rhizoctonia damage at the field and disease patch scale. Appl Soil Ecol 78:37–47.

33. Wenzl H, Demel J. 1967. Bildskalen für die Beurteilung von Kartoffelschorf und Rhizoctonia-Pocken. Der Pflanzenarzt 20:77–78.

34. Sagova-Mareckova M, Cermak L, Novotna J, Plhackova K, Forstova J, Kopecky J. 2008. Innovative methods for soil DNA purification tested in soils with widely differing characteristics. Appl Environ Microbiol 74:2902–2907.

581 35. Lane DJ. 1991. 16S/23S rRNA Sequencing. Nucleic acid Tech Bact Syst 115–175.

582 36. Muyzer G, De Waal EC, Uitterlinden AG. 1993. Profiling of complex microbial
583 populations by denaturing gradient gel electrophoresis analysis of polymerase chain
584 reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700.

585 37. Stach JEM, Maldonado LA, Ward AC, Goodfellow M, Bull AT. 2003. New primers for
586 the class Actinobacteria: Application to marine and terrestrial environments. Environ
587 Microbiol 5:828–841.

588 38. Qu X, Wanner LA, Christ BJ. 2008. Using the TxtAB operon to quantify pathogenic
589 Streptomyces in potato tubers and soil. Phytopathology 98:405–12.

590 39. Franke-Whittle IH, Klammer SH, Insam H. 2005. Design and application of an
591 oligonucleotide microarray for the investigation of compost microbial communities. J
592 Microbiol Methods 62:37–56.

593 40. Greuter D, Loy A, Horn M, Rattei T. 2016. probeBase--an online resource for rRNA-
594 targeted oligonucleotide probes and primers: new features 2016. Nucleic Acids Res
595 44:D586-9.

596 41. Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T,
597 Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S,
598 Hermann S, Jost R, König A, Liss T, Lüssmann R, May M, Nonhoff B, Reichel B,
599 Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A,
600 Schleifer K-H. 2004. ARB: a software environment for sequence data. Nucleic Acids Res
601 32:1363–71.

602 42. Sanguin H, Herrera A, Oger-Desfeux C, Dechesne A, Simonet P, Navarro E, Vogel TM,
603 Moënne-Loccoz Y, Nesme X, Grundmann GL. 2006. Development and validation of a
604 prototype 16S rRNA-based taxonomic microarray for Alphaproteobacteria. Environ

Microbiol 8:289–307.

43. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590-6.

44. Bruce KD, Hiorns WD, Hobman JL, Osborn AM, Strike P, Ritchie DA. 1992. Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. Appl Environ Microbiol 58:3413–6.

45. Stralis-Pavese N, Sessitsch A, Weilharter A, Reichenauer T, Riesing J, Csontos J, Murrell JC, Bodrossy L. 2004. Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers. Environ Microbiol 6:347–63.

46. R Core Team. 2017. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria.

47. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522.

48. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.

49. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl Environ Microbiol 79:5112–5120.

50. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: Open-source, platform-independent, community-

supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541.

51. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648.

52. Yue JC, Clayton MK. 2005. A similarity measure based on species proportions. Commun Stat - Theory Methods 34:2123–2131.

53. Martin AP. 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. Appl Environ Microbiol 68:3673–82.

54. White JR, Nagarajan N, Pop M. 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol 5:e1000352.

55. McArdle BH, Anderson MJ. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology 82:290–297.

56. Venerables WN, Ripley BD. 2002. Modern applied statistics with S. Springer, New York.

643 **Figures**

644

645 **FIG 1** Severity of common scab of susceptible cultivar Agria and resistant cultivar Kariera in
646 suppressive and conducive soils (means ± standard deviations, n = 4). Statistical significance
647 between treatments (ANOVA) are shown with letters a and b.

648

649 **FIG 2** Bacterial communities in tuberosphere of CS-susceptible cultivar Agria (circles) and CS-
650 resistant cultivar Kariera (squares), and in bulk soil (pentagons) assayed by 16S rRNA taxonomic
651 microarray (A) and 16S rRNA gene Illumina amplicon sequencing (B) in CS-suppressive (open
652 symbols) and CS-conducive soils (grey symbols). Non-metric multidimensional scaling of
653 distance matrices was based on Yue-Clayton theta calculator.

654

655 **FIG 3** Differences between bacterial communities in tuberosphere of CS-susceptible cultivar
656 Agria (circles) and CS-resistant cultivar Kariera (squares), and in bulk soil (pentagons) assayed
657 by 16S rRNA taxonomic microarray (A) and 16S rRNA gene Illumina amplicon sequencing (B)
658 in CS-suppressive (L, open symbols) and CS-conducive (H, grey symbols) soils. Numbers
659 indicate the numbers of probes (A) and OTUs (B) significantly contributing to the difference
660 between samples in pairwise comparisons (Metastats, $p < 0.05$).