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Soil bacterial community in potato tuberosphere following repeated applications of a common scab suppressive antagonist

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ABSTRACT

Disease suppressive soils are important for managing soil-borne diseases that cannot be controlled with chemicals. One such disease is the potato common scab caused by *Streptomyces* species. Suppressiveness against common scab can develop spontaneously in fields where potato is grown for years without interruption, and this has been attributed to non-pathogenic *Streptomyces* strains. *Streptomyces* spp. have been used as inoculants in biological control, but their long-term effects have gained less attention. In our previous studies, a non-pathogenic *Streptomyces* strain (Str272) isolated from a potato common scab lesion suppressed common scab in field trials lasting over 5 years. In this study, bacterial communities in the tuberosphere i.e. in the soil adjacent to potato tubers, were analysed by next generation sequencing (NGS). The aim was to compare bacterial communities in untreated control plots to those in which seed tubers were treated with Str272 in one or several growing seasons. Str272 applications increased soil bacterial diversity and affected the bacterial composition in the potato tuberosphere. The most pronounced differences were observed between the untreated control and the treatments in which the antagonist had been applied in three or four consecutive years. The differences remained similar until the following growing season. Bacterial composition after repeated antagonist applications was associated with lower common scab severity. The antagonist applications had no or only slight effect on the number or abundance of OTUs belonging to *Actinobacteria* or *Streptomyces,* and no differences in quantities of pathogenic *Streptomyces* populations were detected by qPCR. This indicates that suppression of common scab by Str272 may not be based on direct effect on the common scab pathogens but is more likely to be associated with the alterations of the soil bacterial community. The most abundant bacteria phyla in the potato tuberosphere were *Actinobacteria*, *Proteobacteria* and *Acidobacteria*. However, the OTUs responding greatest to the antagonist treatments belonged to *Bacterioidetes* and *Gemmatimonadetes.* Results indicate that repeated applications of Str272 can change the bacterial community in the potato tuberosphere and lead to development of soil that is suppressive against potato common scab for several growing seasons after the last application.

1. Introduction

Disease suppressive soils are important in managing soil-borne pathogens that cannot be controlled with chemicals. Therefore, it is important to develop agricultural practices promoting the growth of soil microbes able to suppress the growth of plant pathogens. During the recent decades many studies have been conducted in order to investigate the long-term effects of soil quality, soil microbiome and soil management on soil disease suppressiveness (for example, Bruggen and Semenov, 2000; Bongiorno et al., 2019, 2020; Mao et al., 2019; Döring et al., 2020). Still, microbes and mechanisms involved in disease suppression are often unknown. Diverse bacterial communities in soil are considered necessary for the ability of soil to suppress plant diseases (Garbeva et al., 2004; Raaijmakers et al., 2009), however, specific groups of microbes that are antagonistic to certain pathogens may also exist in soil (Mazzola, 2002). If these antagonists can be identified and

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propagated under controlled conditions, it may be possible to use them as biological control agents to boost the suppressive properties of soil.

Common scab is a bacterial disease of potato (*Solanum tuberosum*) caused by several *Streptomyces* species (Dees and Wanner, 2012), which beside being saprophytes, can occupy lenticels on potato tubers and cause the development of scab symptoms. Control of common scab has proven difficult. Agronomical measures, such as irrigation during the early tuber formation, or optimizing soil pH can reduce common scab. However, only few reliable methods that can be used at the field scale exist. Many biological agents have been tested and found to suppress common scab under controlled conditions in the greenhouse (Liu et al., 1996; Neeno-Eckwall et al., 2001; Han et al., 2005; Hiltunen et al., 2009) and/or in field trials (Liu et al., 1995; Bowers et al., 1996; Beauséjour et al., 2003; Singhai et al., 2011; Wanner et al., 2013; Arseneault et al., 2015, 2016; Sun et al., 2015; Lin et al., 2018), but have eventually shown variable efficiency in control of common scab symptoms.

Suppressive soils that can reduce common scab disease in potatoes may develop spontaneously in fields, where potatoes are grown without interruption as a monoculture over many years (Lorang et al., 1995; Wiggins and Kinkel, 2005). This phenomenon is known to be of biological origin (Menzies, 1959) and can be attributed to strains of non-pathogenic *Streptomyces* spp. (Liu et al., 1995, 1996; Lorang et al., 1995; Meng et al., 2012). The pathogenic species represent a minority of all *Streptomyces* spp., of which the majority have a saprophytic lifestyle (Hopwood, 2007). An important difference between the pathogenic and saprophytic *Streptomyces* spp. are cyclic dipeptides (thaxtomins) that are essential for development of scab lesions and produced only by the pathogenic species (King et al., 1989). The pathogenicity island contains the genes needed for invasion of the tuber and thaxtomin synthesis (Lerat et al., 2009; Aittamaa et al., 2010).

We isolated a non-pathogenic *Streptomyces* strain (Str272) from a potato scab lesion (Lindholm et al., 1997). Strain 272 consistently reduced scab symptoms in field trials carried out over five years (Hiltunen et al., 2017). These promising results are consistent with *Streptomyces* strains being potential, and in some cases already commercialized, biocontrol agents used against various plant diseases (Doumbou et al., 2001; Palaniyandi et al., 2013). However, in addition to disease suppression, the antagonist may also influence the soil bacterial community more broadly. In previous studies, when antagonists were added into soil at the time of planting the crop, some changes, albeit mostly small, in the bacterial community structure were observed during the same growing season (Prévost et al., 2006; Schreiter et al., 2014; Sun et al., 2015). However, little is known of the long-term effects that antagonist applications may have on the soil microbiome.

In recent years, 'next generation sequencing' (NGS) has revolutionized bacterial ecology studies (Clooney et al., 2016 and refs. therein) by enabling acquisition of large sequence datasets that cover the whole bacterial community and by allowing detailed analysis of the data. The method has allowed for more in-depth studies on interactions between common scab and soil microbiome and has shed light on differences between potato common scab suppressive and conducive soil microbiomes (Sarikhani et al., 2017; Tomihama et al., 2016; Kopecky et al., 2019; Shi et al., 2019).

In this study the diversity and composition of bacteria prevailing in the soil adjacent to potato tubers (tuberosphere) was characterized by NGS. The aim was to investigate i) how a single or multiple applications of the Str272 applied over several growing seasons affect the bacterial communities and ii) if the possible differences in the bacterial communities persist in the soil until the following year(s) after the applications, and furthermore, iii) to find out, if the bacterial community composition is associated with the incidence and severity of common scab.

2. Materials and methods

2.1. Field experiment

The impact of the Str272 on soil bacterial composition in the soil adjacent to the potato tubers, i.e. the tuberosphere (Sagova-Mareckova et al., 2017), was investigated in two consecutive years (2012−2013). The study utilized a long-term field trial, which has been set up to investigate the effects of repeated applications of Str272 on common scab (Hiltunen et al., 2017). The experiment was carried out in a grower's field in Lumijoki (64° 85′ N 25° 17′ E), Northern Finland. The weather data was obtained from the nearest Finnish Meteorological Institute's observation station (Table A.1). Precipitation during the potato growth period was 181 mm and 194 mm in 2012 and 2013, respectively.

The field (Table A.2) was naturally infested with scab-causing *Streptomyces* spp. and had documented problems with common scab (Hiltunen et al., 2011). The experiment included four treatments with the following applications: i) untreated control (C0), ii) one application of Str272 in 2012 (A1), iii) three applications of Str272 in the previous three years, but no application in 2012 (A3), iv) three applications of Str272 in the previous three years and with a further application in 2012 (A4) (Fig. 1). In 2012, the treatments that did not receive an antagonist application (C0, A3), were treated with an equivalent volume of water. In 2013, no antagonist treatments were applied. Each treatment was repeated in four replicate plots nested within the treatments (Fig. 1). The experiment and treatments were fixed to the same position in all years. Care was taken not to move the soil between the plots. Each plot included two 5-m long rows. At planting, certified healthy seed potatoes of the scab-sensitive potato cultivar Bintje were placed in the 5-m long furrows at 0.28 m intervals, resulting in 35 ± 1 plants per replicate. The experiment was maintained as described in Hiltunen et al. (2017).

The antagonist was grown for 3 days in a glucose-yeast-malt extract broth containing 4.0 g glucose, 4.0 g yeast extract and 10 g malt extract added to 1000 ml of distilled water (pH 7–7.2). The culture was diluted 1:10 with distilled water and sprayed on the seed tubers placed in the furrow at the rate of 0.1 l/m^2 (1×10^6 to 1×10^8 CFU/m^2). In the control plots (C0), the tubers were sprayed with water only. The tubers were immediately covered with soil and the furrows were closed (Hiltunen et al., 2017).

In both years (2012 and 2013), the soil samples were collected from the potato tuberospheres at early tuber development, 6 weeks after planting. This is the stage, when the tubers are most susceptible to infection (Loria et al., 2006; Khatri et al., 2010). The tuberosphere was reached by carefully uncovering the topsoil surrounding the plant growing in the ridge. Soil samples were collected by a spoon no further than 1 cm away from potato tubers. Five subsamples were collected from each plot (plot size 8 m^2), pooled to form a composite sample per plot (combined volume of approximately 1 l) and mixed carefully. The samples were handled as described in Wallenius et al. (2010) and Wallenius (2011); samples were transported in a cool box to the laboratory and stored at 5 °C overnight. On the following day, the samples were sieved (4 mm) and homogenized by careful manual mixing. In order to avoid contamination between the samples, the tools that were used were disinfected after each sample. From each composite sample three specimens (1 g) were taken. The three specimens per replicate were processed individually through DNA extraction and subsequent sequencing. Samples were stored at −80 °C. In total, 32 composite samples were analysed (4 replicate samples/treatment × 4 treatments × 2 years).

2.2. DNA sequencing

DNA was extracted using the E.Z.N.A. Soil Extraction Kit (Omega Bio-Tek, Norcross, Georgia, USA). The isolated and resuspended DNA was further purified from phenolic compounds with two HTR Reagent treatments, followed by standard DNA recovery using DNA-binding

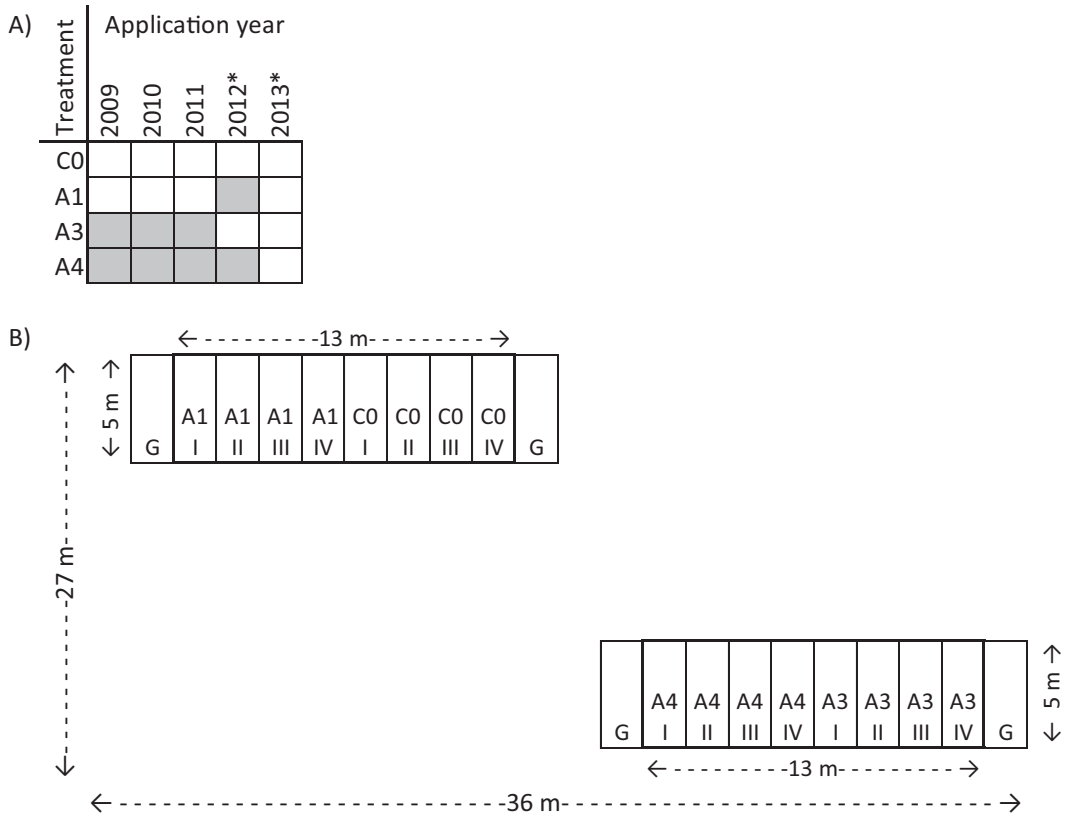

Fig. 1. The layout of the field experiment. (a) Treatment history: application of the Str272 (gray), or water (control) (white). A1, Str272 was only applied once, in 2012; A3, Str272 was previously applied in three consecutive years, but not in 2012; A4, Str272 was previously applied in three consecutive years and in 2012. The untreated control (C0) obtained no antagonist application. The soil samples were taken in 2012 and 2013 (*). (b) The layout of the experimental field (potato cv. Bintje) showing the field dimensions, plot size and the distances between the treatment areas. Roman numerals indicate the replicate number. Each replicate (plot) included two rows of potatoes. G, untreated guard row (potato).

columns (E.Z.N.A. Soil Extraction Kit). A 364-base pair (bp) long region of the bacterial 16S rRNA genes, including the hypervariable regions V3 and V4, was amplified from the soil DNA samples and sequenced using Illumina MiSeq paired-ends 250-bp (Illumina) in the Institute of Molecular Medicine Finland (University of Helsinki, Finland). The forward primer 340F (5′-TCCTACGGGAGGCAGCAGT-3′) and reverse primer 704R (5'-TCTRCGMATTYCACYKCTACAC-3′) were optimized for amplification of the 16S rRNA gene sequences of soil bacteria (Vasileiadis et al., 2012). Sequences were deposited to the European Nucleotide Archive (PRJEB40435).

2.3. Quantitative PCR

The quantification of the pathogenic *Streptomyces* spp. in the tuberosphere soils was carried out by qPCR using the phytotoxin biosynthetic gene *txtA*. The primers txtA_F (5′-CATGTCGTGGTCCGGCAGAC-3′) and txtA_R (5′-GTGGAGCGACTGTCCTTCATG-3′) amplifying the thaxtomin synthesis gene *txtA* were added to the LightCycler 480 SYBR Green I master mix (Roche Diagnostics GmbH) according to manufacturer's instructions, and 25 ng of sample DNA (14–54 ng/μl) was added last. A dilution series of DNA (10-fold intervals) of *S. scabies* strain 87.22 (NC_013929.1) was included in each run to calculate the *txtA* gene copy number in unknown samples. Each sample was analysed in duplicate. After a 10 min initial denaturation step, PCR amplification was carried out using 45 cycles of 10 s at 98 °C and 20 s at 72 °C. At the end of the reaction, a disassociation curve was done to ascertain that a single correctly sized product was obtained. The PCR efficiency and subsequently *txtA* gene copy numbers per sample were calculated with LinRegPCR program (Ruijter et al., 2009). The standard curve efficiencies were 95–96%, the slope −3.42 to −3.43, intercept 17.9–18.8, and coefficient of determination > 0.99.

2.4. Scab symptoms at harvest

The incidence and severity of common scab symptoms in the harvested potatoes from the sampling years 2012 and 2013 (data from Fig. 4 in Hiltunen et al., 2017) were used to compare the relationship between the bacterial community and the repeated antagonist applications. Disease incidence was expressed as the percentage of tubers with scab symptoms and disease severity as the mean percentage of tuber surface area covered with scab (Fig. A.1).

2.5. Bioinformatic analysis of the sequence data and statistical analysis

The paired sequences from the three specimens of each replicate were pooled. The pooled sequence data were then processed using Mothur version 1.42.3 (Schloss et al., 2009; Kozich et al., 2013; López-García et al., 2018). Chimeras and unique sequences were removed, the sequence data was classified using the default method (naïve Bayesian classifier; Wang et al., 2007), and non-bacterial sequences were removed. Finally, closed-reference OTUs were picked from SILVA database (Quast et al., 2013, release 138) and representative sequences with a 97% similarity were kept. The OTUs were summarized at phylum, class, order, and family level.

The total number of sequence reads was 9,652,002 distributed into 5104 OTUs (Appendix B). The original sequence read data was standardised by the total number of reads per sample to reflect relative abundances of the OTUs in each sample (Peršoh and Borken, 2017). We also rarefied the original data to maximum reads of 170,850 using the rarefy function (*vegan* package, Oksanen et al., 2019). The rarefaction

resulted in 5,467,200 sequence reads and 5046 OTUs covering 56.6% of the original sequence data.

The abundance of most common phylogenetic groups was calculated by summarising the standardised read proportions per sample of all OTUs belonging to that group. Furthermore, the number of OTUs belonging to each phylogenetic group was counted.

All statistical analyses were performed on both standardised and rarefied data using R 3.6.0 (R Core Team, 2019). Both data gave similar results. The results are presented using the standardised data. Species richness (*S*) and Pielou's evenness (*J*) were calculated for the bacterial community data (*vegan* package, Oksanen et al., 2019).

The effects of antagonist treatment (C0, A1, A3, A4) and sampling year (2012,2013) on soil bacterial phylogenetic groups were analysed using linear and generalised linear mixed models (*lme4* package, Bates et al., 2015). The fixed factors in the models were 'treatment' and 'year' as ordered factors. The study plot was used in the random part of the model in order to consider the fact that each plot was sampled in consecutive years. Pairwise differences between treatments were tested separately for sampling year using analysis of variance (*lm* or *glm* function, *stats* package, R Core Team, 2019). Transformations were used when necessary to fulfil the model demands for normality and heterogeneity.

The differences in soil bacterial community composition between treatments and the two sampling years (Fig. 1) were visualised using non-metric multidimensional scaling (NMDS) with Bray-Curtis coefficient as a dissimilarity measure (*metaMDS*). The permutational multivariate ANOVA (*adonis*) was used in the comparisons of bacterial species composition in different categories, and *envfit* function was used to identify which of the variables were significantly associated with bacterial community composition (*vegan* package, Oksanen et al., 2019). The variables indicating 1) common scab incidence and severity, 2) most common/important bacterial phyla and 3) species richness or diversity were used in correlation analysis. Furthermore, pairwise comparisons between the bacterial communities in the treatments were analysed by *pairwise.adonis* function (Martinez, 2017). The *cor.test* function was used for the separate correlation analyses, and the result was considered significant if the $r > 0.5$ and $p < 0.05$.

The relationship between bacterial OTUs and different treatments was examined by indicator species analysis (Dufrêne and Legendre, 1997) using the *multpatt* function available in the *indicspecies* package (De Caceres and Legendre, 2009, De Caceres et al., 2010). Indicative bacterial OTUs were identified for different groups if their indicator value was ≥ 0.7, $p < 0.01$. The analyses were carried out with four treatment groups (C0, A1, A3, A4) as described in Fig. 1. All the information regarding this analysis can be found in Appendix B. The figures and tables present the original values averaged at treatment level separately for both sampling years.

3. Results

Altogether, 5104 bacterial OTUs were present in the samples collected from the potato tuberospheres in the two consecutive years after Str272 applications (Appendix B). The OTUs belonging to four major phyla (*Actinobacteria, Proteobacteria, Firmicutes* and *Acidobacteria*) covered over 94% of the identified OTUs and approximately 70% of the sequence reads in the data (Table A.3). One OTU representing *Gemmatimonadetes* and seven OTUs belonging to *Nitrospira* covered almost 10% of the sequence reads. The phyla with a relative abundance of less than 1% were considered rare. They included *Bacterioidetes* and twelve other phyla. The portion of organisms with unidentified taxonomy ranged from 16% to 21% of the sequence reads in the samples.

Only 172 (3.4% of all OTUs) bacterial OTUs were present in all samples. However, these OTUs covered 86% of the sequence reads in the data. Most of these OTUs belonged to four major phyla, *Proteobacteria* (59 OTUs), *Actinobacteria* (35 OTUs), *Firmicutes* (25 OTUs) and *Acidobacteria* (22 OTUs).

3.1. Bacterial community

Diversity indices showed differences in bacterial communities between treatments (Fig. 2). Bacterial OTU richness (*S*) was greater in the treatments with repeated antagonist applications (A3 and A4) as compared to the untreated control (C0). The difference was statistically significant in 2013, but not in 2012. Also, the treatment with a single application (A1) differed from the untreated control in the second sampling year in 2013. Evenness (*J*) of the bacterial community declined with the number of antagonist applications ($F = 14.9$, $p < 0.001$) and from year 2012 to year 2013 ($t = -3.79$, $p = 0.003$). There were no significant differences in richness or in evenness between treatments with three (A3) or four (A4) antagonist applications in either year.

The impact of the antagonist applications on soil bacterial community was evident in both sampling years (Fig. 3, Table A.4). In 2012, the differences between the treatments followed the number of applications. In 2013, the soil bacterial compositions in the C0 and A1 were similar but differed from those in the A3 and A4 with repeated antagonist applications (Table A.4b). However, the differences in the bacterial community compositions between A3 and A4 were small in both years (Fig. 3). In 2012, bacterial OTU richness (*S*) and the relative proportion of OTUs belonging to phylum *Bacterioidetes* and unidentified OTUs increased with the number of applications. (Fig. 3, Table A.4). The bacterial community composition was most even (evenness, *J*) in the untreated control in 2012, while the opposing trend was seen in the relative proportion of OTUs belonging to phyla *Acidobacteria* and *Nitrospira*, which increased from 2012 to 2013.

3.2. Bacterial community and scab symptoms

The bacterial community composition in the potato tuberosphere after antagonist applications was associated with the incidence and severity of common scab (Fig. 3). The disease incidence and severity were at the highest level in 2013 in the untreated control (C0) and in the treatment with a single antagonist application (A1) (Fig. 3). The relative abundance of OTUs belonging to phyla *Proteobacteria and Gemmatimonadetes* were high in those microbial communities (Fig. 4, Table A.5). However, only *Gemmatimonadetes* correlated significantly positively with common scab incidence and severity ($r = 0.67$ and 0.68, $t = 4.98$ and 5.03, $p < 0.001$ and 0.001, respectively). The relative abundance of *Bacterioidetes* was low. However, their abundance increased significantly by the repeated antagonist applications (A3 and A4) (Fig. 4, Table A.5) and correlated negatively with the common scab incidence and severity ($r = -0.61$ and -0.58, $t = -4.20$ and -3.86, $p < 0.001$ and 0.001, respectively). There was no correlation between the abundance of *Actinobacteria* and disease symptoms.

In total, 83 OTUs belonging to genus *Streptomyces* were identified. However, the number of *Streptomyces* OTUs per sample and their relative abundance were low (Fig. 5). The antagonist applications did not have a significant impact on the number of *Streptomyces* OTUs or their relative abundance. Only in 2013, in the plots with three repeated antagonist applications (A3) the abundance was lower than in the other treatments.

No significant differences were detected in the copies of the *txtA* gene in the DNA extracted from the tuberosphere soil from the differently treated plots indicating that there were no differences in quantities of pathogenic *Streptomyces* populations. The number of *txtA* gene copies ranged from 1.90–6.97 $\times 10^4$ and 4.30–6.32 $\times 10^4$ g^{-1} dry soil in 2012 and 2013, respectively.

3.3. Indicator OTUs

Fifty-one (<1% of all OTUs) of the bacterial OTUs had a high indicator value according to the indicator species analysis (Iv > 0.7; Table 1). Forty of these OTUs were indicative of treatments with multiple antagonist applications (A3 and/or A4) and mostly belonged to phyla *Proteobacteria, Actinobacteria and Firmicutes.* Nine OTUs belonging

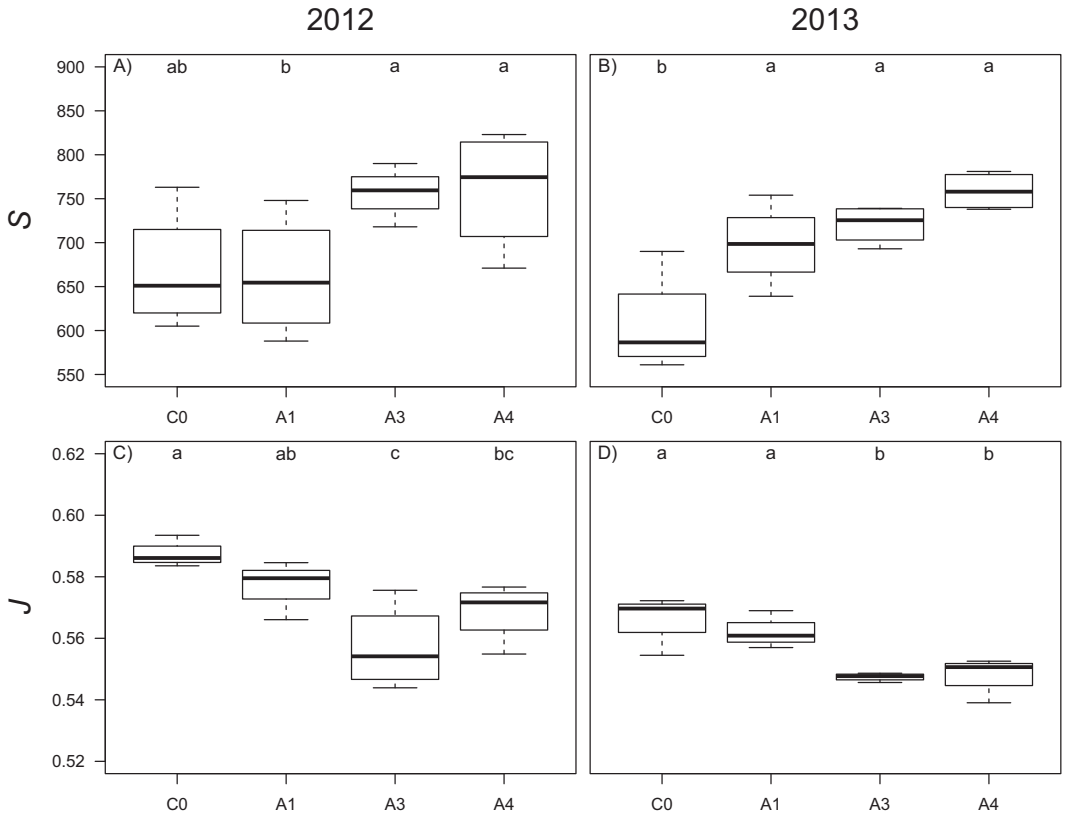

Fig. 2. Mean (±sd) bacterial OTU richness (*S*) (A, B) and evenness (*J*) (C, D) in the soil bacterial community after antagonist applications, respectively for 2012 and 2013. Samples were collected in two consecutive years from plots with no application (C0) and with one (A1), three (A3) or four (A4) antagonist applications. The groups with different letters are significantly ($p < 0.05$) different within the sampling.

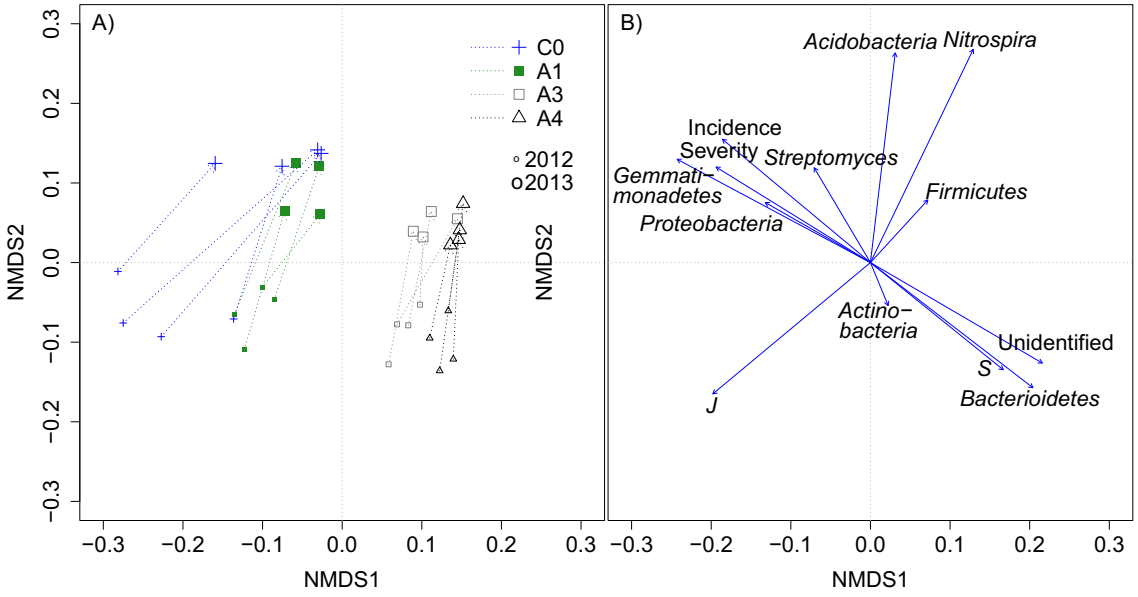

Fig. 3. A) Differences in soil bacterial communities after repeated antagonist applications (NMDS, stress = 7.23). The samples were collected in two consecutive years from plots with no applications (C0) or with one (A1), three (A3) or four (A4) antagonist applications. The treatments (C0, A1, A3, and A4) are expressed with different symbols. See Fig. 1 for a description of the treatment history. The sampling year is indicated with the size of the symbol. The dashed arrows represent the change in bacterial OTU composition between sampling years. B) The fitted vectors describe the association of potato common scab incidence and severity in 2012 and 2013 with bacterial OTU richness (*S*), evenness (*J*) and relative proportions of major bacterial phyla. The length of each vector indicates the strength of correlation between the vector and the NMDS ordination (Table A.4).

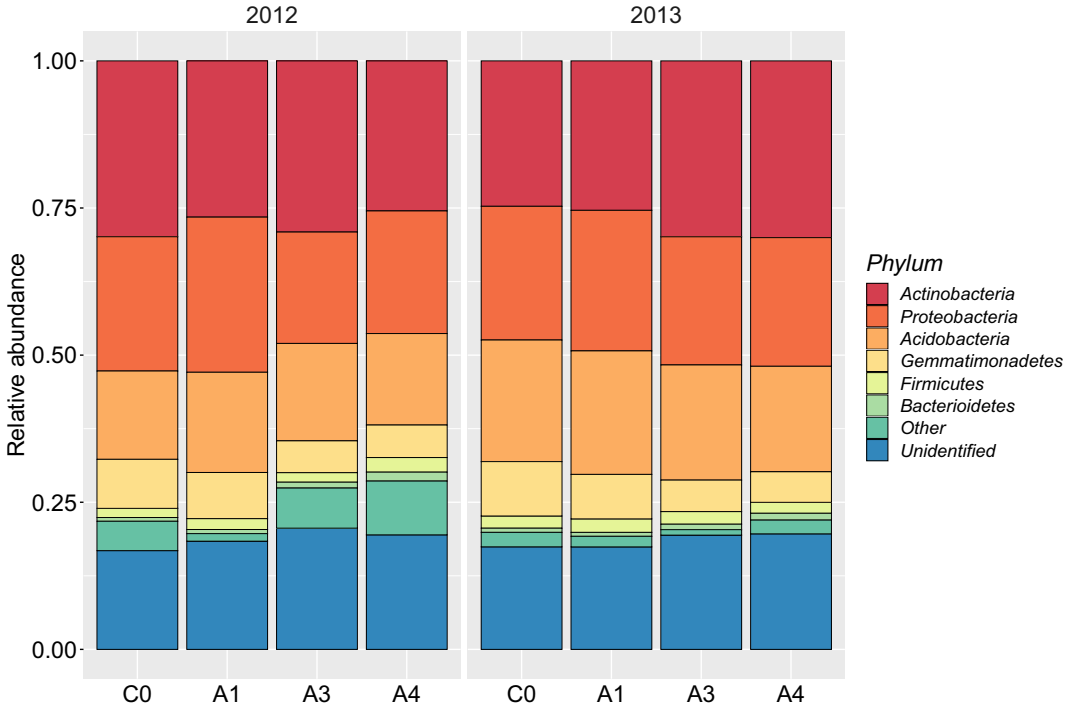

Fig. 4. Mean relative abundance of bacterial OTUs by major phyla in the soil samples after antagonist applications. The samples were collected in two consecutive years in 2012 and 2013 from plots with no application (C0) or with one (A1), three (A3) or four (A4) antagonist applications. See Table A.5 for standard deviations.

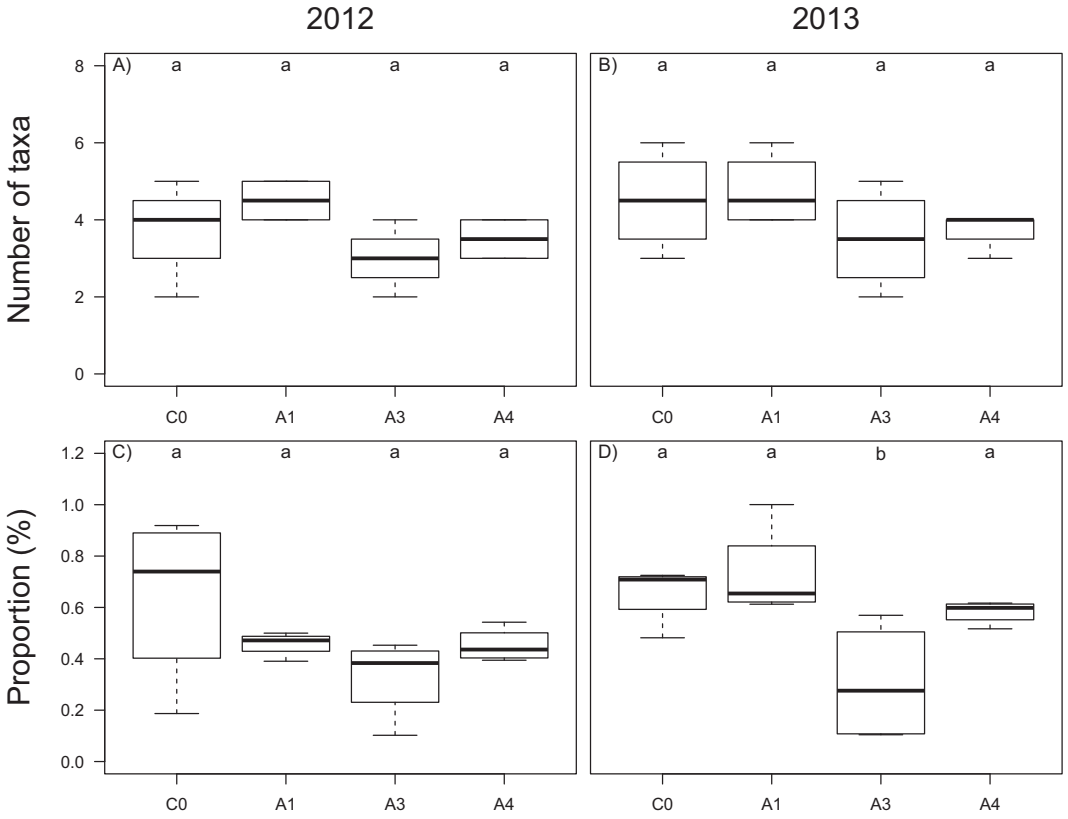

Fig. 5. Mean (±sd) number of OTUs belonging to the genus *Streptomyces* (A, B) and their relative abundance as proportion of all the sequence reads (C, D) in the soil samples after repeated antagonist applications, respectively for 2012 and 2013. The samples were collected in two consecutive years from plots with no applications (C0) or with one (A1), three (A3) or four (A4) antagonist applications. The groups with different letters are significantly ($p < 0.05$) different within the sampling year.

Table 1
Indicator OTUs for the treatments with different antagonist applications. Standardised data was used for the analysis and the grouping was done by the treatment. The Indicator value (>0.7) given for each OTU after 1000 permutations are presented. The treatments are as follows: C0, untreated control without antagonist application; A1, the antagonist was only applied once, in 2012; A3, the antagonist was previously applied in three consecutive years, but not in 2012; A4, the antagonist was previously applied in three consecutive years and also in 2012. Information for the OTUs see Appendix B.

Indicator taxa (otuID)	Phyla	Indicator value	Abundance	Frequency	Taxa indicating the treatment			
					C0	A1	A3	A4
*Acidobacteria*_Gp1_family_incertae_sedis (otu0001)	*Acidobacteria*	0.89	159760	32				
*Solirubrobacterales*_unclassified (otu1790)	*Actinobacteria*	0.87	30171	32				
Gemmatimonas (otu2955)	*Gemmatimonadetes*	0.85	628536	32				
Dokdonella (otu4856)	*Proteobacteria*	0.84	37862	32				
Glycomyces (otu0485)	*Actinobacteria*	0.82	432	25				
Kribbella (otu1277)	*Actinobacteria*	0.77	225	27				
*Actinomycetales*_unclassified (otu0299)	*Actinobacteria*	0.76	82225	32				
Rhizomicrobium (otu2970)	*Proteobacteria*	0.73	211	29				
Mycobacterium (otu1040)	*Actinobacteria*	0.69	54397	32				
*Acidobacteria*_Gp25_family_incertae_sedis (otu0017)	*Acidobacteria*	0.81	2529	32				
*Acidobacteria*_Gp2_family_incertae_sedis (otu0012)	*Acidobacteria*	0.90	21671	32				
Caldilinea (otu2080)	*Chloroflexi*	0.70	560	32				
*Rhodocyclaceae*_unclassified (otu4389)	*Proteobacteria*	0.91	35672	32				
Thiobacillus (otu4298)	*Proteobacteria*	0.90	15942	32				
*Coriobacteriaceae*_unclassified (otu1716)	*Actinobacteria*	0.88	19583	32				
*Holophagaceae*_unclassified (otu0031)	*Acidobacteria*	0.85	1288	31				
*Gammaproteobacteria*_family_incertae_sedis_unclassified (otu4679)	*Proteobacteria*	0.83	3275	32				
*Anaerolineaceae*_unclassified (otu2072)	*Chloroflexi*	0.83	785	32				
*Chloroflexi*_unclassified (otu2085)	*Chloroflexi*	0.83	7256	32				
*Bacteria*_unclassified (otu1839)	*Bacteria_unclassified*	0.81	1789260	32				
*Acidobacteria*_Gp7_family_incertae_sedis (otu0024)	*Acidobacteria*	0.80	161748	32				
Acetobacterium (otu2732)	*Firmicutes*	0.80	1058	32				
*Acidobacteria*_Gp10_family_incertae_sedis (otu0002)	*Acidobacteria*	0.78	18441	32				
*Acidobacteria*_Gp11_family_incertae_sedis (otu0003)	*Acidobacteria*	0.78	9492	32				
Dethiobacter (otu2882)	*Firmicutes*	0.78	129	19				
*Gammaproteobacteria*_unclassified (otu4696)	*Proteobacteria*	0.76	268240	32				
Bryobacter (otu0019)	*Acidobacteria*	0.76	244	24				
*Acidobacteria*_Gp18_family_incertae_sedis (otu0010)	*Acidobacteria*	0.75	1235	32				
Nocardia (otu1221)	*Actinobacteria*	0.75	915	32				
*Acidobacteria*_unclassified (otu0025)	*Acidobacteria*	0.75	6116	32				
Sphaerobacter (otu2089)	*Chloroflexi*	0.74	1557	32				
Stella (otu3427)	*Proteobacteria*	0.74	139	21				
Ohtaekwangia (otu1841)	*Bacteroidetes*	0.74	22151	32				
Proteiniclasticum (otu2650)	*Firmicutes*	0.73	496	29				
*Ruminococcaceae*_unclassified (otu2850)	*Firmicutes*	0.73	2349	32				
Desulfocapsa (otu4466)	*Proteobacteria*	0.72	267	23				
*Desulfobulbaceae*_unclassified (otu4464)	*Proteobacteria*	0.72	79	21				
*Betaproteobacteria*_unclassified (otu3603)	*Proteobacteria*	0.72	830449	32				
*Clostridiales*_unclassified (otu2719)	*Firmicutes*	0.72	9870	32				
Haliea (otu4628)	*Proteobacteria*	0.72	124	17				
*Firmicutes*_unclassified (otu2886)	*Firmicutes*	0.72	3195	32				
BRC1_genera_incertae_sedis (otu2021)	BRC1	0.72	585	32				
*Chromatiales*_unclassified (otu4639)	*Proteobacteria*	0.71	413	30				
Trichococcus (otu2564)	*Firmicutes*	0.70	587	25				
*Bacteroidetes*_unclassified (otu1844)	*Bacteroidetes*	0.69	29571	32				
Sporacetigenium (otu2818)	*Firmicutes*	0.78	137	18				
Truepera (otu2094)	*Deinococcus-Thermus*	0.77	31	9				
Inhella (otu3674)	*Proteobacteria*	0.77	642	17				
Acidovorax (otu3788)	*Proteobacteria*	0.72	2267	29				
Pseudoxanthomonas (otu4925)	*Proteobacteria*	0.71	12030	32				
Microcella (otu0764)	*Actinobacteria*	0.71	22	8				

to *Proteobacteria, Actinobacteria* and *Gemmatimonadetes* were indicative of the untreated control (C0) and the single antagonist application (A1). Only one bacterial OTU was specific to the treatment with a single antagonist application(A1) and another one to the treatment with three repeated applications (A3). Both these belonged to phylum *Acidobacteria*. None of the OTUs indicated the untreated control alone.

OTUs indicative of repeated antagonist applications and with the highest indicator values (0.9) belonged to families *Rhodocyclaceae* or *Coriobacteriaceae* or genus *Thiobacillus,* whereas the most abundant OTUs were unclassified and belonged to phyla *Acidobacteria*, or classes *Gammaproteobacteria* and *Betaproteobacteria*. All these OTUs were also found in samples from untreated plots.

4. Discussion

Results from this study showed that the introduction of the antagonistic *Streptomyces* strain Str272, which can suppress development of potato common scab (Hiltunen et al., 2017), had an impact on the bacterial community in the potato tuberosphere. A single application of Str272 did not significantly alter bacterial diversity. However, the

application of Str272 in three or four consecutive growing seasons increased the bacterial diversity and resulted in significant differences in bacterial community structure, which could be documented still one or two growing seasons after the last application of the antagonist. Many previous studies have also shown that single applications of antagonists applied to the soil to control plant diseases do not have an impact on the soil bacterial composition (Prévost et al., 2006; Schreiter et al., 2014; Sun et al., 2015). Comparison of the bacterial communities with the common scab symptoms observed on potato tubers in the same experiment (Hiltunen et al., 2017) revealed that higher bacterial diversity in the tuberosphere was associated with lower severity and, in many cases, incidence of common scab. This is in accordance with Shi et al. (2019) who suggested that higher soil bacterial diversity in the tuberosphere is associated with lower common scab severity and may be explained by the more complex co-occurrence network of the bacterial communities in common scab suppressive soils. Stability of soil microbial community driven by resistance and resilience is important for explaining community response to disturbance (Shade et al., 2012). It depends not only on species richness but also on the evenness or composition of the soil microbial community (Griffiths and Philippot, 2013). In our study, multiple applications were necessary to break the resistance, and consequently, response of the soil bacterial community was dependent on the number of antagonist applications.

Several studies have investigated the association between potato common scab and soil microbiome (Rosenzweig et al., 2012; Kobayashi et al., 2015; Sagova-Mareckova et al., 2017; Sun et al., 2015; Tomihama et al., 2016; Sarikhani et al., 2017; Roquigny et al., 2018; Kopecky et al., 2019; Shi et al., 2019; Nahar et al., 2020). However, ccomparison of the results is problematic, because methods vary regarding sample analysis, sampling time, and most importantly, regarding spatial locations, where samples are taken. Shi et al. (2019) sampled four different soil-root zones (geocaulosphere, rhizosphere, root zone, furrow), but found significant differences in soil microbial community composition and function between common scab suppressive and conducive soil only in geocaulosphere soil. In contrast, some studies have found differences also in the rhizosphere (Rosenzweig et al., 2012; Tomihama et al., 2016) and bulk soils (Kopecky et al., 2019).

As with any study, experimental methods, environmental factors and analysis methods can affect the results. This is especially true with microbiome studies (Knight et al., 2018). In our study, the methods of collection, preservation and storage remained consistent across all samples. The DNA extraction, primers used, amplification by PCR as well as sequencing process were controlled so that the results were comparable within the study. Another concern is the potential bias concerning species richness and evenness in functional and compositional responses of microbial community (Shade et al., 2012; Willis, 2019). This bias is often corrected by using rarefaction. We got similar results using both the data standardised within each sample and the data rarefied in order to control the impact of the rare OTUs. However, it is suggested that the sample richness and rarefied richness are both driven by artifacts of the experiment (Willis, 2019). Thus, we argue that the results using standardised data provided a good estimate of the bacterial community in our study.

Soil suppressiveness against common scab is often found associated with increased numbers of antagonistic streptomycetes, but not necessarily with the total amount of *Streptomyces* (Schottel et al., 2001; Wiggins and Kinkel, 2005; Meng et al., 2012). Some studies (Tomihama et al., 2016; Rosenzweig et al., 2012), but not all (Sarikhani et al., 2017; Kopecky et al., 2019), have associated *Actinobacteria* with common scab suppressive soils. In our study, the antagonist applications had no or only slight effect on the number or abundance of OTUs belonging to *Actinobacteria* or *Streptomyces*. However, strain-specific primers would be required for quantitative detection of Str272 by qPCR, because Str272 is closely related to the potato scab pathogen *S. europaeiscabiei*, which is common in Finland (Hiltunen et al., 2014, 2017).

Our results indicated that Str272 had no direct effect on pathogenic *Streptomyces* spp., as no significant differences were found in the absolute numbers of *txtA* gene copies in the samples from tuberosphere soil from Str272 treated plots and untreated control plots. *txtA* is required for thaxtomin production and infection and found in all *Streptomyces* spp. causing common scab (Lerat et al., 2009; Hiltunen et al., 2011), and has been used to quantify the pathogenic streptomycetes in soil. Our results are consistent with some previous studies suggesting that the amount of pathogenic *Streptomyces* bacteria in the potato tuberosphere may not be related to soil suppressivity to common scab (Sarikhani et al., 2017; Kobecky et al., 2019). It has been suggested that suppression of scab-causing populations can result from interactions among soil microbes that are not dependent on pathogen populations thus reflecting possible general suppressive capacity within soil microbial communities (Schlatter et al., 2017). However, it seems that pathogen quantity in the tuber periderm or in the soil adhering to tubers positively correlates with common scab severity (Tomihama et al., 2016; Kopecky et al., 2019; Shi et al., 2019). It is also possible that the changes in the bacterial community are associated with antagonists that can suppress gene expression in pathogenic *Streptomyces* as previously demonstrated by St-Onge et al. (2011).

The most common bacterial OTUs in the potato tuberosphere were those belonging to phyla *Proteobacteria* and *Actinobacteria*, whereas most abundant phyla were *Actinobacteria* followed by *Proteobacteria* and *Acidobacteria*. These phyla have also been found in potato growing soils in other studies (Rosenzweig et al., 2012; Kobayashi et al., 2015; Tomihama et al., 2016; Kopecky et al., 2019). We did not find any major differences in proportions of these phyla between treatments, but the greatest differences were in proportions of *Bacterioidetes* and *Gemmatimonadetes*. These bacteria are commonly found in agricultural soils (Wolińska et al., 2017; Semenov et al., 2020). In our study, the abundance of *Bacterioidetes* was low (<1.5%), but both the number of their OTUs and relative abundance increased with the repeated antagonist applications. This might reflect increased activity, as according to Semenov et al. (2020) the abundance of *Bacteroidetes* can be high in active bacterial communities of agricultural soils and potato rhizosphere. In contrast, the abundance of *Gemmatimonadetes* in our samples was relatively high (5–9%), as they have previously been reported to comprise only ca. 2% of soil bacterial communities (Janssen, 2006). However, our results are in accordance with Hou et al. (2020), who recently reported a similar abundance of *Gemmatimonadetes* (5–10%) in the potato rhizosphere during potato growth.

The relative abundance of *Gemmatimonadetes* decreased with the repeated antagonist applications. In addition, an OTU belonging to *Gemmatimonadetes* was identified as one of the potential indicative species of the untreated control i.e. common scab conducive soil. In contrary to our findings, Cha et al. (2016) reported *Gemmatimonadetes* to be more abundant bacteria in suppressive soil for Fusarium wilt. *Gemmatimonadetes* have previously been linked to drier soils (DeBruyn et al., 2011) and it is known to benefit from organic amendments (Shen et al., 2014), but other than that little is known of their ecology. Neither of these findings explain the increased abundance of *Gemmatimonadetes* in common scab conducive soils, as found in our experiments, and therefore, would warrant further studies.

OTUs indicative of repeated antagonist applications and with the highest indicator values belonged to families *Rhodocyclaceae* or *Coriobacteriaceae* or genus *Thiobacillus*. A few species of genus *Thiobacillus* are involved in sulfur cycling in soil. *T. thiooxidans* mixed with elemental sulfur was the first effective treatment used to control *S. scabies* (known as *Actinomyces chromogenus* at that time) (Martin, 1921). The bacterium oxidizes sulfur to sulfuric acid, which lowers pH in the microenvironment and reduces growth of many *Streptomyces* spp. (Lindholm et al., 1997; Tashiro et al., 2012). Recently, members of *Rhodocyclaceae* were commonly detected in soils suppressive to tomato bacterial wilt and their role as potential biocontrol agents was linked to nitrogen cycling in soil (Lee et al., 2017). However, further research is required in order to better understand the possible roles *Thiobacillus* and *Rhodocyclaceae* may

play in common scab suppressive soils.

In natural conditions suppressiveness against potato common scab is a slow process and has been attributed to non-pathogenic *Streptomyces* species. However, there is increasing evidence that interactions among a broad range of bacterial and fungal taxa contributes to scab suppression (Schlatter et al., 2017). We demonstrated that repeated applications of the non-pathogenic *Streptomyces* strain Str272 can result in changes in the microbial community in the potato tuberosphere by increasing bacterial diversity and lead to development of suppressive soil that is able to control common scab for several growing seasons after the last antagonist application. Rather than having a direct effect on the common scab pathogens, suppression of common scab by Str272 may be associated to its capacity to impose selection on soil indigenous microbial communities thus shifting soil microbial characteristics as suggested by Kinkel et al. (2012) and Sun et al. (2015). We only focused on soil bacterial communities, but it is likely that also other factors such as soil chemical or physical properties, the function of soil bacteria or other soil microbes contribute to the suppression of common scab induced by Str272. Investigating the role these factors may play could further help shed light on the mechanisms of common scab suppression by Str272.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

LHH and JPTV supervised the project and designed the field experiments; JK and LHH collected the samples; JK analysed the samples; JT, JKA and OT were responsible for the bioinformatics; OT and JKA analysed the data; JK, OT, JPTV, LHH contributed to the writing of the manuscript. All authors read the final version of the manuscript and gave their approval for publication.

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