Bacterial communities in the potato tuberosphere share similarities with bulk soil and rhizosphere communities, yet possess distinct features

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Abstract

Background and Aims

Bacterial communities in tuberospheres (soil attached to potato tubers) and levels of bacterial sulfur cycling in this compartment have been overlooked in healthy potato plants to date. Here, we aimed to describe the structure and composition of tuberosphere bacteriomes and its sulfur cycling capability using the rhizosphere and bulk soil bacterial community characteristics as a reference.

Methods

We collected rhizosphere and tuberosphere soil along with bulk soil from two field grown potato varieties with different properties. Bacterial communities were characterized by 16S rRNA gene amplicon sequencing. Bacterial sulfur cycling indicators were evaluated with cultivation dependent and independent methods and were correlated (Spearman) with the relative abundance of bacterial families.

Results

The structure of tuberosphere bacterial communities either overlapped with the bulk soil or had similarities with the rhizosphere. Relative abundance of specific bacterial families increased or decreased from bulk soil to tuberosphere and rhizosphere continuum. Tuberospheres had a tendency for higher arylsulfonate utilization compared to bulk soil. The families *Sphindomonadaceae, Sphingobacteriaceae* and *Rhizobiaceae* which presented a decline in their relative abundances from the rhizosphere to tuberosphere and bulk soil had positive correlations with sulfur cycling indicators.

Conclusion

Potato variety and soil characteristics played a role in structuring the tuberosphere bacterial communities. Tuberospheres represent a transitional environment between bulk soil and rhizosphere indicative from the intermediate relative abundances of specific bacterial families. A moderate stimulation of bacterial sulfur cycling activity in tuberospheres suggests that this microbial function may serve specific biological roles for potato tubers.

Introduction

The plant microbiome carries out vital processes such as nutrient mobilization and suppression of plant diseases that stimulate plant growth and improve plant productivity (Berendsen et al. 2012; Leff 2017). Microbial communities colonize specific microhabitats of the host plant such as the rhizosphere (soil surrounding the root system), phyllosphere (aerial plant parts) and endosphere (internal plant tissues). Each of these microhabitats are unique environments with different physiochemical properties and they

influence the composition of their microbial communities (Bulgarelli et al. 2013; Berg et al. 2016; Dastogeer et al. 2020)

Most studies have focused on the rhizosphere microbiome due to the significance of this compartment in plant nutrient uptake and in defense against soil pathogens (Berendsen et al. 2012; Philippot et al. 2013). Compared to the bulk soil (root free soil), rhizosphere has higher microbial populations and activities due to the secretion of root exudates by the plant roots (Bais et al., 2006 Hartmann et al., 2009). Root exudates contain a repertoire of nutrients, carbon rich compounds and phytochemicals that boost microbial growth and enables plants to select for beneficial microbes from a broader pool of microorganisms in the bulk soil (Hartmann et al. 2009; Bakker et al. 2013).

Intensive research has been conducted in the past couple of decades on the potato rhizosphere microbiome with the aim to enhance potato yield. Several factors have been identified as determinants of diversity in potato bacterial microbiota, including the plant developmental stage (Van Overbeek and Van Elsas 2008; Andreote et al. 2010; İnceoğlu et al. 2010; İnceoğlu et al. 2011; İnceoğlu et al. 2013b; Pfeiffer et al. 2017; Hou et al. 2020), environmental conditions (Rasche et al. 2006), soil types (Dedourge et al. 2004; Van Overbeek and Van Elsas 2008; İnceoğlu et al. 2012) and the potato genotype (Van Overbeek and Van Elsas 2008; Andreote et al. 2010; İnceoğlu et al. 2010; İnceoğlu et al. 2011; İnceoğlu et al. 2013b).

Apart from the rhizosphere, potato plants host rich microbial communities in another belowground compartment, the tuberosphere (Weinert et al. 2010; Diallo et al. 2011) (also termed geocaulosphere), which is defined as the soil in contact with the surface of potato tubers. This compartment is characterized by carbon limitation and lower nutrient content due to the lack of root exudate production. However, it is still enriched with carbon and nutrients released from damaged tuber tissues or cells detached from tubers during their growth (Lottmann et al. 1997). In addition, lenticels on the surface of potato tubers participate in CO_2 and O_2 exchange and they are permeable to water (Lendzian 2006). These factors may also influence the tuberosphere microenvironment and subsequently the microbial communities in this compartment.

Recently, an increasing number of studies examined the tuberosphere, rhizosphere, and bulk soil bacterial communities under common scab infection (Kobayashi et al. 2015; Kopecky et al. 2019; Shi et al. 2019; Nahar et al. 2020; Marketa et al. 2021). These studies yielded different results regarding the similarity of the tuberosphere microbiome with the two other soil compartments, dependent on the resistance level of potato genotype to common scab (Marketa et al. 2021), or no effect of the genotype was reported (Kobayashi et al. 2015; Nahar et al. 2020). Therefore, it remains obscure where the tuberosphere microbiome is positioned relative to the rhizosphere and bulk soil in potato plants not affected by common scab disease.

Microbial nutrient mobilization has been intensively investigated in the rhizosphere (Berendsen et al. 2012) and to some extent in the phyllosphere (Abril et al. 2005; Xiang et al. 2020; Zhang et al. 2022) and

endosphere (Oliveira et al. 2020; Liu et al. 2022). Two recent studies have reported different levels of nutrients in the tuberosphere of resistant and susceptible potato genotypes under common scab infection (Kopecky et al. 2019; Marketa et al. 2021). These findings highlight the need for further research to be conducted on bacterial nutrient cycling in the tuberosphere. To the best of our knowledge, to date, there are no studies examining the levels of microbial nutrient cycling including sulfur in this compartment and how it compares to the rhizosphere and bulk soil nutrient cycling capabilities.

Sulfur is an essential element for the metabolism and growth of both plants and microorganisms (Heinze et al. 2021). The major sulfur pool in soil is organically bound sulphate esters and sulfonates. Microbial activity significantly increases the level of inorganic sulfur in soil by mineralizing these organically bound sulfur forms (Autry and Fitzgerald 1990; Kertesz and Mirleau 2004). Mobilization of sulfur from aromatic sulphate esters is accomplished in soil by arylsulfatases (Deng and Tabatabai 1997) and can be measured via a soil enzymatic test (Tabatabai and Bremner 1970). A wide variety of sulfonates can be mineralized by a group of bacteria through a multicomponent enzymatic complex in which enzymes encoded by the *ssu* gene cluster are of importance, while for aromatic sulfonate desulfurization the *asf* gene cluster equally plays a major role (Vermeij et al. 1999; Kertesz and Mirleau 2004). Measuring the bacterial enzymatic activity for mineralization of sulfonates has failed up to now due to the multienzyme complex needed for the reaction, but the population of bacteria that participate in this process provides an estimation of sulfonate utilization in soil (Schmalenberger et al. 2009; Fox et al. 2016). Therefore, organic sulfur mobilization can be used as a proxy of microbial nutrient cycling in the different soil compartments. In addition, sulfur plays important roles in potato crop as it improves potato yield and reduces tuber defects (Sharma et al. 2023) while also increases the resistance to common scab (Pavlista 2005). Furthermore, sufficient sulfur decreases the potential of acrylamide formation during potato processing (i.e. frying, baking). Acrylamide is a carcinogenic substance which is produced during high temperature processing of potatoes (Muttucumaru et al. 2013).

Deciphering the processes that shape tuberosphere bacteriomes as well as its activity is important for a better management of potato plant health and productivity through beneficial soil microorganisms. To expand upon the small body of research on this topic, the present study profiled the bacterial communities of the bulk soil, rhizosphere and tuberosphere with 16S rRNA gene sequencing and compared the three soil compartments to determine their relationships and the origin of tuberosphere microbiome. Furthermore, we investigated the potential of bacterial communities in each soil compartment to carry out microbial sulfur cycling, a key nutrient cycling process in soil. To examine whether our results can be generalized, two varieties grown in soils with different physicochemical properties were analysed. We hypothesized that due to the distinct properties of the tuberosphere microenvironment it should uniquely shape the bacterial communities and therefore affects microbial sulfur cycling differently to the rhizosphere and the bulk soil.

Materials and Methods

Sample Collection Two commercial potato cultivars (*Solanum tuberosum* L.) Kerr's Pink and Rooster were grown in two adjacent agricultural field sites (T1 and T2) respectively, under standard agricultural practices in South-west of Ireland (Castletownroche area, County Cork). The general soil chemical characteristics of the two field sites (T1 and T2) at the time of sampling were as follows. The soil type of both field sites was sandy loam but the other soil parameters differed considerably between the two sites. At the T1 field site, the pH was 6.3 and the nutrient content was for phosphorus Morgans 11.8 (mg/L), potassium Morgans 117 (mg/L) and sulfur 8 (mg/L). At field site T2 the pH was 5, phosphorus Morgans 4.2 (mg/L), potassium Morgans 58 (mg/L) and sulfur 24 (mg/L) (as determined by Lancrop Laboratories Ltd., York, UK).

Four healthy plants of each genotype were selected at random from the field and sampled at the senescence stage on September 6th, 2019. The plants were carefully removed from the soil with a shovel and transported to the lab. The soil that was loosely attached to the roots and tubers was removed by shaking and was discarded. The tightly attached soil was collected by carefully brushing the roots (rhizosphere) and potato tubers (tuberosphere). The bulk soil was collected near the sampled plants (within 1m distance). Samples were stored either at − 80°C for DNA extraction or at 4°C for enzymatic and cultivation dependent analysis.

Evaluation of nutrient (sulfur) cycling in the three soil compartments of potato

Arylsulfatase activity measurement. Arylsulfatase activity was assayed in the three soil compartments by the method described by Tabatabai and Bremner (1970). Briefly, 1 g of soil was incubated for 1 h at 37°C with 4 ml of 0.5 M acetate buffer (pH 5.8), 0.25 ml toluene, and 1 ml of 50 mM p-nitrophenyl sulfate (Sigma-Aldrich, St. Louis, MO) as a substrate. After sample filtration with Whatman filter paper and dilution within the range of the standard curve, the p-nitrophenol color intensity was measured with a spectrophotometer at 400 nm.

MPN analysis of aromatic sulfonate utilizing bacteria. The estimation of the population of aromatic (aryl)sulfonate utilizing bacteria in the three soil compartments was performed in microtiter plates with minimal media (MM2TS) following the most probable number method (Fox et al. 2014; Fox et al. 2016; Ikoyi et al. 2020).

DNA extraction and quantitative PCR of the asfA **gene.** DNA was extracted from 0.25 g of frozen soil with the PowerSoil DNA Isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The DNA concentration was measured with a Qubit Fluorometer (Life Technologies) following the instruction of the Qubit dsDNA HS Assay kit (Life Technologies, Carlsbad, CA).

The quantification of *asfA* was performed in the microbial communities of the three soil compartments. The universal *asfA* primers, asfAF2 (5'-TACATGCGSCTGATGCGCAA-3'(Schmalenberger and Kertesz 2007) and asfAR1all (5'-GARAACTCCATGCCBGASA-3'; this study) were used to amplify a fragment of

282 bp. Quantitative PCR (qPCR) was performed on a Roche LightCycler® 96 (Roche Diagnostics, Mannheim, Germany). Reactions of 10 µl were prepared with 1x KAPA SYBR FAST qPCR Master Mix (2.5 mM MgCl2) (KAPA Biosystems, Wilmington, MA), 0.3 mM of each primer, 1µL (5M) betaine and 1 µL of DNA template. Samples, standards and non-template controls were tested in technical replicates of three. The qPCR conditions were as follows: a 5 min preincubation at 95°C, followed by 40 cycles of denaturation at 95°C for 3 s, annealing at 60°C for 20 s and extension at 72°C for 20 s. The normalization of the raw copies was performed relative to the DNA concentration of each sample and expressed as copies g^{-1} soil with the help of a standard curve (Schmalenberger et al 2008).

Statistical analysis of microbial sulfur cycling measurements MPN, arylsulfatase activity and the number of *asfA* copies were analyzed by one-way ANOVA. Shapiro–Wilk's and Levene's tests were used to check data for normality and homogeneity of variance respectively. Data from the MPN analysis violated the model assumptions of ANOVA and were logarithmically transformed to obtain normality. LSD (least significant difference) post hoc test was applied for pairwise comparisons to assess any significant differences ($P < 0.05$) in arylsusfatase activity and MPN between the three soil compartments with SPSS (IBM, Armonk, NY; 28.0.1.1) and the same test (library agricolae) was also applied to compare *asfA* copy numbers in R studio (V 4.1.1).

Analysis of Soil Bacterial Communities (16S rRNA gene NGS)

16S rRNA gene PCR amplification, library construction, sequencing and data processing. DNA from extractions as outlined above were used for sequence analysis. The 16S rRNA gene PCR amplification, library construction and sequencing were performed at the Novogene Bioinformatics Technology Co., Ltd. The bacterial 16S rRNA gene V3-V4 regions were amplified (barcoded primers 341F: 5'- CCT AYG GGR BGC ASC AG -3' and 806R: 5'-GGA CTA CNN GGG TAT CTA AT -3') using a Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA). Amplicon libraries were constructed with the NEBNext® DNA Library Prep Kit for Illumina (NEB,USA) following manufacturer's recommendations and ligated with index codes. Resulting libraries were assessed with the Agilent 2100 Bioanalyzer and quantified (qPCR). Library sequencing was performed on the Illumina NovaSeq 6000 platform to generate 470bp bp paired-end reads. A total of 2,879,485 raw reads were produced in total. Demultiplexed paired-end sequence data were analyzed with DADA2 (Version 1.16) in R (Version 4.0.2) Briefly, primers were removed and sequences were trimmed based on the quality plots. After denoising the paired end sequences were merged and the chimeras were removed. The sequence reads were aligned with the SILVAv138 database. One tuberosphere sample was excluded from the analysis due to the low number of non-chimera sequences (32,689). Plastid, mitochondrial and Cyanobacteria sequences as well as those not classified in the domain of Bacteria were excluded from the analysis.

Statistical analysis of the 16S rRNA gene data. Analysis of the amplicon sequencing data was performed in R studio (V 4.1.1). Alpha diversity indexes (Chao1, Shannon and Simpson) were calculated (function

estimate_richness; Phyloseq package). Kruskal Wallis test was applied (Kruskal_test function) to check significant differences between the compartments.

Dataset normalization was performed by rarefication to 18,921 reads (depth of the smallest dataset after filtering) per sample and 359 ASVs were removed. Beta diversity analysis was performed on the rarefied data to explore the structural differences in microbial communities among the three soil compartments. The ordinate function (physoseq package) was applied to perform PCoA with Bray-Curtis distance and PERMANOVA for the comparison of the three soil compartments. Pairwise comparisons between the bacterial communities were performed with the pairwise.adonis function (Vegan package). Differences in the relative abundance of the ten most abundant families were determined between the three soil compartments of each variety with Kruskal Wallis test (Kruskal.test function). Pairwise comparisons among the three soil compartments were performed with the Dunn's test (Dunn_test function, library FSA). Sequences were deposited at the Sequence Read Archive (BioProjectID: PRJNA961088; Accession numbers: SRR24309801-24).

Finally, Spearman correlation coefficients were calculated between the MPN values of arylsulfonate utilizers, abundance of *asfA* copies, arylsulfatase activity and the relative abundances of the families with significant differences among the compartments. The library corrplot (R studio) was used for conducting the statistical analysis.

Results

Sulfur cycling in the three soil compartments of potato. To assess the impact of the soil compartment on microbial sulfur cycling, the arylsulfatase activity, the most probable number of sulfonate utilizing bacteria and the number of *asfA* copies were assessed (Table 1**)**. The results showed that the rhizosphere communities of the variety Kerr's Pink had 1.4 times significantly higher arylsulfatase activity compared to the tuberosphere and bulk soil ($p < 0.05$) whereas, in Rooster arylsulfatase activity was only marginally higher in the rhizosphere compared to the bulk soil and not significantly different ($p > 0.05$). Rhizosphere in both varieties were significantly enriched with sulfonate utilizing bacterial populations (9.7 and 6.67 fold increase) compared to the tuberosphere as well as (10.22 and 20.59 fold increase) compared to the bulk soil in Kerr's Pink and Rooster respectively ($p < 0.05$) (Table 1). Additionally, in view of the importance of *asfA* as a molecular marker in the arylsulfonate desulfonation process, the absolute abundance of *asfA* copies was quantified via qPCR. Rhizosphere and tuberosphere microbial communities of Kerr's Pink harbour 5.15 and 4.63 times significantly higher copy numbers of the *asfA* compared to the bulk soil respectively ($p < 0.05$). In contrast, in Rooster the abundance of *asfA* was similar among the three compartments (Table 1).

Table 1
Arylsulfatase Activity, MPN values of desulfonating (MM2TS) as well as *asfA* copies in the bulk soil, rhizosphere and tuberosphere of the varieties Kerr's Pink and Rooster. Means with different letters are significantly different from each other ($p < 0.05$);± indicates the standard error and 95% confidence intervals (CI) of the MPN values are shown in brackets.

	Arylsulfatase Activity		**Arylsulfonate utilizers**		**Abundance of *asfA***	
	µg PNP $g^{-1}h^{-1}$	**±**	**MPN g^{-1}**	**95% CI**	**Gene copies g^{-1}**	**±**
Kerr's Pink bulk soil	110.00^{a}	6.43	$2.19\ 10^{5a}$	[$1.62\ 10^{4}$ – $2.95\ 10^{6}$]	$1.22\ 10^{5a}$	$3.14\ 10^{4}$
Kerr's Pink rhizosphere	156.78^{b}	17.49	$2.24\ 10^{6b}$	[$2.60\ 10^{5}$ – $1.93\ 10^{7}$]	$6.29\ 10^{5b}$	$1.39\ 10^{4}$
Kerr's Pink tuberosphere	111.00^{a}	8.9	$2.29\ 10^{5a}$	[$5.56\ 10^{4}$ – $9.44\ 10^{5}$]	$5.66\ 10^{5b}$	$5.62\ 10^{4}$
Rooster bulk soil	206.28^{ab}	7.77	$1.18\ 10^{5a}$	[$4.70\ 10^{4}$ – $2.83\ 10^{5}$]	$3.05\ 10^{5a}$	$7.52\ 10^{3}$
Rooster rhizosphere	220.08^{b}	9.97	$2.43\ 10^{6b}$	[$4.11\ 10^{5}$ – $4.44\ 10^{6}$]	$4.29x10^{4a}$	$6.01\ 10^{3}$
Rooster tuberosphaere	189.22^{a}	4.27	$3.64\ 10^{5a}$	[$2.43\ 10^{4}$ – $7.03\ 10^{5}$]	$3.29x10^{4a}$	$4.93\ 10^{3}$

Bacterial communities in the three soil compartments of potato. A total of 1,192,951 high-quality merged 16S rRNA sequences corresponding to an average of 51,867 per sample were obtained and mapped to 5,671 reference ASV sequences. All of the 5,671 ASVs were classified to the level of phylum, class and family (after filtering) and 58.8% of them were classified to the level of genus/species.

The bacterial alpha diversity was estimated by the Chao1, Shannon and Simpson indexes and were compared over the three soil compartments of both varieties with the Kruskal Wallis test. All estimated alpha diversity indexes didn't differ significantly among the compartments in both varieties ($p > 0.05$) (**Supplementary Figure S1**).

To further characterize the composition of the bacterial communities in three soil compartments of each variety the 10 most abundant bacterial families were identified (Figs. 1 and 2**)**. Only three families *Chitinophagaceae, Xanthobacteraceae* and *Lachnospiraceae* were common between the two varieties (Fig. 1A and 2A). In Kerr's Pink, out of the 10 families, six showed statistically significant differences between the soil compartments (*Xanthobacteraceae*, *Sphingomonadaceae*, *Rhizobiaceae, Nocordiaceae Hyphomicrobiaceae*, and *Gemmatinomonadaceae*) (Fig. 1B), while in Rooster five families had significant

differences among the compartments (*Sphingomonadaceae*, *Xanthobacteraceae, Lachnospiraceae, Acidothermaceae and Sphingobacteriaceae*) ($p < 0.05$) (Fig. 2B).

Pairwise comparisons with Dunn's test was applied to compare the relative abundances of the families with statistical significant differences among the compartments. In the tuberosphere of Kerr's Pink relative abundances of the families *Sphingomonadaceae*, *Xanthobacteraceae*, *Hyphomicrobiaceae, Nocardioidaceae* and *Gemmatinomonadaceae* were similar to the rhizosphere and bulk soil ($p > 0.05$), while significant differences were recorded between the rhizosphere and bulk soil ($p < 0.05$). (Fig. 1B).

In the rhizosphere, the family *Sphingomonadaceae* and *Rhizobiaceae* were enriched 1.25 and 2.47 times respectively compared to the tuberosphere and 1.7 and 2.25 times respectively compared to bulk soil. The families *Hyphomicrobiaceae*, *Xanthobacteraceae*, *Gemmatinomonadaceae* and *Nocardioidaceae* had the highest abundance in the bulk soil and they were 2.03, 2.03, 1.91 and 1.71 times respectively more abundant compared to the rhizosphere as well as 1.32, 1.28, 1.16 and 1.31 times respectively more abundant compared to the tuberosphere which was positioned between the two other compartments and statistically overlapped with both (Fig. 1B).

The relative abundances of the families *Sphingobacteriaceae, Xanthobacteracea and Acidothermaceae* in the tuberosphere of Rooster were similar to the rhizosphere and bulk soil ($p > 0.05$), while they were significantly different between the rhizosphere and bulk soil ($p < 0.05$). *Xanthobacteracea* and *Acidothermaceae* had the highest relative abundance in the bulk soil and the lowest in the rhizosphere (2.33 and 1.75 times lower abundance respectively) while in the tuberosphere their relative abundances lied between the bulk soil (1.29 and 1.65 times lower abundance respectively) and the rhizosphere (1.8 and 1.05 higher abundance respectively). The family *Lachnospiraceae* was 3.76 and 2.1 times more abundant in the tuberosphere when compared to the rhizosphere and the bulk soil but only significantly different to the rhizosphere. Finally, the family *Sphingomonadaceae* was significantly more abundant (1.74 and 1.88 times, respectively) in the rhizosphere soil when compared to the tuberosphere and bulk soil (Fig. 2B).

The similarities and differences in the structure of bacterial communities in the three soil compartments for both varieties were visualized with PCoA and the Bray-Curtis metric (Fig. 3). The first two axis explained 51.6% of the variability in the bacterial communities. Communities from the Kerr's Pink and Rooster were clearly separated on the first axis. Communities of the three compartments of Kerr's Pink were separated along the 2nd axis. The Kerr's Pink rhizosphere was more clearly separated from the other two compartments while the tuberosphere and bulk soil partially overlapped. In Rooster, the tuberosphere and rhizosphere communities appeared to cluster together over a larger section of the biplot, whereas the bulk soil appeared to be more distinct to the other two compartments. PERMANOVA analysis ($p = 0.001$*** and pseudo F test = 0.46540) confirmed the presence of significant differences among the bacterial communities. Subsequent pairwise comparisons between the compartments of Kerr's Pink revealed that the bulk and tuberosphere soil communities were similar ($p = 0.215$) whereas the rhizosphere and bulk soil ($p = 0.0425$) as well as the rhizosphere and tuberosphere ($p = 0.0425$)

communities were significantly different. In Rooster there were significant differences between the rhizosphere and bulk soil (p = 0.045) as well as the tuberosphere and bulk soil (p = 0.0425) communities while rhizosphere and tuberosphere communities were similar (p = 0.292).

Correlation analysis of the three soil compartments of potato. To better understand the relationships between the family's composition in the three soil compartments and bacterial sulfur cycling, a Spearman correlation analysis was performed. In both varieties the arylsulfatase activity, number of arylsulfonate utilizers and *asfA* gene copies were positively correlated ($p < 0.05$). Furthermore, in Kerr's Pink the arylsulfatace activity, number of arylsulfonate utilizers and *asfA* copies were positively and significantly correlated with families *Rhizobiaceae* (r = 0.67, 0.65 and 0.50 respectively) and *Sphingomonadaceae* (r = 0.60 0.40 and 0.85 respectively). A similar pattern was also observed in Rooster with the families *Sphingomonadaceae* (r = 0.32, 0.67, 0.31 respectively) and *Sphingobacteriaceae* (r = 0.26,0.81,0.15) but it was significant only for arylsulfonate utilizers in both families ($p < 0.001$). Contrary to that, the families *Xanthobacteraceae* (r=-0.58, -0.51, -0.66 respectively), *Gemmatomonadaceae* (r=-0.57,-0.81, =-0.44 respectively), *Nocordiaceae* (r=-0.55, -0.45, -0.51 respectively) and *Hyphomicrobiaceae* (r=-0.67, -0.43, -0.73 respectively) had a negative significant correlation with all three sulfur cycling indicators in Kerr's Pink. For Rooster, this was the case for *Lachnospiraceae* (r=-0.77, -0.45, -0.55 respectively) (Fig. 4A and B).

Discussion

Understanding the mechanisms of microbiome assembly and functionality in the different plant compartments constitutes an important step to manipulate the microbiome for the benefit of plant health and productivity. This is of special significance in the potato crop as the tuberosphere microbiome is associated with potato diseases (Van Der Wolf and De Boer 2007; Shi et al. 2019) and premature sprouting during potato storage (Buchholz et al. 2021). No research has been conducted on the concurring exploration of the bacteriome and nutrient cycling in the rhizosphere, tuberosphere and bulk soil of healthy potato plants, although these compartments are physically associated. Therefore, a complete picture of the tuberosphere bacteriome characteristics relative to the two other compartments is lacking. Here, we compared the structure, composition and abundance of the bacterial communities and their potential to perform sulfur cycling in the rhizosphere, tuberosphere and bulk soil.

Alpha diversity indexes Chao1, Shannon and Simpson were statistically similar between the three soil compartments. Hou et al. (2020), reported a decline in rhizosphere diversity as potato plants approaching the harvesting stage. In addition, İnceoğlu et al. (2011) reported that at the senescence stage the rhizosphere had similar diversity to the bulk soil. These findings are in line with the results of our study since the plant collected at the senescence stage (one week before harvest) which might have weakened the differences in alpha diversity indexes between the rhizosphere and bulk soil. The analysed tuberosphere had a diversity similar to the other two compartments, showing that by the time of full development of potato tubers differences in alpha diversity between the tuberosphere, bulk soil and rhizosphere are minimal.

Despite the similarities in alpha diversity between the compartments, we observed differences in the structure of bacterial communities between the three soil compartments, and in the bacteriome assembly in tuberospheres of two varieties. Analysis of beta diversity showed that in both varieties rhizosphere and bulk soil had distinct bacterial communities. This is a well-studied phenomenon known as the rhizosphere effect that has been already confirmed in potato (İnceoğlu et al. 2013b). Interestingly, the potato varieties presented different patterns of bacterial assembly in their tuberospheres showing that both soil type and potato genotype may determine the bacteriome dynamics in this compartment. The structure of tuberosphere communities of the variety Kerr's Pink resemble the bulk soil communities and they were differentiated from those in the rhizosphere, indicating that tuberosphere in this variety acquires its microbiome directly from the bulk soil. The tuberosphere of Rooster on the other hand, selects a bacteriome which is different from that in the bulk soil and partially resembles that of the rhizosphere bacteriome, suggesting that it is influenced by the rhizosphere and to a lesser extent by the bulk soil.

Although we cannot determine whether the differences in tuberosphere bacteriome assembly between the varieties is due to plant genotypic differences, soil effect or the synergy between them, previous studies on the potato rhizosphere microbiome indicate that soil and to a lesser extent the genotype are the main drivers of microbiome assembly in the roots (Van Overbeek and Van Elsas 2008; İnceoğlu et al. 2011). Interaction between the two factors has also been reported and influences the amount and composition of exudates released by the plant roots. Potato tubers are growing in close proximity to the root system, hence in the variety Rooster, this interaction may lead to greater production of exudates which influence the bacterial structure of the tuberosphere. Contrary to that, in Kerr's Pink the rhizosphere effect on the tuberosphere seemed to be weaker. A previous study also reported an effect of the genotype on the tuberosphere microbial communities which was attributed to the influence of root exudates on the soil attached to the potato tubers (Buchholz et al. 2019).

Although the structure of tuberosphere bacterial communities in the variety Kerr's pink were similar to that of the bulk soil, an effect of the tuberosphere microenvironment on the regulation of the top 10 most abundant families was observed. In Kerr's Pink the families *Hyphomicrobiaceae*, *Gemmatinomonadaceae*, *Xanthobacteraceae* and *Nocordiaceae* as well as the families *Xanthobacteraceae* and *Acidothermaceae* in Rooster were reduced in abundance, whereas the families *Sphingomodaceae* in both varieties and *Shpingobacteraceae* in Rooster were increased from the bulk soil to tuberosphere and rhizosphere These results indicate that regardless of the potential root exudates influence on tuberosphere, this compartment is able to affect the abundance of particular families. Consequently, the tuberosphere can be considered as an environment with transitional physicochemical characteristics between the rhizosphere and bulk soil, resulting in intermediate bacterial abundances for some bacterial families.

Nutrient cycling is an important microbial process that has been studied mainly in the rhizosphere. In the tuberosphere, there are only indirect evidences for active microbial regulation of nutrient cycling under common scab infection (Kopecky et al. 2019; Marketa et al. 2021). Here, we used the predominantly

bacterial sulfur cycling activity as a representative measurement of microbial activity or potential, in disease free potato plants to investigate this further. Apart from the significant role of sulfur in suppressing fungal and bacterial potato diseases, it is also particularly important for improved potato tuber yield and quality (Klikocka et al. 2005).

Our results showed that arylsulfatase activity and the number of bacterial cells participating in arylsulfonate cycling as well as the *asfA* copy number were higher in the rhizosphere of both varieties compared to the bulk soil and tuberosphere. The present study also observed a tendency for higher sulfonate sulfur cycling capacity in the tuberosphere compared to the bulk soil although this trend did only reach statistical significance for *asfA* copy numbers in Kerr's Pink. Our results are in accordance with previous studies which have shown that the plant capacity to control organic sulfur transformations in the soil is primarily associated with the higher microbial biomass that is present in the rhizosphere compared to the bulk soil (Castellano and Dick 1991). In addition, Diallo et al. (2011) reported similar levels of bacterial densities between the tuberosphere and rhizosphere, which might partly explain the higher sulfonate utilization potential observed in the tuberosphere relative to the bulk soil.

The positive association between *asfA* copies and the number of arylsulfonate utilizers (MPN) suggests that these two bacterial organic sulfur cycling activities are indeed largely the same function that is quantified cultivation independently and dependently. In addition, these bacterial sulfur cycling indicators were positively correlated with the family *Sphingomonadaceae* in both varieties as well as with *Rhizobiaceae* in Kerr's Pink and *Shpinogobactereaceae* in Rooster, suggesting a potential role of these families in bacterial sulfur cycling. Indeed, *ssuD* has been identified in members of the family *Sphingomonadaceae* (Aylward et al. 2013) while members of the families *Rhizobiaceae* (Gopalakrishnan et al. 2015) and *Sphingobacteraceae* (Mehnaz et al. 2007; Marques et al. 2010; Ahmed et al. 2014) are also associated with general plant growth promotion properties. However, the three families in question haven't been associated with known sequences of *asfA* to date (Gahan et al. 2022). It is also evident that these families are specifically selected by the roots given that they presented the highest relative abundance in the rhizosphere and they were negatively associated with the families which had the highest relative abundance in the bulk soil and negative association with the sulfur cycling indicators. The gradual reduction in abundance of the families *Rhizobiaceae Shpingomonadaceae* and *Sphingobacteraceae* from the rhizosphere to tuberosphere and bulk soil might also partially explain the same pattern of reduction that was observed with the absolute quantities of arylsulfonate utilizers (MPN and *asfA* copies) in the three soil compartments.

Contrary to microbial sulfonate cycling, the tuberosphere microbial communities had almost identical or lower arylsulfatase activity compared to the bulk soil. It seems that sulphate ester utilization although positively associated with MPN and *asfA* played a less important role in the enrichment of the tuberosphere with sulfur. Previous studies have indicated that sulfonates are oftentimes more important for plant growth (Kertesz and Mirleau 2004; Kertesz et al. 2007) and hence this might explain the differences observed in the present study among the two microbial processes in the tuberosphere.

The mechanism that promotes microbial nutrient cycling in the tuberosphere is largely unknown to date. Lenticels on the tuber surface may not only participate in gas exchange but may also take in sulfur from the soil solution and reduce its levels in the surrounding soil. Consequently, even a modest increase in microbial sulfur mobilization in the tuberosphere may be beneficial to the plant, while at the same time depletion of nutrients in the tuber vicinity would be likely. This phenomenon is well described in rhizospheres where nutrient uptake by the roots results in a depletion zone (Wang et al. 2007) while at the same time the rhizosphere effect results in enhanced microbial nutrient cycling/mobilization (Kuzyakov and Razavi 2019).

Furthermore, the role and functional importance of sulfur cycling on the surface of the potato tubers is not clearly determined. It has been reported though that sulfur participates in metabolic pathways involved in plant resistance to pathogens (Sagova-Mareckova et al. 2017; Kopecky et al. 2019; Marketa et al. 2021). In addition, higher levels of sulfur were reported in the tuberosphere of potato cultivars resistant to common scab (Kopecky et al. 2019; Marketa et al. 2021). Consequently, enhanced microbial sulfur mobilization in the tuberosphere may take place as a plant defence mechanism. This could also explain why in the present study, where no disease incidence occurred, only a modest increase of sulfur cycling potential was observed in the tuberosphere compared to the bulk soil. Furthermore, in our study we detected up to $6x10^6$ *asfA* copies g^{-1} soil in the organic sulfur utilizing bacterial communities while in a previous study the *asfA* copies of *Variovorax* alone reached this number in soils from the Netherlands (İnceoğlu et al. 2013a). Given that in our study *Variovorax* wasn't detected in the 16S rRNA gene dataset (data not shown) we speculate that *Variovorax* is not a crucial contributor in sulfur cycling in the different soil compartments of the present study and that other bacteria, not yet identified, have taken over this role.

In conclusion, our results show that the tuberosphere is not just an extended environment of the bulk soil but has distinct microbial properties. Indeed, the tuberosphere putatively regulates specific characteristics of the structure and composition of the bacteriome as well as nutrient cycling, dependent on the soil characteristics and plant variety. This observation raises new questions regarding the bacteriomes of the seed tubers and of the newly emerging potato roots. Potatoes are planted as tubers (seed tubers) and following the findings from the present study, their microbiome could also influence the establishment of the plant microbiome during the stage of potato development. In addition, the root system of potato is emerging from the tuber surface and consequently the newly developed roots may acquire the microbiome from the tuber surface and the endosphere. If that is the case, the soil may be less important as a microbial reservoir at the early stages of root development in potatoes. These two hypotheses need further examination as the first stages of plant development and their interaction with soil microbes are critical for the plant vigour.

Finally, it seems that potato genotypes affect sulfur cycling in the tuberosphere to potentially increase potato resistance to common scab. Consequently, the evaluation of microbial sulfur cycling in this compartment could be incorporated in plant breeding programmes as a trait of selection, for picking genotypes with resistance to common scab and other soil pathogens.

Declarations

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Author contributions: Author AS secured the research funding, provided the concept and design of the study, contributed to sampling and analysis and revised the manuscript draft. Author GV was leading the material preparation, sampling and analysis and wrote the first draft of the manuscript as well as contributed to the revision of the manuscript to its current form.

Data availability: Sequencing data analysed in the present study are available from the Sequence Read Archive via the following SubissionID: SUB13162729 and BioProject ID: PRJNA961088; Accession numbers: SRR24309801-24.

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Figures

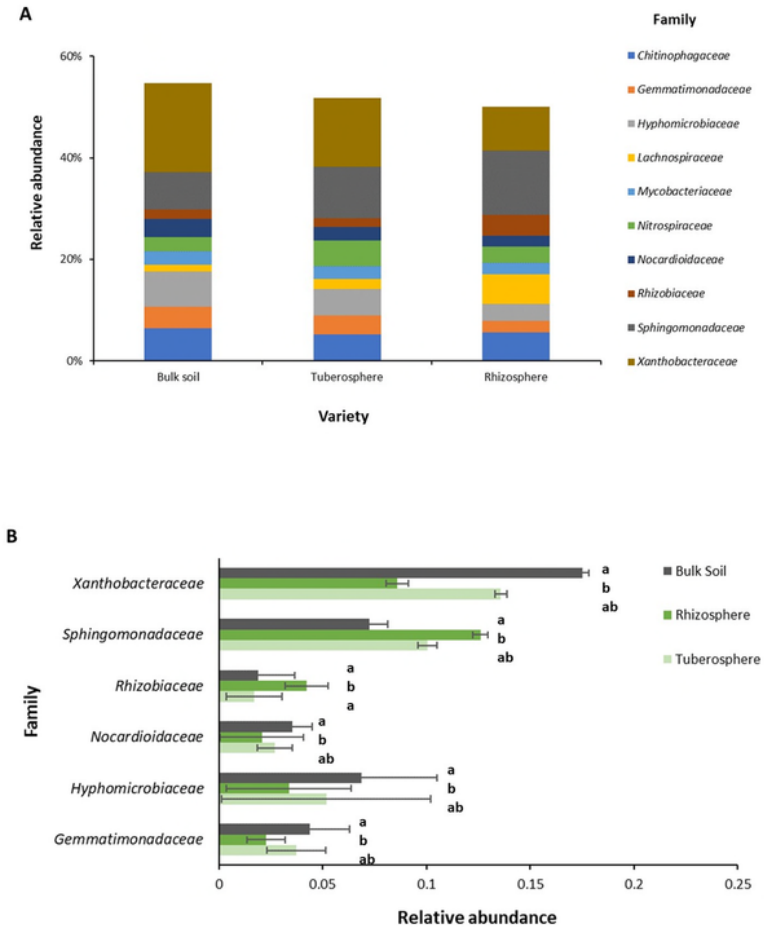

Figure 1 The ten most abundant bacterial families (**A**) and mean relative abundances of significantly different families (**B**) in the three soil compartments of the Variety Kerr's Pink. Letters (a, b, ab) indicate significant differences between Bulk Soil (dark green), Rhizosphere (green) and Tuberosphere (light green). Sequence data were obtained through NGS of the 16S rRNA gene.

Figure 1

See image above for figure legend

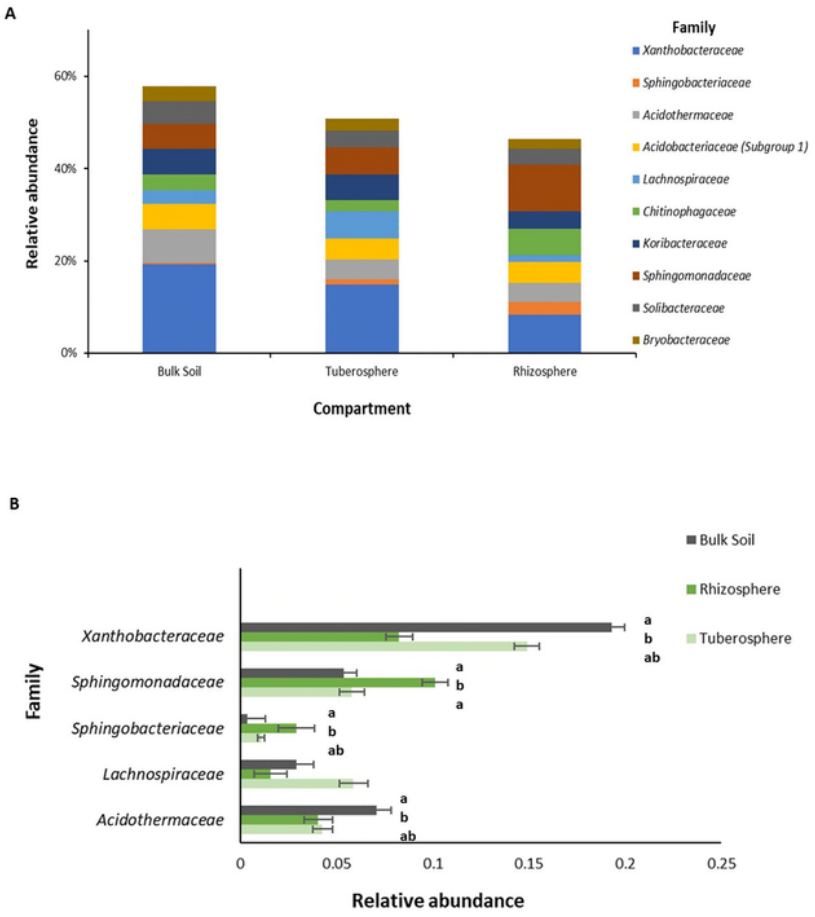

Figure 2 The ten most abundant bacterial families (**A**) and mean relative abundances of significantly different families (**B**) in the three soil compartments of the Variety Rooster. Letters (a, b, ab) indicate significant differences between Bulk Soil (dark green), Rhizosphere (green) and Tuberosphere (light green). Sequence data were obtained through NGS of the 16S rRNA gene.

Figure 2

See image above for figure legend

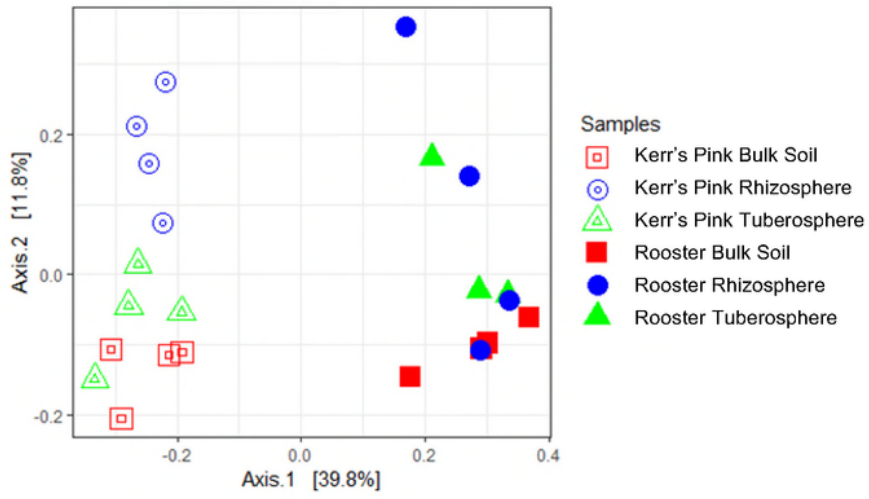

Figure 3 PCoA plots of the bacterial community composition in the three soil compartments Bulk Soil, Rhizosphere and Tuberosphere of the varieties Kerr's Pink and Rooster with Bray-Curtis distance metric. Sequence data were obtained through NGS of the 16S rRNA gene.

Figure 3

See image above for figure legend

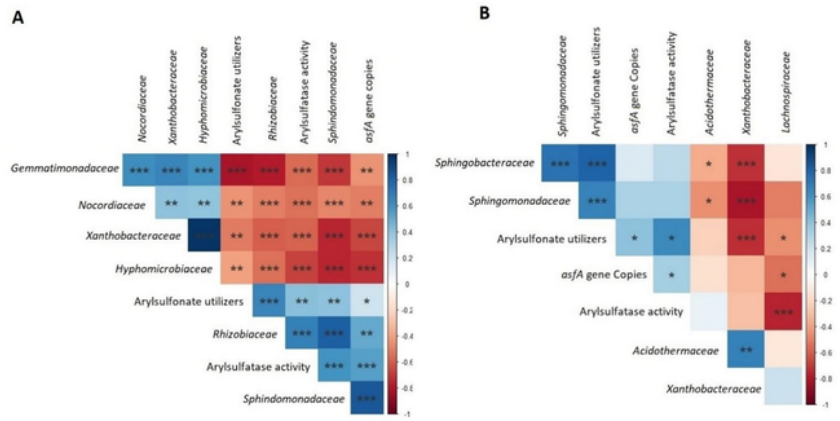

Fig 4. Spearman correlation of the sulfur cycling indicators. Arylsulfatase activity, number of Arylsulfonate utilizers, abundance of *asfA* gene copy number and bacterial families with significant different relative abundances among the compartments in Kerr's Pink (**A**) and Rooster (**B**). Asterisks indicates significant differences (p-values: *** = 0.001, ** = 0.01, * = 0.05). Blue color scale indicates positive and red color scale negative r values.

Figure 4

See image above for figure legend

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