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Metagenomic survey of the bacterial communities in the rhizosphere of three Andean tuber crops

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Abstract

Microbes colonizing the rhizosphere are important drivers of plant health, supplying nutrients and antagonizing pathogens, among other beneficial activities. Tubers are important staple crops in the Andean highlands, produced by thousands of small-farmers and consumed by millions. Here we report the composition of the bacterial communities colonizing the rhizospheres of three Andean tuber crops (ATCs), namely oca (*Oxalis tuberosa*), ullucu (*Ullucus tuberosus*) and mashua (*Tropaeolum tuberosum*). We used high throughput sequence analysis of 16S rRNA genes to describe the bacterial diversity of rhizospheric soils associated to thee crops. Between 4862 and 5080 OTUs were exclusively detected in each one of the ATCs' rhizospheres; the majority of the 100 most abundant OTUs belonged to the Bacteroidetes and Proteobacteria phyla. Beta diversity indices revealed a low similarity between the three communities, suggesting differences in their specific composition. Only 566 bacterial OTUs were shared by all three tuber's rhizospheres and absent from the surrounding bulk soil. Apart from studies in potato, this is the first report concerning the diversity and abundance of bacterial taxa associated with the rhizosphere of other important and traditional ATCs.

Keywords Andean tuber crops · Rhizobacteria · 16S rDNA · Rhizosphere microbiome · Taxonomic survey

1 Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), agricultural production must have to be increased by at least 60% (relative to 2005–2007 levels) in 2050 in order to sustain the needs of the world human population (Alexandratos and Bruinsma 2012). Reaching this goal will demand a greater intensification of current agriculture within the constraint of being unable to expand the agricultural frontier while also reducing and mitigating past impacts of agriculture on the environment (Doran and Zeiss 2000). Unfortunately, in many densely populated mountainous regions of the world —such as the Himalayas or the Andes Mountains—, agriculture intensification is subject to even more challenges, due to the competing uses of agricultural land, growth-limiting constant low temperatures, unpredictable changes in weather, severe erosion of soils, low nutrient availability, low organic matter content, and lack of irrigation among others (Poulenard and Podwojewski 2006; FAO 2015). All this is further complexed by the greatly fragmented organization of farmers, mostly small family farmers, often impoverished, but who are still key contributors to the food security of their countries.

In the past, advances in agricultural intensification relied on the development of improved plant varieties, and the use of synthetic pesticides and chemical fertilizers, giving rise to the so-called Green Revolution (Pingali 2012). Among the current paradigms, intensification of agriculture can be achieved in a sustainable way by making rational use of an often-neglected resource: the microbes that naturally colonize the rhizosphere of crop plants. Many of these microbes exhibit plant-growth promoting traits and are called thus Plant Growth Promoting Microorganisms or PGPM (Fuentes-Ramirez and Caballero-Mellado 2005;

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Berg and Smalla 2009; Singh et al. 2011; Velivelli et al. 2014a). In fact, many rhizosphere-inhabiting PGPM are paramount players in some major biogeochemical cycles; for instance, they mobilize and transform minerals containing important plant nutrients like P and S. Further, rhizosphere microorganisms promote plant growth and plant health through direct- (e.g. production of phytohormones) and indirect mechanisms (e.g. induction of plant-systemic resistance) (Glick 2012). Altogether, these microbial activities increase plant productivity (De-la-Peña and Loyola-Vargas 2014).

In the past 15 years, scientists have started to explore the possibility of making use of indigenous PGPM colonizing mountainous soils and crops, to develop efficient bioinoculants to be used in these regions (Pandey et al. 2004, 2006; Yarzábal and Chica 2017). Bioprospection of these environments has led to the discovery of potentially useful PGPM, some of which have been tested successfully in the field (Trivedi et al. 2012; Ghyselinck et al. 2013; Yarzábal and Chica 2017). Among the natural environments bioprospected stand out the rhizosphere of a few native Andean crops, like potato and quinoa. However, in terms of food security, other native Andean tuber crops (ATCs), like oca (*Oxalis tuberosa*), ullucu (*Ullucus tuberosus*) and mashua (*Tropaeolum tuberosum*), are as important as potatoes, especially for small and impoverished farmers of these highlands.

Together with potato, ATCs play an important role as staple crops, produced and consumed by thousands smallholder families in the Andean highlands. ATCs produce some of the highest yields of calories produced per cultivated area, which make them important as food security crops in these communities (Flores et al. 2003). Furthermore, these ATCs show great adaptation to grow in extremely inhospitable areas for agriculture where other crops normally fail (Roca et al. 2007), making them an excellent alternative (if not the only one) for to grow food under the harsh conditions of the high mountains.

Notwithstanding their importance as food-security crops, with a high nutritional value and several medicinal properties, no studies have been conducted so far to survey the microbes colonizing the rhizosphere of these ATCs. Therefore, to expand our knowledge about the microbial species colonizing the rhizosphere of ATCs, and as a first step towards the identification of potentially useful microbes to support options for their sustainable production, we explored the ATC rhizospheric microbiomes by massive parallel sequencing of 16S rRNA genes (metabarcoding).

Our main goals were i) to characterize the bacterial community of the rhizosphere of three ATC species, namely oca, mashua and ulluco; ii) to investigate significant compositional differences among these communities; and, iii) to define a shared rhizosphere microbiome for these three ATCs..

2 Materials and methods

2.1 Site description and soil sampling

Soil samples were collected at the end of the dry season from one *chacra* (e.g. small plots used by traditional Andean farmers to grow cereals, leguminous vegetables, roots and tubers) located near Cañar (−2575442S, −78.971689 W) at ~3700 masl in Ecuador (Electronic Supplementary Material, Fig. S1). At sampling time, the *chacra* had been cultivated for almost ten months with oca, mashua and ullucu, without chemical fertilization. Prior to this crop, the plot had remained almost undisturbed for several years, as customary for growers in this area.

Tubers from plants belonging to three ATC species (oca, mashua and ullucu) (Electronic Supplementary Material, Fig. S2) were collected at different sites at approximately 5–10 cm depth by pulling out the plants with their complete root system. Several tubers and the soil adhering to them were pooled in the same collecting bag. Rhizospheric soil and tubers were transported to the lab (< 2 h) and stored at −80 °C until DNA extraction. Bulk soil samples were collected the same way at three different points in the chacra and at the furrow between rows. The samples were pooled in the same collecting bag and processed as described above.

2.2 Environmental DNA extraction from soil samples

DNA was extracted from 250 mg of unfrozen soil using the PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, USA), following the manufacturer's protocol, as it has been shown to be a robust method for DNA extraction from soils (Mahmoudi et al. 2011). Quality and size of DNA were checked by electrophoresis on 1% agarose. Further quality checking of extracted DNA was performed spectrophotometrically by calculating 260 nm/280 nm and 260 nm/230 nm ratios. Concentration of DNA was determined using Qubit Fluorometric Quantitation (Life Technologies, Carlsbad CA).

2.3 Amplification and Illumina MiSeq sequencing of DNA

Polymerase chain reaction (PCR) amplification of the hypervariable V3–V4 regions of the 16S rRNA gene was performed on each individual soil DNA sample using universal primers (Klindworth et al. 2013) joined to a multiplex identifier sequence, following standard procedures recommended by the manufacturer (Illumina documents 2019). For each sample, amplicons were generated in several replicate PCRs using mixtures (25 μl) that contained 25 pmol of each primer, 1x KAPA HiFi Hotstart Ready Mix (Kapa Biosystems, Wilmington, MA USA) and 10 ng of the DNA template. The PCR program consisted of an

initial denaturation step at 95 °C for 3 min, 25 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final step of heating at 72 °C for 5 min. Amplicons of the same treatment were pooled to reduce per-PCR variability and purified using AMPure XP beads (Beckman Coulter, IN USA) according to the manufacturer's instructions. After PCR clean up, Illumina sequencing adapters were attached by a second PCR step using Nextera XT Index Kit (Illumina Inc., Sand Diego CA USA). The mixture contained Nextera Index Primers 1 and 2 (5 μl), 2× KAPA HiFi Hotstart ReadyMix (25 μl), DNA (5 μl) and PCR grade water (10 μl) for a total volume of 50 μl. The PCR program in this step consisted of an initial denaturation step at 95 °C for 3 min, followed by 8 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s and extension at 72 °C for 30 s, and a final step at 72 °C for 5 min. The amplicons were cleaned-up as previously described. The amplicon libraries were quantified using Qubit (Invitrogen, CA, USA), the samples were combined in equimolar amounts (4 nM each) and sequenced in an Illumina's MiSeq platform at Macrogen (Seoul, South Korea) according to the manufacturer's instructions.

2.4 Taxonomic assignment of sequence reads and diversity indexes

Paired-end read sequences generated from Illumina MiSeq were processed using Mothur (Schloss et al. 2009) following Schloss' lab standard operating procedure (Kozich et al. 2013) adjusted to amplicons from the V3-V4 hipervariable region. Briefly, sequences were paired into contigs, screened for quality (ambiguity = 0, homopolymers = 0, maximum length = 475 bp) and preclustered (diffs = 4); chimeras were identified using Vsearch (Rognes et al. 2016) and removed. The resulting sequences were aligned to the Silva 132 database (Quast et al. 2013) and classified with a cutoff value of 97% similarity; after classification, non-bacterial taxa were removed. The taxonomy and shared files produced in Mothur were then imported into R (R Core Team 2018) using the Phyloseq package (McMurdie and Holmes 2013) where diversity indices were calculated and the number of shared- and unique OTUs identified in the samples.

Data availability High throughput sequencing datasets were deposited in the NCBI Biosamples database under accession numbers SAMN10414050, SAMN10414052, SAMN10414053, and SAMN10414056 for the Sani Mashua, Gallo Ullucu, Cambray Oca and soil 16S DNA metabarcoding libraries, respectively.

3 Results

Between 84,318 and 99,643 good quality sequences were obtained after quality control of the sequenced V3-V4 16S amplicons from rhizospheric soil of three ATC species (namely "cambray" oca, "sani" mashua and "gallo" ullucu) and the surrounding bulk soil. These sequences were grouped into 26,016 unique OTUs (97% sequence identity). Depending on the soil sample, the number of OTUs identified in these sequences ranged between 9032 and 9578 OTUs (Electronic Supplementary Material, Table S1).

Alpha diversity indices of bacterial OTUs detected in rhizospheric soil from oca, ullucu and mashua were similar to the diversity of bacterial OTUs detected in uncultivated soil (Electronic Supplementary Material, Table S1). In contrast, beta diversity measures revealed low similarity between the four samples, suggesting differences in the specific composition of the bacterial communities present in each rhizosphere (Fig. 1a). A lower proportion of OTUs was shared by all samples (1810), whereas a larger number of OTUs were unique to each tuber or soil samples.

Pairwise analysis of the taxonomic composition and relative abundance of the OTUs among soil- and tuber samples revealed that communities in the soil/ullucu and soil/mashua samples were the most similar (~28% similarity, Fig. 1a), whereas communities in the oca/ullucu and oca/mashua samples were the most dissimilar (~16–18% similarity).

Between 4862 and 5080 OTUs were exclusively detected in each one of the four samples. The number of OTUs shared by all four samples (i.e. ATCs rhizospheres plus soil samples) reached 1810. From these, less than one-third OTUs (566) were shared by the rhizosphere of the three tuber species (shared OTUs) and were absent in the surrounding bulk soil (Fig. 1b).

The taxonomic diversity of the OTUs detected in each sample was explored at the level of Phylum and family. OTUs from all four samples belonged to 38 unique Phyla as annotated in the Silva database; from these, the 100 most abundant OTUs in the four samples belonged to 10 phyla, being Proteobacteria, Actinobacteria and Acidobacteria the most abundant, accounting for at least 50% of the total relative OTU abundance in these microbiomes (Fig. 2a). Strikingly, Proteobacteria, Actinobacteria and Bacteroidetes were less abundant in the bulk soil than in the rhizosphere of ATCs. On the contrary, the abundances of Acidobacteria and Chloroflexi was higher in the bulk soil.

The relative abundance of the phyla of the 100 most abundant OTUs in the four samples was similar in all four samples (Fig. 2a). However, when removing from this set

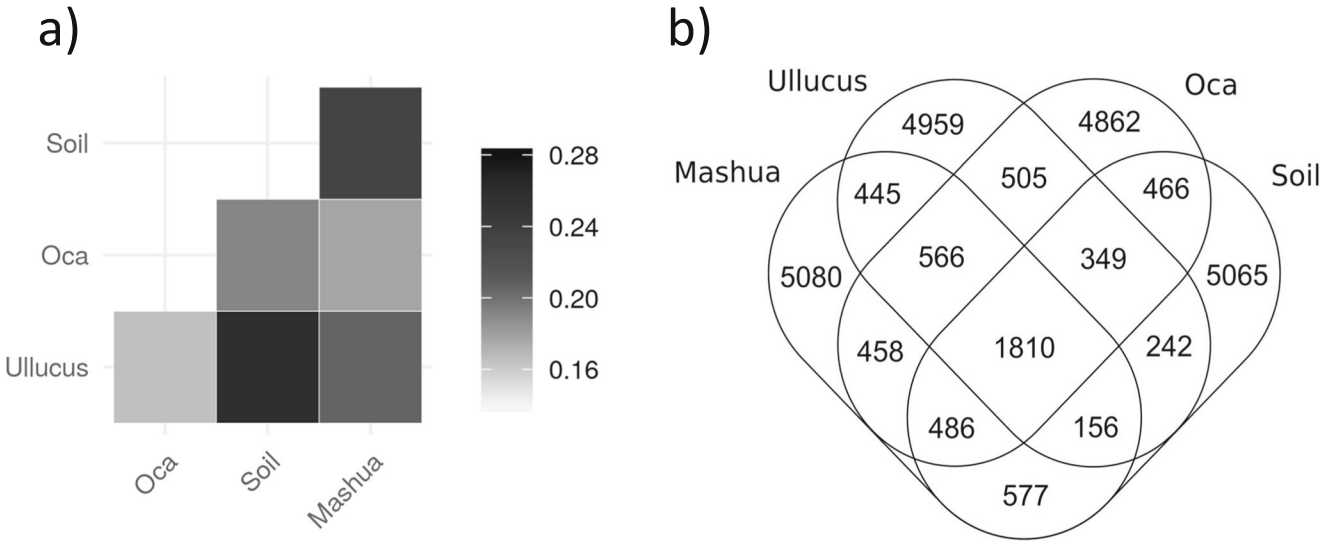

Fig. 1 **a** Jaccard's similarity matrix of the bacterial OTU composition considering relative OTU abundance in oca's, ullucu's and mashua's rhizospheric soil and in uncultivated soil; **b** Number of unique and shared bacterial OTUs in oca's, ullucu's and mashua's rhizospheric soil and in uncultivated soil

of data OTUs shared by the ATCs rhizosphere and the bulk soil, the taxonomic composition was different (Fig. 2b). Indeed, most of the 100 most abundant OTUs in the rhizosphere of the three ATCs belonged to the Bacteroidetes and Proteobacteria phyla, whereas Acidobacteria, Chloroflexi and WPS-2 related OTUs were barely represented. Interestingly, as opposed to oca's and mashua's rhizospheres, the relative abundance of Actinobacteria-related OTUs in ullucu's rhizosphere remained almost unchanged after removing OTUs shared with the surrounding bulk soil (Fig. 2a, b).

The relative abundances of a few phyla in the rhizosphere of ATCs showed important variations when OTUs present also in the bulk soil were removed from the analysis. This was the case of Bacteroidetes, a taxonomic group that was more abundant in the rhizosphere of ATCs when OTUs shared with the bulk soil were not considered in the comparison (see Fig. 2a, b); at the opposite side, Chloroflexi and Acidobacteria were barely present in the rhizosphere of ATCs when OTUs shared with the bulk soil were excluded from the analysis (See Fig. 2a, b). Surprisingly, the distribution of taxa remained

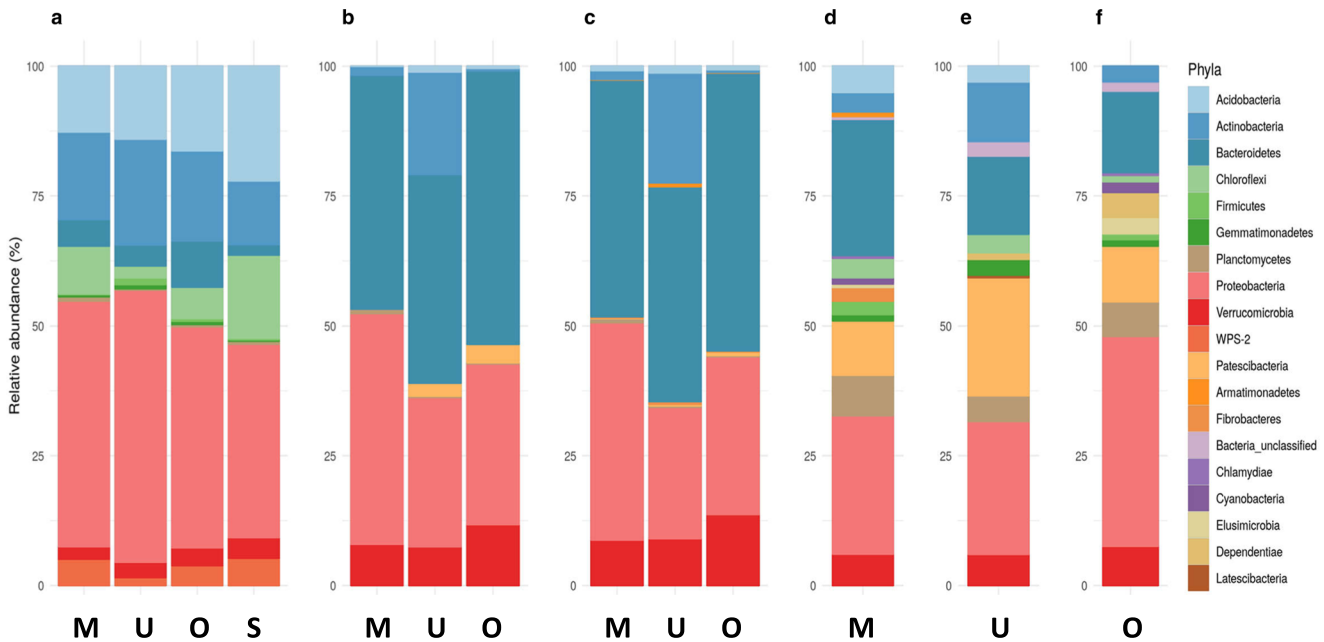

Fig. 2 Relative abundance of the phyla to which the 100 most abundant OTUs in the complete dataset belong (**a**). OTUs exclusively present in ATC rhizosphere soil samples (**b**). OTUs shared by the three ATCs (**c**). OTUs exclusively detected in mashua's (**d**), ullucu's (**e**) and oca's (**f**) rhizosphere soil samples. M: mashua; U: ullucu; O: oca; S: soil

almost unchanged if only the 100 most abundant OTUs shared by the three ATCs (the "shared bacteriomes" see below) were considered in the analysis (see Fig. 2b, c). As highlighted above, the proportion of Actinobacteria-related OTUs remained almost unchanged and was much higher in ullucu's rhizosphere.

While the number of phyla represented by the 100 most abundant OTUs of each sample ranged between 7 and 10 when considering OTUs from all four samples (Fig. 2a), ATC-exclusive OTUs (Fig. 2b) and ATC-shared OTUs (Fig. 2c), the number of phyla exclusive to mashua's, ullucu's and oca's rhizospheres reached 16, 12 and 14 respectively (Fig. 2d–f). Noticeably, OTUs related to the Planctomycetes and Patescibacteria phyla —barely detected in the 100 most abundant OTUs shared by all samples—, were present at a higher relative abundance when considering tuber-exclusive OTUs (Fig. 2b).

When interrogating the dataset at a lower taxonomic level, some common patterns appeared. For instance, OTUs belonging to the Nocardiaceae were present in the rhizospheric soil of the three ATCs, but absent from the surrounding bulk soil (Fig. 3). Another two families, namely Flavobacteriaceae and Steroidobacteriaceae, were less represented in the bulk soil than in the rhizosphere of the ATCs.

When excluding from the analysis the OTUs shared between the ATCs and the surrounding bulk soil, other patterns emerged. For instance, while two families (Oligoflexaceae and Saccharimonadales) were absent from mashua's and oca's rhizospheres, Porticoccaceae was not present in ullucu's rhizosphere (Electronic Supplementary Material, Fig. S3). On the other side, Parcubacteria and Rhodanobacteraceae were absent in mashua's rhizosphere, while Rhodociclaceae was absent in oca's rhizosphere.

Seven families were detected in the three ATCs rhizospheres and present at higher abundances. These families are Opitutaceae, Methilophilaceae, Sphingobacteraceae, Chitinophagaceae, Flavobacteraceae, Sphingomonadaceae and Burkholderaceae (Electronic Supplementary Material, Fig. S3).

As stated before, 1810 OTUs were present in all four samples (ATC rhizospheres and bulk soil), but only 566 were exclusively present in three tuber's rhizospheres (Fig. 1b). From these, only a few exhibited an abundance above 0.001% (Electronic Supplementary Material, Fig. S4) and will be referred therefore as the "shared ATCs bacteriome". Among the genera represented in this shared bacteriome, the following stand out: *Ferruginibacter* (>0.003%), *Pedobacter* (=0.003%), *Methylotenera* (=0.003%), *Lacunisphaera* (=0.003%), *Flavobacterium* (=0.003%), and *Aquabacterium* (=0.003%). Finally, members belonging to some other genera were also present at all three rhizospheres, but with a lower abundance (including *Sediminibacterium*, *Rhodoferax*, *Luteolibacter*, *Dyadobacter* and *Chitinophaga*).

4 Discussion

Andean tuber crops (ATCs) are highly nutritive staples produced and consumed by millions in the high Andes. As a first step towards understanding the ecological interactions that occur in the rhizosphere of ATCs, we used a high-throughput sequencing method to depict the composition and structure of the microbial communities colonizing this habitat. We show here that the species richness of the rhizosphere bacteriomes of oca, mashua and ullucu was similar. Our data also revealed some important differences in the taxonomic composition of the bacterial communities present in each tuber's rhizosphere. Moreover, only a few OTUs were common to all three ATCs, whereas a large number of OTUs were present exclusively in the rhizosphere of each tuber.

We explored the taxonomic diversity of the rhizospheric bacteriomes at the level of Phylum and family, since it has been established that the assignment of sequences to lower taxa (i.e., genus and species level) with the classifiers in use drops off under a certain level, thus making inappropriate finer taxonomic resolution (Tessler et al. 2017). Noticeably, the composition of the bacterial community represented by the 100 most abundant OTUs was mainly influenced by the surrounding bulk soil. This suggests that bacteria present in bulk soil are major contributors to the diversity of the rhizospheric soil in the three tuber crops. In fact, it has been proposed that soil act as a source *reservoir* from which plant roots sample their microbiota (Schlaeppi et al. 2013; Zarraonaindia et al. 2015; Qiao et al. 2017).

OTUs belonging to three phyla, namely Proteobacteria, Actinobacteria and Acidobacteria, accounted for at least 50% of the 100 most abundant OTUs in all samples (Fig. 2a). As previously established, bacteria belonging to those phyla are the most frequent colonizers of soils in general (Janssen 2006; Fierer et al. 2012), and those showing the highest relative abundances across soils from all around the globe (Delgado-Baquerizo et al. 2018). Furthermore, bacteria belonging to those phyla are also frequently detected in the rhizosphere of crop plants such as potato (Inceoğlu et al. 2011; Barnett et al. 2015; Marques et al. 2014; Pfeiffer et al. 2017), maize (Garcia-Salamanca et al. 2013; Peiffer et al. 2013; Correa-Galeote et al. 2016), and wheat (Donn et al. 2015; Ai et al. 2015), among many others.

Some OTUs were exclusively detected in the rhizosphere of the ATCs studied (while absent from the surrounding soil), and very few of these were shared by the three ATCs species. This is in line with previous studies showing that root exudates can tailor-shape the taxonomic composition of the microbial communities they support, by producing and excreting complex mixtures of compounds that serve as energy sources but also as chemoattractants (Shi et al. 2013; Biedrzycki and Bais 2013). Furthermore, some phyla (i.e. Proteobacteria, Bacteroidetes and Verrucomicrobia) were more abundant than others, suggesting

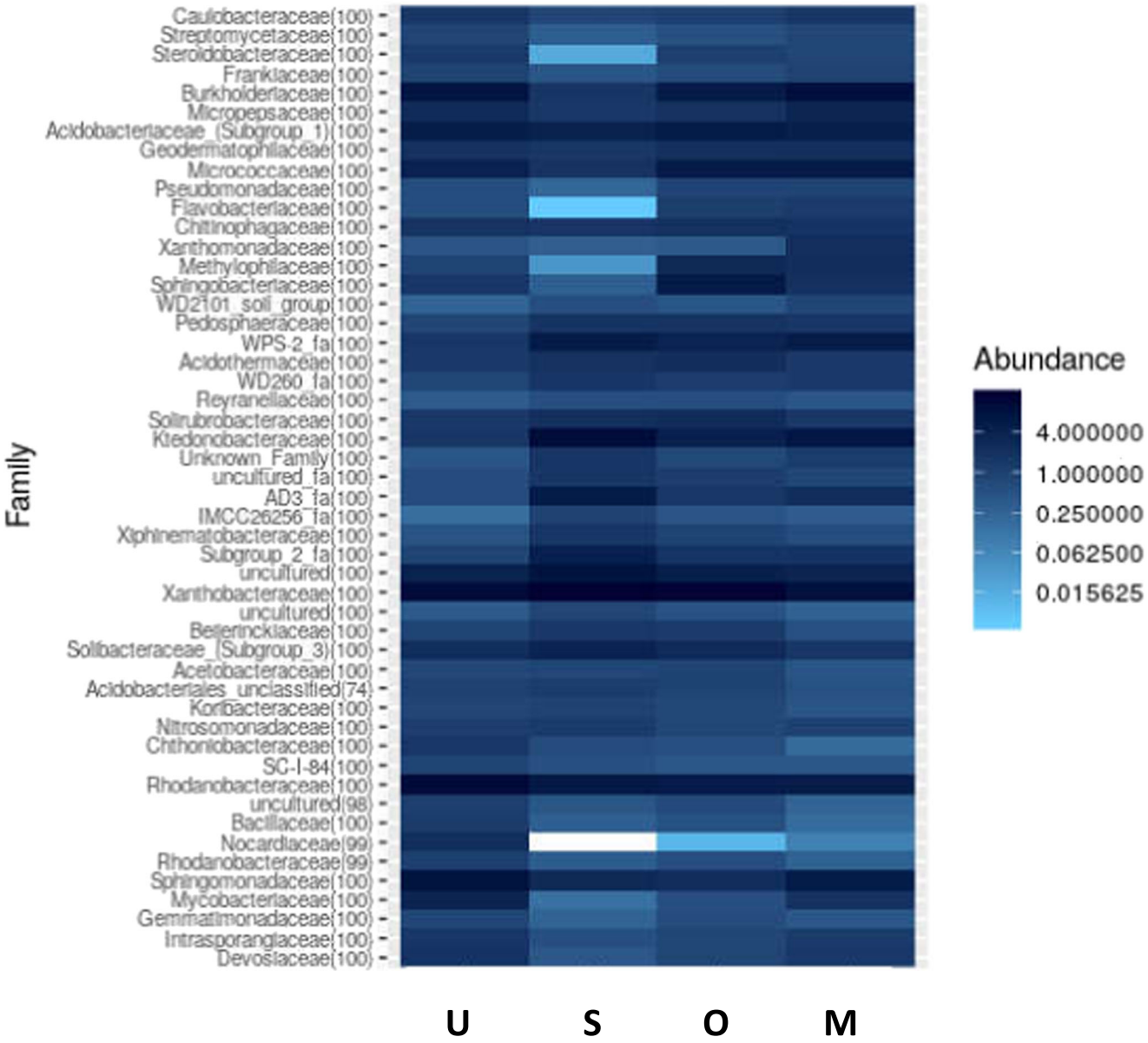

Fig. 3 Relative abundance of the top 100 bacterial families detected in ATCs rhizospheric- and non-rhizospheric soil by 16S DNA metabarcoding. U: ullucu; S: soil; O: oca; M: mashua

that they were promoted in this environment, a result that confirm previous observations made by Lundberg et al. (2012), Ling et al. (2015), Bulgarelli et al. (2015) and Qiao et al. (2017).

When the composition and abundance of the rhizobacterial OTUs were compared pairwise, the communities in oca/ullucu and oca/mashua samples were the most dissimilar. Incidentally, *O. tuberosa* ("oca") plants produce and excrete high amounts of fluorescent exudates through their roots, both in vitro and in the field (Bais et al. 2002a, 2003, 2010). Although the identity of these compounds remains to be firmly established, some of them are biologically active and exhibit a strong phototoxicity against oca predators (Larson et al. 1988; Flores et al. 1999; Walker et al. 2003). These toxic exudates also affect a wide range of soil-borne microorganisms (Bais et al. 2003), a result that suggest a potential role for these products as chemical *selectors* in the rhizosphere environment, allowing the growth of particular strains while inhibiting others. Interestingly, neither ullucu nor mashua have been reported to produce these fluorescent compounds in vitro (Bais et al. 2002a).

Some OTUs, assigned to the Proteobacteria, Actinobacteria and Bacteroidetes phyla, were more abundant in the rhizosphere of ATCs than in the surrounding bulk soil. The reasons explaining these differences might be related to i) the ability of Proteobacteria as effective rhizosphere and root colonizers (Uroz et al. 2010); ii) the efficient use of root exudates by members of the Proteobacteria and Actinobacteria (Fierer et al. 2007; Ai et al. 2015); and, iii) the ecological role played by these bacteria as promoters of plant growth, a trait

that favors their recruitment from the surrounding soil by the plant host, through modification of their root exudates, as proposed by several authors (Rudrappa et al. 2008; Berg and Smalla 2009; Bakker et al. 2013; Philippot et al. 2013). Besides, it is well documented that Actinobacteria are highly resilient towards the environmental challenges imposed by edaphic- and climatic conditions; therefore, they are successful in colonizing different types of soils, including extreme soils like those characteristic of the high mountains (Lauber et al. 2009; Basilio et al. 2003). Incidentally, Proteobacteria and Actinobacteria are also amongst the dominant groups in permanently frozen soils, like those characteristic of high elevation permafrost (Yun et al. 2014; Hu et al. 2015, 2016; Frey et al. 2016).

In the case of Bacteroidetes, their abundance in the rhizosphere of ATCs can be a consequence of their copiotrophic mode of life, as already proposed by Trivedi et al. (2013). Indeed, members of this Phylum grow at a high rate in the presence of adequate amounts of nutrients (R-strategists) and play a fundamental role as decomposers and communicators in the rhizospheric environment, promoting the flow of energy and the cycling of materials (Wu et al. 2018). Interestingly, Bacteroidetes and Proteobacteria are enriched in the rhizosphere of senescent Andean potatoes, as recently shown by Pfeiffer et al. (2017). On the contrary, Acidobacteria and Chloroflexi were more abundant in the bulk soil than in the rhizosphere of ATCs. This can be related to the oligotrophic mode of life of these bacteria (Koch 2001; Fierer et al. 2007), which are able to degrade ancient- or older soil organic matter, including cellulose (Lauber et al. 2009; Bruce et al. 2010), usually present in the soil.

The relative abundance of the Actinobacteria phylum remained almost unchanged in ullucu's rhizosphere, after removing OTUs shared with the surrounding bulk soil. This was particularly intriguing, since oca's and mashua's rhizospheres did not exhibit the same behavior (see Fig. 2a, b). Actinobacteria are recognized as K-strategists, able to effectively colonize resource-limited environments (Atlas and Bartha 1998). Additionally, since members of some orders form spores, they can withstand unfavorable conditions (Tang et al. 2016). For those reasons, they are frequently found in soil microbial communities. To what extent ullucu's exudates are able to sustain a specific community of Actinobacteria, which do not colonize efficiently the bulk soil, remains to be established.

The distribution of bacteria belonging to Planctomycetes and Patescibacteria was particularly intriguing. Both were barely detected in the 100 most abundant OTUs shared by all four samples; however, when considering only tuber-exclusive OTUs, they were present at higher relative abundances (see Fig. 2b). Planctomycetes are frequently detected in low-temperature environments, like cold soils from the Himalaya (Stres et al. 2014), the Kunlun Mountains in the Tibetan Plateau (Yang et al. 2016), the Arctic and Sub-arctic tundra (Steven et al. 2007; Wagner et al. 2009; Kim et al. 2014; Hultman et al. 2015) and the polar deserts (Steven et al. 2013). Bacteria belonging to this phylum can degrade efficiently exopolysaccharides produced by other soil bacteria (Wang et al. 2015), an ability that may allow them to grow in heavily colonized habitats, like the rhizosphere of plants.

Patescibacteria, on the other hand, are small-genome bacteria presumed to lead a semi-parasitic or ectosymbiotic lifestyle, owing to their limited biosynthetic abilities (He et al. 2015; Nelson and Stegen 2015). Initial studies identified members of this superphylum in anoxic environments, but it was recently showed that their distribution is more widespread than previously established (Sánchez-Osuna et al. 2017). Interestingly, members of this group were recently shown to be present in the rhizosphere of amylaceous maize grown in Andean chacras at Huancavelica (Peru) (Correa-Galeote et al. 2016). The relative abundance of sequences assigned to Patescibacteria (Parcubacteria phylum) was higher in the rhizospheric soil of maize than in the bulk soil.

Some important aspects concerning the rhizosphere microbiomes of ATCs still need to be addressed. For example, we did not compare variations of this microbiome among different varieties of the same ATC, a challenging task if one considers the enormous phenotypic diversity of such tuber crops (Malice et al. 2010). On the other hand, we did not address changes in this microbiome related with plant growth stage or the season of the year. In a recent study, Pfeiffer et al. (2017) identified a dynamic microbiome that change as the plants progressed through different growth stages and seasons, a stable microbiome that do not, and another microbiome considered as "opportunistic". Consequently, more studies are needed to determine the likely effects of plant age and seasonal variations on the microbiome we have described here.

However, we provide here –for the first time- important information concerning the diversity and abundance of bacterial taxa associated with the rhizosphere of several of important food security crops, which are at the basis of nutrition for a large population (i.e. several million) of Andean people. Additionally, since several ATCs have been introduced a long time ago in other latitudes, including Australia, New Zealand, Europe and North America, (Flores et al. 2003), studying their rhizosphere microbiomes may be of more relevance and interest for wider audiences than initially thought.

OTUs belonging to three phyla, namely Proteobacteria, Actinobacteria and Acidobacteria, accounted for at least 50% of the 100 most abundant OTUs in all samples. Species belonging to these phyla, isolated from the rhizosphere of potato (another well-known ATC) in different Andean countries, have been shown to exhibit plant-growth promoting activities —including antagonism against phytopathogens, production of phytohormones, solubilization of mineral

phosphates and production of NH_3 (Ghyselinck et al. 2013; Ogata-Gutiérrez et al. 2017). It is thus tempting to propose that several of these OTUs might play important ecological roles as promoters of ATC growth. It is also possible that some of these OTUs would be of help, in the near future, as valuable tools to develop biofertilizer inoculants and/or biocontrol products. The results from preliminary studies seem to confirm that this potential can be turned into reality in the field (Velivelli et al. 2014b).

The search for PGPM in selected rhizosphere habitats and particular plant crops can provide more opportunities to identify the basic ingredients for novel products, that could be useful to develop sustainable cropping technologies as well as to improve organic- or marginal agricultural systems (Dimkpa et al. 2009). A better understanding of the agroecology of ATCs production systems could help improving the functioning of these systems and contribute to maintain food security and sovereignty in the region, especially in the face of predicted changes in climate in the high Andes.

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Author contributions L.A.Y. designed the study, collected samples, analyzed and discussed the results, and wrote the paper. E.C. collected samples, performed bioinformatics analyses from metagenomics data and co-wrote the paper. L.B. collected samples, isolated, purified and characterized rhizobacterial strains. A.V. extracted the metagenome and performed quality control analyses. D.P and P.V. extracted and purified bacterial genomic DNA, amplified 16S rDNA and performed bioinformatic analyses.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest

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