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ORIGINAL PAPER

A first insight into the structure and function of rhizosphere microbiota in Antarctic plants using shotgun metagenomic

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Abstract

Antarctic vascular plants such as *Deschampsia antarctica* (*Da*) could generate more suitable micro-environmental conditions for the establishment of other plants like *Colobanthus quitensis* (*Cq*). Although positive plant–plant interactions have been shown to contribute to plant performance and establishment, little is known about how microorganisms might modulate those interactions, particularly in stressful environmental conditions. Several reports have focused on the possible ecological roles of microorganisms on vascular plants, but if rhizospheric microorganisms can impact positive interactions among Antarctic plants has been seldom studied. Here, we assessed the physical–chemical characteristics of rhizospheric soils from *Cq* growing alone or associated with *Da* (*Cq* + *Da*). In addition, we compared the rhizosphere microbiomes associated with *Cq*, either growing alone or associated with *Da* (*Cq* + *Da*), using a shotgun metagenomic DNA sequencing approach and using eggNOG for comparative and functional metagenomics. Overall, there were no differences among rhizospheric soils in terms of physical–chemical characteristics. On the other hand, our results show significant differences in terms of taxonomic diversity between rhizospheric soils. Functional annotation and pathway analysis showed that microorganisms from rhizospheric soil samples also have significant differences in gene abundance associated with several functional categories related to environmental tolerance and in metabolic pathways linked to osmotic stress, among others. Overall, this study provides foundational information which will allow to explore the biological impact of the rhizobiome and its functional mechanisms and molecular pathways on plant performance and help explain the concerted strategy deployed by *Cq* to inhabit and cope with the harsh conditions prevailing in Antarctica.

Keywords Functional symbiosis · Vascular antarctic plants · Rhizobiome · Gene ontology

Introduction

Diverse mutualistic bacteria and fungi thrive on plant surfaces and inhabit most plant tissues. Many of these microorganisms interact with their plant hosts intimately; they can influence plant metabolism and hormonal pathways in

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Impact statement Functional symbiosis can be a key strategy used by plants to cope with the harsh Antarctic environment by activation of functional pathways.

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addition to providing novel nutritional or biosynthetic capacities, stimulating plant growth, and conferring enhanced resistance to different stressors (Lugtenberg and Kamilova 2009; de Zelicourt et al. 2013). Several studies have shown that microorganisms can have a direct effect on plants capacity to resist biotic and abiotic stress such as herbivory, drought, extreme temperatures and high salinity (Redman et al. 2002; Márquez et al. 2007; Giauque and Hawques 2013; Acuña-Rodríguez et al. 2019). Many bacteria and fungi have been found in association with plant roots, facilitating the establishment, spread and/or increasing plant fitness in stressful environments (Frey-Klett et al. 2007; Bano and Fatima 2009; Hoffman and Arnold 2010; Torres-Díaz et al. 2016). On the other hand, it has been documented that some microorganisms can modulate the interaction between plants or filter the establishment of new species in a given community (Amsellem et al. 2017). Thus, if microorganisms can impact plant–plant interactions, studying the diversity and composition of microbial communities is key for understanding how vascular plants interact and survive. This could be especially relevant in stressful environmental conditions, such as those found in Antarctic habitats, where positive interactions, in particular, could be essential for survival (He et al. 2013; Atala et al. 2019).

The Antarctic ecosystem is one of the most stressful natural habitats, especially for terrestrial plants (Convey et al. 2014; Pointing et al. 2015). In such harsh environment, only two vascular plants; *Colobanthus quitensis* (*Cq*, Caryophyllaceae) and *Deschampsia antarctica* (*Da*, Poaceae), have colonized the ice-free zones (Moore 1970). Although both plants are found in the Antarctic Peninsula, *Cq* is mainly found growing in association with *Da* in more stressful areas, and alone in more favorable sites (Atala et al. 2019). *Da*, on the other hand, is capable of growing alone in areas with higher abiotic stress (Alberdi et al. 2002; Atala et al. 2019). *Da* is a grass that form tussocks where micro-environmental conditions above and below their canopy could be milder than outside, acting like a "nurse species" for other less tolerant species (e.g., *Cq*) in Antarctica (see Molina-Montenegro et al. 2013). In fact, some native and invasive species increase its physiological performance and fitness-related traits when growing in association with *Da* compared with those growing alone (Atala et al. 2019). Although it is clear that positive interactions can determine the performance and survival for some less tolerant species, the underlying mechanisms are not clear and whether microorganisms mediate this positive interaction remains unknown.

It has been proposed that positive inter-specific interactions between plants and microorganisms play a pivotal role in the structure and functioning of several plant communities (i.e. Smith and Read 2008; Sielaff et al. 2018). Plants harbor a wide diversity of microorganisms, which play a crucial role in their growth, survival and establishment by conferring enhanced resistance to abiotic stress, allowing plants to grow in extreme conditions (De Zelicourt et al. 2013; Louca et al. 2018) such as those from polar environments (Hughes et al. 2015). Despite microorganisms having positive impacts on plant fitness in stressful environmental conditions (Vandenkoornhuyse et al. 2015; Torres-Díaz et al. 2016; Ramos et al. 2018), little is known about how Antarctic rhizospheric microorganisms might affect plant performance. Although several reports have focused on the occurrence, type of association, diversity and possible ecological roles of microorganism interactions with vascular plants (Upson et al. 2009; Torres-Díaz et al. 2016), there are very few studies regarding microorganisms' interactions with Antarctic vascular plants (but see Rosa et al. 2009; Ramos et al. 2018). For example, Teixeira et al. (2010) showed no clear differences in terms of diversity between rhizospheric bacterial communities from the rhizospheres of *Cq* and *Da* in Admiralty Bay, by 16S rRNA analysis. However, and to the best of our knowledge, there is no evidence regarding the metabolic capacity and functional traits of the rhizospheric microbiomes related to *Cq* and *Da*, nor if the rhizospheric microorganisms could modulate positive interactions among these vascular Antarctic plants.

In this study, we compare the rhizosphere microbiomes associated with *Cq*, either growing alone or in association with *Da* (*Cq* + *Da*) by using an approach based on shotgun metagenomic DNA sequencing technology. This strategy allowed us to gain insight into the rhizospheric microbial taxonomic diversity (including non-culturable organisms), and to compare similarities and differences in terms of the observed functional attributes (Jovel et al. 2016). Specifically, we asked if the rhizosphere microbiome of *Cq* when grow associated to *Da* have higher taxonomic and functional diversity than when grow alone. Finally, we analyzed the physical–chemical characteristics of the rhizospheric soil in order to assess if differences in microbiome or edaphic characteristics induced by *Da* can help explain the greater performance and abiotic tolerance of *Cq* when grow associated to with *Da*.

Materials and methods

Site description and soil sample processing

Rhizospheric soil samples were collected from Devils Point, Byers Peninsula, Livingstone Island, Antarctica (62°40′S, 61°10′W) during the growing season in the Summer (February) of 2016 (Fig. 1). *Colobanthus quitensis*' rhizospheric soil (*Cq*) and rhizospheric soil of *Cq* growing associated with *Da* (*Cq* + *Da*) were sampled at sea level. Plants and surrounding soil were dug out at 7 cm of depth using a sterilized shovel, bulk soil and fine

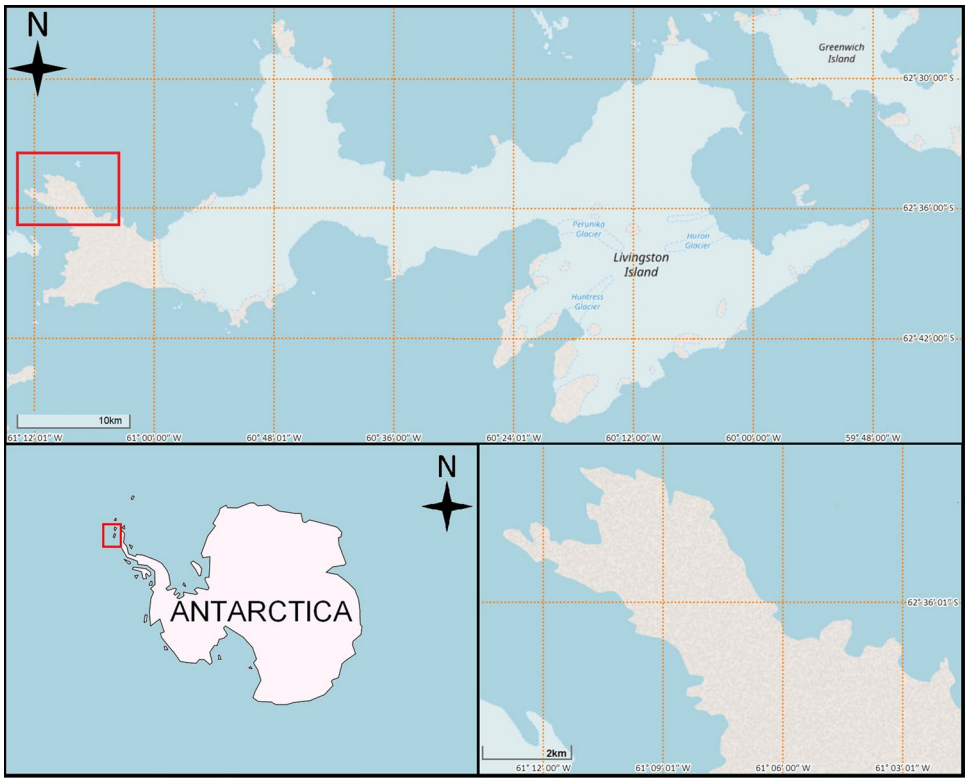

Fig. 1 Map of Antarctica (below left image) highlighting in a red square the study site "Devils Point" in the Livingstone Island (above image). Close-up of the study site "Devils Point", Byers Peninsula (below right image)

roots were discarded by shaking the plants by hand until non-adhering particles were completely removed. Then, for each sample, fine soil particles (≤ 2 mm), tightly adhered to the roots (rhizospheric soil), were carefully separated from the roots and stored in 50 mL sterile screw cap tubes at – 20 °C until DNA extraction and processing of soil samples. All plant and soil samples were collected under permission of the Chilean Antarctic Institute (INACH; Authorization Number 1060/2014).

Physical–chemical analysis of rhizospheric soil

To assess whether physical–chemical characteristics of soil can modify the rhizospheric soil biodiversity we measured nitrogen, phosphorus, potassium, organic material, pH, and sand, silt and clay percentages in the rhizospheric soil of *Cq* growing alone and associated with *Da*. A rhizospheric soil sample at 5–7 cm depth (ca. 50 g) was taken beneath 12 *Cq* growing alone and beneath other 12 *Cq* individuals growing associated with *Da*. All samples were collected inside of patch of 25×25 m and each pair of samples (i.e., *Cq* and *Cq* + *Da*) were distanced no more than 100 cm. Soil samples were stored in hermetic plastic bags at – 20 °C and then sent for analyses to Centro Tecnológico de Suelo y Cultivos at the Universidad de Talca (Order of Analysis R-PT-09–01).

DNA extraction and sequencing

For a total of six rhizospheric soil samples (three replicates per condition, *Cq* or *Cq* + *Da*), total DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc.). DNA integrity was checked with capillary electrophoresis using a Fragment Analyzer (AATI) and DNA quantification was performed using fluorometry (Qubit 2.0; Qubit DNA Broad Range Assay Kit, Invitrogen). After QC, all samples were subjected to library construction for Illumina sequencing. Briefly, DNA was fragmented by Covaris ultrasonicator (average fragment size of 550 bp), and size-selected using AMPure XP purification beads. Libraries were constructed using the TruSeq LT kit following manufacturer instructions (Illumina), ligated to indexed adapters for cluster generation and sequenced using the Illumina MiSeq reagent kit (v3) in an Illumina MiSeq sequencer (600 cycle; 300 bp, Paired-End sequencing) (Illumina, San Diego, CA). Demultiplexing and fastq generation were performed automatically using Illumina's built-in software. Raw metagenomic datasets were deposited in NCBI's Sequence Read Archive database under BioProject ID PRJNA419970.

Statistical and bioinformatics analyses

To assess the differences in physical–chemical characteristics of rhizospheric soils beneath *C. quitensis* plants growing

alone (*Cq*) and *C. quitensis* associated with *Da* (*Cq* + *Da*) a *t*-test was used.

Raw sequences obtained from six metagenomic samples were subjected to a quality check using FastQC v0.11.3 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Next, the sequences were run through Trimmomatic 0.36 (Bolger et al. 2014) to remove low-quality base pairs, sequencing adapters and reads shorter than 100 bp; the following parameters were used: SLIDINGWINDOW 4:15 MINLEN: 36. After trimming, all libraries were interleaved for downstream analyses.

For taxonomic assignment, libraries were aligned using DIAMOND BLASTX algorithm ver. 0.9.30.92 (using default parameters; Buchfink et al. 2015) to the NCBI-NR database (April 2018). Only alignments with an *e*-value of 10^{-3} or lower were included in our analysis. Alignment result files were imported in MEGAN v6.11.5 (MEGAN6; Huson et al. 2016), which parsed results using the Lowest Common Ancestor Algorithm (LCA) and NCBI's taxonomy under default values. All samples were normalized by using MEGAN6′s built-in normalization tool. Comparison between *Cq* and *Cq* + *Da* rhizospheric soil samples were carried out using MEGAN6′s Taxonomic abundance estimates (phylum, genera and species levels) and were imported into STAMP (Statistical Analysis of Metagenomic Profiles, version 2.1.3; Parks et al. 2014) for statistical analysis, using a Welch's *t*-test with correction for multiple comparisons (Benjamini–Hochberg false discovery rate correction approach, q-value < 0.05). Further, taxonomic diversity analysis was carried out in R using the ggplot2, phyloseq, vegan, and DESeq2 packages (Dixon 2003; McMurdie and Holmes 2013; Love et al. 2014). Finally, comparison between samples was performed using the software STAMP (Statistical Analysis of Metagenomic Profiles, version 2.3.1).

To analyze metabolic profiles and perform functional annotations, all interleaved, filtered libraries per condition ($n = 3$) were pooled in silico and assembled using MEGAHIT v1.1.3 (minimum contig length 400 bp; default parameters) (Li et al. 2015). Each representative, assembled metagenome was aligned using DIAMOND BLASTX algorithm ver. 0.9.30.92 (using default parameters; Buchfink et al. 2015) to the NCBI-NR database (April 2018). Functional assignment was performed by mapping aligned contigs to eggNOG database using MEGAN6 in long-read mode. For sample comparisons, relative abundance of aligned bases binned to eggNOG clusters between samples (based on aligned contig coverage, long-read mode) were normalized using MEGAN6 built-in normalization tools. Statistical analysis of samples was performed in STAMP, using a Fisher exact test with correction for multiple comparisons (Benjamini–Hochberg false discovery rate correction approach, q-value < 0.05). Additionally, for each assembled metagenome, all protein-encoding genes were predicted with Prodigal v2.6.2 in metagenomics mode (Hyatt et al. 2012) and were used as queries for functional module and pathway analysis [as defined by Kyoto Encyclopedia of Genes and Genomes (KEGG)]. This analysis allows estimating the percentage of module components filled with input genes [module completion ratio (MCR)] by mapping genes to all functional modules/pathways/complexes using the single-directional best-hit method, implemented in the Metabolic and Physiological potential Evaluator system v2.3.0 (MAPLE; Arai et al. 2018). If all genes are assigned to all KEGG orthology IDs in each condition, the MCR is 100%, thus allowing comparison in terms of MCR between metagenomes (Takami et al. 2015).

Results

Physical–chemical analysis of rhizospheric soil

The chemical characteristics of soil samples (nitrogen, phosphorus, potassium, organic material and pH) were not different between rhizospheric soils beneath *Cq* and *Cq* + *Da* (Table 1). Similarly, physical characteristics of soil samples (sand, silt and clay percentages) were not different between rhizospheric soils from *Cq* and *Cq* + *Da* (Table 1).

Summary of sequencing data and de novo assembly

For the *Cq* metagenomes, a total of 20,081,770 reads were obtained, while for *Cq* + *Da* samples, 19,348,212 reads were obtained (Online Resource 1). Read sizes ranged from 35 to 300 bp, although the majority of reads (~97% of total sequenced reads per library) had a length equal or over 290 bp. Filtering steps reduced library sizes in approximately

Table 1 Physical–chemical analysis of rhizospheric soils of *Colobanthus quitensis* growing alone (*Cq*) and associated with *Deschampsia antarctica* (*Cq* + *Da*)

Variable	*Cq*	*Cq* + *Da*	*F*	d.f	*p*-value
Nitrogen (mg/kg)	71.7 (±2.4)	73.3 (±3.7)	2.32	22	0.18
Phosphorus (mg/kg)	35.2 (±2.1)	33.9 (±2.8)	2.76	22	0.11
Potassium (mg/kg)	305.3 (±11.3)	302.1 (±14.3)	1.59	22	0.45
Organic material (%)	4.9 (±0.3)	5.1 (±0.4)	1.21	22	0.76
pH	5.8 (±0.1)	5.7 (±0.1)	1.25	22	0.72
Sand (%)	79.1 (±2.2)	77.9 (±2.7)	1.49	22	0.46
Silt (%)	16.3 (±1.5)	16.7 (±1.6)	1.09	22	0.89
Clay (%)	5.6 (±1.8)	5.4 (±1.7)	1.11	22	0.86

Values are means (±SD)

18%. After trimming, two representative metagenomes were assembled by combining *per-condition* libraries (*Cq* and *Cq* + *Da*); a total of 1,013,395 and 676,033 contigs were obtained, respectively (Online Resource 2). Additionally, separate DIAMOND alignments of each trimmed library were used for taxonomic analysis; approximately 56% of reads per library were successfully assigned into a taxonomic category (Online Resource 1).

General prokaryotic taxonomic analysis and rhizospheric soil diversity

Results of the MEGAN taxonomic analysis for both metagenomes revealed that Bacteria, followed by Eukaryota and Archaea dominated both samples (96.8%, 2.8% and 0.2%: *Cq*, 99.2%, 0.58%, and 0.24%: *Cq* + *Da*). The differences in relative abundances for Eukaryota detected between samples may be related to a higher number of sequences aligned to *Viridiplantae* in *Cq* compared to *Cq* + *Da* (mean difference between samples: 58,542.3; p-value = $1.4e^{-3}$), suggesting that plant DNA was also recovered and sequenced from the rhizospheric soil samples. Therefore, all non-bacterial aligned reads were in silico filtered and removed from the following analysis.

Microbiome analyses revealed a diverse microbial community at the phylum level for both soil samples and showed that Proteobacteria was the most abundant phylum (34.1%), followed by Actinobacteria (23.7%) and Bacteroidetes (16.4%) (Fig. 2; Core Microbiome is shown in Online Resource 3). The clustering analysis based on Bray–Curtis dissimilarities calculated from the relative abundance of each genus, indicated higher similarity values within the three sampled replicates per rhizosphere (within *Cq* and *Cq* + *Da*, respectively), but dissimilarities between *Cq* and *Cq* + *Da* rhizospheres, respectively (Online Resource 4). Furthermore, comparative taxonomic analysis at genus-level showed differences in the relative diversity between *Cq* and *Cq* + *Da* rhizospheric soil samples (222 vs. 215 genera; genus listed in Online Resource 4), while significant differences were detected

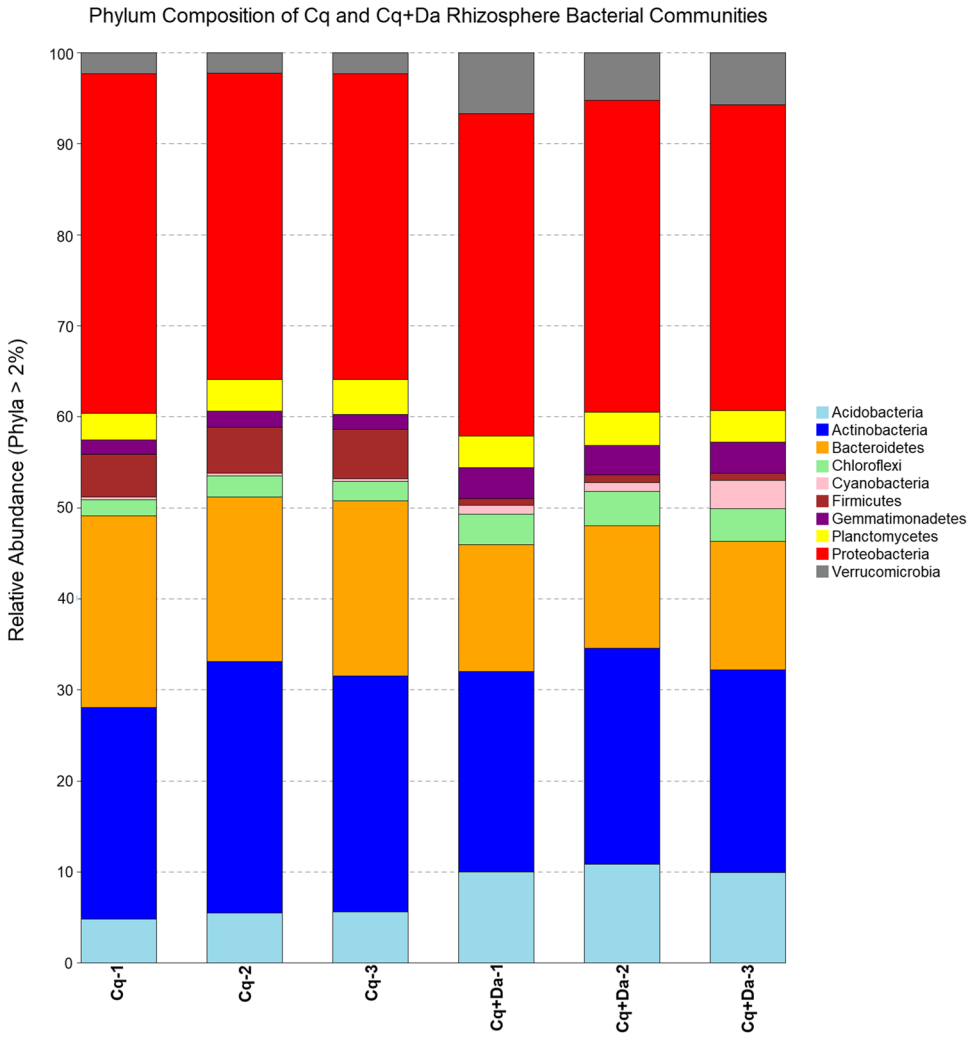

Fig. 2 Percentage distribution of bacterial phylum from rhizospheric soil samples of *C. quitensis* (*Cq*) and *C. quitensis* + *D. antarctica* (*Cq* + *Da*)

for 89 bacterial genera (Online Resource 5), being *Sporosarcina* and *Chryseobacterium* the more represented genus in *Cq* and *Cq* + *Da*, respectively (Fig. 3).

At bacterial species level (considering presence/absence) we found that 46.1% species were shared between rhizospheric soil samples; 25.4% were exclusively found in *Cq* rhizospheric soil and 28.5% were found only in *Cq* + *Da* rhizospheric soil (Online Resource 6). Alpha diversity analysis revealed that all *Cq* + *Da* samples were consistently found to be more diverse than *Cq* samples, both in richness [observed and corrected (Chao1)] and evenness (ACE, Shannon, Simpson, Inverse Simpson) (Online Resource 7). While interquartile ranges did not overlap under any metric, suggesting significant differences, these are sensitive to low sample sizes for which more samples need to be collected and evaluated to obtain precise alpha diversity estimates. However, median values between Observed and Chao1 estimates coincide (~755 taxa), which suggests that sequencing depth was adequate for sampling these rhizosphere communities. It is worth noting that the taxonomic analyses performed in this study are based on tools relying on sequence alignments using as reference known/classified microorganisms. However, a high proportion of the observed lineages are either from unknown or unclassified bacteria, suggesting that they are unique to Antarctic soils (Bottos et al. 2014) and warrant further study.

Functional categories analysis and functional rhizospheric soil sample comparison

In order to gain functional insights into microorganisms abundantly present in these rhizospheric libraries, soil metagenomics samples were pooled by condition, assembled to construct contigs and aligned against NCBI's NR database using DIAMOND. A total of 839,980 and 598,467 contigs (82.9–88.5%) had homologous sequences within this database (*Cq* and *Cq* + *Da* assembles, respectively). However, as the alignment results included matches with hypothetical/predicted proteins, not all aligned contigs could be annotated. Thus, 265,414 and 278,276 sequences (26.2–41.2% of all contigs per assembly) were annotated and functionally categorized using the eggNOG database in MEGAN6 and associated with at least one cluster of orthologous genes of the following top hierarchies: cellular processes and signaling, information storage and processing, and metabolism. Subsequent distribution analysis of putative genes with general functions indicated that overall, the most represented functional categories at eggNOG level 2 in both rhizospheres were linked to aminoacid transport and metabolism (11.4%), replication, recombination, and repair (11.1%), energy production and conversion (9.8%) and carbohydrate transport and metabolism (7.9%) (Online Resource 8).

We also compared the number of assigned contigs to eggNOG categories between rhizospheric soil samples,

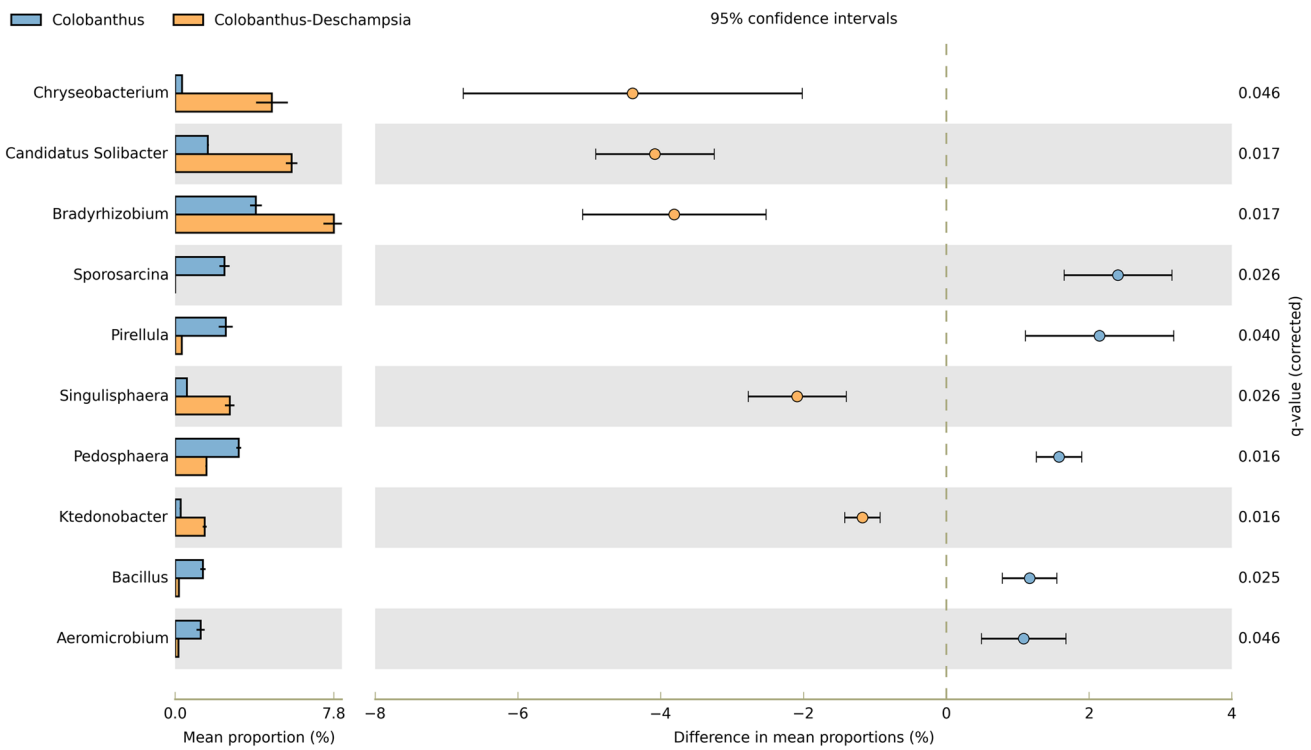

Fig. 3 Alpha diversity measure for both *C. quitensis* (*Cq*) and *C. quitensis* + *D. antarctica* (*Cq* + *Da*)

which revealed a varying degree of similarity between the two groups in all categories (Online Resource 8). However, there was significant differences in terms of relative abundance for 16 categories (out of 22), with mean differences ranging between 0.08 and 2.24% (q-value < 0.05; Fig. 4; Online Resource 8). Of these 16 categories, 11 were more represented in *Cq* (Fig. 4, blue dots) while the other 5 were more represented in *Cq* + *Da* (Fig. 4, orange dots). A global, deep comparison of genes binned to different eggNOG categories at level 3 (using MEGAN6 visualization tool) showed that 372 categories displayed differences in terms of relative abundance between both samples (Online Resource 8). These results suggest an overall enrichment of several molecular functions and biological process linked to these eggNOG categories, such as Serine Threonine protein kinases, which in bacteria have been linked to phosphorylation of serine or threonine residues in proteins, thus being considered as a key mechanism in regulation of protein activity and control of cellular functions, such as stress response (Pereira et al. 2011) (Online Resource 8). However, our results also indicate that many categories with similar molecular functions such as "ABC transporter" display opposite patterns of enrichment: for example, "ABC transporter, permease (ENOG410XP9H)" is enriched in *Cq* + *Da*, while "ABC transporter, permease (COG0577)" is enriched in *Cq*. Hence, in this case, evaluation and differentiation

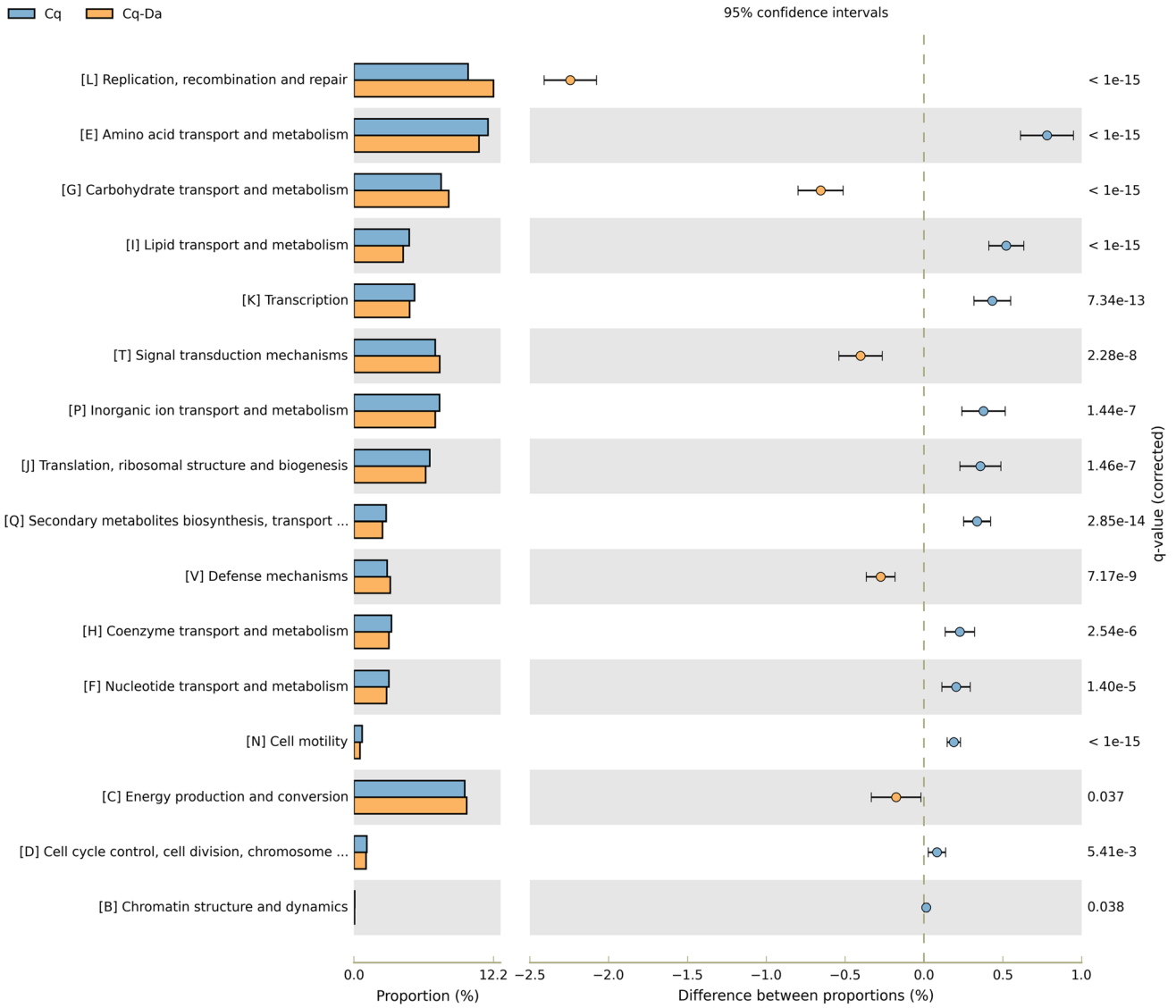

Fig. 4 SEED functional categories found in rhizospheric soil bacteria communities. Bar plot shows mean proportion (%) of functional categories found in rhizospheric bacterial communities based on the SEED database level 2 categories. Points indicate the differences between *C. quitensis* and *C. quitensis* + *D. antarctica* soils (blue and orange bars, respectively). Corrected p-values (q-values) were derived from a Fisher's exact test with Benjamini–Hochberg correction for false discovery rate. (Color figure online)

of the functional potentials between different metagenomes based on functional annotation of genes and eggNOG clustering/classification tools would be a difficult task (Takami et al. 2015). Thus, as we were interested in characterizing the functional genomic features derived from both rhizospheric metagenomes ("Functionome", sensu Fujinawa et al. 2016), we comparatively studied the potential functionomes using a method based on the presence and completeness of KEGG functional modules and metabolic pathways (Takami et al. 2015; Fujinawa et al. 2016). The predicted aminoacid sequences from both metagenomes were loaded in MAPLE 2.3.0 (Arai et al. 2018). This analysis revealed that, in both samples, 116 metabolic pathways have 100% MCR, thus were considered as shared among rhizospheres. We also found 76 pathways with differences in their MCR between rhizospheres (Online Resource 9). In the case of *Cq*, 23 out of 33 pathways linked to secondary metabolism had higher MCR compared to *Cq* + *Da* (e.g. biosynthesis of secondary metabolites, aromatics degradation). Contrastingly, in the case of *Cq* + *Da*, 33 out of 43 pathways with higher MCR were related to "carbohydrate and lipid metabolism" category, while no pathways related to these categories had a high MCR in *Cq* (Online Resource 9).

Discussion

We explored the taxonomic and functional diversity of microbial communities in two rhizospheric soils (*Cq* and *Cq* + *Da*) of the vascular species from Antarctica using shotgun sequencing. Our metagenomic analyses revealed that significant differences were observed between samples in terms of bacterial abundance, although bacterial communities shared some similarities in terms of taxonomic composition. In addition, some functional categories also showed differences in terms of abundance between rhizospheres, suggesting that these microbial communities could have different functional activities. This could ultimately have an effect on colonization and plant growth and environmental tolerance.

Results showed that bacterial species had the highest relative abundance in both habitats (98%) compared to Archaea (0.22%) and Eukaryota (1.77%), suggesting that a highly complex bacterial community is present in these rhizospheric soil samples. Interestingly, the rhizospheric core microbiome (shared Phyla between both samples; Online Resource 6) obtained in this study indicates that the most abundant bacterial Phyla were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Verrucomicrobia, accounting for approximately 85% of the sequences in rhizospheric soil samples. Thus, they may constitute a core, functional microbial community structure, capable of performing several biochemical functions related to plant growth promotion, nutrient acquisition, and abiotic stress tolerance, among others (Chen et al. 2017; Louca et al. 2018). However, our findings in terms of bacterial Phyla abundance and diversity were rather unexpected, as they contrast with the previously reported bacterial diversity from Antarctic rhizospheric bacterial communities associated with vascular plants, where Firmicutes were the most abundant phylum in almost all of the samples, and Acidobacteria were rarely found (Teixeira et al. 2010). Congruently, the bacterial Phyla detected in our study are similar to those detected in soil rhizospheres worldwide (Delgado-Baquerizo et al. 2018) and agree with other studies on microbial diversity in Antarctica (Wang et al. 2015). Nevertheless, it must be noted that differences in methodological approaches (i.e., amplicon vs. shotgun) may explain disparities in terms of phylum abundance between our study and those previously reported.

Another possible explanation for the observed differences is that other environmental factors, such as physical/chemical soil characteristics or vegetative cover could participate in shaping soils' microbial diversity (Teixeira et al. 2010; Bottos et al. 2014; Wang et al. 2015), as bacterial community structures of Antarctic soils are highly heterogeneous (Yergeau et al. 2012; Bottos et al. 2014) and may display unique biochemical adaptations, depending on habitat conditions (González-Rocha et al. 2017). For example, several studies have demonstrated that soil characteristics, like pH or carbon mineralization rate, can help explain the relative abundance of dominant Phyla and/or bacterial community structure (Fierer et al. 2007; Lauber et al. 2009). Previous studies conducted in the Antarctica have shown differences in the presence and concentrations of nutrients in Antarctic soils, but majority of this studies have compared sites spatially distanced or with presence and absence of ornithogenic or mammals effects, suggesting that big vertebrates are the main driver for the variation of nutrient soil amount, more than plants itself (see Bokhorst et al. 2019). On the other hand, other studies have shown that under different type of Antarctic vegetation the nutrient and edaphic characteristics can variety significantly (see Roberts et al. 2009). Nevertheless, this study compared different functional groups (vascular plants vs. mosses or plants vs lichens) but no between vascular plants. In our study, no differences among physical–chemical characteristics of rhizospheric soils from *Cq* and *Cq* + *Da* were found. This result could be explained since we assessed the rhizospheric soil, that is, the portion that is in direct contact with roots. In addition, we compared the rhizospheric soil the same species, although in two conditions, but only in the glued soil to roots of *Cq*. On the other hand, Beyer et al. (2000) suggested that other factor as moisture, temperature or wind are most important traits to explain colonization and performance in Antarctic plants compared with nutrients. Similar microclimatic modifications as those

provided by *Da* to *Cq*. In addition, rhizospheric soil samples were taken in a patch of 25 × 25 m, where each pair (*Cq* and *Cq* + *Da*) was less than 1 m away. So, the probability to collect soils samples with external influences rather than the root effect of *Cq* was low. Thus, the evident variation both in diversity and structure of microorganism community could be mainly determinate by the effects that *Da* is able to exert on soil microorganisms. Future studies should also consider other aspects of the interaction among plant and microorganisms (e.g., energetic costs, vertebrates or vegetation presence surrounding) in order to explain other ecological and functional aspects of this symbiosis.

Regarding the phylum composition between the rhizospheric communities of *Cq* growing alone compared to *Cq* growing with *Da*, we also found differences in their relative abundance. For example, Bacteroidetes, were higher in *Cq* compared to *Cq* + *Da* samples (19.08% and 13.53%, respectively). These bacteria are involved in degradation of plant material and related organic molecules such as starch and cellulose (Aislabie et al. 2013) and their abundance correlates with soil pH, available nitrogen/phosphorus content and water content (Zhang et al. 2014). We also observed significant differences in the taxonomic distinctiveness of the rhizospheric soil samples observed at the genus level and in terms of unique and shared bacterial species present in both rhizospheres, suggesting the existence of complex microbial assemblages associated with each rhizospheric type. Furthermore, the Bray–Curtis dissimilarity index suggests that *Cq* and *Cq* + *Da* samples are dissimilar in terms of diversity of bacterial genera. A previous study reported no clear differences between microbial communities from the rhizospheres of *Cq* and *Da* (Teixeira et al. 2010). However, a recent study found evidence of different bacterial strains from the rhizospheres of these Antarctic plants (Gallardo-Cerda et al. 2018).

Rhizospheric microorganisms may play a crucial role in growth and stabilization of plants in extreme environmental conditions such as those found in Antarctica (Hughes et al. 2015). Thus, characterizing the number of biochemical functions or genes present in Antarctic rhizospheric communities is an important step towards the understanding of the complex ecological aspects and biotechnological potentials of Antarctic bio-resources (Wang et al. 2015; Molina-Montenegro et al. 2016; Gallardo-Cerda et al. 2018; Louca et al. 2018; Acuña-Rodríguez et al. 2019). Based on functional analysis using eggNOG categories, all categories had at least 3 genes assigned, although mean gene abundance per category varied widely; for example, the most abundant category was "Replication, recombination and repair" (61,814 summed genes) while the least abundant category is "extracellular structures" (3 summed genes). These results in terms of gene abundance indicate a specific enrichment of some eggNOG categories, which could be related to microbial adaptation to cold Antarctic environment. This could include energy production, nutrient transportation, cellular membrane functions and tolerance to abiotic stress (Barrientos-Díaz et al. 2008), suggesting selection of particular functional traits that could be adaptive in a given environment. Therefore, we propose that differences observed both in the completion rates of functional modules and in the eggNOG functional categories from *Cq* and *Cq* + *Da* rhizospheres could be a consequence of microorganism selection by presence of specific functional traits (Yan et al. 2017). Specific genera/species of bacteria, uniquely present in a certain sample/condition (but under- or not-represented in the other sample/condition), could be responsible for these functional traits which, in turn, could explain the observed differences in terms of microbial diversity/abundance. However, it must be noted that the differences/similarities in terms of functional modules/categories described in this study, do not necessarily reflect the actual working probability of each functional module (Takami et al. 2015).

Finally, our results suggest that Antarctic plants may shape their rhizosphere communities, by differentially altering both the abundance and diversity of bacteria. This is especially relevant when comparing sites where *Cq* grows alone to sites where is found associated with *Da*, possibly being a key element in the positive effect of the latter on *Cq* and even on some invasive species (Atala et al. 2019). Furthermore, it is plausible that these different rhizospheric microbial communities encode different molecular mechanisms and express different functional pathways. However, the results in this study do not show the actual working probability of these functional pathways, nor indicate whether they are actually being expressed and/or functional in the soil. Hence, additional research is required to uncover the biological impact of these different microbiomes on plant performance and survival in order to shed lights about the strategy deployed by *Cq* to inhabit and cope with the harsh abiotic conditions prevailing in Antarctica.

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Compliance with ethical standards

Conflict of interest This study has been conducted in absence of conflicts of interest.

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