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Insights into quinoa endophytes: core bacterial communities reveal high stability to water stress and genotypic variation

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Abstract

Background Plant endophytes, comprising non-pathogenic bacteria, fungi, and archaea, inhabit various plant parts, including roots, stems, leaves, and seeds. These microorganisms play a crucial role in plant development by enhancing germination, growth, and stress resilience. Seed endophytes, in particular, represent the most adapted and conserved segment of plant microbiota, significantly influencing the initial stages of plant growth and microbial community establishment. This study investigates the impact of environmental and genotypic factors on the endophytic communities of *Chenopodium quinoa* Willd. (quinoa), a crop notable for its adaptability and nutritional value.

Results We aimed to characterize the core endophytic communities in quinoa seeds and roots from two distinct genotypes under well-watered (WW) and water-deficit (WD) conditions, utilizing various soil infusions as inoculants to explore potential changes in these endophytes. Our findings reveal distinct changes with quinoa seeds exhibiting a high degree of conservation in their endophytic microbiome, even between maternal and offspring seeds, with specific bacterial taxa showing only minor differences. Tissue specificity emerged as a key factor, with seeds maintaining a stable microbial community, while roots exhibited more pronounced shifts, highlighting the tissue-dependent patterns of microbial enrichment.

Conclusions The results highlight the stability and conservation of endophytic communities in quinoa seeds, even under varying water conditions and across different genotypes, emphasizing the role of tissue specificity in shaping microbial associations. These findings suggest that quinoa-associated endophytes, particularly those conserved in seeds, may play a crucial role in enhancing drought resilience. Understanding the dynamics of plant-microbe interactions in quinoa is vital for developing stress-resilient crop varieties, supporting sustainable agricultural practices, and ensuring food security in the face of climate change and environmental challenges.

Keywords Seed endophytes, Root endophytes, Quinoa, Drought, Microbial diversity

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Background

Plant endophytes are understood as a diverse group of non-pathogenic microorganisms (including bacteria, fungi, and archaea) capable of inhabiting various parts of the plant, such as roots, stems, leaves, and seeds [1, 2]. They constitute a vital component of plant microbiomes, significantly influencing plant health and development. Their diverse interactions and mechanisms provide numerous benefits such as growth promotion, stress tolerance, disease resistance, and improved nutrient acquisition [3–5]. Understanding and harnessing these endophytic relationships holds great potential for sustainable agriculture, as they can reduce reliance on chemical inputs and enhance crop resilience and productivity Seed endophytes represent the most adapted and conserved part of plant microbiota compared to other plant habitats or plant tissues such as the rhizosphere, roots, and leaves, which are more susceptible to colonization by microbial communities recruited from the surrounding environment [6–10]. Seed endophytes play a pivotal role in plant development, positively impacting germination and growth by protecting against pathogens and mitigating abiotic stressors [3, 8, 11–15]. Moreover, seed endophytes act as the initial inoculum for the germinating seed, rapidly colonizing the rhizosphere and critically shaping the establishment and composition of the root microbiome, thus determining the final stage of microbial community assembly [16].

Long-term evolutionary selection has been observed to strongly influence the loss of endophyte diversity, including the loss of key beneficial seed endophytes [7, 17–21].

Besides the changes produced by domestication processes, other factors such as environmental conditions, particularly drought and salinity, have been shown to impact the composition of root and seed endophyte communities, resulting in significant changes in alpha and beta diversity community analyses [22–26]. Considering that environmental conditions can significantly influence the composition of microbial communities, there has been growing interest in studying plant-seed microbe interactions in the context of climate change [6, 25, 27]. This interest is particularly relevant when analysing these interactions under water-stress conditions. The increasing frequency and severity of drought episodes have a profound negative impact on crop yields [28–33]. Understanding how microbial communities respond to and potentially mitigate the effects of such stressors is crucial. Specifically, research has focused on how changes in the rhizosphere and seed microbiomes can enhance plant resilience to drought, potentially leading to the development of more drought-tolerant crop varieties [34]. By examining the dynamic interactions between plants and their associated microbes under various environmental stressors, scientists aim to uncover strategies to sustain agricultural productivity and ensure food security in the face of ongoing climate change [35–37].

Wild relatives of traditional crops and Neglected and Underutilized Species (NUSs); some of which can survive in extreme environments, hold special interest due to their endophytic microbial composition [38–40]. Underutilized crops and resilient plant species are often related to unique microbiomes associated with their tissues, which might play a critical role in stress resilience in their host [41–44]. Thus, microorganisms isolated from plants grown in semiarid environments hold special interest as promoters of growth and improving resilience to drought stress [38–40, 44–46].

Overall, the number of studies focused on analysing plant endophytes has increased significantly over the last decade [47]. Among these studies, those examining stress-resilient plant species and underutilized crops with exceptional nutritional properties are particularly relevant. Such research may provide insights into the role of microorganisms in controlling plant stress responses and seed nutritional quality [38, 48–50]. Additionally, these crops may harbour beneficial microorganisms with important applications in agriculture [38, 40, 50, 51].

Chenopodium quinoa Willd. (commonly known as quinoa) cultivation has expanded widely in recent years [52]. However, very few works have analysed the seed and root endophytic communities associated with this crop, and none have described the quinoa microbiome nor specifically determined the impact of drought on structuring these plant-hosted microbial communities. Interestingly, some studies isolating endophytic fungi from quinoa grown in arid regions highlight the agronomic potential of these fungi in counteracting drought [53]. This suggests that quinoa-associated endophytes could play a crucial role in enhancing drought resilience, thereby supporting sustainable agriculture in the face of climate change.

Quinoa holds potential adaptability to a wide range of environments due to its large genetic diversity [54], constituting a facultative halophyte capable of growing in marginal lands and stressful environments [55–58]. Quinoa is also an interesting crop due to the nutritional composition of its seeds, being an exceptional source of nutrients [59, 60], which includes a high content of proteins of outstanding quality containing all the essential amino acids in a proper balance [61–63] Other interesting quinoa seed features include a high content of minerals, highlighting iron (Fe), magnesium (Mg), and potassium (K) [64, 65], as well as bioactive compounds with antioxidant capacities like polyphenols, carotenoids, and flavonoids [61, 62, 65, 66].

Previous studies have identified that the environmental conditions, the genotype, and their interaction (GXE) affect the nutritional composition of quinoa seeds [63,

67–69]. These results aligned with a group´s former work showing key changes in bacterial composition in quinoa rhizosphere were linked to drought and genotypic differences [44]. Such changes correlated well with alterations in seed nutritional content and plant physiological traits [44], as previously observed in other crops including tomato, rice, or alfalfa [70–72]. Considering that understanding differences in early colonization processes linked to seed endophytes may lead to significant bacterial community changes during later life stages [73], and that environmental factors may impact the composition of root and seed endophytes [6, 22, 23, 27], the study of these microbial communities is especially relevant in the context of plant-microorganism interactions [42, 51, 73].

In this study, our main objectives were to characterize the core endophytic communities in quinoa, representing the conserved microbial composition of this crop, and to investigate the bacterial endophytic communities present in the seeds and roots of two distinct genotypes of quinoa, each exhibiting contrasting water use efficiency strategies, under well-watered (WW) and water deficit (WD) conditions. This was done to determine the impact of environmental and genotypic factors on these communities. Additionally, we analysed the impact of exogenous inoculation on these endophytic communities. We also examined the potential heritability of certain bacterial taxa collected from the rhizosphere, as they may serve as root endophytes, potentially altering the original microbial composition of the seed. Furthermore, we discuss the potential roles of these endophytes in promoting plant growth and preserving plant health in quinoa offspring, also mitigating the effects of drought stress events. Overall, our results indicate good conservation of endophytic microorganism communities in quinoa seeds, with no significant differences observed in alpha and beta diversity between the maternal and the offspring seeds, except for particular bacterial taxa. These specific differences warrant further analysis, considering their functionality, as they may represent microorganisms specialized in coping with water stress, selected by plants to improve conditions for future generations.

Methods

Acronyms used in this study are listed in Table 1.

Experimental design

This experiment was performed using the same plants and conditions as described in previous work by Maestro-Gaitan et al. 2023 [44]. Samples were collected from three different plants per genotype, water treatment, and soil infusion ($n = 3$)0.2023 [44]. **Seeds of two contrasting quinoa cultivars** in terms of water use efficiency (WUE), the F15 genotype, which uses water less efficiently, and the F16 genotype, which is more efficient in the use of water resources [74], were sowed in 1.6 L pots (using a mixture of peat: vermiculite (3:1) at a bulk density of 0.153 g/cm^3, supplemented with a controlled release fertilizer Nutricote® (following manufacture recommendations). The plants were grown in a greenhouse located at the Centre for Plant Biotechnology and Genomics (GBGP) in Madrid, Spain (40°24'20.2"N 3°49'56.8"W), under natural light conditions supplemented with high-pressure sodium lamps and oscillating temperatures ranging between 15°C and 20°C. Quinoa seeds were provided by the company Algosur S.L. (Lebrija, Spain).

The **pots** were **inoculated with bacterial infusions** at the beginning of the experiment when the quinoa plants had already emerged from the soil but had not yet fully established their root systems. Bacterial infusions were obtained from two different agricultural soils in Badajoz, Spain. Soil 1 (S1) corresponded to an agricultural soil (38°50'41.1"N 6°40'31.2"W), and Soil 2 (S2) corresponded to an agricultural soil where quinoa has been grown for the last 10 years (38°51'51.4"N 6°40'12.3"W). Each soil infusion was divided into two equal volumes: the MB infusion, which contained both microorganisms and nutrients from the soil, and the E infusion, which was sterilized in an autoclave for 30 min at 121°C (15 psi), thus only containing nutrients from the soil (Autester ST, P.Selecta, Spain autoclave). The inoculants were prepared as described in Maestro-Gaitan et al. 2023 [44], adding 50mL of inoculant per pot.

Once the plants reached the branching stage (20 on the BBCH scale according to Sosa-Zuniga et al. 2017 [75]), they were subjected to **two different water treatments: well-watered (WW)** conditions with pots maintaining around 65% soil water content (SWC) **and long-term water stress (WD) conditions**, irrigated until reaching 30% SWC (moderate but long-term stress), following the protocol standardized in Maestro-Gaitán et al. 2022 [74].

Root and seed sampling, and DNA extraction

Root sampling was performed once quinoa plants started the grain filling stage (81 at BBCH scale), following the protocol described by Edwards et al. [10],. Approximately, 10 g of roots were placed in 50 mL Falcon tubes. For the DNA extractions, root surface was sterilized following an adaptation of the protocol described by Barra et al. [76], with serial washes of 3 min in ethanol 70%, followed by 5 min in NaClO 2.5% and five rinses with sterilized MiliQ water. On the other hand, quinoa seeds were sterilized first in ethanol 70% (2 min), followed by a wash in bleach 50% with a droplet of Tween-20 (2 min), and then rinsed five times in sterile distilled water, as described in Granado-Rodríguez et al. [67]. During the root and seed sterilization, the water supernatant from the final rinse was streaked onto LB medium. The procedure success was

assessed after 48 h, during which no bacterial colonies were detected in any of the samples (*data not shown*).

Genomic DNA from root and seed endophytes was extracted using 250 mg of root/ seed tissue. The tissue (roots or seeds) was homogenized by maceration in liquid nitrogen and the DNeasy® PowerSoil® Pro (QIAGEN) was employed for the DNA extraction, following the manufacturer's instructions.

Sequencing

The 16 S rRNA gene metabarcoding (V3-V4 region) was used to evaluate the impact of the genotypic factor (quinoa cultivar), the water treatment (optimal irrigation or water stress), the soil inoculants (obtained from different soils) and the tissue analysed (seeds or roots) on the diversity and abundance of quinoa endophytic bacterial communities. After the extraction, DNA concentrations were determined spectrophotometrically (Nanodrop ND-1000 Spectrophotometer, USA). Before sequencing, 20 ng of the extracted DNA was amplified by PCR using 341 F (5-CCTAYGGGRBGCASCAG-3) and 806R (GGACTACNNGGGTATCTAAT) specific primers [77], to check the DNA quality. Then, DNA samples (including a negative control, that did not yield DNA, to check for possible DNA extraction kit contamination) were sent for sequencing analysis. Libraries were constructed following Novogene specifications, and the amplicon was subsequently sequenced by Novogene company using Illumina Novaseq PE 2 × 250 bp reads (50 K tags per sample), yielding an average total of 126,144 raw reads per sample. The number of raw reads, the filtered reads together with the denoised sequences, the merged sequences, and the non-chimeric reads obtained per cultivar, water condition, and inoculant are included in Supplementary File 1, with an average of 119,149 filtered reads for quinoa root samples and an average of 131,765 filtered reads for quinoa seed samples.

ASVs determination

Demultiplexed files containing raw reads were processed as described in DADA2 Pipeline Tutorial (1.30.0) [78]. In the first place, the adapters and primers regions were removed from the raw reads using CutAdapt program [79] (fastq file reads without adapters can be found in Bioproject PRJNA1129450). The output reads were quality filtered, denoised, clustered into ASVs (amplicon sequence variant), and chimeras were removed using the DADA2 v.1.30.0 package in R [78], with an average of 81.47% conserved reads after filtering and chimeras' removal. Then, raretons were removed using 1:1000 of the average number of sequences per sample as the threshold value, as well as filtering through prevalence (deleting any read that did not appear at least in three samples), leaving a final number of 656 ASVs. The taxonomic assignment was carried out with *assignTaxonomy* DADA2 function using DADA2 preformatted SILVAnr99 138 SSU database [80]. The filtered feature table and the taxonomic assignation were used for subsequent analysis of the community's diversity.

Bacterial diversity and composition analyses

Phyloseq v 1.46.0[81] package was used to study bacterial communities by generating a Phyloseq object in R version 4.3.1 [82] integrating the ASVs sequences, their abundances table, taxonomic assignation, and the samples metadata description. Mitochondrial, chloroplast, and archaea-associated ASVs were removed, as well as low-depth samples, obtaining the final ASVs count and Taxonomy tables (Supplementary File 2 and Supplementary File 3. respectively). We employed a widely accepted bioinformatics approach to remove chloroplast and mitochondrial sequences during data processing. This method has been validated in numerous studies analyzing endophytic bacterial communities (e.g [83–85])., and is sufficient to minimize host DNA interference while ensuring accurate microbial community characterization. After the filtering, data normalization was performed using the function *rarefy_even_depth* (phyloseq), at 95% of the sample with the lowest number of sequences. The normalization process was performed by separating the samples depending on the tissue factor (root samples and seed samples). Total ASVs obtained per sample were represented as a rarefaction curve using the function *rarecurve*, from vegan package v2.6-6.1 [86] to test the sequencing effort as total read counts and as normalized read counts (Supplementary Fig. 1A-B and Supplementary Fig. 1C-D respectively). Relative bacterial abundances at the family taxonomic rank level were calculated using *trans_abund* function from *microeco* package v1.7.1 [87].

Alpha diversity analysis was performed using Shannon and Chao1 diversity indexes, both indexes analysed using the *plot_richness* phyloseq function. Statistical differences in alpha diversity were analysed by performing a one-way ANOVA test followed by a multiple comparison with Tukey post hoc test at a $p\text{-}value < 0.05$, using *rstatix* version 0.7.2 package [88]. Alpha diversity index was analysed considering the cultivar and water treatment factors, as well as among soil inoculants for each cultivar and treatment conditions (applying nested comparison), and between maternal and offspring seeds ($p\text{-}value < 0.05$).

To further explore the differences in the bacterial communities found in the samples, a beta diversity analysis was performed. The data were transformed to centered log-ratio counts (*clr*) using the ordinate function from the phyloseq R package, followed by representation in a PCA ordination plot. A Permutational Analysis of Variance

(PerMANOVA) was performed using the *adonis2* function from the *vegan* package, with 999 permutations and a *p-value* threshold of < *0.05*, to assess the influence of different factors based on *clr* Euclidean distances (calculated using the distances function from the *phyloseq* package). The analysis followed a 3-way design including cultivar, water treatment, and soil inoculant factors (and their interactions) for each tissue, as well as between the roots and maternal and offspring seeds. Subsequently, the *pairwise.adonis2* function from the *pairwiseAdonis* package (v0.4.1 [89]) was used to examine specific differences among the groups analyzed, with 999 permutations and BH correction at a *p-value* threshold of < *0.05*.

To determine which ASVs were differentially abundant between cultivar, watering treatments, and tissues, differential abundance analysis ALDEx2 was performed using *trans_diff* function from *microeco* package. This analysis involved performing a Wilcoxon test with BH correction on *clr*-transformed data. The per-feature technical variation of each sample was estimated using Monte Carlo instances drawn from a Dirichlet distribution, correcting for differences in sequencing depth and allowing for comparisons between seed and root tissues.

All data visualization was performed with the R package ggplot2 v3.5.1 [90].

Results

The 16 S rRNA amplicon sequencing yielded an average total of 126,144 raw reads per sample, with an average of 119,149 reads in root samples and 131,765 reads in seed samples after filtering and removing chimeras (Supplementary File 1). After the aforementioned filtering, an average of 81.47% of reads per sample were retained. These reads were associated with a total of 9,726 Amplicon Sequence Variants (ASVs), ultimately leaving a final number of 656 ASVs after removing raretons and low-abundance microorganisms. Subsequently, sequences associated with non-bacterial microorganisms (mitochondria, chloroplasts, and archaea) and low-depth samples were removed, reducing the number of ASVs from 656 to 599 and lowering the average depth from 101,683 reads per sample to 5,820 reads per sample (Supplementary File 1). However, the depth was not equally distributed across all samples, with an average of 10,432 reads per sample in root-associated endophytes and an average of 618 reads per sample in seed-associated endophytes. Consequently, the normalization process through rarefaction was conducted separately based on tissue type, resulting in a final count of 85 normalized reads per sample in seed samples and 512 normalized reads in root samples, associated with 239 and 529 ASVs, respectively (Supplementary File 1 and 2). This normalization process ensured that the rarefaction curves reached a plateau in both tissues (Supplementary Fig. 1).

Distinct abundance patterns of unclassified bacteria and key families in quinoa seeds and roots

The analysis of bacterial family abundance among different quinoa tissues (maternal seeds, offspring seeds, and roots) revealed that tissue type is the primary factor influencing bacterial community structure. When analysing the relative abundance of the top 30 families in seed and root samples, we observed the varying presence of *Unclassified Bacteria* within F15 seed samples. Their seed relative abundance ranged from 0.59% in F15 water deficit (WD) S2 E to 18.82% in F15 well-watered (WW) S1 MB (Fig. 1A). These *Unclassified Bacteria* (which corresponded to three different ASVs) were also present in F16 WD S1 MB seeds with an abundance of 4.71%, which was the only F16 seed sample in which this group appeared (Fig. 1A). Notably, F15 WW seed was the sample type with the highest accumulation of this group compared to the other seed samples (with an average relative abundance of 9.9%).

F16 offspring seed samples showed enrichment in the relative abundance of *Unclassified Proteobacteria* (which corresponded to one ASV), also displaying variable levels varying from 0.39% in F16 maternal seeds up to 15.29% in F16 WD S1 MB seeds (Fig. 1A). This group exhibited a higher prevalence in F16 WW seed samples (4.71% relative abundance) when compared to the rest of cultivars and water treatments, while being absent in F16 WD S2 MB seed samples (Fig. 1A).

Maternal seeds of both genotypes (F15 and F16) presented a large abundance of *Micrococcaceae* family comprising 28.24% in F15 maternal seeds and 31.77% in F16 maternal seeds, with F16 seed samples displaying the highest presence of *Micrococcaceae* family, particularly the *Pseudarthrobacter* genus (Fig. 1A, Supplementary File 2 and Supplementary File 3). Remarkably, this bacterial family was not present in the seeds obtained from pots inoculated with S1 MB soil infusion, except for F15 WD S1 MB seeds, which showed a relative abundance of 2.35%, the lowest relative abundance for this bacteria taxon (Fig. 1A). Seeds obtained from plants inoculated with S1 MB soil infusion also lacked *Planococcaceae*, *Saccharimonadaceae* and *Hymenobacteraceae* families, which appeared in the other seed samples except for *Hymenobacteraceae* family, which was also absent in F16 WW S2 MB seed samples (Fig. 1A).

Focusing on root endophytes, the *Micrococcaceae* family (the most abundant Actinobacteria in seed samples) exhibited a low percentage of relative abundance, ranging from 0.13% in F15 WD S1 MB up to 2.34% in F15 WD S2 E root samples, and was absent in F16 WW S1 E, F16 WD S2 MB and F16 WD S2 E root samples (Fig. 1B). Root samples also lacked *Unclassified bacteria* and *Unclassified proteobacteria*, as well as *Sacharimonadia* bacterial endophytes (Fig. 1B), although these three

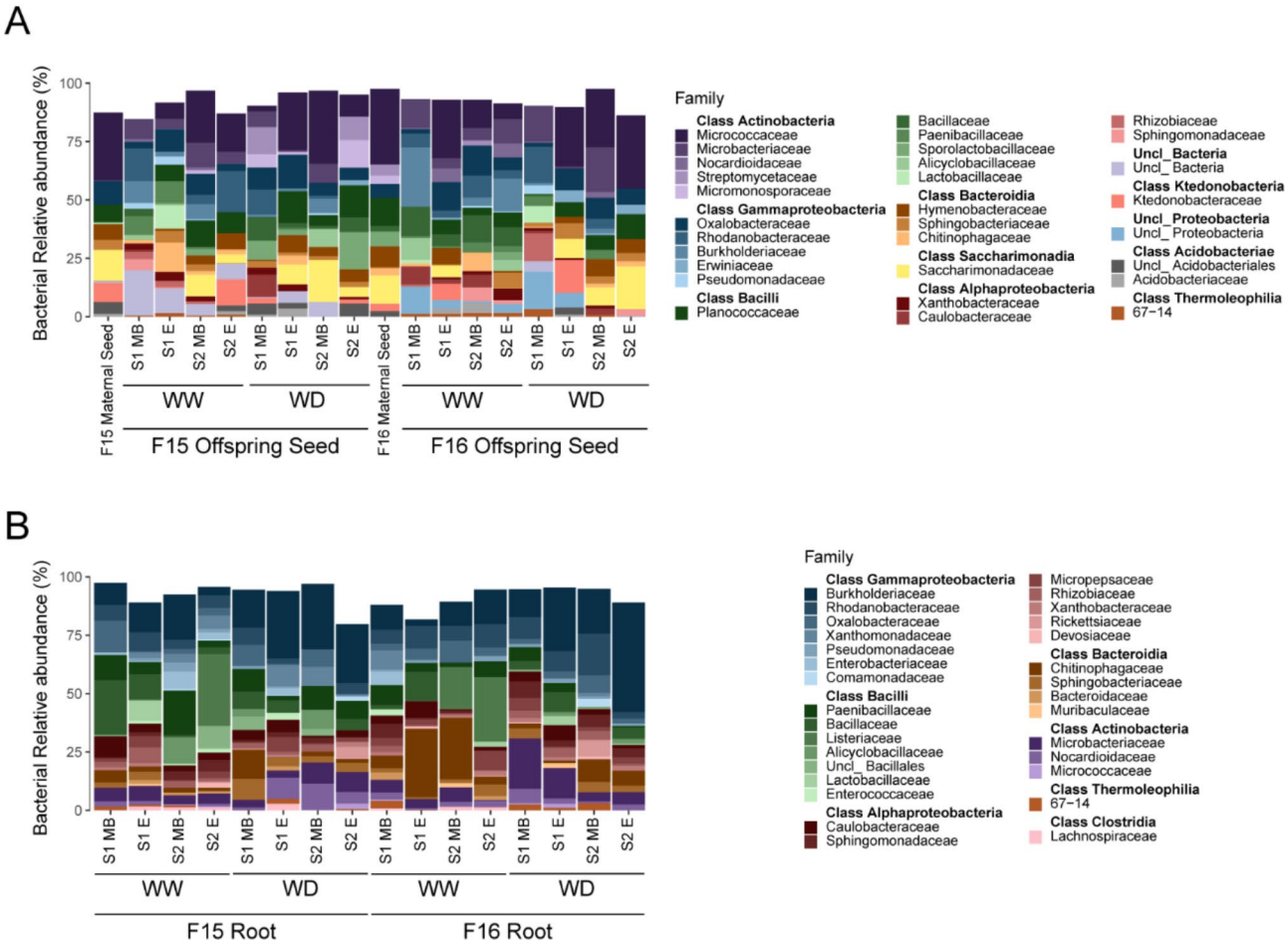

Fig. 1 Relative abundance of the top 30 bacterial taxa at family level. The graph shows the relative abundance of the most prevalent bacterial ASV at the family level, with less abundant families classified as "Other" (not included in the graph). (**A**) Bar plots representing the relative abundance of maternal and offspring seed samples (**B**) Bar plots representing the relative abundance of root samples. Both panels show the relative abundances among different cultivars (F15 and F16), water treatments (WW and WD), and type of soil infusion inoculated (S1 MB, S1 E, S2 MB, and S2 E). A gradient using the same colour is employed for all families present in each class

bacterial families were relatively abundant in the seed samples (Fig. 1A).

Root samples featured a high presence of *Burkholderiaceae* family, particularly under WD conditions, with an average of 23.88% in F15 WD samples and 24.73% in F16 WD samples. The roots of the cultivar F16 grown under WD conditions had the highest presence of *Burkholderiaceae* family (25.59% relative abundance) compared to F15 and F16 WW samples (10.79 and 9.13%, respectively) (Fig. 1B). Additionally, there was a high relative abundance of *Listeriaceae* family in the roots of both cultivars grown under WW conditions inoculated with S2 E soil infusion (30.08% in F15 and 26.95% in F16) (Fig. 1B).

Quinoa root endophytic bacteria displayed higher diversity than seeds, with differences between cultivars

Both Shannon and Chao1 indexes were calculated to assess the impact of the cultivar, water treatment, and tissue type (specifically comparing maternal seeds and offspring seeds) and their interactions on the diversity of endophytic bacterial communities in quinoa seeds and roots (Fig. 2). The findings emphasized the complex interactions between tissue type, cultivar, water treatment, and inoculation in shaping quinoa's endophytic bacterial communities.

Although no differences were found when using the Shannon index among seed samples considering cultivars and water treatments (Fig. 2A), a significant influence of the inoculant and the interaction between inoculant and water treatment was observed using the Shannon index in seed samples ($Pr\ (>F) = 0.028$ *and* $Pr\ (>F) = 0.025$, respectively) (Supplementary Fig. 2A). Nonetheless, no significant differences were found after multiple comparisons using a Tukey post hoc test among the different soil inoculants (Supplementary File 4). Indeed, nested comparisons among samples considering the soil inoculant factor for each cultivar, water treatment, and tissue, revealed significant differences in the Shannon index for

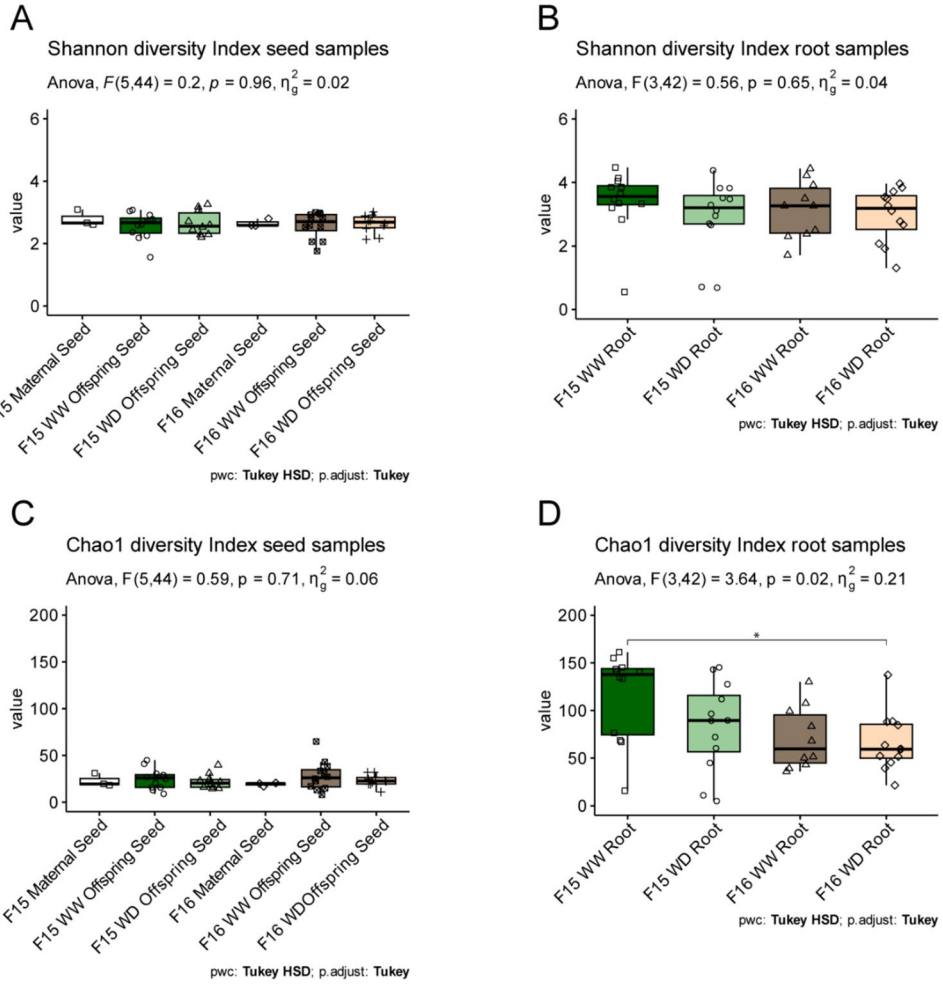

Fig. 2 Alpha diversity richness among cultivars, water treatments and tissues. (**A**) Shannon diversity index for maternal and offspring seed samples. (**B**) Shannon diversity index for root samples. (**C**) Chao1 diversity index for maternal and offspring seed samples. (**D**) Chao1 diversity index for root samples. Differences among cultivars and water treatments for each tissue were analysed through ANOVA and Tukey post-hoc test, at *p-value* < 0.05

F16 seeds grown under WW conditions. Specifically, F16 WW seeds from plants inoculated with S1 MB soil infusion exhibited lower bacterial diversity compared to those obtained from other soil inoculants (S1 E, S2 MB, and S2 E) (*p-value = 0.006*) (Supplementary Fig. 2A, Supplementary File 4). Statistical differences were also identified when comparing F16 maternal seeds to F16 WW S1 MB seeds, with the latter group exhibiting lower alpha diversity (*p-value < 0.001*). In contrast, the F16 WW S1 MB sample showed significantly higher bacterial diversity in the roots, as indicated by the Shannon index, compared to F16 plants grown under WW conditions and inoculated with S1 E and S2 MB (*p-value* = 0.028 and *p-value* = 0.01, respectively) (Supplementary Fig. 2B, Supplementary File 5).

Furthermore, the Shannon index exhibited slightly higher values in root samples compared to seed samples (T-test, *p-value* < 0.001), with Shannon index values of 2.54 ± 0.35 and 3.10 ± 0.98, respectively (Fig. 2A and B). These differences were much more pronounced when using the Chao1 index, which was 267.49% higher in root samples compared to seed samples (82.35 ± 42.23 and 22.43 ± 3.96, respectively) (Fig. 2C and D) (T-test, *p-value* < 0.001).

While the Chao 1 index revealed no influence by any factor nor statistical differences in bacterial diversity of endophytes among seed samples (Supplementary Fig. 2C and Fig. 2C, Supplementary File 6), an influence of the cultivar factor was found in root samples (*Pr (> F) = 0.0109*), with F15 showing greater bacterial diversity compared to the cultivar F16. Besides, differences within the root bacterial diversity were found between the F15 cultivar grown under WW conditions and the F16 cultivar grown under WD conditions, with Chao1 index values of 116.03 ± 44.73 and 67.39 ± 29.77, respectively (*p-value = 0.021*) (Fig. 2D). Additionally, F16 grown under WD conditions showed greater diversity when inoculated with S1 MB compared to S2 E

(*p-value* = 0.031) (Supplementary Fig. 2D, Supplementary File 7).

2.3 Tissue type shapes endophytic bacterial composition in quinoa with large stability of communities between maternal and offspring seeds.

Focusing on beta diversity indexes, we analysed our data using a Euclidean distance matrix calculated through a centred log-ratio (*clr*) count transformation. This data was visualized through principal component analysis (PCA), which accounted for 19.6% and 9.4% of the total variation in seed and root samples (PC1 and PC2, respectively) (Fig. 3). Maternal seed samples were grouped in the negative section of Axis 1 and the positive section of Axis 2, clustering separately from root samples, and partially overlapping clustering with offspring seed samples (Fig. 3). The tissue was one of the main factors explaining differences in beta diversity across samples (*PerMANOVA, Pr(> F) = 0.001*, Supplementary File 8), with significant differences in bacterial endophytic communities between maternal seeds and roots and between offspring seeds and roots, but not between maternal seeds and offspring seeds (*pairwise Adonis, Pr(> F) < 0.001, < 0.001, and = 0.157*, respectively; Supplementary File 8).

Other factors influencing the beta diversity of quinoa endophytic bacterial communities included the water conditions applied (*PerMANOVA, Pr(> F) = 0.001*, Supplementary File 8). However, the main differences depending on the water treatment factor occurred indirectly through the tissue factor, as the unique differences that appeared were those between maternal seeds and WW and WD samples (considering offspring seeds and roots) (*pairwise Adonis, Pr(> F) = 0.002, 0.018*), but not between WW and WD samples (considering offspring seeds and roots) (*pairwise Adonis, Pr(> F) = 0.079*, respectively; Supplementary File 8). More precisely, we found differences between F15 maternal seeds and all root samples (F15 WW, F15 WD, F16 WW, and F16 WD with a *pairwise Adonis Pr(> F) = 0.008, 0.004, 0.025, and 0.003*, respectively), and between F16 maternal seeds and all root samples (F15 WW, F15 WD, F16 WW, and F16 WD roots with a *pairwise Adonis Pr(> F) = 0.01, 0.004, 0.036, and 0.002*, respectively).

On the other hand, the soil inoculant factor showed a significant influence on quinoa endophytic bacterial composition (*PerMANOVA, Pr(> F) = 0.006*, Supplementary File 8). Further analysis of the differences associated with this factor revealed that seed samples from pots inoculated with S1 MB infusion showed differences compared to S1 E, S2 MB, S2 E and maternal samples (*pairwise Adonis Pr(> F) < 0.001, = 0.0216, = 0.004* and *< 0.001*,

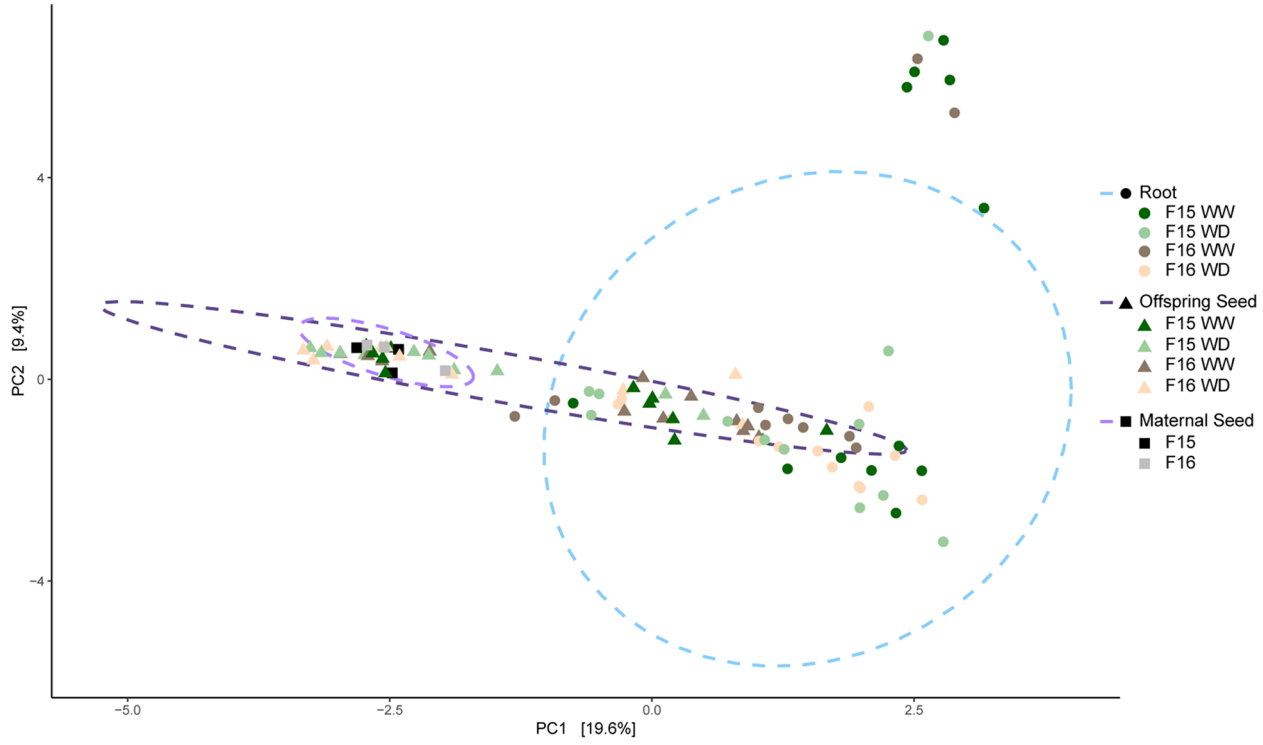

Fig. 3 Clustering analysis of endophytic bacterial communities through maternal and offspring seeds and roots. Principal Component Analysis (PCA) plot performed using *clr* transformed ASV counts matrix. With *x-axis* / Principal Component 1 (PC1) explaining 19.6% of the total variance and *y-axis* / Principal Component 2 (PC2) explaining 9.4% of the total variance among groups. The colour of the dots represents the different cultivars and water treatments analysed, and the shapes represent the different samples considering the tissue factor. The lilac ellipse corresponds to the cluster of maternal seed samples, the dark purple ellipse corresponds to the offspring seed samples, and the blue ellipse the cluster of the root samples

respectively, Supplementary File 8), and, in the case of root samples, between S1 MB and S2 E samples (*pairwise Adonis Pr(> F) = 0.005*; Supplementary File 8).

The interaction between the cultivar and tissue factors, and the interaction between water treatment and tissue (*PerMANOVA, Pr(> F) = 0.046 and Pr(> F) = 0.031*, respectively), were also significant factors explaining variations in the beta diversity among samples. Despite the lack of influence and interactions between cultivar and water treatment in the PerMANOVA analysis, significant differences in the bacterial community composition among cultivars and treatments appeared through pairwise Adonis comparisons (Supplementary File 8). Specifically, significant differences appeared in root samples between F16 WD and F15 WW root samples (*pairwise Adonis Pr(> F) = 0.006*) and in seed samples between F15 WD and F16 WW offspring seeds (*pairwise Adonis Pr(> F) = 0.024*, Supplementary File 8). Additionally, no differences in bacterial composition through beta diversity analysis were found in nested comparisons among soil inoculants for each cultivar and water condition (*pairwise Adonis Pr(> F) > 0.05*, Supplementary File 9).

2.4 Bacterial family endophyte enrichment in quinoa reveals tissue-specific patterns and complex interactions between cultivar, water treatment, and inoculant.

Performing an ALDEx2 analysis on differentially abundant bacteria (at the family taxonomic level) revealed distinct enrichments among the samples. Tissue type emerged as the primary driver of bacterial community enrichment although significant interactions with cultivar, water treatment, and soil inoculant were also observed, further shaping the diversity and composition of endophytic bacterial populations. Thus, when comparing bacterial family enrichment among tissues (maternal seeds, offspring seeds, and roots) (Fig. 4A), the main differences appeared between maternal seeds and roots and between offspring seeds and roots, showing a more pronounced effect in the first comparison. On the contrary, no enrichment was found between maternal seeds and offspring seeds, consistent with the conserved beta diversity analysis between these samples (Figs. 3 and 4A, Supplementary File 8 and Supplementary File 9).

The most notable differences included the enrichment of *Burkholderiaceae*, *Caulobacteraceae*, and *Rhodanobacteraceae*, with more than a 1.8 effect size enrichment in root samples compared to maternal seed samples, and more than a 1 effect size enrichment in root samples compared to offspring seed samples (except for *Rhodanobacteraceae*, which showed 0.78 effect size enrichment). Conversely, significant enrichment was observed for *Hymenobacteraceae*, *Planococcaceae*, and *Saccharimonadaceae*, with more than 2 effect size enrichment in maternal seed samples compared to root samples and

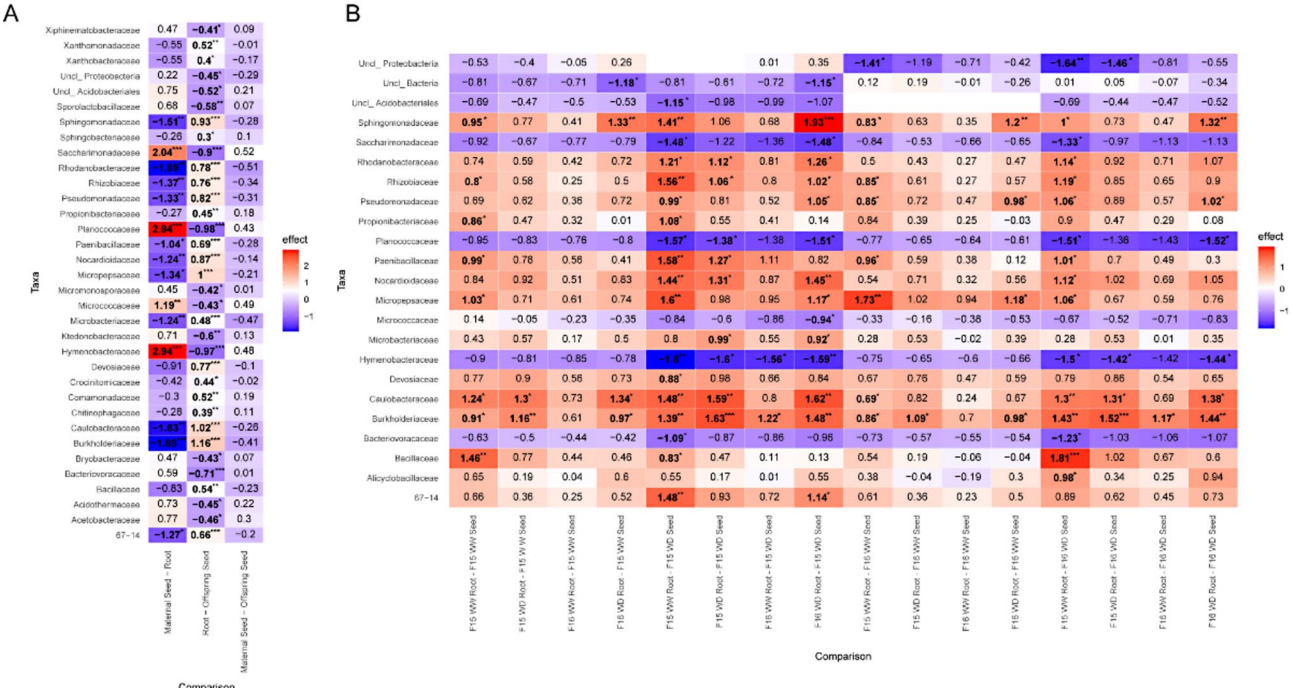

Fig. 4 Differential abundance analysis of high throughput sequencing count compositional data (*Aldex2*). (**A**) Bacterial enrichment dependent on tissue factor. (**B**) Bacterial enrichment in the comparisons among different offspring seeds and root samples. Groups compared pairwise are shown on the *x-axis*, while the *y-axis* shows the different bacterial families enriched in the comparisons. The level of enrichment between groups is shown as the *effect* in a colour gradient ranging from red (positive values) to blue (negative values)

more than a 0.9 effect size enrichment in offspring seed samples compared to root samples.

When comparing bacterial family enrichment among soil inoculants for each tissue (root and seed) (Supplementary Fig. 3A and B), the main differences appeared between non-inoculated samples (maternal seeds) and S1 MB seed samples, with a higher presence of *Planococcaceae*, *Hymenobacteraceae*, *Saccharimonadaceae*, and *Micrococcaceae* in the maternal seeds. No significant differences in bacterial enrichment among different soil inoculants were observed in root samples (Supplementary Fig. 3B).

Moreover, when analyzing differences among cultivars, water treatments, and tissues, samples collected from the roots of the F15 cultivar grown under WW conditions showed enrichment in the families *Paenibacillaceae*, *Caulobacteraceae*, *Sphingomonadaceae*, *Micropepsaceae*, and *Rhizobiaceae* compared to all offspring seed endophytes (F15 WW, F15 WD, F16 WW and F16 WD) (Fig. 4B). Additionally, F15 WW root samples were enriched in *Rhodanobacteraceae* and *Nocardioidaceae* families compared to WD offspring seeds from both cultivars (F15 and F16). F15 WW root samples were also enriched in *Propionibacteriaceae* compared to F15 offspring seeds (WW and WD), and in *Burkholderiaceae* compared to all offspring seeds and F15 maternal seeds (Fig. 4B and Supplementary Fig. 3C). Besides, F15 WW root samples were enriched in the family *67–14* compared to F15 WD seed samples (Fig. 4), in *Pseudomonadaceae* compared to all offspring seed samples (except for F15 WW seeds), and in *Bacillaceae* compared to all seed samples (except for F16 WW offspring seeds) including F15 maternal seeds (Fig. 4B and Supplementary Fig. 3C).

In contrast, F15 WD root samples were enriched in *Paenibacillaceae*, *Caulobacteraceae*, *Rhodanobacteraceae*, *Microbacteriaceae*, *Nocardioidaceae*, and *Rhizobacteriaceae* compared to F15 WD seed samples (Fig. 4B). They were also enriched in the family *Burkholderiaceae* compared to seed samples (including maternal seeds) from both cultivars (Fig. 4B and Supplementary Fig. 3C).

This *Burkholderiaceae* enrichment pattern was also observed in the root endophytes of the water-saving cultivar F16 under WW conditions, with a larger presence of this family than in the maternal seeds of both cultivars and when compared to the offspring seeds from plants grown under WD treatment (in both cultivars) (Fig. 4B and Supplementary Fig. 3C).

For the F16 cultivar grown under WD conditions, root endophytes were enriched in *Rhodanobacteriaceae*, *Microbacteriaceae*, *Micropepsaceae*, 67–14, *Nocardioidaceae*, and *Rhizobiaceae* families compared to F15 WD seed samples (Fig. 4B). F16 WD root samples were also enriched in *Caulobacteraceae* compared to all seed samples (except for F16 WW seeds, including F15 maternal seeds) and enriched in *Sphingomonadaceae* and *Burkholderiaceae* compared to all seed samples (including F15 maternal seeds) (Fig. 4B and Supplementary Fig. 3C). Furthermore, F16 WD root samples were also enriched in *Pseudomonadaceae* compared to all offspring seed samples (except for F15 WW seeds) (Fig. 4B).

Furthermore, F16 maternal seeds were also enriched in *Saccharimonadaceae*, *Planococcaceae*, and *Hymenobacteraceae* compared to F15 WW root and F16 WD root samples and in *Micrococcaceae* only compared to F15 WW root samples (Supplementary Fig. 3C).

Lastly, specific differences appeared in some bacterial families among samples. For instance, enriched bacterial families in seeds collected from plants grown under WD conditions included the family *Bacteriovoracaceae* (enriched in F15 WD offspring seeds compared to F15 WW roots and in F16 WD offspring seeds compared to F15 WW roots), the family *Saccharimonadaceae* (enriched in F15 WD offspring seeds compared to F15 WW and F16 WD roots, and, also, in F16 WD seeds compared to F15 WW roots), and the family *Planococcaceae* (in F15 WD offspring seed samples compared to F15 WW, F15 WD, and F16 WD root samples, and in F16 WD offspring seed samples compared to F15 WW and F16 WD root samples) (Fig. 4B). Under these WD conditions, it was also found an enrichment in the family *Hymenobacteraceae* in F15 WD offspring seeds compared to all root samples and in F16 WD seeds compared to all root samples (excluding F16 WW root samples) (Fig. 4B). These findings suggest that water deficit conditions may promote the growth of specific bacterial families in seeds, as most differences in bacterial endophytes were observed in seeds from plants grown under WD conditions compared to roots from both WW and WD plants.

In seeds, the family *Burkholderiaceae* and the *Unclassified Proteobacteria* were enriched in F16 WW offspring seeds compared to F15 WW Roots (Fig. 4B). The latter was also enriched in F16 WD offspring seeds in comparison to F15 roots under both water treatments. The *Unclassified Bacteria* group was enriched in F15 WD and F15 WW offspring seeds compared to F16 WD root samples (Fig. 4B), and the *Unclassified Acidobacteriales* were enriched in F15 WD offspring seeds compared to F15 WW roots (Fig. 4B). These unclassified groups may represent novel bacterial communities that play specific roles in seed development and stress responses, warranting further investigation.

Overall, these results underscore the critical role of tissue type in shaping the microbial communities associated with quinoa plants, with clear distinctions between bacterial families present in seeds and roots. Thus, the tissue type emerged as the primary driver of bacterial

community composition, with significant enrichment of distinct bacterial families in roots compared to both maternal and offspring seeds. These findings suggest that root-associated microbiomes could be specialized to meet the functional demands of root tissues, such as nutrient uptake and soil interactions, while seed microbiomes are more conserved, likely contributing to seed development and protection.

Discussion

The necessity of implementing approaches to enhance water use efficiency and drought stress resistance is particularly critical in the current climatic context, which is characterized by increasing temperatures and irregular precipitation patterns [91]. Many traditional crop species often struggle to cope with these conditions, leading to reduced yields and threatening food security. To address this, one promising strategy is the diversification of agriculture through the introduction and cultivation of underutilized plant species such as quinoa [92]. Quinoa is highly resilient to drought and poor soil conditions, making it an excellent candidate for sustainable agriculture in arid and semi-arid regions. Its introduction not only helps to mitigate the effects of climate change on agriculture but also contributes to the stability and diversification of food systems [93].

On the other hand, given the importance of endophytic microorganisms in seed germination and seedling establishment as well as in coping with stressful factors (biotic and abiotic), identifying bacterial communities has become of great interest [94–97]. This is particularly crucial in emerging and understudied crops like quinoa, as, despite some initial studies, the exploration of endophytes in this crop remains limited [43, 97–100]. Moreover, the effects of water stress on endophytic microorganism communities have been underexplored in this crop, with existing research focusing predominantly on fungal endophytes [53]. Understanding and leveraging these endophytic communities can enhance the stress resilience and overall performance of quinoa, further supporting its role in agricultural diversification and sustainability. Thus, this study focused on evaluating and characterizing these bacterial taxa to provide foundational insights into the composition of the microbial community.

Interestingly, a previous study focusing on the bacterial communities of its rhizosphere observed significant differences in bacterial composition and diversity between genotypes and water regimes highlighting genotype-specific variations in the abundance of stress-responsive bacterial taxa [44]. In line with this, the current study focused on analysing the endophytic bacterial communities in quinoa roots and seeds, investigating the impact of water stress, genotypic factor, and the addition of soil infusion inoculants on shaping their structure. To do so, a metabarcoding analysis was performed on the roots and seeds of plants subjected to water stress (WD) and optimal irrigation (WW), as well as on their maternal seeds from two different quinoa varieties, which previously showed contrasting water-saving strategies under drought [74] (F15 and F16). Additionally, at the beginning of crop development, the plants were inoculated with soil infusions obtained from two distinct localities, a non-cultivated agronomical soil (S1) and a quinoa-cultivated soil (S2). This approach was used to evaluate the potential incorporation of exogenous bacteria into the endophytic communities or the possible displacement of the original communities. By introducing these soil-derived bacterial communities, we aimed to determine how local environmental microbiota could influence the composition and dynamics of quinoa endophytes (from roots and seeds) under different irrigation regimes, a topic that has been more extensively studied in other plant species [101–103].

Focusing on the heritability of certain bacterial taxa by analyzing differences between maternal and offspring seeds of both genotypes under both water conditions, we observed no enrichment of any specific bacterial family (Fig. 4A, and Supplementary Fig. 3D). Additionally, there were no significant differences in bacterial enrichment when comparing offspring seeds based on genotype and water conditions (Supplementary Fig. 3D), indicating strong conservation of seed endophytic bacterial communities across genotypes and environmental conditions in quinoa as previously stated [99]. Supporting this, the alpha and beta diversity analyses performed in the current study (Figs. 2 and 3) revealed a high degree of bacterial conservation in terms of diversity and composition between the two quinoa varieties and water treatments compared to that observed in the rhizosphere of this plant species [44]. This is consistent with previous studies on other plant species, indicating that endophytic communities are usually more conserved and share a core set of bacteria between varieties of the same plant species or even between different plant species [104]. As stated, the plant genotype seems to play an insignificant effect in shaping quinoa endophytic communities, and the environmental factors likely played a key role in structuring soil microbial communities, though their influence on root and seed endophytes is weak [99].

Analysis of root endophyte communities similarly revealed no significant differences in bacterial enrichment between varieties or water treatments (Supplementary Fig. 3E). Therefore, even root endophytes, which are known to be more susceptible to change due to their close contact with the rhizosphere (a highly variable and dynamic zone with diverse microbial communities [7, 9]), mostly remain well conserved in a species-dependent

manner [105]. Although generally more conserved, endophytic communities can undergo changes due to various factors such as environmental conditions, genotypic differences, or the introduction of external bacterial communities [106, 107]. Hence, despite the overall lack of significant genotype and water regime effects on root microbial endophytic composition, specific differences emerged within these endophytic communities. In our study, these specific differences were observed in the alpha diversity results, particularly when using the Chao1 diversity index, which emphasizes bacterial ASVs present in smaller proportions. In line with this, changes were observed between the F15 variety under optimal irrigation (WW) and the F16 variety under water deficit (WD) conditions in root samples (Fig. 2). This result was also supported by the beta diversity analysis, as the pairwise Adonis comparisons among cultivars, water treatments, and tissues, yielded differences between roots of F15 WW and F16 WD. However, this interaction between genotype and environment (GxE) was not significant in the ANOVA analysis (Supplementary File 7) nor in the PerMANOVA, and thus can be considered a specific variation. Moreover, this lack of significance was supported by the Aldex2 results, which did not yield enrichment when comparing these samples (Supplementary Fig. 3E). Thus, our findings revealed limited genotypic variation in the endophytic bacterial communities between the two quinoa varieties studied, with only a few significant differences observed. This may reflect the adaptability and stability of these endophytic microbial communities across different genotypes or suggest that environmental factors exerted a stronger influence on their composition. The environmental conditions in this study may have masked subtle genotypic effects, highlighting the need to investigate endophytic communities under a wider range of environmental conditions, such as salinity stress or varying soil types, where these differences may become more pronounced. Additionally, the limited number of varieties examined constrains the generalizability of our results. Future research should explore a more diverse set of quinoa genotypes and other crops to better understand the relationship between plant genetics, environmental factors, and the assembly of endophytic communities. Such studies will provide valuable insights into the adaptability of these microbial communities and their potential roles in plant growth and stress resilience, contributing to the broader understanding of plant-microbiome interactions.

The introduction of soil infusions had varied effects on the alpha diversity of quinoa endophyte communities. While significant differences were observed in very specific cases, the overall influence of this parameter was not pronounced (Supplementary Fig. 2, Supplementary Files 4–8). This is consistent with several works that have demonstrated limited effects of inoculants on altering plant-associated microbiota [44, 108]. However, an exception was observed in offspring seeds from plants inoculated with S1 MB, which exhibited a decrease in the families *Planococcaceae*, *Hymenobacteraceae*, *Sacchari-monadaceae*, and *Micrococcaceae* compared to maternal seeds (Supplementary Fig. 3A). This S1 MB inoculant effect was not observed in root endophytes (Supplementary Fig. 3B). This contrasting response may reflect differences in the compatibility between soil-derived bacteria and endophytic communities in seeds versus roots stressing the complex interactions between soil-derived microbiota and endophytic communities. Besides, the observed changes suggest a potential selective pressure exerted by a particular soil infusion on the seed endophytic community composition, leading to an alteration in the species diversity, composition, and enrichment [108]. This further underscores the stability of root-associated bacterial communities in quinoa and opens the possibility of exploring seedling inoculants as an effective strategy to produce changes in the bacterial composition of seeds. Inoculant application has proven to be an effective technique for enhancing agricultural productivity [109–111]. Although numerous studies demonstrate the temporary impact of these targeted microorganism applications, the long-term effects remain unclear, and there is a lack of evidence regarding their potential impact on endophytic communities over extended periods. This study demonstrates the effect of inoculation on the displacement of certain bacterial families, opening the possibility for future research to develop control applications that positively impact crops in both the short and long term.

Nonetheless, the influence of added inoculants was generally limited as mentioned, which reflects, on one hand, that plant reinoculation with greater concentrations of bacterial infusions could lead to significant effects on plant-associated microorganisms [112], and, on the other, that further insight is required into the mechanisms by which inoculants influence the host endophytic microbiome, and into the colonization dynamics of microbial inoculants within the plant environment [113]. Further investigations are necessary to elucidate the underlying mechanisms driving these differential responses and to assess the long-term effects of soil infusions on the stability and function of quinoa endophyte communities.

Another important aspect to consider is the analysis of quinoa bacterial communities, considering the huge genetic diversity of this crop and wild relatives with more than 16,422 accessions available [114], which suggests that quinoa could potentially serve as a source of plant growth-promoting bacteria (PGPB) [44, 98, 100, 115]. Thus, analysing the diverse bacterial communities associated with different tissues (including roots, maternal

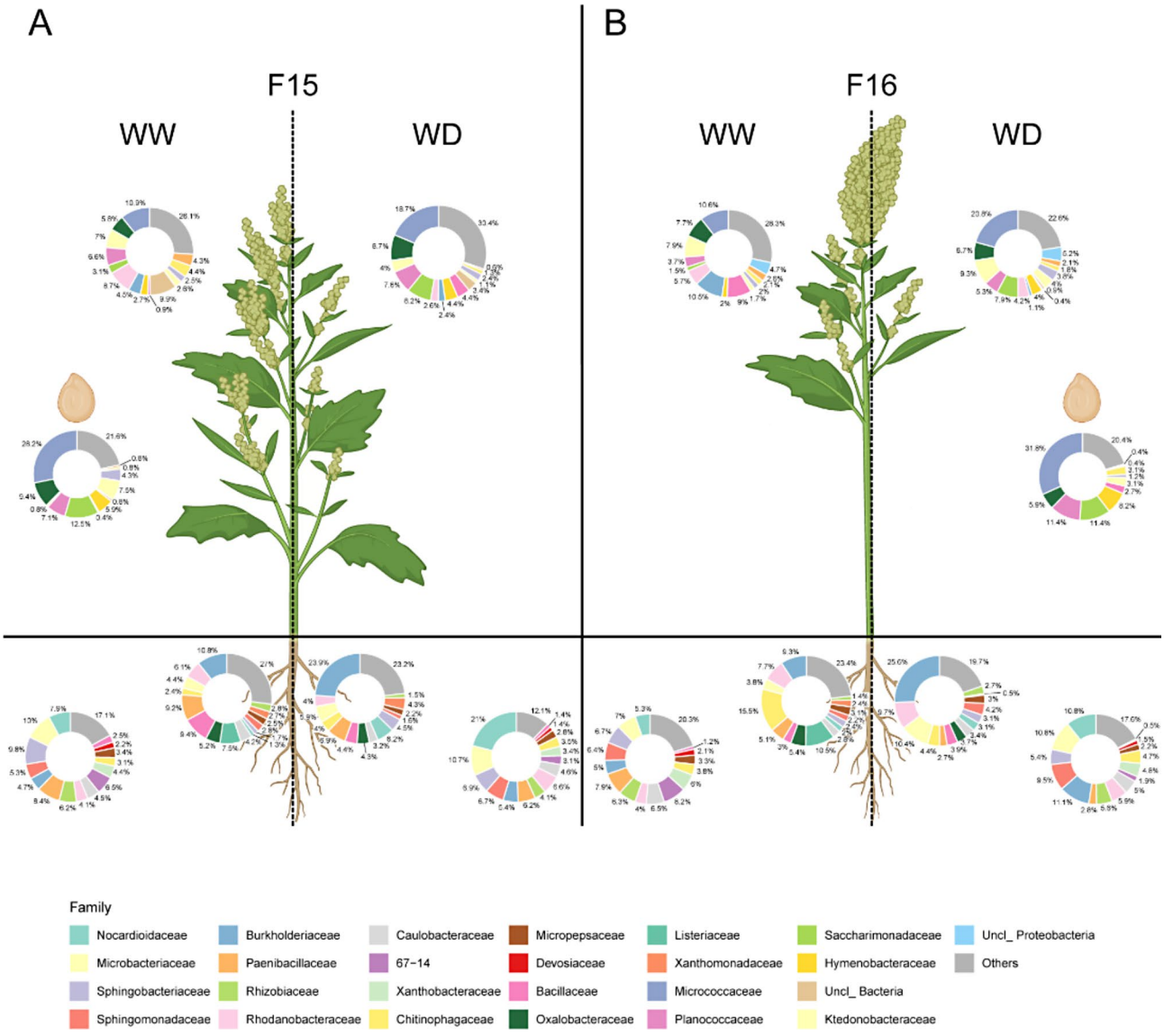

Fig. 5 Pie-Donut chart showing the relative abundance of the top 15 most abundant families in each quinoa tissue (endophytic maternal and offspring seeds and root bacterial communities, as well as rhizosphere), dependent on cultivar and water conditions. Based on this work and previous data from Maestro-Gaitan et al. 2023 [44]

seeds, and offspring seeds) as stated in this and former works [44, 99], and the information provided by their relative abundances and enrichments under varying environmental conditions, provides a rich repository and knowledge for further identifying PGPB candidates in this crop. Indeed, considering this and a previous study on quinoa [44], tissue-dependent variations (including the rhizosphere) in the relative abundance of key bacterial families in response to the genotype and water conditions were found (Fig. 5). To perform this, a comparative analysis for each tissue type (rhizosphere, root, and seed) was applied, considering the 15-most abundant bacterial families per genotype (F15 and F16) and water treatment (WW or WD). The *Micrococcaceae* family was prevalent in seeds, but less so in the rhizosphere and roots, indicating tissue-specific adaptations (Figs. 4A and 5). Members of the *Micrococcaceae* family which were also found in this work (i.e. *Pseudarthrobacter* Supplementary File 3) have been involved in the degradation of organic matter and nutrient cycling and have been shown to promote growth under salinity stress [116–118]. Similarly, the *Saccharimonadaceae* family, though present in all tissues, showed higher abundance and enrichment in seeds. In contrast, the *Burkholderiaceae* family was more prominent in root endophytes and contributed to differentiating the F16 variety under water deficit conditions in the rhizosphere (Fig. 5).

These bacteria, associated with pathogen defence mechanisms [119], highlight the potential of quinoa's endophytic communities in enhancing plant resilience against biotic stress [100]. This was also supported by the finding of a larger abundance and enrichment of *Rhodanobacteraceae* and *Caulobacteraceae* families in root bacterial communities (Figs. 4A and 5), which have also been reported to promote plant growth [120, 121] as can help plants cope with fungal pathogens promoting their growth [120]. Furthermore, our findings suggest that root-associated bacteria such as *Nocardioidaceae*, which promote root elongation and regulate bacterial density through *quorum quenching* [122, 123], are well-conserved in water-stressed conditions. Also, the presence of *Pseudomonas*, some members well known as PGPBs [124], mainly in roots under certain irrigation regimes, underscores their role in promoting plant growth and stress resilience. Overall, the consistent presence and specific enrichment of these bacterial taxa in quinoa's endophytic communities may indicate that this crop's genetic diversity can be harnessed to further identify and select effective PGPBs. However, it is important to note that drawing functional conclusions at the bacterial family or even genus level may have limitations, as members of the same family or genus can exhibit a range of interactions with plants, from beneficial to pathogenic. Therefore, while we discuss potential roles based on differential abundance, these generalizations may oversimplify the underlying complexity, which can only be fully disentangled through experimental validation at the species level. Indeed, further experimental studies are necessary to validate these potential associations. While 16 S rRNA amplicon sequencing is a powerful tool for identifying bacterial taxa and understanding microbial diversity, it has inherent limitations in inferring functional roles. Addressing these functional aspects requires complementary approaches such as shotgun metagenomics, which can provide information about functional pathways related to plant protection or bioaugmentation, such as those associated with growth promotion, nutrient cycling, or pathogen resistance, and offer valuable insights into microbial contributions to plant health. Future research should develop these advanced methodologies to explore the roles of quinoa-associated bacterial taxa in plant development, stress tolerance, and other beneficial interactions, building on the baseline data presented in this study. Considering these aspects, by exploring the extensive genetic diversity of quinoa and its relatives [114], researchers can uncover a wide array of beneficial microorganisms adapted to various environmental conditions. This potential can be leveraged to develop microbial inoculants that enhance growth and stress resilience in other crops [115], thereby contributing to sustainable agricultural practices [125].

Conclusions

This study highlights the crucial role of tissue type in shaping the endophytic microbial communities associated with quinoa, with distinct bacterial families enriched in roots compared to seeds. Root microbiomes were significantly influenced by water availability and cultivar, with specific families like *Burkholderiaceae* and *Paenibacillaceae* showing notable enrichments under particular conditions. Seed microbiomes, on the other hand, were more conserved. Soil inoculation primarily impacted seed-associated microbiomes, with limited effects on root communities. These findings underscore the complex interplay of tissue type, water availability, cultivar, and inoculation in determining quinoa's microbial composition. Furthermore, based on the results presented here and the extensive genetic diversity of quinoa, it can be inferred that these crop-associated microbial communities offer a promising avenue for selecting and utilizing PGPBs. These microorganisms could be instrumental in improving crop performance and resilience, demonstrating the value of quinoa as a potential source of agricultural innovations. Nonetheless, further research is needed to fully understand and harness this potential, with this work establishing the basis for exploring quinoa's microbial potential as a source of PGPB. Thus, we have defined here the core of endophytic bacteria inhabiting quinoa roots and seeds, which may also be of interest as PGPBs for their applicability in other crops that are less resilient to various stress types than quinoa.

Abbreviations

F15	Water stress-sensitive quinoa genotype
F16	Water stress.tolerant quinoa genotype
WW	Well-Watered conditions
WD	Water deficit conditions
S1 MB	Pots inoculated with microorganisms and nutrients obtained from an agricultural soil
S1 E	Pots inoculated with nutrients obtained from an agricultural soil
S2 MB	Pots inoculated with microorganisms and nutrients obtained from an agricultural soil in which quinoa was cultivated
S2 E	Pots inoculated with nutrients obtained from an agricultural soil in which quinoa was cultivated
PGPB	Plant growth-promoting bacteria
ASV	Amplicon sequence variant
PCA	Principal component analysis
Aldex2	ANOVA-Like Differential Expression tool for high throughput sequencing data

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00673-x.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

Supplementary Material 13

Supplementary Material 14

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Author contributions
IMG: Data curation; formal analysis; investigation; methodology; software; validation; writing– original draft; writing– review and editing. MRN: Data curation; formal analysis; methodology; writing– review and editing. SGB: Formal analysis; investigation; methodology; writing– review and editing. LCR: Methodology; writing– review and editing. JM: Conceptualization; investigation; methodology; writing– review and editing. LB: Writing– review and editing. Maria Reguera: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing– original draft; writing– review and editing.

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Data availability
Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information (NCBI) with the primary accession Bioproject PRJNA1129450BioProject and associated SRA metadata are available at: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1129450According to NCBI, please forward this email to the publisher to share with the reviewer(s) or send them the URL above. It will remain active and reflect all metadata associated with your BioProject until your BioProject is released to the public.Best, Maria Reguera.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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