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Diversity and community structure of rhizo- and phyllosphere bacterial assemblages in tomato cultivars of Santorini Island.

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I hereby declare that the work submitted is mine and that where I have made use of another's work, I have attributed the source(s) according to the Regulations set in the Student's Handbook.

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Abstract

This dissertation was written as part of the MSc in Bioeconomy: Biotechnology and Law at the International Hellenic University.

The aim of this study was to identify the bacterial assemblages, the diversity and the relative abundance and dominance of the taxonomic groups found in the phyllosphere and rhizosphere of *Solanum lycopersicum* plants in two fields. This cultivar was grown in arid volcanic soil of Santorini island of the Mediterranean basin which is characterized by its unique properties. The study was approached with the use of 16S rRNA gene high-throughput amplicon sequencing and a downstream pipeline of bioinformatic analysis. The results showed overall, high bacterial richness with Proteobacteria and Actinobacteria displaying dominance in terms of the number of OTUs. Furthermore, Proteobacteria, Actinobacteria and Cyanobacteria display higher relative abundance in both the plant compartments. Vlichadas' phyllospheric communities generally differed in composition while also displayed the lowest number of OTUs in the dataset. In conclusion, the bacterial assemblages of both sampling sites consisted of common generalist taxa which had a key role in the survivability of the plants, along with the abundant specialist taxa which were driven by the environmental pressure, while composition was overall similar, the abundance of species differed between samples.

Keywords: High-throughput Sequencing; Bioinformatic analysis; Microbiome; Plant Growth Promoting Bacteria

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1. Introduction

The soil is a very complex and diverse ecosystem which is estimated to house approximately 30% of all species on Earth. All three domains of life, Archaea, Bacteria and Eukaryota are hosted in this ecosystem (Bach and Wall, 2018). Soil contains a large diversity of microorganisms such as bacteria, fungi, protozoa, and algae, however the most common group of these are bacteria which comprise approximately 95% of all microbial life in the soil (Glick, 2012). According to the United States Department of Agriculture, soil bacteria are divided into four groups; Decomposers, which can break down harmful elements in the soil and retain nutrients within their cells, mutualists, which form symbionts with plants, pathogens, which can harm plants and crops and finally, lithotrophs or chemoautotrophs which utilize various nitrogen, sulphur, iron or hydrogen compounds for energy intake instead of carbon compounds (Ingram, Soil bacteria- USDA).

Bacterial compositions of soil observed on a global scale, form similar assemblages but different abundances depending on the geographical location and the ecosystem. The most abundant species in soil according to a study were Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Verrucomicrobiota, Bacteroidetes, Gemmatimonadetes and Firmicutes (Delgado-Baquerizo et al., 2018). In the Mediterranean basin, bacterial diversity was distributed among 17 taxonomic groups where the most common were Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Firmicutes and Verrucomicrobiota (Siles et al., 2014). The bacterial composition of the various ecosystems hosted in the Mediterranean basin is very similar. Arable soils, however, have shown different assemblages which are shaped by the plant and the soil properties (Alami et al., 2020). Additionally, there is evidence that bacterial composition of arable soils can be altered in cases where there are constant crop cycles, and the phenomenon is rendered as soil fatigue (Wolińska et al., 2018). In these cases, Proteobacteria remained highly dominant and Bacteroidetes had lower abundance compared to controlled samples of non-cultured soil. Agricultural fields display major distinctions compared to natural occurring ecosystems which results in lower microbial diversity due to the human interference for the plants nutrition and

protection, such as fertilization, agrochemicals, and pH correction (Andreote and Pereira e Silva, 2017).

Plants interact with external bacteria through two major compartments: the phyllosphere and the rhizosphere, whereas bacteria that inhabit the plant are found in the endosphere. The composition of the two external routes of microbial interaction seem to overlap, and the bacterial community structure has no significant shifts between the phyllosphere and the rhizosphere of the same plant according to a study performed on three different plant species (Bao et al., 2020). The phyla of bacteria associated with plants include the groups of: Aquificae, Thermotogae, Thermodesulfobacteria, Deinococcus-Thermus, Chrysiogenetes, Chloroflexi, Thermomicrobia, Nitrospiria, Deferribacteres, Cyanobacteria, Chlorobi, Proteobacteria, Firmicutes, Actinobacteria, Planctomycetes, Chlamydiae, Spirochaetes, Fibrobacteres, Acidobacteria, Bacteroidetes, Fusobacteria, Verrucomicrobia, Dictyoglomi, and Gemmatimonadetes (Beattie, 2006).

The main taxonomic groups found in the phyllo- and rhizosphere of tomato cultivars grown in a greenhouse were Proteobacteria, Actinobacteria, Chloroflexi, Firmicutes, Acidobacteria, Gemmatimonadetes, Bacetroidetes, Nitrospirae, Cloacimonetes, and Spirochaetae (Dong et al., 2019). The distribution of these taxonomic groups, however, vary in abundance from phyllosphere to rhizosphere. Studies conducted on tomato cultivars displayed consistent dominance of the same taxonomic species under greenhouse growth, as in the studies of Lee and Dong, dominance was observed in Proteobacteria followed by Actinobacteria, Bacteroidetes, Acidobacteria and Planctomycetes (Lee et al., 2016; Dong et al., 2019). On the other hand, dominance in the phyllospheric microbiome was attributed solely to Proteobacteria with very low presence other phyla such as Actinobacteria, Bacteroidetes, and Firmicutes (Bulgarelli et al., 2013; Chaudhary et al., 2017; Parasuraman et al., 2019; Dong et al., 2019).

The phyllosphere compared to the rhizosphere is comparatively nutrient poor, however, the microbial communities of phyllospheres are critical in the physiological development, nitrogen fixation, phytopathogen protection, phytohormone and metabolite production. The large and diverse microbial communities of the phyllosphere vary based on their specific ecological niche such as the nutritional

availability, geographic location, water availability, pH conditions. Phyllospheric bacterial assemblages can regulate the interactions between the plant and the atmosphere by controlling attributes such as the gas composition in the air, the effects of climate and other physical and chemical properties of the atmosphere (Bringel and Couée, 2015). These bacteria can also protect the plant from incoming sunlight or by presenting gateways for penetration to the endosphere which enhance the survivability of the plant in stressed environmental ecosystems (Bringel and Couée, 2015).

The microbial process of phyllosphere and rhizosphere inhabitation differ from one another. Rhizosphere microbiota is originated from the soil adjacent to the plant root system, however there is evidence that suggests that the composition, structure and abundance of these communities are influenced by the plant's needs depending on the growth stage, soil properties, plant strain and potential environmental stress (Genitsaris et al., 2020; Schlemper et al., 2017). On the other hand, the phyllospheric microbiota is influenced by different phenomena, such as rainfall, animals, bioaerosols and other potential water and soil sources which end up in the phyllosphere. There is also evidence that bacterial assemblages in the phyllosphere of a single species of plant can share a common core (Genitsaris et al., 2020; Noble et al., 2020). Another factor that configures bacterial assemblages in different plant compartments, including the phyllosphere and the rhizosphere are the interactions of the microbes within their community (Hassani et al., 2018).

The development of -omic tools in recent years has given the opportunity for a broader understanding of the composition, diversity, and abundance of the microbes in environmental samples. Classic culture techniques are not applicable in all the bacteria found in the phyllosphere and the rhizosphere of plants, hence profiling is conducted with metagenomic tools. Additionally, agar-based cultures mediums can grow up to 10% of nutrient rich soil bacteria, with many taxa being excluded for various reasons, such as the slow growth of some species. Nevertheless, culturable soil bacteria techniques was immensely improved with the use of an *in-situ* culture apparatus ("iChip"), however, analyses performed with marker genes (e.g., the 16S rRNA gene) have allowed for the identification of complex plant-microbiome interactions (Gurusinghe et al., 2019). Moreover, even if bacteria are cultured, the

composition is defined through microscopy which can preclude some species that under normal circumstances display low abundance.

The development of High-Throughput Sequencing (HTS) or Next-Generation Sequencing (NGS) in the last years has given the ability to simultaneously, independently, and reliably sequence and analyse billions of DNA, RNA, and protein molecules. Metagenomics have allowed the bypassing of several limitations which microbiologists faced throughout the years such as the definition of composition of different microbiota (Shuikan et al., 2019). These tools have been used to reveal complete phyllosphere and rhizosphere bacterial communities and assist in the understanding of the interactions between microbes and plants. The 16S rRNA gene sequences used with the NGS instruments, has revealed the existence of the complex functions and interactions within plant microbiota (Genitsaris et al., 2020).

Microbes that are beneficial to plant growth (PGPB) can improve significant aspects of a plant's life including nutrient intake, defence response via metabolite secretion and the forming of complex soil matrices. The use of PGPB by plants in natural occurring ecosystems allow the plant to adapt to the environment stresses that may occur. For example, evidence shows that plant growth promoting bacteria alleviate salt stress (Mokrani et al., 2020; Kumar et al., 2020), and can enhance tolerance of crops against drought stress (Vurukonda et al., 2016; Armada et al., 2018). Additionally, PGPBs are capable of improving tolerance in various adverse environmental conditions including weed infestations, nutrient deficiency and heavy metal contamination (Pandey et al., 2019). Some bacteria that ultimately promote plant growth, have the ability in some cases to colonize a portion of plant's interior tissues and others form symbiotic relationships with the plants by colonizing the rhizosphere. PGPB promote the growth of the plants either directly or indirectly. Direct paths of growth promotion include resource acquisition and plant hormone modulation, while indirect promotion includes the inhibition of potential pathogenic agents in the soil where PGPB perform as biocontrol bacteria (Glick, 2011; Glick, 2012). There is also evidence that PGPB can be used as biopesticides against invertebrate pests (Ruiu, 2020). Some examples of the bacteria that exist in these rhizosphere communities and enhance plant growth include the taxa of *Pseudomonas*, *Bacillus*,

Enterobacter, *Streptomyces*, *Klebsiella*, *Agrobacterium*, *Erwinia*, *Azotobacter*, and *Serratia* (Genitsaris et al., 2020).

Tomato cultivars which had inoculated PGPB, showed improvement in nutrient uptake and crop production (Zuluaga et al., 2021), while in a different study, tomato plants benefited in growth and disease management by certain of these bacteria, namely, *Pseudomonas fluorescens*, *Bacillus sp*., *Serratia*, *Micromonospora* and *Azotobacter* (Singh et al., 2017). Numerous studies have been conducted which investigate the genomes of plant associated bacteria and particularly PGPB, of chickpea (*Cicer arietinum L*.) (Khan et al., 2019), coconut, cocoa, and areca nut of plantations (Gupta et al., 2014), and maize (Esmaeel et al., 2018).

Identifying beneficial bacterial strains in stressed, and in this case arid, ecosystems can drive sustainable methods of agriculture due to their evolutionary adaptations which have been developed through the constant environmental stresses (Leontidou et al., 2020). Metaproteomic analysis of these useful microbes and especially in the microbiome of commercial crops can push agricultural sustainability to mainstream and result in the use of less pollutant and chemical fertilizers which are still used in mass (Ramakrishna et al., 2019).

The aim of this research is to identify the taxonomic composition of the phyllosphere and rhizosphere of the native tomato cultivar in Santorini. The tomato cultivars of Santorini Island, *Solanum lycopersicum* 'Santorini', grows without any major human interference and under the conditions created by the volcanic soil in the area. The phyllospheric and rhizospheric microbiomes are analysed in this study, belonged in plants that were not irrigated but watered by rain, and were exposed to high temperatures during the day. NGS and bioinformatic analysis are used to identify any potential specialist bacteria that contribute to the survival and growth of this cultivar in a volcanic environment without human interaction and to compare the taxonomic similarities between the two sampling sites which according to studies on different plants should share a common core microbiota. Moreover, the data generated through the bioinformatic analysis will be used to calculate biodiversity indices which will allow for the definition of which samples show higher diversity, abundance, and equitability.

2. Materials and Methods

2.1 Samples

Samples were collected from the phyllosphere and rhizosphere of *Solanum lycopersicum,* from two different tomato fields in the volcanic island of Santorini, namely Vlichada and Emporio (Figure 1). The samples were collected from eight individual plants of each tomato field where they were grown under drought conditions. These samples were provided for the purpose of this dissertation. (Table 1)

Table 1. Number of samples provided for the dissertation per sampling site and plant compartment.

Sampling Sites	Number of samples per Phyllosphere	Number of samples per Rhizosphere
Vlichada	8	8
Emporio	8	8

Figure 1. Location of sampling sites Vlichada and Emporio in Santorini

2.2 Sample processing and sequencing

Samples collected from the phyllosphere and rhizosphere were transferred into phosphate saline buffer (PBS; NaCl 137 nmol L^{-1}, KH2PO4 1.8 nmol L^{-1}, KCl 2.7 nmol L^{-1} and Na2HPO4 1.42 nmol L^{-1}, pH = 7.4). The bulk soil and other external material were removed by manually shaking the roots and leaves. The buffer solution was sonicated for 10 min with *Transonic 460* and were subsequently centrifuged at 10,000 rpm for 20 min. The pellet containing the bacterial communities was preserved in -20 ^{0}C until DNA extraction (Genitsaris et al., 2020).

DNA extraction was performed with the use of NucleoSpin® Soil DNA Isolation Kit (MACHEREY-NAGEL, Bethlehem, PA, USA). The samples were subjected to cellular lysis and centrifuged, where the supernatant of each was transferred to a different tube, respectively. Contaminants were precipitated and the lysate was filtered from the samples. DNA was bound with the kit and eluted to be checked for concentration and quality by a NanoDrop™ spectrophotometer (ThermoScientific™, Waltham, MA, USA).

Extracted DNA from the samples was subjected to PCR using primers targeting specifically the V6-V8 hyper-variable region of the 16S rRNA gene (B969F = ACGCGHNRAACCTTACC and BA1406R= ACGGGCRGTGWGTRCAA) (Comeau et al., 2011). The primers have been proven to amplify successfully approximately 470bp of all major high-level bacterial taxonomic groups with coverage of up to 83% when analyzed in silico via the SILVA TestPrime 1.0, performed at the Integrated Microbiome Resource (IMR) of Dalhousie University in Canada. A high-throughput Hamilton Nimbus Select robot was used to verify visually the PCR products which were subsequently normalized with the high-throughput Charm Biotech Just-a-Plate 96-23ll Normalization Kit (Charm Biotech, Lawrence, MA, USA). The amplicon samples were sequenced with the Illumina MiSeq (Illumina, San Diego, CA, USA) using 300+300 bp pair-end chemistry which allowed for overlap and stitching together of paired amplicon reads into single full-length reads (https://imr.bio/protocols.html) The PCR amplification and sequencing steps were conducted at the Integrated Microbiome Resource (IMR) of Dalhousie University (Halifax, NS, Canada).

2.3 Read Processing

In order to denoise the produced raw sequences from the Next-Generation Sequencer, the data retrieved was subjected to a series of downstream processing steps in the *mothur v.1.44.1* software. The program was developed by Dr. Patrick Schloss, of the University of Michigan. The files obtained and introduced to the *mothur* software were FASTQ files and the standard operating procedure (SOP) (Kozich et al., 2013, Schloss et al. 2011) was followed through to transform the raw sequences in the FASTQ files into refined operational taxonomic units (OTUs), corresponding to bacterial phylotypes.

The first step of the downstream bioinformatic analysis consists of the *make.file* command which allows the software to create *stability.files* that include the sample names and the forward and reverse reads of each sample in a table form. Then, forward and reverse reads were combined with the *make.contigs* command. This command aligns the pairs of sequences from the FASTQ files of every sample based on the quality of the sequences, gaps in any sequences and extra bases in both sequences.

Once the sequences were paired and combined to contigs, the *screen.seqs* command removed any sequences which had ambiguous bases with read length < 200 bp. To reduce the size of the data file, identical multiplicate sequences were merged with the *unique.seqs* command. This created a dataset of 2,745,798 unique sequences. Following the merge of the multiplicates, the *count.seqs* generated a table which identified the name of each unique sequence, the amount of repeats that were merged into each group and the names of the groups where the sequences were derived from.

The *pcr.seqs* command was used to modify the dataset for the subsequent read aligning. The primers used began at the position 31145 and ended at 42546 of the 16S rRNA gene as is in the Bacterial SILVA 16S rRNA database (https://mothur.org/wiki/silva_reference_files/), and therefore the dataset was customised to these coordinates. Using the *align.seqs* command, the sequences were aligned with the SILVA database and the quality of the sequences. After the alignment, the reads required to be filtered with the *filter.seqs* command which removed any gap characters from within the sequences without the loss of any information. In order to remove any possible multiplicate sequences, the *unique.seqs* command was ran again.

Following the alignment and filtering of the dataset, the sequences that were nearly identical were combined with the *pre.cluster* command. Since the sequences' size should be about 450 bp according to the primers used, the guidelines allow for sequences to be merged with each other when the sequences are within 4 nucleotides distance from each other. (1 nt /100 bp).

At this point, with the use of the *VSEARCH* tool, any potential chimeric reads were identified through the *chimera.vsearch* command (Rognes et al., 2016). A total

of 21,971 chimeric sequences were subsequently removed with the *remove.seqs* command from the fasta file leaving 2,661,506 total sequences.

In order to reduce data files size, and remove any remaining reads suspected of being erroneous sequences, the singletons were eliminated from the dataset by using the *split.abund* command, which splits the sequences in a rare inclusive fasta file containing all singletons, and the abundant fasta file. In order for the dataset to be translated into OTUs, first, the *dist.seqs* command is needed to identify any unrectified pairwise distances between the paired DNA sequences. With the *cluster* command the sequences were clustered into OTUs. To calculate the number of sequences in each OTU and the number of OTUs per sample, the *make.shared* command was used.

A fasta-formatted sequence file was created with the *get.oturep* command which then was normalized with the *sub.sample* command. The initial fasta-formatted file left the dataset with 10,321 OTUs and the normalized files limited the number of sequences to samples with more than 6,000 reads. The pool initiated with 32 samples (16 Emporio – 16 Vlichada) and after the normalization, 6 (5 Vlichada- 1 Emporio) samples were excluded due to their low sequencing depth, as they had less than 6,000 reads. Minimal subsampling leads to coverage heterogeneity, while austere subsampling reduces the proportion of initial sequences and may result to diversity loss (Cárcer et al., 2011). Therefore, the selected subsampling value of 6,000 reads per sample was the best compromise as a relatively homogenous spread of OTUs and satisfactory depth of coverage was achieved along the dataset. A list of the downstream analysis commands is displayed in Table 2.

The taxonomic classification was determined with the use of BLASTN searches against SILVA 132. The lowest acceptable level of similarity to the closest relative was set to 80% (Quast et al., 2013).

2.4 Data analysis

The taxonomic groups considered to have high OTU richness in this study were the groups that had >200 OTUs in the complete dataset. The taxonomic groups which were calculated to have <20 OTUs in the complete dataset, were considered to have low OTU richness. Abundant taxonomic groups were taxonomic groups that comprised at least 2% of the total reads in both the phyllosphere and rhizosphere of both sample sites.

The reads of all samples were analyzed with the use of the PAST v4.03 software in order to calculate alpha-biodiversity indices. For this study, the diversity index of Simpson 1-D was calculated (Simpson, 1949). This index values from 0 to 1 with the lower value indicating lower diversity and the value 1 indicating that every species in the sample is found in the same number of occurrences. Simpson index gravitates more on the evenness of species in the community and the normalization of data is necessary before the calculation of the index to avoid biased results (Lemos et al., 2011; Kim et al., 2017). The species abundance distribution index of Berger-Parker was also calculated which indicates the number of reads of the dominant OTU compared to the total number of reads in each sample where the value 0 indicates high diversity and the value 1 low diversity (Caruso et al., 2008). Additionally, the richness of rare species per sample was calculated with the Chao-1 index (Chao and Lee, 1992). This index estimates the number of expected species by using the number of singletons and doubletons as Chao developed the formula on the concept that rare species extrapolate the most information about the number of missing species (Christaki et al., 2014; Kim et al., 2017). The Equitability J index was used to calculate the evenness of abundance across the communities as it refers to how similar the number of reads of each species is found in a sample. The values are constrained between 0 and 1 with higher evenness in assemblages when the value is closer to 1 and vice versa (Pielou, 1966).

Lastly, the Jaccard similarity coefficient was calculated in the PRIMER v6 software in order to compare the compositional similarity and beta-diversity of the samples. (https://primer.software.informer.com/6.0/)

Table 2. List of commands used in the *mothur v.1.44.1* software and the subsequent use of each command.

Command	Description
make.file	Creates a file which includes sample names with their forward and reverse read
make.contigs	Alligns the forward and reverse read of every sequence creating to paired sequences
screen.seqs	Filters the paired sequences that do not abide to the base pair length of the given primer
unique.seqs	Merges any multiplicate sequences
count.seqs	Generates a table where unique sequences are listed along with the number of repeats and the names of every group
pcr.seqs	Customizes the selected database (SILVA) to the starting and ending point of the primers in use.
align.seqs	Aligns sequences from the sample pool with the reference database sequences
filter.seqs	Removes any overhung bases at both ends of the pair sequences
unique.seqs	Merges any multiplicate paired sequences
pre.cluster	Combines nearly identical sequences with up to 4 mismatches (1 nt mismatch per 100bp)
chimera.vsearch	Identifies chimeric sequences
remove.seqs	Removes chimeric sequences from the fasta file
split.abund	Splits the fasta file into rare and abundant sequences
dist.seqs	Identifies uncorrected pairwise sequences
cluster	Clusters sequences into OTUs
make.shared	Calculates the number of OTU repeats per group
get.oturep	Creates a fasta-formatted file
sub.sample	Normalizes the data by sampling from the total sequences. (Minimum number of reads selected: 6000)

3. Results

3.1. Bacterial Communities and Composition

According to the bioinformatic analysis pipeline, rhizospheric samples proved to be sequenced with greater success than phyllospheric samples, as during the data normalization, only one rhizospheric sample was excluded whereas five phyllospheric samples were excluded. Specifically, the pipeline depicts a higher sequence success rate in the Emporio samples in comparison to the Vlichada samples as depicted in Figure 2. In detail, samples: VLF2, VLF3, VLF6, VLF7, VLR5, and EmF1 produced 5,672, 3,490, 4,270, 2,175, 4,680 and 4,051 reads, respectively.

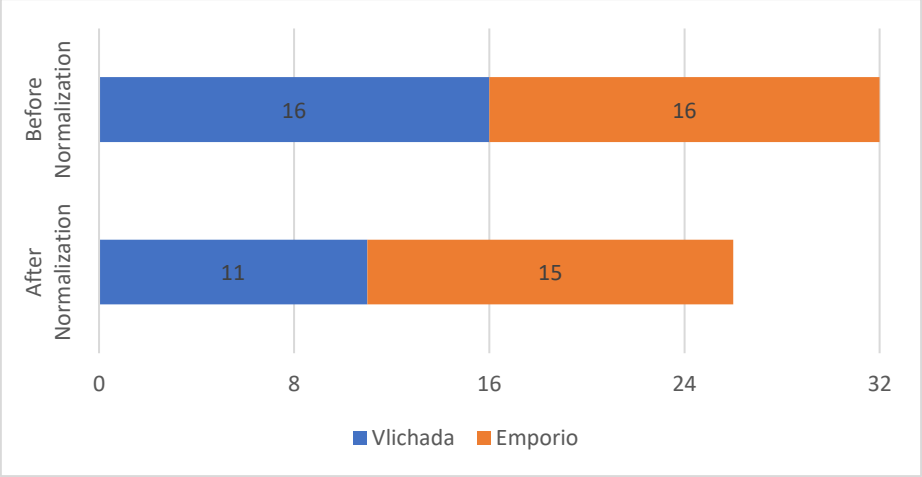

Figure 2. The number of samples before and after data normalization through the sequencing pipeline.

A total of 3,703 OTUs were identified in both the rhizosphere and phyllosphere of the 26 samples sequenced. 2,400 OTUs were recovered from phyllospheric samples in both sites and 2,760 OTUs from subsequent rhizospheric samples. (Figure 3)

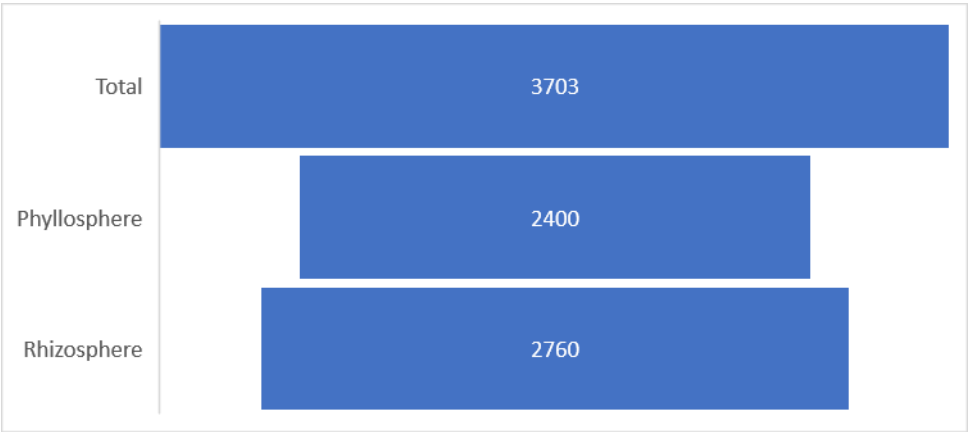

Figure 3. Distribution of OTUs in phyllospheric and rhizospheric samples of both sites combined.

Overall, 85% of the total identified OTUs recovered from all samples were affiliated to 8 taxonomic groups and 15% was affiliated to 27 less occurring taxonomic groups. (Figure 4) High OTU richness was displayed in the taxonomic groups of Proteobacteria, Actinobacteriota, Bacteroidota, Acidobacteriota, Myxococcota and Firmicutes. 1,581 OTUs with a total of 39,706 reads were not identified by the BLASTN searches against the SILVA 132 database and therefore were excluded from the final dataset. The OTUs in the Vlichada samples amounted to 1,963, whereas in the Emporio samples, 2,902 OTUs were identified through the BLASTN searches against the SILVA 132 database. Of the 1,963 OTUs sequenced in Vlichada samples, 423 of them were found in exclusively phyllospheric samples, 1,199 were exclusive to the rhizospheric samples and 341 OTUs were found both in the phyllospheric and rhizospheric samples. Of the 2,902 OTUs sequenced in Emporio samples, 707 OTUs were exclusive to the phyllospheric samples, 976 OTUs were exclusive to the rhizospheric samples and 1,269 OTUs were found both in phyllospheric and rhizospheric samples. (Figure 5)

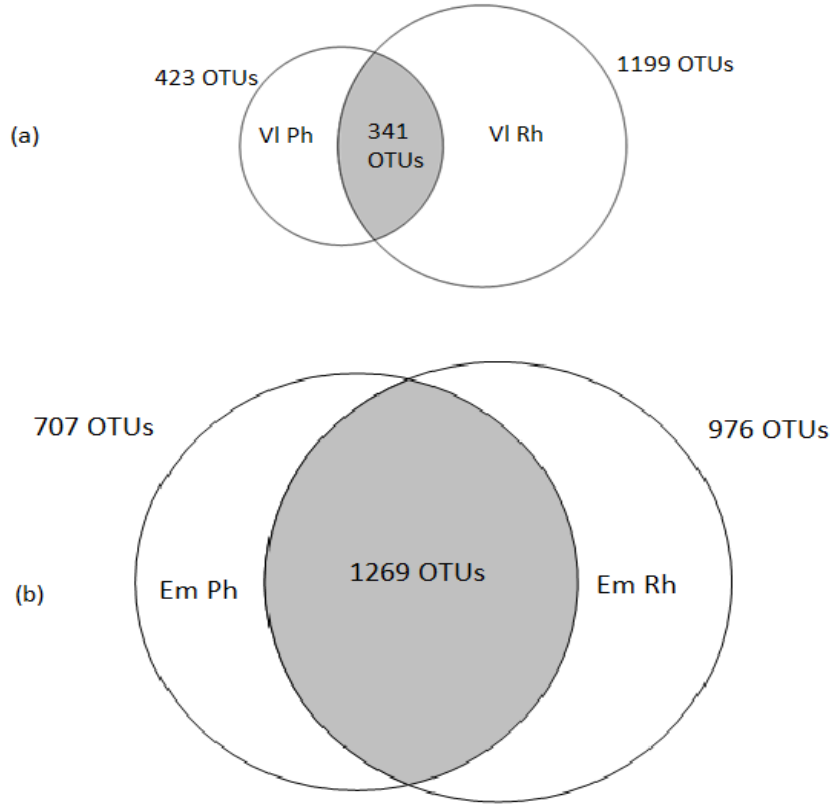

Figure 5. (a) OTU distribution between phyllosphere and rhizosphere samples of Vlichada. (b) OTU distribution between phyllosphere and rhizosphere samples of Emporio

The downstream analysis showed that the most common reads in Vlichadas' phyllosphere samples were OTU_04 and OTU_14 which correspond to the Genus of *Pseudomonas* and *Staphylococcus* correspondingly. In Vlichadas' rhizosphere samples, the most common reads were OTU_05 and OTU_08 which correspond to the Genus of *Bacillus*. In Emporios' phyllosphere samples, the most abundant reads were OTU_07 and OTU_06 which correspond to the Genus of *Microvirga* and *Bacillus*, respectively. In Emporios' rhizosphere samples, the most common reads were, OTU_03 and OTU_02 which correspond to the Genus of *Lechevaliera*, and *Sphingobium* respectively.

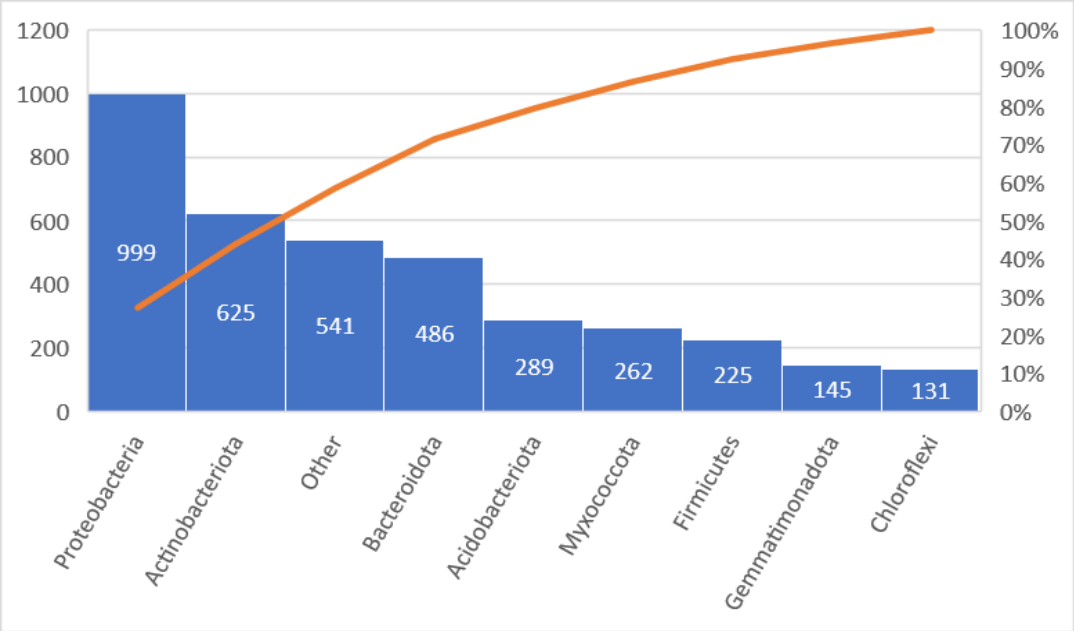

Figure 4. Distribution of the 3,703 OTUs and their bacterial taxonomic correspondence.

The sample with the highest OTU richness was a rhizospheric sample of Emporio with 994 OTUs (EmR8), and the one with the lowest was a phyllospheric sample of Vlichadas' site with 79 OTUs (VLF5). The average number of OTUs in Vlichadas' phyllosphere samples was 228 OTUs and 487 OTUs in the rhizosphere samples. The average number of OTUs in Emporios' phyllosphere samples was 619 OTUs and 713 OTUs in the rhizosphere. The majority of Vlichadas' samples exhibited high bacterial diversity (>300 OTUs), with the only exception of samples VLF4, VLF5 and VLF8, whereas all samples of Emporio, exhibited high bacterial diversity. Notably, rhizospheric samples displayed higher OTU richness compared to the adjacent

phyllospheric samples in both sites. Some taxonomic groups had no OTU representation in the samples of Vlichada, while different taxonomic groups were not represented in the samples of Emporio. VLF1 exhibits a different taxonomic composition compared to the rest of the samples as higher OTU richness in that sample can be observed in the taxonomic groups of Cyanobacteria and Verrucomicrobiota, compared to the other phyllospheric samples of Vlichada, while it also had the highest number of OTUs in that sample team (391). (Figure 6)

In general, Emporio had higher OTU richness than Vlichada, in both phyllospheric samples as, phyllospheric OTU richness in Vlichada averaged at 230 OTUs, whereas in Emporio 621,71, and similarly in Vlichadas' rhizospheric samples, OTU richness averaged at 489,43, whereas in Emporio 715,13 OTUs were found on average. (Figure 7)

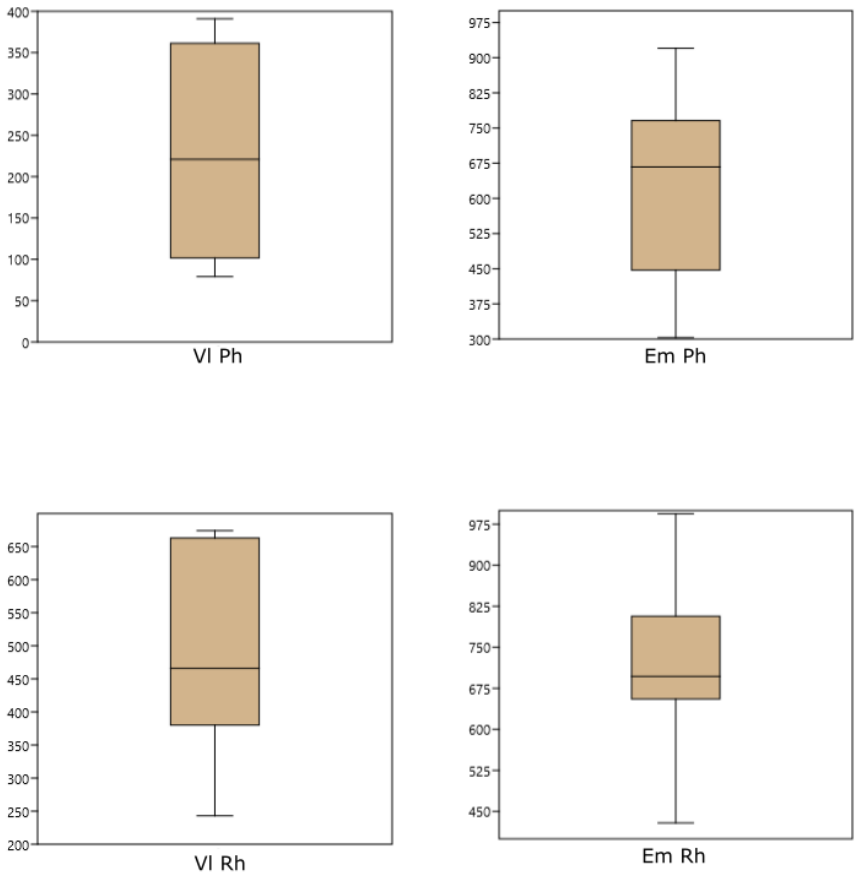

Figure 6. OTU richness of both sampling sites and plant compartments depicted on boxplots with the lowest, highest, and median values.

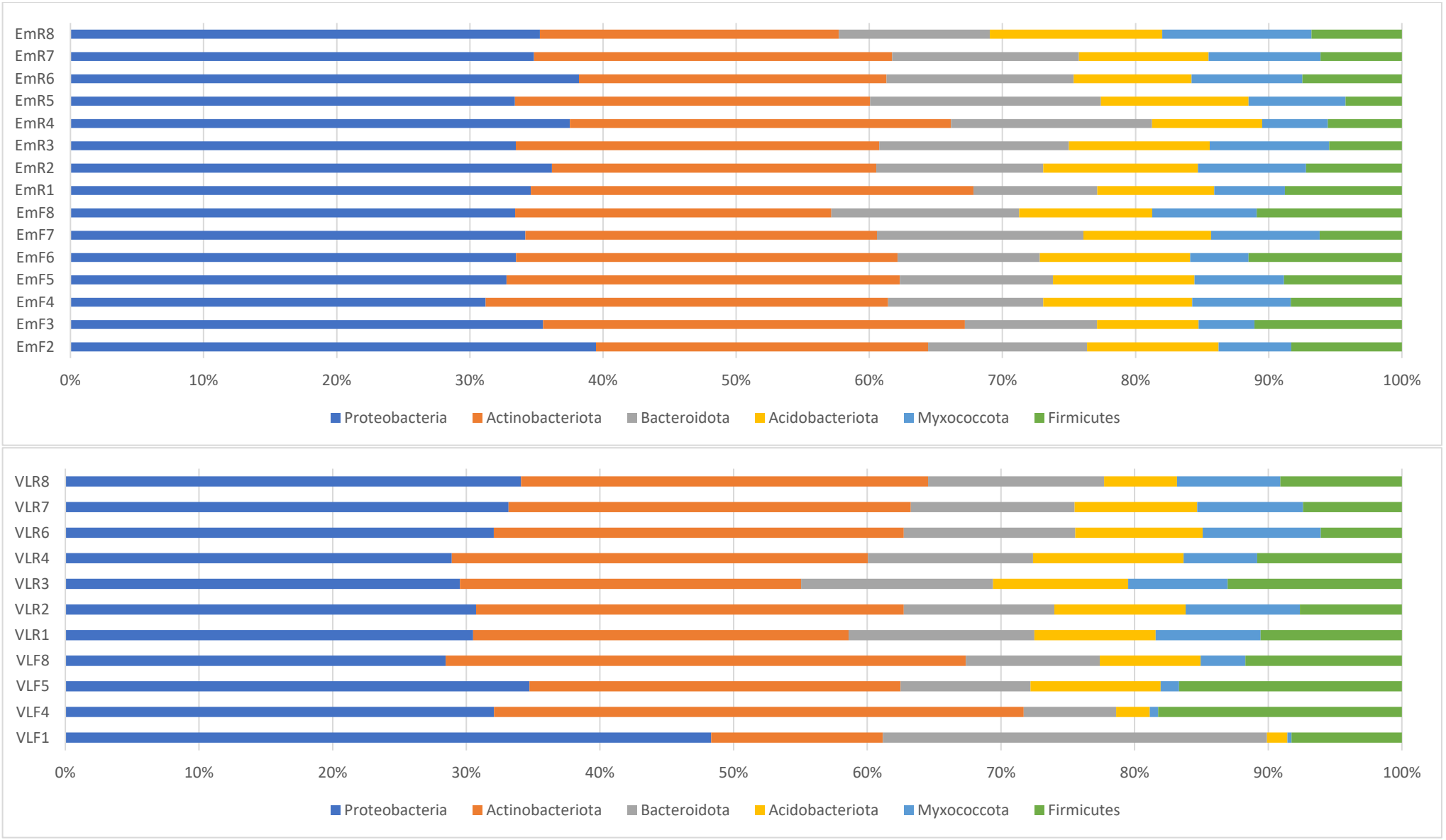

Figure 6a. Top: Taxonomic groups with high OTU richness in samples taken from Emporio, Bottom: Taxonomic groups with high* OTU richness in samples taken from Vlichada
*-Except VLF1 where a unique assemblage was observed and VLF5 which displayed the lowest OTU richness

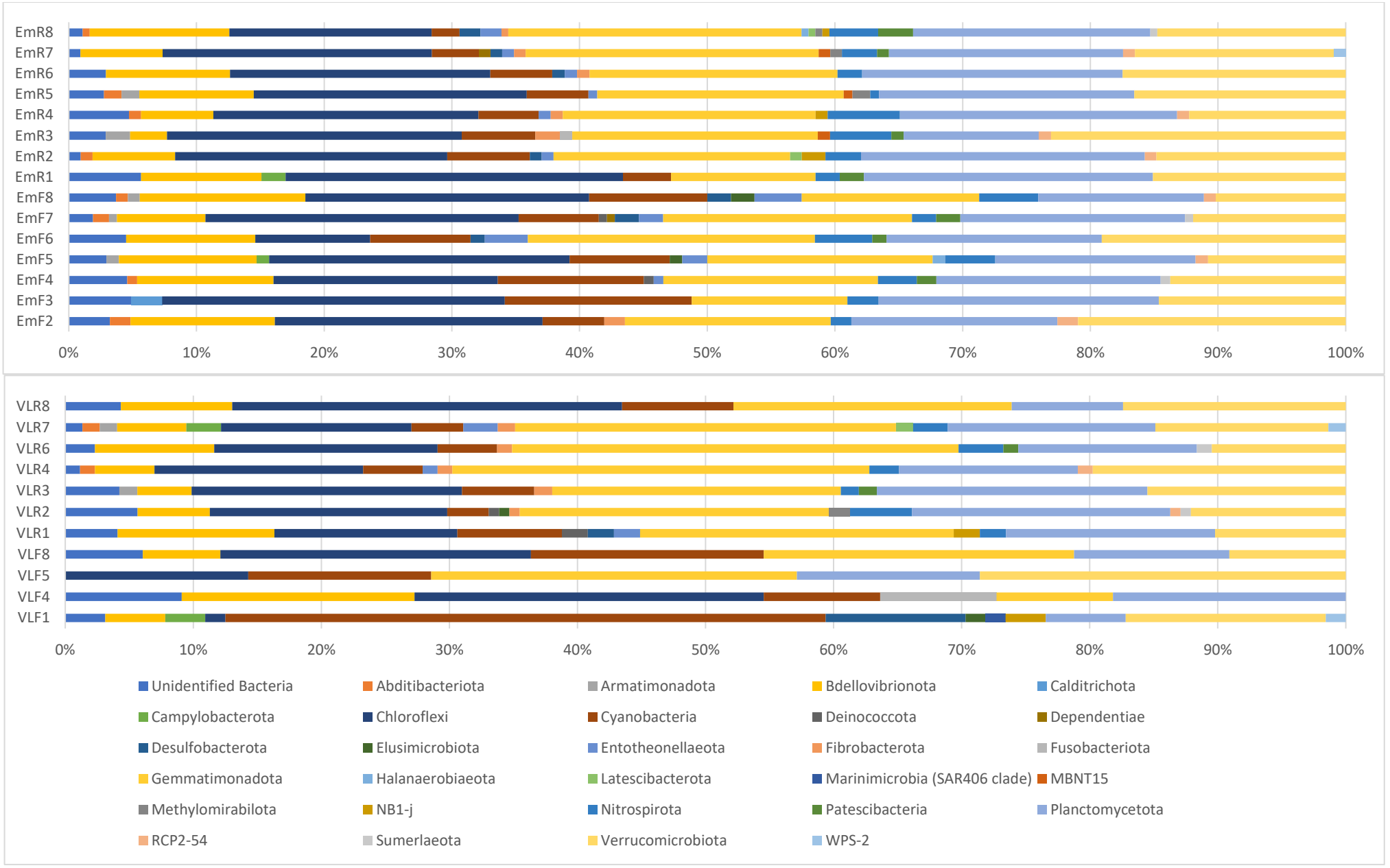

Figure 6b. Top: Taxonomic groups with low OTU richness in samples of Emporio. Bottom: Taxonomic groups with low* OTU richness in samples of Vlichada
*-Except VLF1 where a unique assemblage was detected

3.2 Relative Abundance

The final dataset included a total of 136,147 OTU reads where 77,716 (57%) were found in the rhizospheric samples, and 58,431 (43%) were found in phyllospheric samples. (Figure 8)

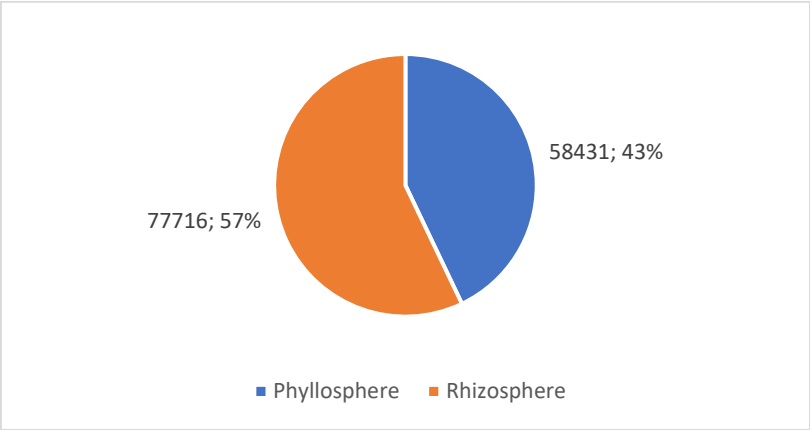

Figure 8. OTU richness of total phyllospheric and rhizospheric samples of both sites combined.

Overall, the abundant taxonomic groups after analysis of the dataset were Proteobacteria, Actinobacteriota, Cyanobacteria, Firmicutes, Bacteroidota and Acidobacteriota. It should be noted that Myxococcota had almost the same number of reads with Acidobacteriota in the rhizospheric sample set, however, the number of reads in the phyllospheric sample set comprised less than 2%, thus were not considered abundant. Additionally, these six abundant groups comprised 95% of the phyllospheric reads and 93% of the rhizospheric reads. The remaining 5% phyllospheric reads were distributed to 26 different less abundant taxonomic groups, some of which generated single reads along the whole phyllosphere dataset. Similarly, with the remaining 7% rhizospheric reads were distributed in 27 different less abundant taxonomic groups, where like the phyllosphere samples, some groups generated single reads. In both phyllospheric and rhizospheric samples, Proteobacteria were the dominant taxonomic group as there were 18,818 OTU reads in phyllospheric samples and 21,347 OTU reads in rhizospheric samples. This is a result of the rarefaction, as the subsampling excluded more phyllosphere samples than rhizosphere samples from the final dataset resulting in the existence of more reads in that group. (Table 4, Figure 9)

Table 4. Number of OTU reads in all normalized phyllosphere and rhizosphere samples respectively

Phyllosphere	OTU Reads / %	Rhizosphere	OTU Reads / %
Proteobacteria	18818 / 32,21%	Proteobacteria	21347 / 27,47%
Cyanobacteria	14410 / 24,66%	Actinobacteriota	16546 / 21,29%
Actinobacteriota	10744 / 18,39%	Cyanobacteria	16122 / 20,74%
Firmicutes	6638 / 11,36%	Firmicutes	10037 / 12,91%
Bacteroidota	3667 / 6,28%	Bacteroidota	6037 / 7,77%
Acidobacteriota	1169 / 2,00%	Acidobacteriota	2232 / 2,87%
Myxococcota	866 / 1,48%	Myxococcota	2231 / 2,87%
Chloroflexi	424 / 0,73%	Gemmatimonadota	976 / 1,26%
Other	1695 / 2,90%	Other	2188 / 2,82%
Total Reads	58431 / 100%	Total Reads	77716 / 100%

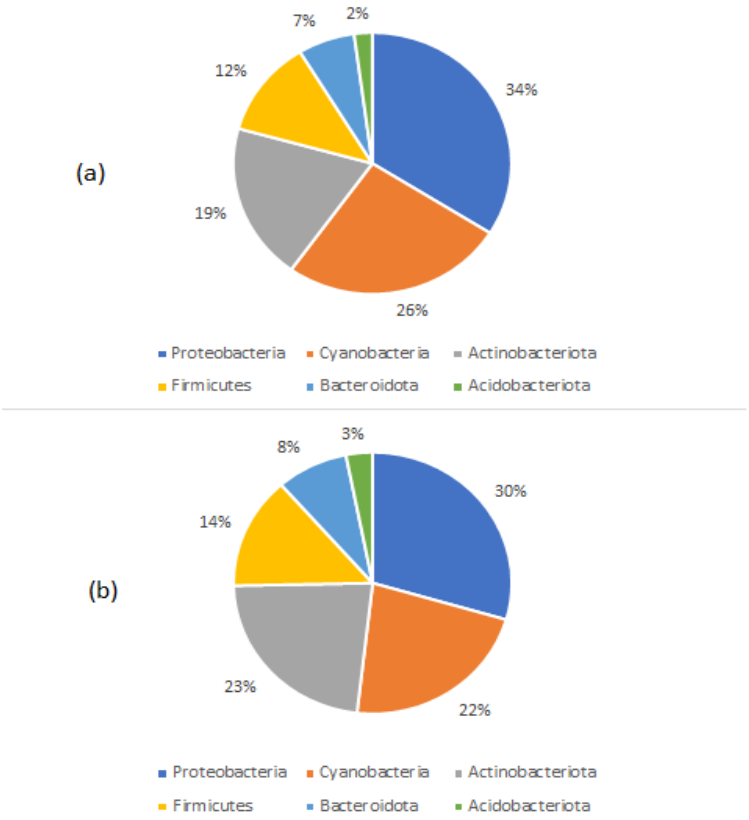

Figure 9. (a) Distribution of the 6 abundant taxonomic groups in all normalized phyllosphere samples. (b) Distribution of the 6 abundant taxonomic groups in all normalized rhizosphere samples.

In Vlichada, Cyanobacteria and Firmicutes had higher relative abundance compared to Emporio and contrary, in Emporio, Actinobacteria and Proteobacteria were relatively more abundant than in Vlichada. In both rhizospheric areas, Myxococcota are relatively abundant with 2,231 reads while this taxonomic groups did not display the same abundance in phyllospheric samples.

The most prominent dominance was detected in VLF5, where proteobacteria approximately accounted for 95% of all the reads in the sample. *Myxococcota*, *Gemmatimonadota*, *Verrucomicrobiota* and *Chloroflexi* comprised a larger number of reads compared to the other less prominent taxonomic groups. In most phyllosphere samples with the exception of EmF3, *Bdellovibrionota* are detected on a higher rate compared to the corresponding rhizosphere samples of the plants. VLF1 displays relative abundance in *Desulfobacterota* which cannot be observed in any other sample, as there are 109 reads in VLF1 (1,88% of total reads in this sample) and the average occurrence of the closest relative to *Desulfobacteriota* across the rest of the dataset is 0,6%. These bacteria have been found to be abundant in ecosystems which are contaminated with heavy metals (Di Cesare et al., 2020). VLF8 also displayed a different assemblage with Cyanobacteria dominating in this sample and with Actinobacteria and Proteobacteria having a relatively lower abundance. The comprehensive percentage rate of every taxonomic group generated by the downstream analysis per sample are presented in figures 10 and 11.

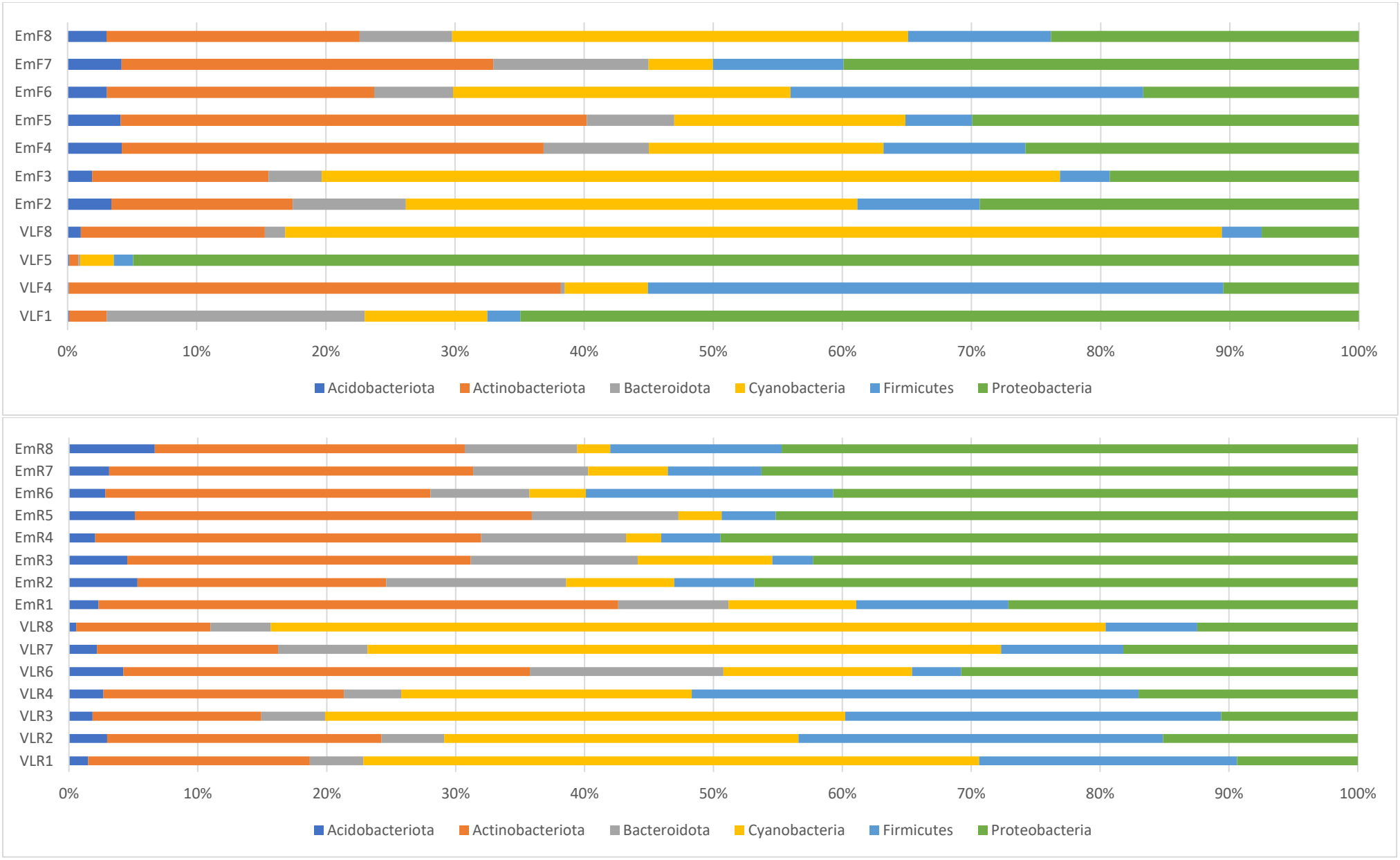

Figure 10. Top: Distribution of the six relative abundant taxonomic groups in phyllosphere samples, Bottom: Distribution of the six relative abundant taxonomic groups in rhizosphere samples. (95% of total phyllosphere reads, 93% of total rhizosphere reads)

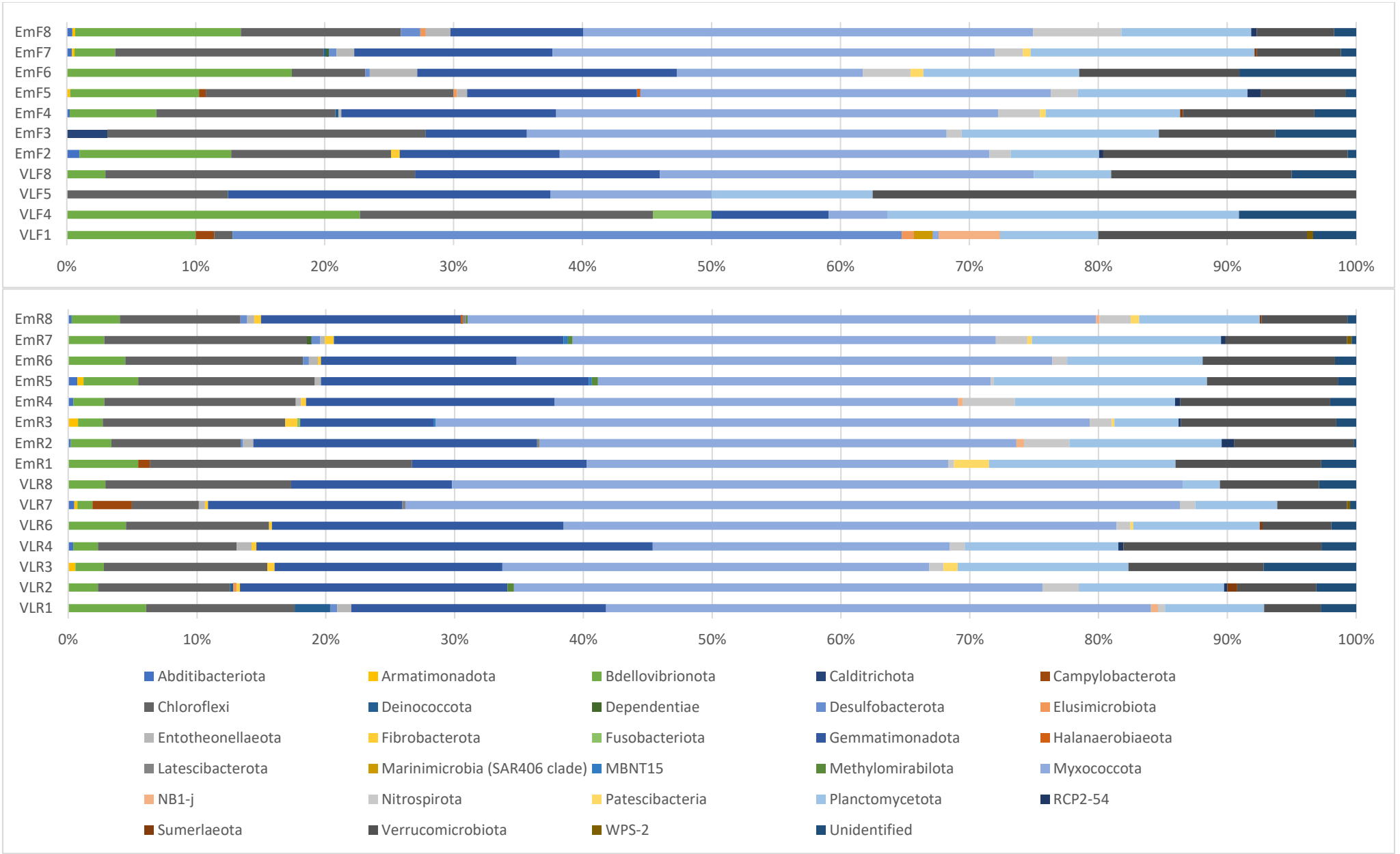

Figure 11. Top: Distribution of taxonomic groups with low relative abundance in phyllosphere samples, Bottom: Distribution of taxonomic groups with low relative abundance in rhizosphere samples. (5% of total phyllosphere reads, 7% of total rhizosphere reads)

3.3. Biodiversity Indices

The Simpson 1-D index indicated the dominance of only a few OTUs in VLF5 as it was the lowest in all the samples (VLF5 1-D= 0,53). The rest phyllosphere samples of Vlichada showed moderate diversity according to the Simpson index. The rhizosphere samples of Vlichada, displayed consistent and relatively high diversity with the exclusion of VLR8 which was the lowest of Vlichadas' rhizosphere samples (VLR8 1-D= 0,67).

In comparison to Vlichada samples, both phyllosphere and rhizosphere samples of Emporio, were more diverse according to the index, with the lowest diversity observed in EmF3, however, higher than most phyllosphere samples of Vlichada. (EmF3 1-D= 0,71) The highest 1-D index was calculated for the sample EmR8 even though most 1-D values of Emporios' rhizospheric samples indicate high taxonomic diversity. (Emr8=0,8) (Figure 12)

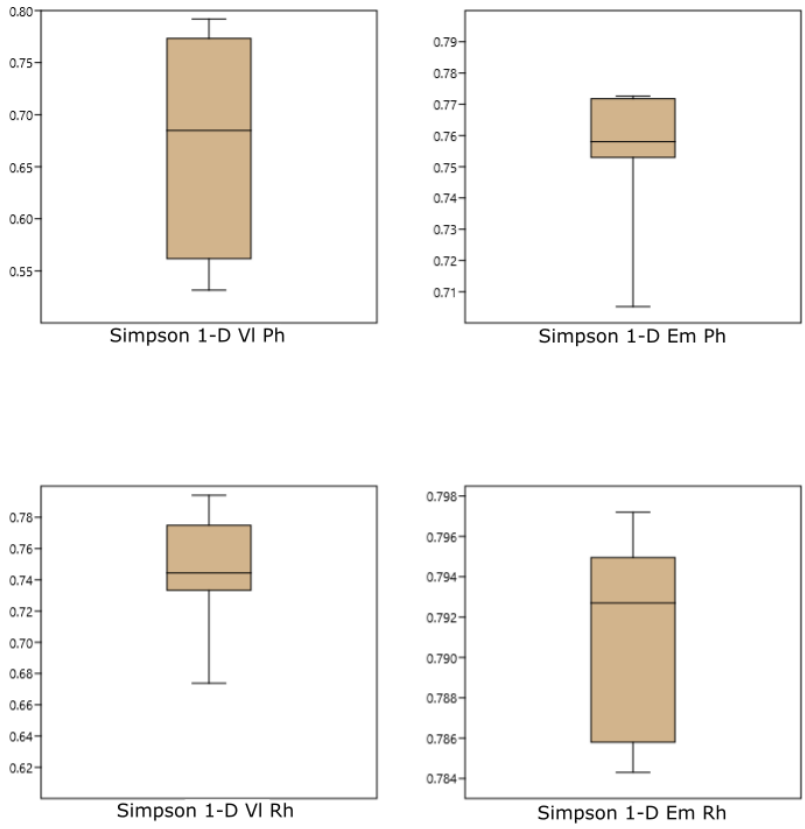

Figure 12. Box plots of the Simpson 1-D index generated with the PAST software with calculated standard error of the mean error rates.

The Berger-Parker index indicated that the sample with the lowest Species Abundance Distribution (SAD) was VFL1 with a value of 0,4. The SAD of the rest phyllospheric samples in Vlichada range in a small spectrum depicting a similar distribution of species. The Berger-Parker index of the rhizospheric samples in Vlichada were similar to each other with the exception of VLR6 which was calculated to be the lowest in this sample pool. (Figure 13).

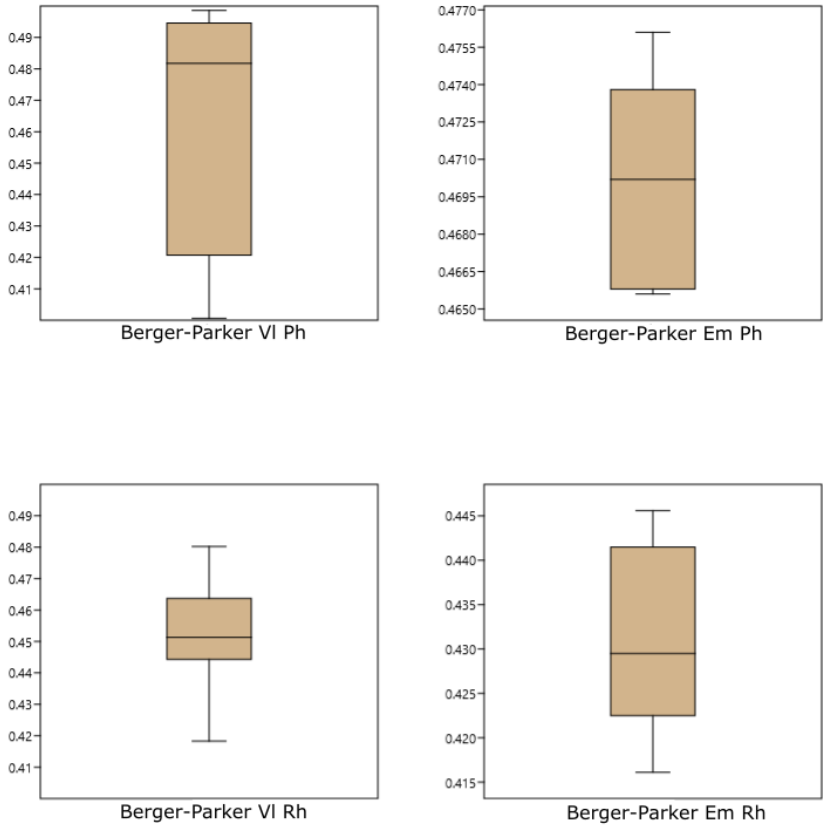

Figure 13. Box plots of the Berger- Parker (SAD) index generated by the PAST software with calculated standard error of the mean rates.

The Chao-1 index calculations for Vlichadas' phyllospheric samples displays the lowest value of the complete dataset in sample VLF5 which had the lowest number of taxa. The estimated number of OTUs provided by the Chao-1 calculation in the PAST software are higher than the actual number of OTUs calculated in the samples. This is a result of the high number of rare reads found in the dataset.

Similar to Vlichadas' Chao-1 index calculations, Emporios' indices depict a higher estimation of OTUs compared to the actual OTU richness of every sample, due to the high number of rare reads in the dataset. Additionally, the highest value was displayed in EmF7 with an estimate of 1735 OTUs, however, there were 922 total OTUs in the dataset. It should be noted that EmF7 did not have the highest number of OTUs, even though it had the highest Chao-1 value due to its rare reads. (Figure 14 & 15)

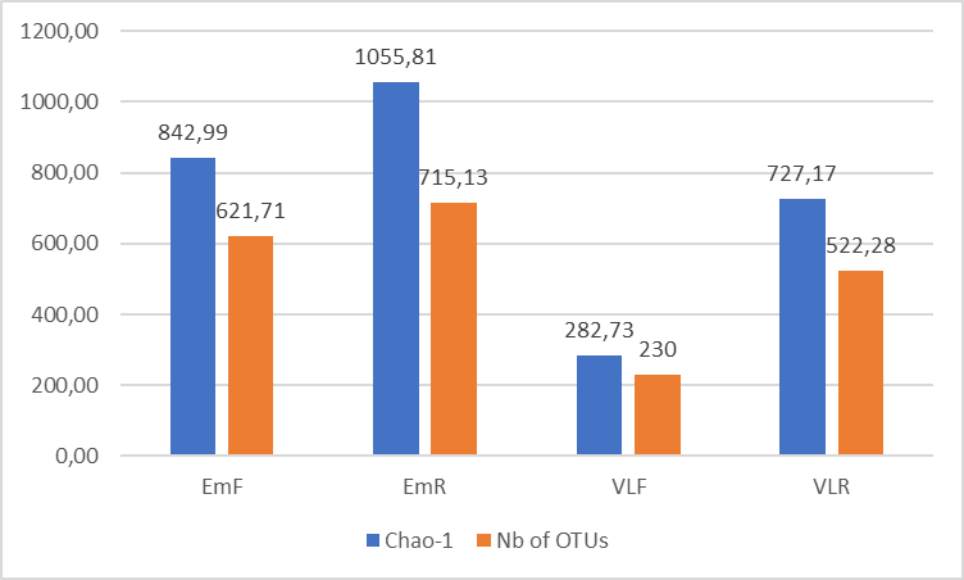

Figure 14. Comparison of average expected OTU richness per sampling site and plant compartment calculated with the Chao-1 index and the corresponding average detected OTUs per site and plant compartment.

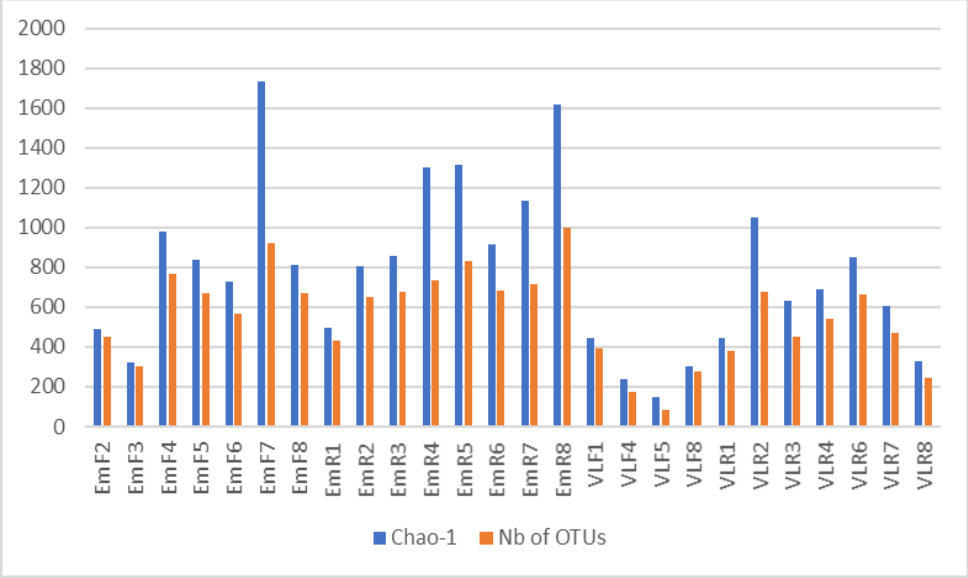

Figure 15. Comparison of the estimated value of OTUs calculated with the Chao-1 index and the corresponding detected number of OTUs per sample

The equitability index (J) in Vlichada displayed low abundance distribution in VLF5 (VLF5 J=0,21), and relatively low distribution among the rest phyllospheric samples of this site. The abundance distribution of the rhizospheric samples is low similar to the phyllosphere, however the index values indicate a slightly higher equitability of species.

In Emporio, the equitability of species as higher than Vlichada, however, abundance was limited to few OTUs as the values range from 0,4 to 0,5 along the whole Emporio sample spectrum. (Figure 16)

The biodiversity indices and each value are depicted in Table 5.

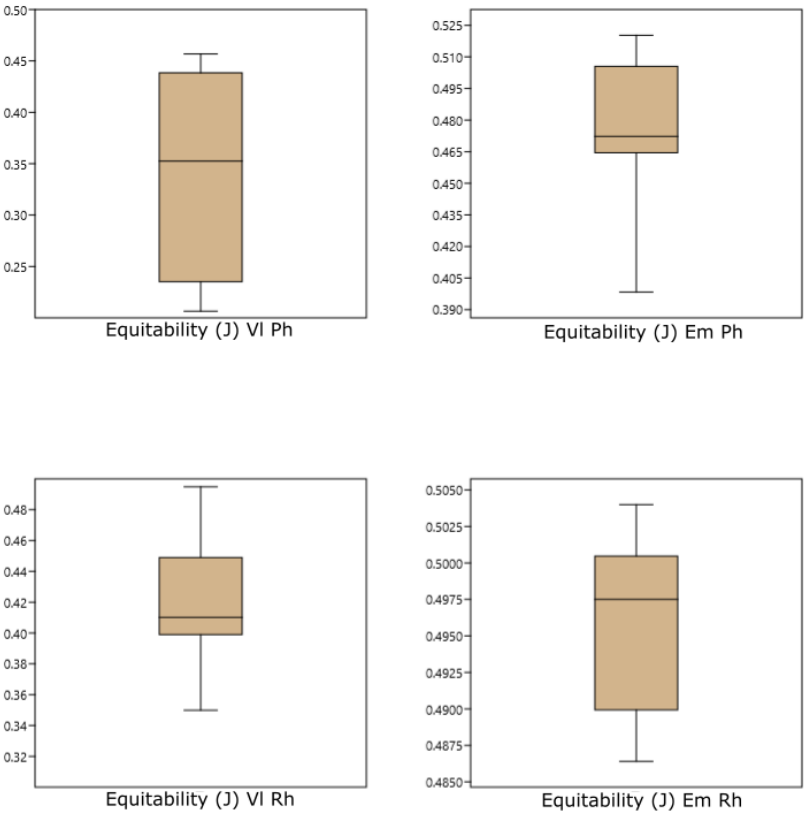

Figure 16. Box plots of the equitability (J) index generated by the PAST software with calculated standard error of the mean error rates.

Table 5. Biodiversity indices of each sample calculated in the PAST software

Sample ID	*Simpson (1-D)*	*Berger-Parker*	*Equitability J (H/H_{max})*	*Chao-1*	*Nb of OTUs*	*Observed/ Expected OTUs*
EmF2	0,75	0,47	0,47	489,5	449	0,92
EmF3	0,71	0,48	0,40	320,4	305	0,95
EmF4	0,77	0,47	0,51	978,5	768	0,78
EmF5	0,77	0,47	0,50	836,6	669	0,80
EmF6	0,76	0,47	0,47	728,1	569	0,78
EmF7	0,77	0,47	0,52	1735	922	0,53
EmF8	0,75	0,47	0,46	812,8	670	0,82
EmR1	0,78	0,44	0,50	497,6	431	0,87
EmR2	0,80	0,42	0,50	805,8	652	0,81
EmR3	0,79	0,43	0,50	854,4	674	0,79
EmR4	0,79	0,44	0,49	1303	737	0,57
EmR5	0,79	0,43	0,49	1316	833	0,63
EmR6	0,79	0,43	0,50	916,7	681	0,74
EmR7	0,79	0,45	0,49	1133	717	0,63
EmR8	0,80	0,42	0,50	1620	996	0,61
VLF1	0,79	0,40	0,46	442,2	393	0,89
VLF4	0,72	0,48	0,38	241,5	172	0,71
VLF5	0,53	0,50	0,21	145,7	81	0,56
VLF8	0,65	0,48	0,32	301,5	274	0,91
VLR1	0,73	0,46	0,40	443,3	382	0,86
VLR2	0,77	0,44	0,44	1050	676	0,64
VLR3	0,74	0,46	0,41	630	449	0,71
VLR4	0,77	0,45	0,45	687,7	541	0,79
VLR6	0,79	0,42	0,49	847,7	665	0,78
VLR7	0,74	0,45	0,41	608	468	0,77
VLR8	0,67	0,48	0,35	326	245	0,75

The Jaccard similarity coefficient depicted on the non-metric multi-dimensional scaling plot (nMDS), that the bacterial communities' structure of the samples of Emporios' phyllosphere and rhizosphere, and Vlichadas' rhizosphere are similar. On the contrary, the phyllospheric samples of Vlichada, were apparently dissimilar to the rest dataset. (Figure 17)

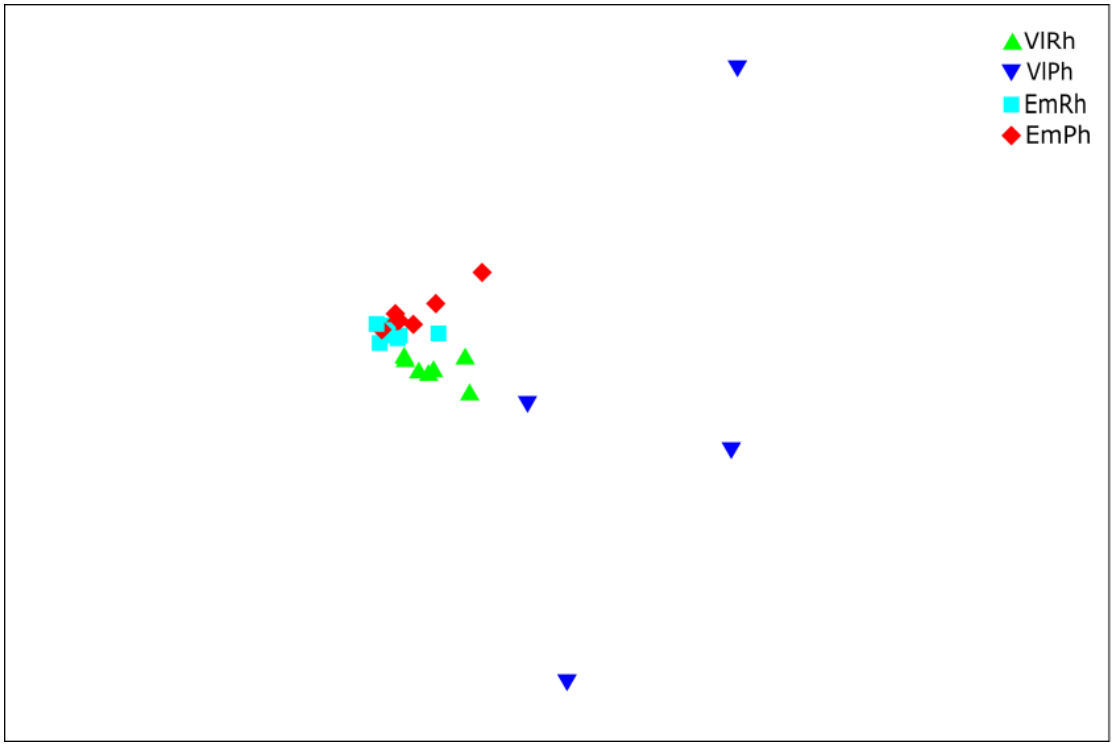

Figure 17. Non-metric Multi-Dimensional Scaling plot, generated by the PRIMER software, indicating the similarity and diversity of samples according to the Jaccard similarity coefficient.

4. Discussion

In this study, the phyllosphere and rhizosphere of the local tomato cultivar in Santorini were examined via the 16s rRNA gene amplicon sequencing. The diversity, variability and read abundances of the bacterial assemblages were analyzed with the use of a downstream analysis. The stressed volcanic and arid environment of the sampling sites according to similar studies assimilate a different microbiota compared to soils unaffected by environmental stress (Hernández et al., 2020; Cockell et al., 2011). With climate change, food shortage, spreading aridity and an increase of the global populace, research in the taming of stressed ecosystems could be the key to agricultural sustainability (Nadeem et al., 2019). Genomic studies have allowed for the detailed analysis of any microbiota and the interactions between those bacteria and plants of which phyllosphere, rhizosphere and even endosphere they inhabit.

The samples consisted of 8 phyllospheres and rhizospheres of 8 plants from Vlichada and Emporio respectively. However, after normalization, 6 of 32 samples were not included in the data analysis due to the low sequencing success. The quality of the extracted DNA in rhizospheric samples led to the successful sequencing of 15 out of 16 rhizospheric samples, consequently also providing these samples with higher OTU richness. Since the sequencing of phyllospheric samples was not a successful as rhizospheric samples, it could be deemed as an indication that phyllospheric bacterial assemblages have lower abundance or DNA extraction was not always successful.

The diversity observed in most samples was high and in both sampling sites, as seen on Figure 4, rhizospheric bacterial communities display a higher species richness than phyllospheric assemblages. Studies have shown similar findings of high species richness in the rhizosphere in comparison to the adjacent phyllosphere of a plant (Zhou et al., 2019; Lambais et al., 2014; Knief et al., 2012).

Microbial survivability in the phyllosphere is inherently hard under normal environmental circumstances. In any given plant growing in an uncontrolled environment, phyllospheric bacteria are exposed to high levels of UV exposure from the sun, temperature variances, water stress (Lindow and Brandl, 2003) and heterogenous nutrient availability (Leveau and Lindow, 2001). In the case of the Santorini *Solanum lycopersicum* studied here, only basic fertilization was applied in

both fields of Vlichada and Emporio. This cultivar has adapted over the years to the volcanic properties of the soil, a non-irrigation scheme and intense temperature and sunlight exposure (Genitsaris et al., 2020). The microbiome of the phyllosphere has been considered to have originated from various sources including the air, neighboring plants and even from the seed (Fahlgren et al., 2010; Maignien et al., 2014; Vorholt, 2012). Other factors that modify the phyllospheric microbiome are the pollinating insects, and animal activity in the surrounding phyllospheric area (Ushio et al., 2015; Aleklett et al., 2014). The phyllospheric bacterial assemblages of the cultivars in this study however, had to adapt to the harsh environmental stresses as they had a pivotal role in the survivability and adaptation of the plant itself to the environment. According to Vorholt, resistance of the bacteria towards the abiotic stresses in the phyllosphere can be displayed with the DNA protection during starvation protein (Dps) for low nutrient adaptation, various catalases, and superoxide dismutases for reactive oxygen species (ROS) to mediate potential damage to nucleic acids, lipids, and proteins. Bacteria also establish formations and excrete bioactive compounds to combat evaporation (Monier and Lindow, 2003). It has been argued that the intrinsic interactions the phyllosphere microbiota with the plant have, determine the plants' adaptive ability and responses to the environmental changes, which initiates a feedback loop that reinforces the mutualistic and coevolutionary relationship of the plant and phyllosphere microbiome (Liu et al., 2020).

In both phyllospheric and rhizospheric samples, the highest relative abundance was observed in the taxonomic group of Proteobacteria. Proteobacteria generated 40,165 reads which constituted 29,5% of the whole rarefied dataset. Moreover, the highest OTU richness was calculated as well with 999 OTUs which constituted 27% of the total OTUs recovered from the analysis. Proteobacteria have been shown to be one of the most abundant taxonomic groups of plant microbiota in many studies (Cheng et al., 2020; Bulgarelli et al., 2012; Edwards et al., 2015). Their utility is vast and many of the Proteobacteria found in the aforementioned studies including this one, belong to the group of Plant Growth-Promoting Bacteria (PGPB). A characteristic example of PGPB found in abundance in this study were *Rhizobia* which were calculated to a total of 9,241 reads. This taxonomic group is known for its ability to produce phytohormones such as indole-3-acetic-acid (IAA) (Remans et al., 2008; de

Souza et al., 2015). Additionally, *Rhizobia* express genes which biologically fix soil nitrogen when they form a symbiotic relationship with legume plants.

Pseudomonas spp. is one of the bacterial families of the Proteobacteria phylum that adapt and protect themselves to osmotic stress through the accumulation of choline or trehalose (Chen and Beattie, 2008; Freeman et al., 2010; Vorholt, 2012). The most repeated OTU in Vlichadas' phyllospheric samples corresponds to the genus of *Pseudomonas*, however, it should be noted that the corresponding OTU (OTU_04) is repeated mostly in sample VLF5. Generally, *Pseudomonas* is not highly abundant in any other sample, even though its genus is present in most samples. This taxon is also a PGPR according to various studies and is used as biofertilizer in many crops (Loper and Gross, 2007; Nihorimbere et al., 2011). In the case of this research however, there was no biofertilizer applied to the plants, thus depicting a symbiotic state between the tomato cultivar and the inherent *Pseudomonas* of the soil. This genus is known for aiding the plant host through the rhizosphere via promoting plant growth through the uptake of Nitrogen, Potassium and Phosphorus, by improving seed germination and by protecting the plant from various fungal diseases (Singh, 2013).

The phylum of Firmicutes consisted 12,25% of all reads in the complete dataset of which 8,71% corresponded to the genus of *Bacillus*. The phylum of Firmicutes established dominance in samples EmF6, VLR2 and VLR4. *Bacillus* spp. has shown great enhancement in the survivability of host plants and even other tomato cultivars according to various studies (Jacobsen et al., 2004; Singh, 2013). In fact, there are bacillus-based biological control agents (BCAs) integrated into pest management systems (IPM). The use of BCAs is a sustainable alternative to potentially toxic, chemical IPM systems and act though antagonizing fungal and bacterial phytopathogens. *Bacilli* are bacteria that belong to the phylum of Firmicutes which are highly present in all the samples that were analyzed and replicate rapidly, have large variance in biocontrol abilities and are resistant to stressed environmental conditions such as the fields of Vlichada and Emporio (Shafi et al., 2017). This taxon produces numerous biocontrol compounds which combat pathogens and induce host systemic resistance and the effect of these compounds have been reported to be similar in both phyllospheric and rhizospheric conditions (Wei et al., 2016). This may suggest that the symbiont of tomato plant and surrounding bacterial assemblages

work in unison as the phyllosphere and rhizosphere utilize naturally the occurring BCAs in order to promote and enhance survivability and growth of the plant.

Cyanobacteria are relatively abundant in the both the phyllosphere and the rhizosphere of the tomato cultivars of this study, as 24,66% of phyllospheric reads were found in 57 OTUs of the phyllospheric samples while 20,75% reads were found in 22 OTUs of the rhizosphere. Cyanobacteria have different effects on the host plant depending on their phyllospheric or rhizospheric origin. In the phyllosphere, the autotrophic cyanobacteria play a key role in nutrient cycling and water relations between the plant and the environment (Fürnkranz et al., 2008). Since the tomato plants in this study were not irrigated, the abundance in cyanobacteria leads to the assumption that the inherent abilities of these autotrophs help the hydration of the plant through condensation. Additionally, cyanobacterial photosynthesis and primary production of compounds can enable or impede the phyllospheric colonization of different heterotrophic organisms according to studies performed on rice plants (Venkatachalam et al., 2016). In the rhizosphere of plants, cyanobacteria are known to provide Nitrogen and Carbon in the form of amino acids and polysaccharides (Venkatachalam et al., 2016). However, other studies underline the production of phytohormones, proteins and vitamins which signal plants to respond correspondingly, promoting growth, disease resistance, phytochemical excretions, and salt tolerance (Mandal et al., 1999; Singh, 2014).

Upon analysis, 20,04% of all reads corresponded to Actinobacteriota in the complete dataset, of which most abundant genus was the class of *Actinobacteria* with 16,77% of all reads. It should be noted that the arid condition of the crops had a key role in the development of actinobacteria as the class of *Thermoleophilia*, a taxon of actinobacteria most commonly found in abundance in harsh environmental ecosystems such as those of hot springs where the temperatures are very high, was calculated with 1,7% reads in the complete dataset and was present in all samples (Hu et al., 2019). Actinobacteriota are ubiquitous in nature and offer a wide spectrum of beneficial mechanisms towards plants, therefore are used on many occasions in agricultural settings. Actinobacteria promote plant growth directly and indirectly either by synthesizing plant growth-promoting hormone such as Indole-3-acetic-acid (IAA), by nitrogen fixation, phosphorous, zinc and potassium solubilization, or via the

production of various enzymes, ammonia, antibiotics, hydrocyanic acid (HCN) and siderophores (Yadav et al., 2018). The existence of actinobacteria however, is affected by various environmental factors such as soil pH, temperature, soil salinity and water availability.

Bacteroidetes are ubiquitous in normal environmental phyllospheric and rhizospheric settings of plants (Arya and Harel, 2019). This taxonomic group did not display dominance over the other relatively abundant taxonomic groups, however, a high percentage of reads of this taxon was calculated with 19,2% of the reads corresponding to this group. Many species of this phylum remain uncultured which was revealed upon analysis of the dataset, as 19,5% of all Bacteroidota reads (72 of 486 OTUs) in the dataset correspond to uncultured variants. A similar study conducted in China on different tomato cultivars showed abundance in the taxonomic group of *Cytophagales* of Bacteroidetes which was calculated in this dataset as well with a total of 2,78% of total reads (Cheng et al., 2020). Another abundant group calculated in the dataset was the family of *Chitinophagales* with 2,86% of all reads. This taxonomic group has the ability to convert complex organic matters such as cellulose and chitin to usable carbon for the plant (Rosenberg, 2014). In a stressed environment where human interference with the tomato plants, the microbiome of the plant played a key role in the nutrient acquisition, which could justify the abundance of this taxonomic group.

The phylum of Acidobacteria (Acidobacteriota) was recently introduced as a separate phylum from Proteobacteria. Acidobacteria were relatively abundant in this study, totaling 2,5% of all reads and 7,8% of all the OTUs in the dataset, and did not display dominance in any sample, contrasting the results of Dunbar where Acidobacteria were the dominant taxonomic group. Their abundance ranges depending on the ecosystem, soil characteristics such as pH and nutrient availability, and finally the plant host (Kielak et al., 2016). There is evidence that Acidobacteria are highly abundant in stressed environmental conditions according to a study that took place in Arizona, where the climate is hot, dry and the soil is rich in volcanic cinders (Dunbar et al., 2002). The environment of the sampling sites in Santorini are similar to Arizona, as the conditions were in both cases arid, had high median temperatures, high UV exposure and the soil had volcanic properties. Additionally, Acidobacterial

survivability in harsh soil conditions has been linked to a list of factors which include their utilization of a variety of carbohydrates as nutrients, antibiotic resistance, metabolite and bacterial polymers production, exopolysaccharide (EPS) production which enables the bacteria to survive for long periods in soil, nitrite metabolism, and lastly high-affinity carbohydrate and metabolite transporter production (Kielak et al., 2016).

In a study performed on three drought stressed plants (*Thymus vulgaris*, *Santolina chamaecyparissus* and *Lavandula dentata*), abundance was observed in various taxa of Actinobacteria. These bacteria were naturally occurring and promoted survival and growth of these plants under drought stress (Armada et al., 2018). In many of the samples of this study, Actinobacteria were highly abundant, which findings lead to the assumption that the taxonomic group of Actinobacteria, have a key role in relieving environmental stresses by rerouting metabolic pathways, even though they are susceptible to environmental stresses themselves. Similar studies about *Solanum Lycopersicum* in stressed and particularly water deficit, in which PGBP were found in abundance with *Bacillus subtilis* and *Bacillus megaterium* having a key role in the survivability of the plants (Mannino et al., 2020). Bacilli were found in abundance in this study as well, indicating that indeed this taxonomic groups plays a significant role in drought stress relief.

The stressed environment of Santorini, which is located in the Mediterranean basin, has been a hub of agriculture for at least a few centuries and the local tomato cultivar has adapted to this type of agriculture. The high temperatures, and the volcanic properties of the land have driven the local plants and their respective rhizospheric and phyllospheric microbiomes to adapt to these stresses. Additionally, in a related study, the biochemical traits of the isolated rhizobacteria were defined and show the elevated levels of various compounds such as IAA, ACC, siderophores and other traits which are expressed by PGPB. (Leontidou et al., 2020). This study suggested that the plant and its microbiome are affected by the environmental stresses and the chemical properties of the soil.

Overall, all the samples had some percentage of their OTUs corresponding to PGPB or even have a relatively high number of reads present in unique phyla. Most notably in all samples a small amount of OTUs corresponded to unidentified bacteria.

This could be an indication of novel bacteria being present in the volcanic soil of Santorini that help the survival of the plant and work in unison with PGPB or even be new bacteria with unknown roles and mechanisms that benefit the plant though their function in both the phyllosphere and rhizosphere of the plants.

5. Conclusions

The identification of the composition, structure, and abundance of phyllo- and rhizospheric bacterial communities of crops similar to the stressed tomato cultivar in this study could assist in the understanding of the mechanics which drive the symbiosis of plant and plant microbiomes. The results of the 16S rRNA gene amplicon sequencing from the tomato samples of the Mediterranean island of Santorini with its arid and volcanic stress properties, suggest that the microbiome of the phyllosphere and rhizosphere have a key role in the survivability of the plants, as it has been linked to benefiting the plants in similar studies. Additionally, the investigation of the taxonomic composition of both plant compartments lead to the conclusion that there is a pool of generalist taxa that inhabit the plants' compartments of both sampling sites even though the abundance of each taxonomic group may vary between individual plants. Further investigation is needed to identify any potential uncultured specialist taxa which may drive the symbiont through the harsh conditions as well as the importance of the interactions of known specialist taxa identified in this study.

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