Bacterial Communities from Extreme Environments: Vulcano Island

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Abstract: Although volcanoes represent extreme environments for life, they harbour bacterial communities. Vulcano Island (Aeolian Islands, Sicily) presents an intense fumarolic activity and widespread soil degassing, fed by variable amounts of magmatic gases (dominant at La Fossa Crater) and hydrothermal fluids (dominant at Levante Bay). The aim of this study is to analyse the microbial communities from the different environments of Vulcano Island and to evaluate their possible correlation with the composition of the gas emissions. Microbial analyses were carried out on soils and pioneer plants from both La Fossa Crater and Levante Bay. Total DNA has been extracted from all the samples and sequenced through Illumina MiSeq platform. The analysis of microbiome composition and the gases sampled in the same sites could suggest a possible correlation between the two parameters. We can suggest that the ability of different bacterial genera/species to survive in the same area might be due to the selection of particular genetic traits allowing the survival of these microorganisms. On the other side, the finding that microbial communities inhabiting different sites exhibiting different emission profiles are similar might be explained on the basis of a possible sharing of metabolic abilities related to the gas composition.

Keywords: bacterial communities; extreme environments; interstitial soil gases

1. Introduction

In ecology, the term "extreme" commonly refers to unfavourable environmental factors that depress the ability of organisms to function [1]. Understanding the mechanisms underlying the adaptation of microbes to extreme environments is of fundamental importance to deeply investigate processes that led to the evolution of Earth [2]; for example, it has been shown that microbial communities are mainly shaped by environmental conditions and the microbes inhabiting extreme habitats evolve faster than those populating benign environments [1]. Bacterial communities from natural extreme environments represent not only a gene reservoir for potential biotechnological applications (e.g., the discovery of the *Taq* polymerase, isolated from the thermophilic bacteria *Thermus aquaticus*), but they can be used as a model system to explore relationships between diversity and environmental factors [3]. Adaptative features of these extremophiles permit them to survive under such extreme and hostile environmental conditions [4].

In this context, volcanic environments arouse interest in the scientific community, as they are considered analogous to some of the earliest environments on Earth [5]. In the last decades, several

studies focused on understanding the distribution of microorganisms in volcanic environments [6], such as Antarctic and Icelandic volcanoes. These studies were motivated by the desire to elucidate how the geochemically extreme conditions of such environments can influence microbial diversity both on the surface and in the subsurface of the Earth. Interest in the microbial diversity of geothermal and volcanic soils has been growing, and high diversity, including novel and rare bacteria, has been detected so far [7].

The Aeolian volcanic archipelago is situated north of Sicily (Italy), along the southern margin of the Tyrrhenian Sea and is underlain by a 15 to 20 km thick continental crust. Lipari, Vulcano, and Salina are three summits of a volcanic pile located along a regional crustal lineament crossing the north-eastern coast of Sicily and continuing as far as the Aeolian Islands. Each island in the Aeolian arc has had a different tectonic and magmatic history and their activity is not always correlated in time [8]. As usually occurs, shallow hydrothermal systems are associated with the Aeolian volcanoes [9]. Vulcano and the other Aeolian Islands have been investigated for the microbial populations [10], leading to the isolation of novel halotolerant and thermophilic species [11–14]. The thermal springs in the bay Baia di Levante of Vulcano Island (Aeolian Islands) host dozens of aerobic and anaerobic, thermophilic, and hyperthermophilic microorganisms belonging to *Bacteria* and *Archaea* domains [9].

Despite the abundance of researches about the microbial communities from these environments, from a scientific viewpoint, very little is known about the possible correlation between interstitial soil gases and soil bacteria.

Due to their detectability in almost every environment and the high information content, volatile (organic) compounds (VOCs) have been widely studied in relation to different organisms, functions, and interactions [15]. VOCs produced by bacteria have been correlated with inhibition of laccase activity [16], alteration of nitrification processes [17], antagonistic interactions with other species [18], and plant growth promotion [19]. Asensio et al. found microorganism, roots, and physio-chemical factors to be involved in VOCs emissions [20].

It is known that geothermal fluxes shape microbial soil communities [21] and that bacteria produce volatile compounds [22], but there is an almost complete lack of information about the possibility that bacteria may actually participate in the production of these interstitial gases.

Volcanoes are extreme environments for plants too. The role of bacteria associated with plants represents, at the moment, a very interesting topic. Symbiotic bacteria can establish a relationship with the host plant that gives benefits to both parts [23]. In an extreme environment, it is reliable to suppose that the role of bacteria for the survival of the plant in harsh conditions (lack of organic matter, high or low values of pH, presence of high concentration of metals and heavy metals) might be of major importance [24]. Microbial communities associated with plants in diverse conditions, including extreme environments, have been characterized for their potential biotechnological applications in industry, medicine, and agriculture [25]. Plants belonging to the *Poa* genus are pioneer plants widely found in volcanic environments [26–29], thus they result in interesting models to study the endophytic communities in extreme environments.

In this study, we aim to investigate the diversity of bacterial communities of Vulcano Island, Aeolian Islands (Italy). Physicochemical characteristics of fluids from the different sampling areas analysed in this study were investigated, in order to establish a possible correlation of these data with the composition of bacterial communities, and to evaluate the possibility that bacteria may be involved in the production of these interstitial soil gases.

2. Materials and Methods

2.1. Study Area: Vulcano Island

Vulcano is the southernmost island of the Aeolian archipelago (Sicily, southern Italy), a volcanic arc consisting of seven islands and nine sea mountains with an extension of 22 km^2. The base of the volcanic cone leans on the Calabro-Peloritano block at an average depth of 1000 m below sea level,

for a diameter of about 15 km [30]. The evolution of the island is linked to a trans-tensional tectonics, related to the subduction process [31].

Sampling Areas

Samples for microbiological and geochemical analyses were mostly collected from two areas marked by intense exhalative activity: La Fossa Crater and the Levante Bay, along the eastern side of the isthmus connecting Vulcano Island to the Vulcanello peninsula.

The north-western sector of the crater rim of La Fossa Crater is currently affected by intense fumarolic degassing, with outlet temperatures ranging from the boiling point of water up to 450 °C. The fumarolic fluids have a typical magmatic composition, with dry gas fraction dominated by CO_2 and with relevant concentrations of HCl, SO_2, H_2S, HF, and CO [32,33]. The mineralogy of the crater fumaroles is dominated by sulfides and sulfosalts, deposited due to a sublimate formation under high-temperature reducing conditions [34–36]. Notable quantities of ammonium minerals were also recognized [37]. Rapidly changing temperatures, magmatic fluids input, soil permeability, etc., offer a multitude of environment in a very narrow area. On the volcanic edifice, pioneer plants belonging to the *Poa* genus start to grow at the altitude of nearly 360 m above the sea level.

The Levante Bay area is characterized by the presence of low-temperature fumaroles ($T < 100$ °C), both subaerial and submerged, that originated by boiling of a shallow geothermal aquifer heated by the uprising hot magmatic fluids. The discharged fluids have a typical hydrothermal composition, being characterized by higher contents of CH_4 and H_2S relative to the crater fumaroles. Pioneer plants are present in Levante Bay, in high CO_2 fluxes areas, and in high salinity environments. This site and, in particular, the submarine gas vents were selected as natural laboratories for research studies regarding, e.g., (i) ocean acidification [38] and (ii) thermophilic bacteria [11].

2.2. Interstitial Soil Gas Sampling and Analyses

Sampling was performed on May 2017 in different sites of Vulcano Island, Aeolian Islands (Sicily, Italy). In this work, analyses were concentrated at La Fossa Crater (38°24′15″ N 14°57′42″ E) and Levante Bay (38°25′02″ N 14°57′34″ E).

Interstitial soil gases were collected from 8 sampling sites, as follows: (i) 3 sites on the northern flank of La Fossa Crater at different distances from fumarolic emissions (CSCS, CSF56, CSF94), (ii) 1 site on the western flank of the volcanic edifice characterized by the presence of a pioneer plant (CPBS), and (iii) 4 sites in the Levante Bay (LSV11, LSV13, LSV14, LPBS), selected according to in situ soil CO_2 flux measurements. Soil CO_2 fluxes were measured according to the accumulation chamber (AC) method [39], using a cylindrical chamber (basal area 200 cm^2 and inner volume 3060 cm^3) and a Licor® (NE, USA) Li-820 Infra-Red (IR) spectrophotometer, as described in Venturi et al. [40].

Gas samples were collected by inserting a stainless-steel probe within the soil down to the sampling depth (5, 10, or 30 cm) connected, through silicon tubing and a PTFE three-way valve, to a 60 mL plastic syringe in order to pump the gas into (i) a 12 mL glass vial equipped with a silicon rubber membrane and (ii) a 1 L Supelco's (Darmstadt, Germany) Tedlar® gas sampling bag. The detailed description of the sampling equipment and procedure is reported in previously published literature [40–43]. After sampling, soil temperature was measured with a thermocouple inserted within the soil down to the sampling depth.

The chemical analyses of major gas constituents were performed on gas samples stored in the glass vials using a Shimadzu (Duisburg, Germany) 15A gas chromatograph (GC), equipped with a thermal conductivity detector (TCD) and either a 10 m long 5A Molecular Sieve column (for N_2, O_2, Ar and H_2) or a 3 m long column filled with 80/100 mesh Porapak Q (for CO_2 and H_2S). The analyses of VOCs were performed using (i) a Shimadzu 14A GC equipped with a flame ionization detector (FID) and a 10 m long stainless-steel column filled with 23% SP 1700 on Chromosorb PAW (80/100 mesh) for the analysis of light hydrocarbons (C_1–C_3), and (ii) a Thermo Trace Ultra GC coupled with a Thermo

DSQ Quadrupole Mass Spectrometer (MS) for the analysis of C$_{4+}$ VOCs. The analytical procedure and instrumental specifications are described in Venturi et al. [43].

The analysis of carbon isotopic composition of CO$_2$ and CH$_4$ (expressed as δ^{13}CO$_2$ and δ^{13}CH$_4$, respectively, in ‰ vs. V-PDB) was performed on gas samples stored in the Tedlar® bags by using a Cavity Ring-Down Picarro G2201-i spectrometer after proper gas sample treatments, as described in Venturi et al. [43].

2.3. Biological Sample Collection

Samples were collected into 50 mL sterile falcon tubes. Then, 20 g of soil samples were taken in the same sites where interstitial soil gases were measured, immediately before the gas sampling.

A pioneer plant, belonging to the *Poa* genus, present both on the western flank of the volcanic crater and in Levante Bay, was included in the study. Each plant sample analysed was composed of a pool of three plants collected in the same site. Plants were divided into two main compartments: Aerial part and roots. Moreover, rhizospheric and bulk soils were collected from each site, resulting in four different samples for each plant (Levante Bay: LPA, LPR, LPRS, LPBS; La Fossa Crater: CPA, CPR, CPRS, CPBS).

Table 1 reports the list of samples collected from each site.

Table 1. List of samples collected on Vulcano Island.

No.	Code	Site	Sample
1	CPA	La Fossa **C**rater	**P**lant **A**erial Part
2	CPR	La Fossa **C**rater	**P**lant **R**oots
3	CPRS	La Fossa **C**rater	**P**lant **R**hizospheric **S**oil
4	CPBS	La Fossa **C**rater	**P**lant **B**ulk **S**oil
5	LPA	**L**evante Bay	**P**lant **A**erial Part
6	LPR	**L**evante Bay	**P**lant **R**oots
7	LPRS	**L**evante Bay	**P**lant **R**hizospheric **S**oil
8	LPBS	**L**evante Bay	**P**lant **B**ulk **S**oil
9	CSF94	La Fossa **C**rater	**S**oil, **94** °C
10	CSF56	La Fossa **C**rater	**S**oil, **56** °C
11	CSCS	La Fossa **C**rater	**S**oil from **C**ap **S**urface
12	LSV11	**L**evante Bay	**S**oil, site **V11**
13	LSV13	**L**evante Bay	**S**oil, site **V13**
14	LSV14	**L**evante Bay	**S**oil, site **V14**

2.4. Extraction of Genomic DNA and Next Generation Sequencing

DNA extraction was performed from each sample by using the PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instruction. Concentration and purity of extracted DNA were checked by 0.8% agarose gel electrophoresis. Bacterial 16S rRNA gene contains conserved sequences and nine hypervariable regions, named V1–V9 [44,45]. Hypervariable regions are used as molecular markers for bacterial identification in HTS analysis [46]. In particular, in this study, we sequenced the V3–V4 regions using the primer 341F and 805R [47] according to the protocol reported in the 16S Metagenomic Sequencing Library Preparation protocol from Illumina [48]. Library preparation and demultiplexing were performed following Illumina's standard pipeline [49]. Libraries were sequenced in a single run using Illumina MiSeq technology with pair-end sequencing strategy with MiSeq Reagent Kit v3. PCR amplification, library construction, and sequencing were performed by an external company (IGA Technology Services, Udine, Italy). Sequence files were deposited in the NCBI sequence read archive (SRA) under the accession PRJNA508599.

2.5. Sequence Analysis

Primers were removed from all the sequences using Cutadapt [50], version 2.4. Sequences were clustered following the DADA2 pipeline (version 1.8) described at https://benjjneb.github.io/dada2/tutorial.html [51], using the R software version 3.4.3 [52]. Sequences were trimmed, quality checked (Figure S1), denoised, and then forward and reverse reads were merged. An amplicon sequence variant (ASV) table was obtained, a higher-resolution analogue of the traditional OTU table [53]. Chimeras were removed; then the taxonomy was assigned to the output sequences through the Silva database (version 128) [54]. Tables produced by the DADA2 pipeline were imported into phyloseq (through the phyloseq R package version 1.22.3), for further analyses of microbiome data. Representative sequences that were not assigned to domain Bacteria were removed from the dataset (Tables S1 and S2).

2.6. Statistical Testing

All statistical analyses were performed into the R environment, version 3.4.3 [52,55]. Good's coverage estimator was calculated using the formula: $1-(n/N) \cdot 100$, where n is the number of sequences found only one time in a specimen (singletons) and N is the total number of sequences assigned to an ASV in that specimen [56].

Bacterial diversity was estimated using the vegan package (version 2.5–4, Dixon 2003). The function 'diversity' was used to compute the Shannon index (H), whereas species evenness (J) was estimated as a function of the Shannon diversity and the number of ASVs detected in the sample (S), according to the Pielou's formula $J = H/\log(S)$ [57]. Differences in bacterial diversity between the different sampling sites were tested using the Student's *t*-test ('*t*.test' R function), and the possible correlation between samples' Shannon diversity and environmental parameters was calculated with the 'cor.test' function through the Pearson method.

Beta-diversity was analysed with the Bray–Curtis dissimilarity, using the 'vegdist' function of the vegan package. Correspondence analysis (CA) and canonical correspondence analysis (CCA) were performed using the 'cca' function of the vegan package.

Different community structures were tested using permutational multivariate analysis of variance ('adonis2' function of the vegan R package) with 1000 permutations.

The possible correlation between taxa distribution and gas composition of soil samples was evaluated with a Mantel test of correlation ('mantel' function of vegan package with Bray–Curtis distance).

The composition of all interstitial gases detected was fitted onto ordination analyses previously produced using the 'envfit' function of the vegan package.

2.7. Isolation of Culturable Bacterial Strains

Isolation of bacteria was performed on Tryptic Soy Agar (TSA) medium using as starting material 1 g of soil (for samples CSCS, CSF56, CSF94, CPBS, CPRS, LSV11, LSV13, LSV14, LPBS, LPRS), and 0.5 g of surface sterilized plant tissue (samples CPA, CPR, LPA, LPR) [58].

Samples were serial diluted and plated on TSA medium, in triplicate. Bacterial plate counts were carried out after 48 h incubation at 30 °C. For each sample, a variable number of colonies (based on the different number of isolates obtained from the samples) was isolated on TSA medium and stored at −80 °C in 20% glycerol for further analysis.

Cell lysates of bacterial isolates were prepared by processing with thermal lysis (95 °C for 10 min, 0 °C for 5 min) an isolated colony dissolved in 20 μL of distilled sterile water.

2.8. Random Amplified Polymorphic DNA (RAPD) Analysis

Random amplification of DNA fragments [59] was performed on DNA samples obtained from thermal lysis. Reactions were performed in a 25 μL total volume, as described in Chiellini et al. [58] using primer 1253 (5'-GTT TCCGCCC-3') [58,60]. Amplicons were analysed by 2% agarose gel

electrophoresis. For each biological sample, isolates showing the same RAPD profile were grouped together into the same haplotype.

2.9. Taxonomical Characterization of Culturable Bacterial Strains

One strain for each RAPD haplotype was chosen for the amplification of the 16S rRNA gene, following the protocol described in Chiellini et al. [58]. Amplification of 16S rRNA genes was performed in a final volume of 20 μL, with DreamTaq DNA Polymerase reagents (Thermofisher Scientific, Waltham, MA, USA), 0.5 μM of P0 and P6 primers (P0, 5′-GAGAGTTTGATCCTGGCTCAG; P6 5′-CTACGGCTACCTTGTTACGA) [61] and 1 μL cell lysate was used as template [61]. Amplification conditions were the following: 90″ denaturation at 95 °C, 30 cycles of 30″ at 95 °C, 30″ at 50 °C, and 1′ at 72 °C, followed by a final extension of 10′ at 72 °C. Sequencing of the 16S rRNA amplicon was performed by and external company (IGA Technology Services) with primer P0. Taxonomic affiliation of the 16S rRNA gene sequences was performed using the Classifier tool of Ribosomal Database Project (RDP) [62]. Each sequence obtained was submitted to GenBank with the accession numbers from MK249884 to MK249979.

3. Results

3.1. Geochemical Features of Interstitial Soil Gases

3.1.1. La Fossa Crater

Sampling sites at La Fossa Crater were characterized by widely varying soil temperatures at 5 cm depth, sharply decreasing as the distance from the fumarolic emission increased, i.e., 94 °C at less than 1 m (CSF94), 56 °C at about 10 m (CSF56), and 31 °C at about 50 m (CSCS). Similarly, the chemical composition of soil gases changed as a function of the distance from the fumarolic vent. Although N_2 was the dominant gas constituent (from 507 to 877 mmol/mol), CO_2, H_2S, and H_2 progressively increased approaching the fumarolic vent, while N_2, Ar, and O_2 showed an opposite behaviour (Table S3). The isotopic composition of CO_2 was slightly heavier than that of the fumarolic gas (−0.86‰ vs. V-PDB; data not reported), with $\delta^{13}CO_2$ values progressively increasing from 1.11‰ to 2.39‰ vs. V-PDB moving away from the fumarolic vent. Considering that the isotopic signature of CO_2 in the fumarolic gases implies an origin related to mixing of magmatic and carbonate sources [32], the $\delta^{13}CO_2$ values of the interstitial gases, which are enriched in ^{13}C, were likely produced by partial consumption of volcanogenic CO_2 occurring in the soil due to microbial activity [43]. CH_4 showed similar concentrations in CSF94 and CSF56 (6.2 and 7.1 μmol/mol, respectively) and markedly decreased in CSCS (2.3 μmol/mol), while the $\delta^{13}CH_4$ values markedly increased from −47.2‰ and −44.8‰ vs. V-PDB in CSF94 and CSF56 to −5.5‰ vs. V-PDB in CSCS. Such a wide range of $\delta^{13}CH_4$ values was likely caused by oxidation, at different degrees, of volcanogenic CH_4 [43], the latter likely being produced by thermogenic processes acting on pre-existing organic matter at relatively high temperatures typical of a volcanic environment [63].

The organic fraction of interstitial soil gases was largely dominated by alkanes (C_2 to C_{11} compounds, mainly represented by ethane), with relative abundances ranging from 61% to 79% of ΣVOCs (Table S3), followed by aromatics, which were characterized by relative abundances varying from 12% to 26% of ΣVOCs and decreasing as the distance from the fumarolic emission increased. A similar trend was observed for cycloalkanes and S-bearing compounds (≤2.96% and ≤8.75% of ΣVOCs), whilst the relative abundance of O-bearing species sharply increased as the distance from the fumarolic vent increased (from 0.26% to 7.40% of ΣVOCs). Alkenes (only represented by iso-butene) were present at relative abundances varying in a narrow range, from 1.06% to 1.99% of ΣVOCs. Overall, ΣVOCs concentrations ranged from 0.15 to 0.53 μmol/mol and decreased moving away from the fumarolic emission.

3.1.2. Levante Bay

Sampling sites at Levante Bay were characterized by soil temperatures ranging from 30.9 to 37.5 °C and strongly different soil CO_2 fluxes (hereafter, ΦCO_2), with values of 6.5, 53, and 346 g m^{-2} day^{-1} in LSV13, LSV11, and LSV14, respectively (Table S3). Similarly, the chemical composition of interstitial soil gases varied widely among the selected sites. N_2 was the dominant gas component (from 537 to 963 mmol/mol), but the CO_2/N_2 ratio progressively increased with increasing ΦCO_2 values (from 0.02 to 0.77), the CO_2 concentrations varying from 15 to 411 mmol/mol. H_2S and H_2 were only detected in LSV11 and LSV14, where O_2 and Ar concentrations were from 36 to 44 mmol/mol and around 7 mmol/mol, respectively, and CH_4 ranged from 1.7 to 1.9 μmol/mol. Differently, LSV13 was characterized by lower O_2 (8.7 mmol/mol) and higher Ar (13 mmol/mol) and CH_4 (5.1 μmol/mol) contents (Table S3). The isotopic compositions of CO_2 and CH_4 (Table S3) were largely different among the selected sites, with heavier values in sites characterized by higher ΦCO_2 (from −3.33‰ to −1.15‰ vs. V-PDB and from 4.30‰ to 6.47‰ vs. V-PDB, respectively) and lighter values in LSV13 (−8.13‰ and −46.2‰ vs. V-PDB, respectively). Methane concentration was 840 μmol/mol, i.e., two orders of magnitude higher than those of the interstitial soil gases. The isotopic compositions of CO_2 and CH_4 in this gas sample were −2.21‰ and −16.2‰ vs. PDB, respectively.

Alkanes dominated the organic fraction of interstitial soil gases (Table S3), being represented by C_2 to C_{11} compounds with overall relative abundances ≥74% of ΣVOCs in high ΦCO_2 sampling sites and by C_2 to C_6 compounds with overall relative abundance of 51% of ΣVOCs in LSV13. Ethane was the most abundant alkane, followed by propane. Aromatics (dominated by benzene) represented the second most abundant organic functional group, with relative abundances ≤19% of ΣVOCs in LSV11 and LSV14 and of 43% in LSV13. Iso-butene was constantly present at ca. 2% of ΣVOCs, whereas cycloalkanes and S-bearing species were only detected in LSV11 and LSV14 (up to 1.37 and 3.5% of ΣVOCs). On the other hand, O-bearing compounds were present at relative abundances ≤0.37% of ΣVOCs in LSV11 and LSV14 and of 3.39% in V13. Overall, ΣVOCs concentrations ranged from 0.12 (in LSV13) to 1.48 (in LSV11) μmol/mol.

3.1.3. Pioneer Plant Sites

The chemical composition of interstitial soil gases from the two pioneer plants bulk soil (temperatures around 30 °C) was largely dominated by N_2 (up to 965 mmol/mol). No relevant difference was observed in terms of inorganic constituents between CPBS and LPBS, being characterized by CO_2 concentrations of 16 and 21 mmol/mol, respectively, and Ar and O_2 of 14 and 15 mmol/mol and 5.2 and 3.1 mmol/mol, respectively. H_2S and H_2 were not detected (Table S3). LPBS, characterized by a low ΦCO_2 value (2.6 g m^{-2} day^{-1}), was slightly enriched in CH_4 (5.7 μmol/mol) with respect to CPBS (2.8 μmol/mol). Although the $\delta^{13}CH_4$ values were similar in the two sites (−45.4‰ and −47.7‰ vs. V-PDB in CPBS and LPBS, respectively), the $\delta^{13}CO_2$ values strongly differed, the interstitial soil gas from CPBS being much heavier (8.60‰ vs. V-PDB) with respect to that from LPBS (−15.5‰ vs. V-PDB) (Table S3).

The chemical composition of the organic fraction of the two interstitial soil gases was different in terms of relative abundances of alkanes and aromatics. The former (C_2 to C_6 compounds) represented 63% and 58% of ΣVOCs in CPBS and LPBS, respectively, whereas the latter accounted for 24% and 37% of ΣVOCs in the two sites, respectively (Table S3). Cycloalkanes and S-bearing species were not detected, whereas iso-butene was present at ca. 1.6% ΣVOCs in both sites. A sharp difference was observed in the relative abundances of O-bearing compounds, accounting for 12% and 3.8% of ΣVOCs in CPBS and LPBS, respectively. Overall, ΣVOCs concentrations were 0.10 and 0.14 μmol/mol in CPBS and LPBS, respectively (Table S3).

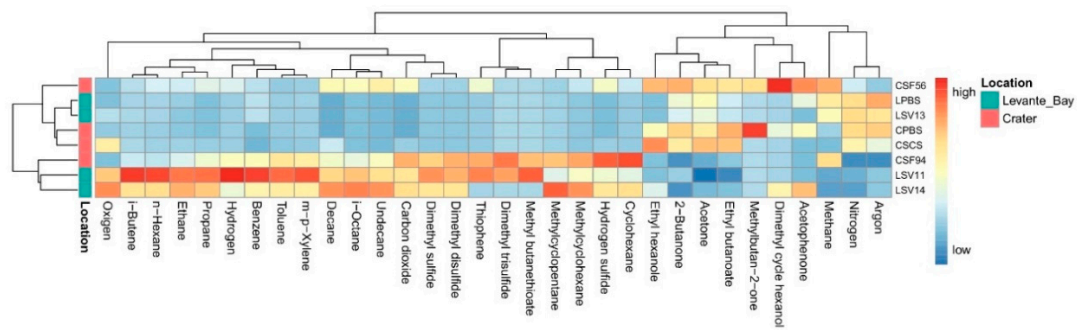An overall view of the interstitial gases composition of every sample site is shown in Figure 1.

Figure 1. Heatmap showing the scaled measures of detected interstitial soil gases.

3.2. Molecular Characterization of Bacterial Communities

Bacterial communities of soil and plant tissues samples collected in both emissive and non-emissive areas of Vulcano Island were examined through NGS analysis. Sequencing yielded 2,360,693 paired sequences (2×300 bp). More than 60% of the initial pairs were correctly merged (1,446,377 sequences) with a mean of 103,313 sequences *per* sample. Quality filtering steps produced 975,139 high-quality sequences that were correctly mapped into 5073 ASVs with an average of 69,653 sequences *per* sample. Samples reported a Good's coverage estimator ranging from 99% to 100% indicating that roughly 1% of the reads in a given sample came from ASVs that appear only once in that sample [64].

Representative sequences for each ASV were correctly classified into 520 bacterial genera belonging to 23 phyla according to the Silva database (see Materials and Methods), with a different distribution across samples. The analysis of the taxonomic composition revealed that more than 83% of the ASVs were classified into five phyla: *Proteobacteria* (32.22%), *Planctomycetes* (16.44%), *Actinobacteria* (13.56%), *Firmicutes* (11.93%), and *Bacteroidetes* (8.95%).

Comparison between La Fossa Crater and Levante Bay Soil and Plant Samples

Bacterial communities of soil samples from La Fossa Crater (at different soil temperatures) and Levante Bay (at different soil CO_2 fluxes) were analysed, and data obtained are shown in Figure 2.
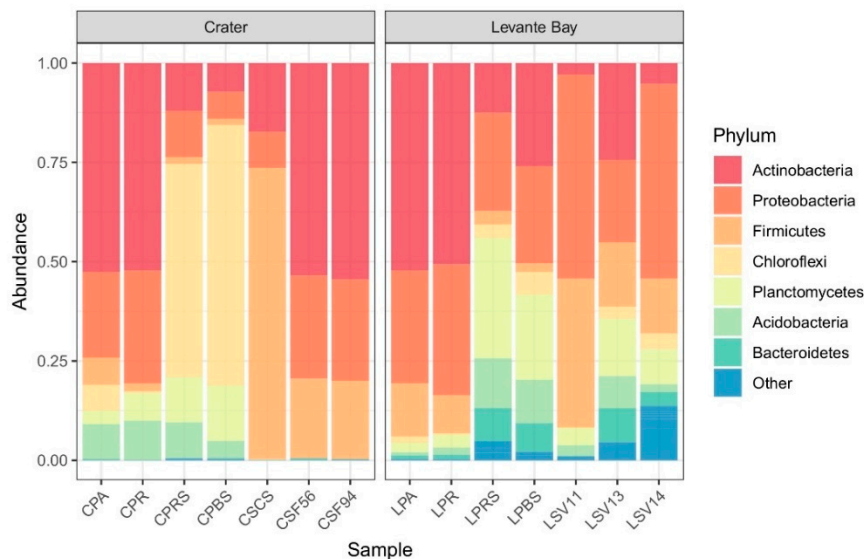

Figure 2. Bar plot showing the relative abundances of bacterial phyla in each sample (from Crater, on the left, and Levante Bay, on the right). Amplicon sequence variants (ASVs) representing <3% of the whole community were reported as "Other".

Exploring the different communities at the phylum level, the Crater soil samples showed the presence of three main phyla (*Actinobacteria*, *Proteobacteria,* and *Firmicutes*) that, in the samples CSCS, CSF56, and CSF94, comprise more of the 99% of the detected ASVs. Both CSF94 and CSF56 showed a frequency of over 50% in *Actinobacteria* (54.5% and 53.5%, respectively), of 25.6% and 25.9% in *Proteobacteria*, and of 19.7% and 20% in *Firmicutes*. The sample CSCS showed frequencies of 73.3% in *Firmicutes*, 17.3% in *Actinobacteria,* and 9.1% in *Proteobacteria*. Conversely, the sample CPBS presented a frequency in *Chloroflexi* equal to 65.6%, and in *Planctomycetes* of 13.9%, followed by *Actinobacteria* (7.3%), *Proteobacteria* (6.8%), *Acidobacteria* (4.3%), and *Firmicutes* (1.6%).

In Levante Bay soil samples, the main phyla detected were *Proteobacteria* (51.3%, 20.7%, 49%, and 24.4%), *Firmicutes* (37.5%, 16.2%, 13.9%, and 2.2%), *Planctomycetes* (4.4%, 14.4%, 8.9%, and 21.4%), and *Actinobacteria* (2.9%, 24.4%, 5.2%, and 26%) in LSV11, LSV13, LSV14, and LPBS, respectively.

Bacterial communities of *Poa* plant aerial part, roots, rhizosphere, and bulk soil from both Levante Bay and La Fossa Crater were analysed too (Figure 2). All the samples presented a high frequency in *Actinobacteria* and *Proteobacteria*, except for the samples CPBS and CPRS in which *Chloroflexi* are predominant (65.6% and 53.7%, respectively).

Levante Bay samples showed a higher biodiversity than Crater samples (Figure 3a,b), and the Student's t-test between the Shannon indices and evenness of the two sampling sites resulted in statistically significant *p*-values (*p*-value = 0.0033 and 0.0028, respectively).
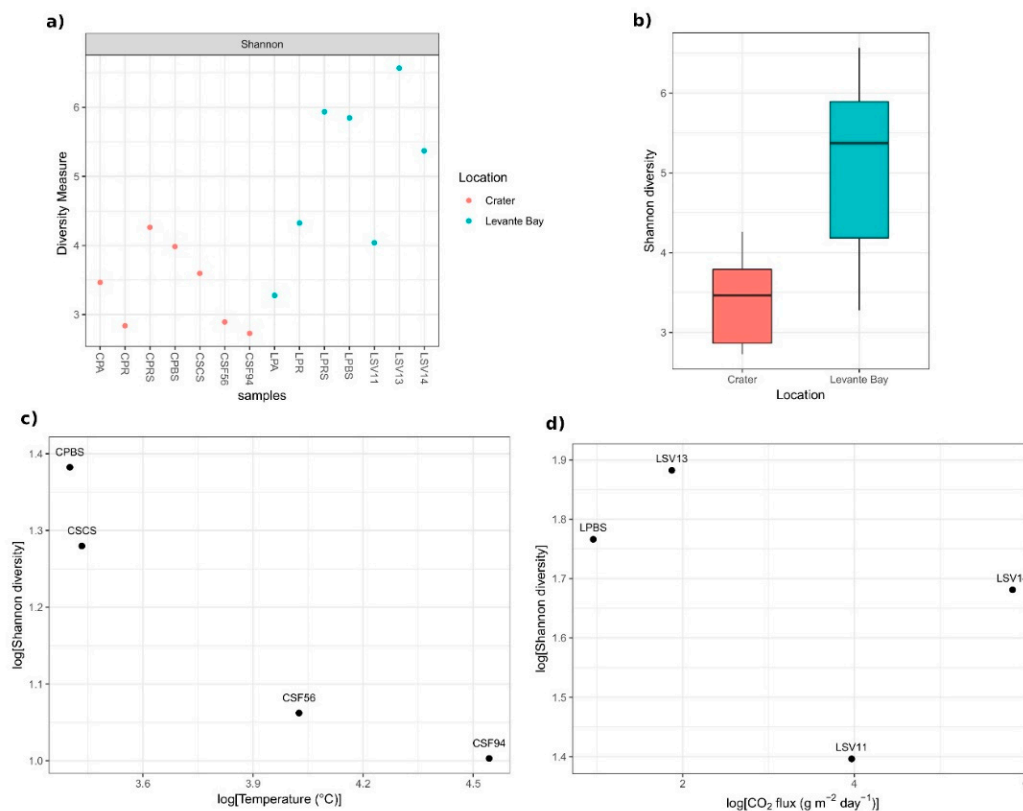

Figure 3. Shannon diversity of samples bacterial communities. (**a**) Samples' Shannon diversity indices, calculated on raw data; (**b**) box-whiskers plot of the indices based on the different sampling area: Ends of the whiskers represent the lowest and the highest point still within 1.5 of the inter quartile range (IQR); (**c**) relation between Crater samples soil temperatures and Shannon diversity indices (log transformation of the data has been performed); (**d**) absence of correlation between Levante Bay samples CO_2 fluxes and Shannon diversity indices (log transformation of the data has been performed).

A reduction in the Shannon diversity of the Crater soil samples was observed as the distance of the sampling site to the fumarolic emission decreases (so, as the soil temperature enhancing) (Pearson's

$r = -0.94$, *p*-value = 0.06) (Figure 3c). The analysis of the Levante Bay soil samples did not reveal a linear correlation between the diversity and the soil CO_2 fluxes (Pearson's $r = -0.50$, *p*-value = 0.50) (Figure 3d).

Correspondence analysis (CA) showed a high similarity between the Crater soil samples CSF94, CSF56, and CSCS, while Levante Bay soil bacterial communities of the different samples resulted in being diverse from each other (Figure 4a). Different community structures were tested using permutational multivariate analysis of variance, resulting in a coefficient of determination $R^2 = 0.24$ and in a non-statistically significant *p*-value (*p*-value = 0.09).
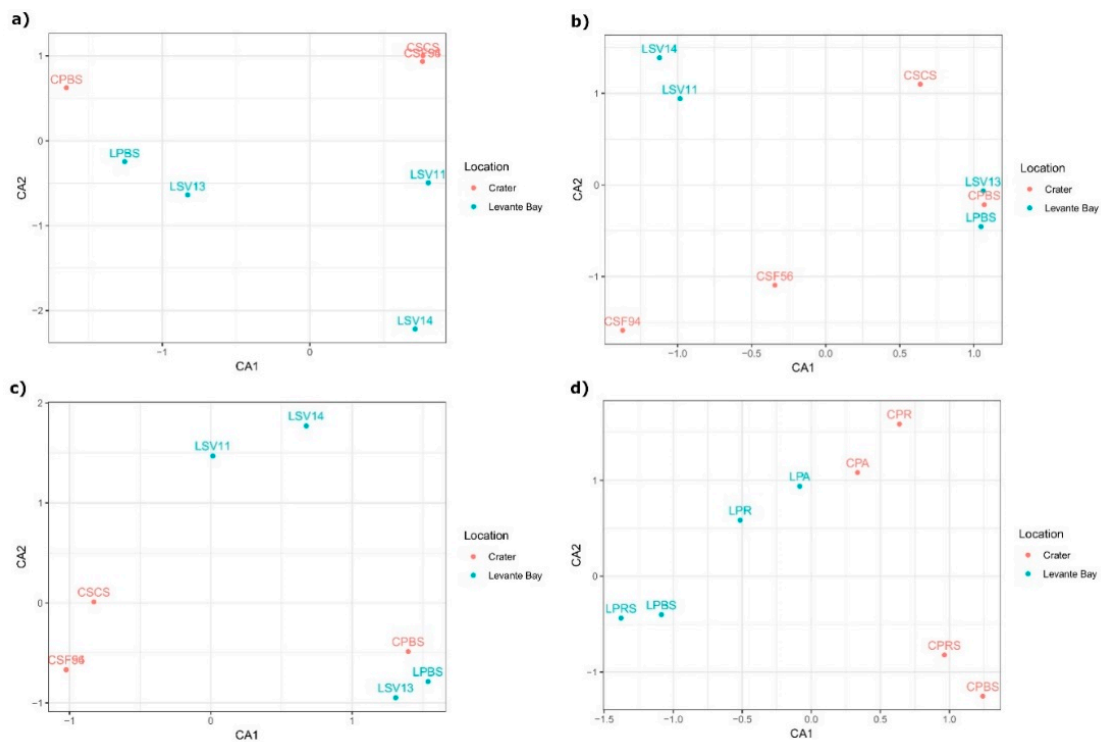
Figure 4. Correspondence analysis (CA) ordination analysis of samples based on (**a**) soil microbiome taxonomic composition; (**b**) soil gas emissions; (**c**) soil microbiome taxonomic composition collapsed at the genus level; (**d**) plant microbiome taxonomic composition.

To evaluate whether the differences between the two sampling sites, in terms of composition of the bacterial community, can be possibly correlated to the gas emissions present in these areas, a CA analysis was performed on the samples on the basis of the gas emissions (Figure 4b). In this case, the samples LSV11 and LSV14 appeared to be similar to each other, as did LPBS, CPBS, and LSV13, whereas the other samples were separated. Permutational multivariate analysis of variance resulted in a *p*-value = 0.97, showing no interstitial gases differences between the sampling sites.

Subsequently, a CA analysis was performed on the microbiome taxa composition collapsed at the genus level (Figure 4c): Both the CA analysis and the CCA analysis constrained on the interstitial gases composition (Figure S2) resulted in the same spatial distribution of the samples, which appeared to be comparable to the distribution of the samples based on the gases composition: In particular, samples LSV13, LPBS, and CPBS, and LV11 and LSV14 formed two clusters that are conserved in both the distributions. However, a contrasting pattern was observed for the samples CSF94, CSF56, and CSCS, when comparing microbiome and gases ordinations. Indeed, a Mantel test showed an overall non-significant correlation between taxa and gases composition (*p*-value > 0.1).

To assess the possibility that one (or more) gases could affect the taxonomic distribution, the fitting of the interstitial gases composition into taxa ordination analysis was performed, resulting in

significant *p*-values for decane ($p = 0.009$), argon ($p = 0.018$), i-octane ($p = 0.029$), and undecane ($p = 0.044$) (Figure S3).

Beta diversity between plant samples was analysed through CA analysis (Figure 4d) and revealed a marked separation between Levante Bay and La Fossa Crater samples, on the CA1. Permutational multivariate analysis of variance between the two sites gave back a $R^2 = 0.23$ and a *p*-value = 0.047, resulting in a statistically significant difference between the plants bacterial communities of La Fossa Crater and Levante Bay.

3.3. Culturable Bacterial Community Analysis

Due to the very scarce amount of microbiological studies concerning the cultivable microbiome of Vulcano Island, we performed the isolation of cultivable bacteria from the samples collected in this study. The analysis of the titer of cultivable bacteria in the samples revealed that they contained from 5×10^2 to 1.5×10^5 CFU/g (Table 2).

Table 2. Table counts of cultivable bacteria in the collected samples.

No.	Code	Site	Sample	Bacterial Plate Count (CFU/g)	Number of Isolated Strains
1	CPA	La Fossa Crater	Plant Aerial Part	$1.5 \pm 0.7 \times 10^4$	14
2	CPR	La Fossa Crater	Plant Roots	$3.8 \pm 0.6 \times 10^4$	5
3	CPRS	La Fossa Crater	Plant Rhizospheric Soil	$2.7 \pm 2.5 \times 10^4$	11
4	CPBS	La Fossa Crater	Plant Bulk Soil	$9.5 \pm 0.7 \times 10^3$	14
5	LPA	Levante Bay	Plant Aerial Part	$1.03 \pm 0.09 \times 10^3$	16
6	LPR	Levante Bay	Plant Roots	$4.9 \pm 0.9 \times 10^3$	16
7	LPRS	Levante Bay	Plant Rhizospheric Soil	1.1×10^4	9
8	LPBS	Levante Bay	Plant Bulk Soil	$6.1 \pm 1.4 \times 10^4$	14
9	CSF94	La Fossa Crater	Soil, 94 °C	0	0
10	CSF56	La Fossa Crater	Soil, 56 °C	0	0
11	CSCS	La Fossa Crater	Soil	$5 \pm 0.007 \times 10^2$	2
12	LSV11	Levante Bay	Soil	$1 \pm 0.7 \times 10^4$	15
13	LSV13	Levante Bay	Soil	$1.2 \pm 0.2 \times 10^5$	46
14	LSV14	Levante Bay	Soil	$1.4 \pm 1.6 \times 10^5$	32

All the isolates were screened through RAPD analysis in order to split them into groups, on the basis of their RAPD profile (hereinafter haplotype), assuming that isolates exhibiting the same haplotype correspond to the same strain. For one strain for each haplotype, a 16S rRNA gene sequencing was performed, in order to taxonomically affiliate the strain. Table S4 reports the list of strains isolated from the samples collected in this study.

4. Discussion

The comprehension of the mechanisms that may shape bacterial communities living in extreme environments is crucial from both evolutionary and ecological viewpoint. In this context, Vulcano Island (Aeolian Islands, Sicily, Italy) represents an interesting field of study for analysing bacterial communities of extreme environments, since it offers a multitude of different situations in a very narrow area. Even though previous studies have been carried out on microbial communities of Vulcano Island, very little is known about the possible correlation between interstitial soil gases and soil bacteria. Thus, in this work, we aimed to analyse bacterial communities from different substrates of Vulcano Island and explore the possibility that these bacteria may participate in the production of interstitial gases.

4.1. Bacterial Communities Study

A wide number of studies on extreme environments microbial communities has been carried out in the last decades. Chan et al. performed a similar bacterial communities' study on Malaysian circumneutral hot springs, finding that *Firmicutes* and *Proteobacteria* dominated this environment.

Medrano-Santillana et al. found that the bacterial communities in volcanic soils were characterized by the prevalence of *Firmicutes*, *Proteobacteria*, and *Actinobacteria* [65,66]. The same phyla were disclosed in this study, along with others, such as *Planctomycetes* and *Bacteroidetes*, in agreement with other studies showing that members of these phyla have been found in a wide variety of environments, confirming their strong versatility.

4.1.1. Soil Samples Analysis

Analysing the composition of the bacterial communities of La Fossa Crater and Levante Bay samples, it was shown that Crater samples exhibited a lower biodiversity with respect to Levante Bay ones (Figure 3a,b). Considering the Shannon diversity of the bacterial communities of La Fossa Crater and Levante Bay soil samples, it was shown that Crater samples exhibited a negative trend between the Shannon diversity index and the proximity to the fumarolic emission, in agreement with previous studies showing that extreme environments are less diverse than other ones [67]. Moreover, Crater samples, with the exception of CPBS sample, showed a major taxonomic uniformity compared to Levante Bay ones, confirming that more emissive environments select only few taxa able to survive under these conditions (Figure 4a).

The correlation between the soil bacterial composition and interstitial soil gases composition was analysed. The ordination analyses resulted in different spatial distribution of samples between the taxa composition CA plot and the gases CA plot (Figure 4a,b). These data did not highlight the existence of a direct influence of bacteria on the interstitial gases' composition, or *vice versa*. However, bacterial communities of Levante Bay samples differentiate from each other on the basis of the sampling sites (they present differences in the relative abundances of the main phyla detected, that are *Proteobacteria*, *Firmicutes*, *Planctomycetes*, and *Actinobacteria*), but belong to the same clusters in the interstitial gases ordination analysis; therefore, we can hypothesize that, in spite of their taxonomical diversity, they could share metabolic abilities related to the gas composition. On the other hand, we can also speculate that sites with similar bacterial communities' composition and different interstitial gases (i.e., Crater samples) could host different genetic repertoire responsible for different functions within the same taxonomic unit.

Performing a CA analysis on the taxa composition collapsed at the genus level (Figure 4c); the distribution of samples obtained appeared to have some similarities to the distribution based on interstitial gases composition. This could suggest the possibility of a direct relation between some soil bacterial genera and interstitial soil gases. However, a Mantel test did not support statistical correlation, possibly in relation to the relatively low number of samples analysed. Anyway, we can hypothesize that some genetic traits, possibly related to the presence of some interstitial gases, could be shared among the same genus.

Further, the fitting of the interstitial gases' composition into the taxa ordination analysis was performed to evaluate the possibility that one (or more) gases could affect the taxonomic distribution. The analysis suggested that decane, argon, i-octane, and undecane could be related with the different composition of the microbial communities; however, data obtained from this study do not allow to understand whether the gases affect the taxa distribution, or if bacteria are involved in the production of such gases. Moreover, since these results do not represent a cause-effect relation, it is not possible to know which group of bacteria is responsible for such evidence.

4.1.2. Plant Samples Analysis

It is known that microbial communities associated with plants can establish relationships with the host, giving benefits to both parts [23]. Accordingly, in an extreme environment, it is plausible that the role of bacteria could be of major importance for the survival of the plant [24].

In this study, we choose a pioneer plant belonging to the *Poa* genus, since they are widely distributed in volcanic environments, as a model to study the bacterial communities associated with it.

The bacterial communities of plants tissues and the surrounding soil from both Levante Bay and La Fossa Crater presented the predominance of *Actinobacteria* and *Proteobacteria*, except for two Crater soil samples (bulk soil and rhizospheric soil) in which *Chloroflexi* are the most abundant phyla detected (Figure 2). In a previous study [68], *Chloroflexi* phylum has been found as dominant group in the Atacama Desert, the oldest and driest desert located in South America. So, it is possible that the aridity might represent a driving force for the development of such communities. At the same time, the absence of this phylum in the Crater soil samples CSCS, CSF56, and CSF94 could be due to the presence of higher fumarolic emissions in these sites.

Evaluating the distances between the samples, ordination analysis resulted in a marked separation between Levante Bay and Crater samples showing that, despite the differences between the plant compartments analysed, the composition of bacterial communities between the two sampling areas is more different than between the plant compartments of the same area (Figure 4d).

4.2. Cultivable Bacteria

Due to the very scarce amount of microbiological studies concerning the cultivable microbiome of Vulcano Island, we also isolated cultivable bacteria from the samples for further analyses and future perspectives. About the soil samples, the vital titer resulted in a difference between sampling areas: In particular, samples collected in La Fossa Crater showed a lower amount of cultivable bacteria, with a total absence in the samples CSF94 and CSF56, collected at the temperatures of 94 °C and 56 °C, respectively, and in proximity of the fumarolic emission. Concerning the plants samples, there are no obvious differences between the sampling sites.

Analysing the 16S rRNA gene of the isolates (Table S4), a prevalence of the *Bacillus* genus in Levante Bay soil and plant tissue samples was observed, while in Crater samples, a higher diversity of cultivable bacteria was detected, with isolates mainly belonging to *Arthrobacter* and *Staphylococcus* genera, and the almost total absence of *Bacillus* genus.

5. Conclusions

Vulcano Island is an interesting place harbouring different geochemical situations able to shape bacterial communities. In this context, our work represents one of the first geo-microbiological approaches for the study of volcanic environments. Data obtained on the composition of the bacterial communities from different sites collapsed at the genus level with different emission profiles suggested the possibility of a correlation between the two parameters. It is plausible to imagine that the ability of different bacterial genera/species to survive in the same area might be due to the selection of particular genetic traits, allowing the survival of these microorganisms. On the other side, the finding that microbial communities inhabiting different sites exhibiting different emission profiles are similar might be explained on the basis of a possible sharing of metabolic abilities related to the gas composition.

Moreover, analysing the gases individually, a possible correlation between decane, argon, i-octane, and undecane and the different composition of the microbial communities emerged, even though it is not possible to know which group of bacteria is responsible for such evidence.

Additional analyses are still required, involving a major number of samples and a targeted analysis on archaea communities, combined with gas composition profiling.

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