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Article

DNA Metabarcoding of Soil Microbial Communities in a Postvolcanic Region: Case Study from Băile Lăzărești, Romania

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Abstract

This study investigates the impact of post-volcanic gas emissions on soil microbial communities in the Băile Lăzărești region (Romania). Nineteen soil samples across a CO_2 gradient ranging from background levels to ≈46,221 ppm. Methane and hydrogen sulfide showed localized peaks (CH_4 up to 8271 ppm; H_2S up to ~10.12 ppm), with CH_4 contributing to outlier community patterns. eDNA metabarcoding identified 3064 OTUs, (2463 bacterial and 601 fungal). Bacteria were dominated by Proteobacteria, fungi by Ascomycota, with Thelebolales nearly ubiquitous. Alpha diversity (Chao1, Fisher) declined significantly in high-CO_2 soils (>3000 ppm), while intermediate concentrations (1000–3000 ppm) showed heterogeneous responses. Beta-diversity analyses (PCoA, clustering) revealed distinct grouping of high-CO_2 soils, with sample P16 (CH_4-rich) forming an outlier. A PCA including CO_2, CH_4, and H_2S confirmed CO_2 as the main driver of variance (>65%), with CH_4 accounting for local effects. At the genus level, *Acidobacterium*, *Granulicella*, *Streptomyces*, and *Nocardia* increased with CO_2, while *Rhizobium* and *Pseudomonas* declined. Fungal responses were mixed: *Thelebolus* and *Cladosporium* increased, whereas *Mortierella* and *Cryptococcus* decreased. Overall, elevated soil CO_2 reduced microbial richness and reorganized communities, while CH_4 shaped local niches. These findings provide key natural analog insights for assessing ecological risks of CO_2 leakage from geological storage.

Keywords: Băile Lăzărești; bacterial diversity; CO_2 emissions; DNA metabarcoding; fungal diversity; soil microbial communities; volcanic gas emissions

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1. Introduction

The Sixth Assessment Report of the [1] attributes the observed increase in global temperatures primarily to anthropogenic emissions of greenhouse gases, emphasizing the urgent need for large-scale interventions to reduce these emissions. Carbon capture and storage (CCS) is one of the promising and recommended [1,2] methods for reducing greenhouse gas levels. However, responsible implementation of CCS and especially of the storage part of the chain requires a comprehensive risk assessment, particularly considering the potential leakage of CO_2 from storage sites to near-surface environments, which could affect ecosystems and human health [3,4].

A number of studies have explored the impact of increased atmospheric CO_2 on ecosystems [5]. However, few have examined the effects on subsurface ecosystems exposed to the upward migration of CO_2 from storage sites. Examples include studies of terrestrial CO_2 vents in Italy [6,7], volcanic emissions in California [8], and the impact of geothermal CO_2 on vegetation in Slovenia [9,10]. These studies reveal how elevated soil CO_2 alters soil chemistry, mineralogy, and affects both vegetation and microbial communities.

CO_2 geological storage has a potential environmental impact if CO_2 leaks from deep storage, possibly altering groundwater quality by reducing pH, increasing salinity, and mobilizing harmful solutes [11–13]. CO_2 exposure also affects soil microbiota, influencing microbial metabolism, community composition and diversity [14]. Research on volcanic vents, natural analogs of CO_2 seepage, has shown that microbial communities adapt to high-CO_2 conditions where acidophiles or anaerobic autotrophs dominate [15].

While short-term studies on CO_2 leakage offer insights, they are limited by short observation periods, typically months to a few years, while CO_2 exposure in CO_2 geological storage can last for centuries. This has led researchers to explore natural analogs—places where CO_2 seeps naturally, such as volcanic gas vents and geysers—providing a model for understanding long-term ecosystem responses to CO_2 exposure [7,16,17]. The responses observed in these analog sites indicate significant changes in microbial populations and soil chemistry under sustained CO_2 exposure.

The Băile Lăzărești site, located near the town of Băile Tușnad, has a long history of research centered on the mineral springs and gas emissions characteristic to post-volcanic activity. As early as the 1950s, scientific studies documented the chemical composition of the mineral waters and the gases emitted from local mofettes. Significant insights are recorded in works such as "Mineral and Thermal Waters of Romania" [18] and "Therapeutic Mineral Substances of Romania" [19] by Artemiu Pricăjan. By 1974, an inventory compiled by Zoltán Rákosy detailed 44 mineral springs in the Lăzărești area alone, underscoring the region's rich hydrogeological diversity [20]. From the 1970s through the early 2000s, geologists and chemists from the Harghita Geological Research and Exploration Company conducted extensive mapping and chemical analyses of the region's acidulated waters and mofettes. These findings, published by Berner, Jánosi, and Péter in 2000, provided comprehensive data on 16 mineral springs on the northern slope and 18 on the southern slope of the Ciomad Massif [21]. Additionally, studies of the Ciomadul region continued, with analyses describing eutrophic and oligotrophic peat bogs, as well as the flora and fauna that thrive in mineral-rich, gas-emission areas.

The geological structure around Lăzărești is shaped by deep fault systems that facilitated the development of volcanic structures and ongoing post-volcanic activity, including significant gas emissions. Geological surveys have identified a complex system of crustal, regional, and local faults, which define the tectonic and hydrogeological framework of Harghita County. These features, combined with the region's unique sedimentary and volcanic deposits, contribute to the geological and ecological significance of the Băile Lăzărești area, offering a valuable site for ongoing studies on mineral springs, gas emissions, and biodiversity.

The aim of this study is to investigate the ecological and microbial responses to naturally elevated soil CO_2 levels in Băile Lăzărești, Romania, an area affected by post-volcanic emissions. By analyzing soil microbiota and geochemical changes in response to CO_2 migration pathways, the study seeks to understand the long-term impacts of CO_2 leakage on soil ecosystems, particularly in terms of microbial community composition, biodiversity, and soil chemistry. These findings will contribute to assessing the ecological risks associated with geological carbon storage and CO_2 leakage, providing insights relevant for environmental monitoring in similar high-emission areas.

2. Materials and Methods

2.1. Study Area

The study area surrounding Băile Lăzărești, approximately 16 km northeast of Băile Tușnad, is renowned for significant post-volcanic activity. The last eruption of the Ciomadul volcano occurred about 27–35 ka ago [22], yet volcanic gases continue to reach the surface. These emissions manifest as dry and wet gas vents (mofettes) in areas like Lăzărești, Băile Tușnad, and Bálványos, where the local population has historically used them for medicinal purposes.

In addition to concentrated emissions, gases diffuse through the soil, impacting vegetation—often causing absence or alteration—and rock formations, marked by secondary mineral precipitations. Within the selected measurement area, locals have developed several springs and wells, notably Nagyborvíz (a calcium–magnesium carbonated mineral water) and Fortyogó and Nyírfürdő (Nyír Baths), which feature outdoor pools, foot basins, and a mofette used for treating rheumatic, vascular, and skin conditions. Residents collect this mineral water for household use [23].

On Borvíztető ridge, additional calcium–magnesium bicarbonate mineral springs dry up in droughts and function as dry gas vents. The area is divided into two zones for research: a northern zone with high-CO_2 emissions, including therapeutic baths with wet and dry gas emissions, and a southern zone with lower, primarily dry emissions.

2.2. Field Campaigns

Two field campaigns have been conducted in the summer of 2022, in July and August for assessing the soil gas flux and for sample collections. Two profiles have been selected as representative, one in the northern zone with 10 sampling points and one in the southern zone with 9 sampling points (Figure 1).

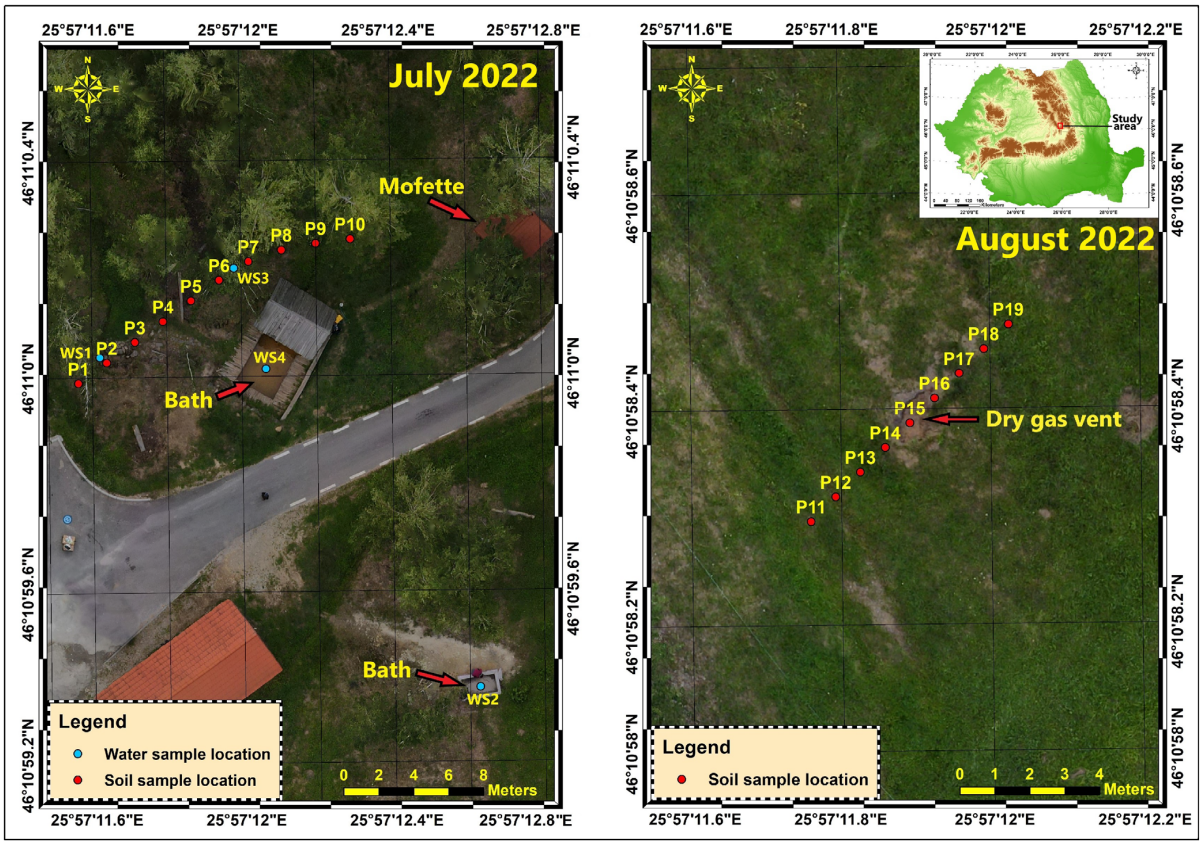

Figure 1. Sampling stations on the Băile Lăzărești area.

Gas flux measurements (Figure 2) were carried out during both field campaigns. Soil gas flux was measured using a portable flux meter from West Systems (Pontedera, Italy), equipped with CO_2 (Li-COR LI-830-3, 0–2%; Vaisala GMP251, 0–20%), CH_4 (WS-CH4-TLD), and H_2S (WS-TOX-H2S) sensors. The flux data was processed using FluxRevision software version 4.11 which resulted in flux values for each measured gas and average gas concentrations. For the current study, correlations were conducted exclusively with CO_2 concentrations, as CO_2 was the dominant gas across the study area and provided the most consistent and spatially extensive dataset. Methane (CH_4) and hydrogen sulfide (H_2S) concentrations were either too low, highly localized, or irregular to support robust statistical comparisons.

Figure 2. Gas measurements in the summer campaign from 2022.

In addition to gas measurements, soil samples were collected (Figure 3) in the same sample points, immediately after measuring gas flux. Samples were taken in sterile plastic containers (50–100 g each, depending on the material's physical state), sealed, and stored to prevent contamination. These samples were then frozen and sent to Nature Metrics, a specialized laboratory in the UK, for DNA sequencing to analyses the microbiome (bacteria and fungi) present, both qualitatively (species identification) and quantitatively.

Figure 3. Soil sampling from the area with high emissions of the Lăzărești site.

The mofetta field at Băile Lăzărești extends over more than 1 ha, with multiple active degassing spots covering several hundred square meters. Because gas emissions are spatially widespread across this area, even the lowest emission points (<1000 ppm CO_2) were considered as internal controls. Establishing an external control would have required sampling soils located far outside the mofetta field, thus removing them from the geological and ecological framework of the study site.

Atmospheric Gas Composition

In addition to soil gas fluxes, the composition of the near-surface atmosphere in the Ciomadul–Băile Tușnad area has been investigated in recent studies using Multi-GAS GFM 436 (Gas Data, Coventry, UK) instruments and direct sampling. Measurements at mofettas and caves (e.g., Stinky Cave, Turia; Băile Tușnad mofetta) consistently show that the gas phase is strongly dominated by CO_2, typically accounting for 95–99% of the composition, with minor fractions of CH_4 and H_2S [24]. Annual CO_2 emissions were estimated at ~1923 tons for the Stinky Cave mofetta [25], while the minimum total CO_2 flux for the youngest volcanic segment at Ciomadul was estimated at 8.7×10^3 t/year [24]. Methane concentrations generally remain below 5% but can locally exceed background levels, while H_2S is usually <200 ppm, with occasional local maxima explained by sulfide alteration processes [26]. By comparison, global background atmospheric values are much lower: ~420 ppm CO_2) [27], ~1.9 ppm CH_4, ~0.1 ppm CO, and ~0.0004 ppm H_2S. This highlights the strong localized enrichment in CO_2 and trace volcanic gases in post-volcanic emission areas compared to global atmospheric averages.

For sample grouping, CO_2 concentration was selected as the primary variable because it represented >95% of the measured gas fluxes and was spatially continuous across the study area. Methane (CH_4) and hydrogen sulfide (H_2S) were recorded only at localized points and in much lower concentrations, and therefore could not support robust stratification.

2.3. DNA Extraction and Sequencing

In this study, environmental DNA (eDNA) refers to the total DNA extracted from bulk soil samples, including DNA from intact microbial cells, dead cells, and extracellular fragments bound to soil particles. This approach provides an integrative snapshot of microbial community composition in the sampled soils.

Total DNA was extracted from approximately 0.5 g of each sample. An extraction blank was processed for each extraction batch. DNA was quantified using a Qubit DNA broad range kit (Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's protocol. Our analysis was based on eDNA metabarcoding targeting the V4 region of the bacterial 16S rRNA gene and the ITS2 region of fungal DNA, which allows both qualitative (taxonomic identification) and quantitative (relative abundance) characterization of soil microbial communities; DNA was amplified in triplicate PCRs with primers specific for these regions.

PCRs were performed with a negative control (the extraction blank) and a positive control (a sample known to amplify with these primers). Amplification success was assessed by gel electrophoresis. PCR replicates were pooled and purified, and sequencing indexes were added. Amplicons were purified and verified by gel electrophoresis, and then quantified using a Qubit DNA broad range kit following the manufacturer's protocol. All purified indexed PCRs were pooled into final libraries, with equal concentrations of each sample. The final libraries were sequenced using an Illumina MiSeq V3 kit (Illumina, San Diego, CA, United States) at 10.5 pM with a 20% PhiX spike-in.

2.4. Bioinformatics

Sequence data were processed using a custom bioinformatics pipeline for quality filtering, OTU clustering (97%), and taxonomic assignment. A total of 2,678,241 high-quality sequences (bacteria: 1,349,217 and fungi: 1,329,024) are represented. Consensus taxonomic assignments for each OTU were made based on sequence similarity searches against two reference databases appropriate for the dataset: one general (NCBI nt) and one specialized: Bacteria: SILVA 16S (v138.1) and Fungi: UNITE (v8.2). The GBIF (http://doi.org/10.5281/zenodo.17279548, accessed on 20 September 2025) taxonomic backbone was used to ensure consistency across databases. Results from both searches were combined, and taxonomic assignments were made to the lowest possible level where matches were consistent. Conflicting results were flagged and resolved manually. Minimum similarity thresholds of 98%, 95%, and 92% were required for species-, genus-, and higher-level assignments, respectively. Identifications based on fewer than three reference matches were flagged for review. The OTU table was filtered to remove low-abundance OTUs from each sample (<0.03% or fewer than 10 reads, whichever threshold was greater for the sample). Results are presented for OTUs identified at the target kingdom level or below.

2.5. Statistical Analysis

Statistical analyses were conducted using a combination of dedicated bioinformatics and ecological software tools. For multivariate statistics, we used PRIMER 7 with the PERMANOVA+ add-on package to test for significant differences in microbial community composition across CO_2 categories [28]. In addition, PAST v.4 (Paleontological Statistics Software; [29]) was employed for ordination and clustering analyses, including Principal Component Analysis (PCA) and hierarchical clustering (Ward's method, Bray–Curtis distances), to visualize community differentiation along the CO_2, CH_4, and H_2S gradients.

3. Results

3.1. Data Analysis

The final dataset included a total of 3064 Operational Taxonomic Units (OTUs) across the 19 soil DNA samples, consisting of 2463 bacterial OTUs and 601 fungal OTUs (Table 1). A greater number of fungal OTUs were identified at the species level compared to bacteria. This difference is attributed to differences in the availability of reference sequences for different organisms in the databases, as well as a higher frequency of assignment conflicts among bacteria, where identical sequences (100% matches) frequently corresponded to multiple species.

Table 1. Summary of the number of OTUs detected and the percentage of OTUs successfully classified at each taxonomic level for each target.

Target	Number of OTUs	Phylum	Class	Order	Family	Genus	Species
Bacteria	2463	68.3%	53.8%	39.3%	27.8%	11.3%	2.1%
Fungi	601	98.8%	89.7%	79.7%	62.6%	31.4%	11.3%

Most abundant phylum:

- Bacteria: Proteobacteria
- Fungi: Ascomycota

OTU with the highest proportion of reads:

- Bacteria: kingdom Bacteria
- Fungi: order Thelebolales

For the purpose of data analysis, we have utilized the publicly available web-based tools provided by MicrobiomeAnalyst [30], latest updated on 21 March 2025, a comprehensive platform designed for statistical, integrative and visual analysis of microbiome data. We have used the raw data (abundance tables) provided by NatureMetrics in a CSV (comma-separated values) format compatible with MicrobiomeAnalyst to generate the graphs. Taxonomy labels were in a non-specific format. For the purpose of better understanding the taxonomy, we have removed the unassigned OTUs. Data filtering involved removal of constant features in order to benefit the differential analysis and of one sample occurrences, since such appearances are often considered artifacts (https://www.microbiomeanalyst.ca/MicrobiomeAnalyst/home.xhtml, accessed on 13–28 February 2025, 8–9 April 2025). The low count filter was set to a minimum count of 4 (the default setting). A 20% prevalence filter was set, which means that at least 20% of values should contain at least 4 counts. A low variance filter was also set in place to remove 10% of features with variance measured using inter-quantile range (the default setting). Data normalization aims to address the variability in sampling depth and data sparsity to ensure more biologically meaningful comparisons. We have used total sum scaling to normalize the data (the default setting).

In order to perform the analysis, the 19 samples were assigned to three categories based on CO_2 concentration (Table 2) C1 with less than 1000 ppm CO_2 ("low"), C2 between 1000 and 3000 ppm CO_2 ("intermediate") and C3 over 3000 ppm CO_2 ("high").

Table 2. Samples and gas concentrations.

Sample	Profile	CH_4 (ppm)	H_2S (ppm)	CO_2 (ppm)	CO_2 Categories
P1	1P	275.2008	7.37361	42,653.92	C3
P2	1P	82.63361	0.453123	13,162.42	C3
P3	1P	4.494262	0.393549	3199.084	C3
P4	1P	2.43252	0.542659	601.6707	C1
P5	1P	36.94797	0.939593	5396.768	C3
P6	1P	15.77377	1.096336	2531.079	C2
P7	1P	318.7418	10.11688	46,220.71	C3
P8	1P	2.33252	0.765073	992.7963	C1
P9	1P	2.1416	0.829536	631.2909	C1
P10	1P	2.135075	0.250425	485.4114	C1
P11	2P	1.963934	0.374443	1505.181	C2
P12	2P	2.024793	0.366752	929.5848	C1
P13	2P	1.887705	0.344893	977.153	C1
P14	2P	2.447934	0.336711	1608.869	C2
P15	2P	19.968	0.44908	3447.992	C3
P16	2P	8271.474	0.384769	1456.66	C2
P17	2P	3.504132	0.33995	1173.752	C2
P18	2P	1.856911	0.349366	1520.143	C2
P19	2P	1.858537	0.382748	785.9752	C1

3.1.1. Bacteria

In the bacterial dataset, OTUs were identified in 25 distinct phyla from the kingdom Bacteria. The average bacterial taxon richness per sample, based on rarefied data, was 418.8, with values ranging from 79 (sample P6) to 745 (sample P9). The overall taxonomy of the detected OTUs is illustrated in Figure 4, indicating that the phylum Proteobacteria exhibited the highest OTU richness. The bacterial OTU with the highest number of reads was detected in 6 of the 18 samples analyzed, while a single bacterial OTU was consistently identified in each sample. In addition, 975 OTUs (39.6%) were identified exclusively in individual samples.

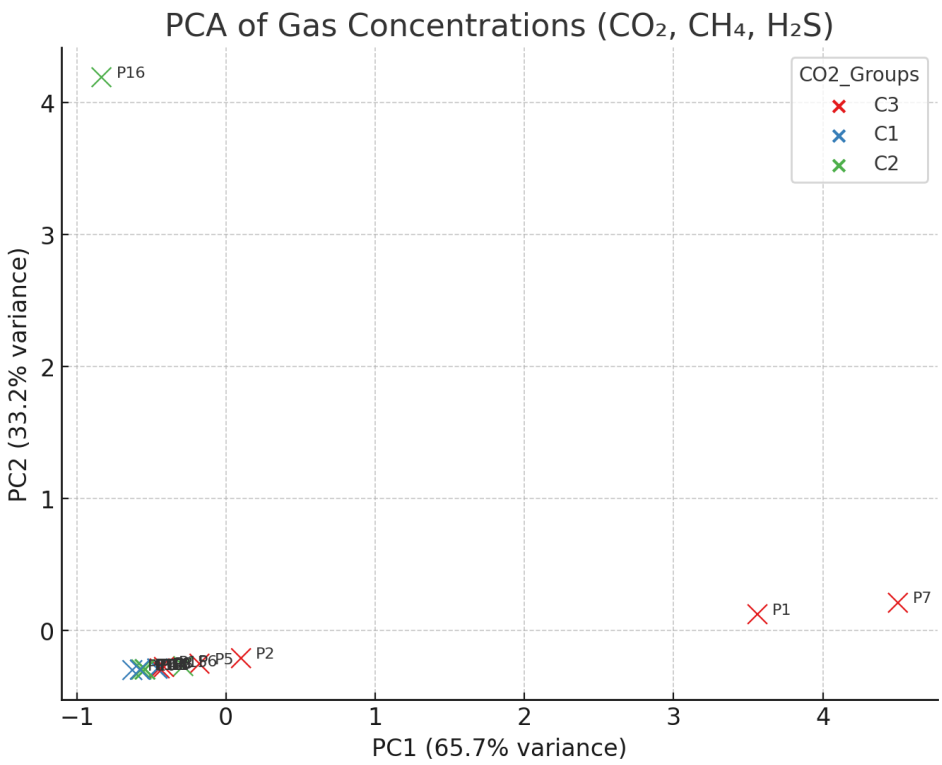

Figure 4. Principal Component Analysis of Gas concentrations (CO_2, CH_4, and H_2S).

A PCA including CO_2, CH_4, and H_2S concentrations (Figure 4) confirmed that CO_2 is the dominant factor explaining gas variability among samples. PC1 (65.7% of total variance) was strongly correlated with CO_2 and separated low-, intermediate-, and high-CO_2 soils. PC2 (33.2%) was associated mainly with CH_4, distinguishing sample P16 with the highest methane concentration. H_2S contributed negligibly, consistent with its low concentrations (<10 ppm).

The taxon richness (number of OTUs) for bacterial communities within each CO_2 concentration group is shown in Figure 5. The composition and variability of soil bacterial communities between samples are shown in Figure 6. Samples with similar community compositions tended to form distinct clusters. Considerable variability in both bacterial abundance and community composition was observed between samples. In particular, sample P16, characterized by the highest methane concentration (8271.5 ppm), represented an outlier with a significantly increased relative abundance of the bacterial classes Actinomycetia and Planctomycetes compared to other samples.

Analyzing the distribution of bacterial classes in relation to CO_2 concentration groups revealed specific trends. The classes Acidobacteriae, Actinomycetia and Bacteroidia increased in relative abundance with increasing CO_2 concentrations, while the classes Gammaproteobacteria, Alphaproteobacteria and Verrucomicrobiae showed a decreasing trend under the same conditions. However, substantial variability within the group was observed. Given this variability and the limited sample size, these trends should be interpreted with caution and further research with additional samples is needed to confirm the observed patterns. The unique microbial composition of sample P16 highlights the potential influence of elevated methane levels on bacterial community structure (Figures 7 and 8).

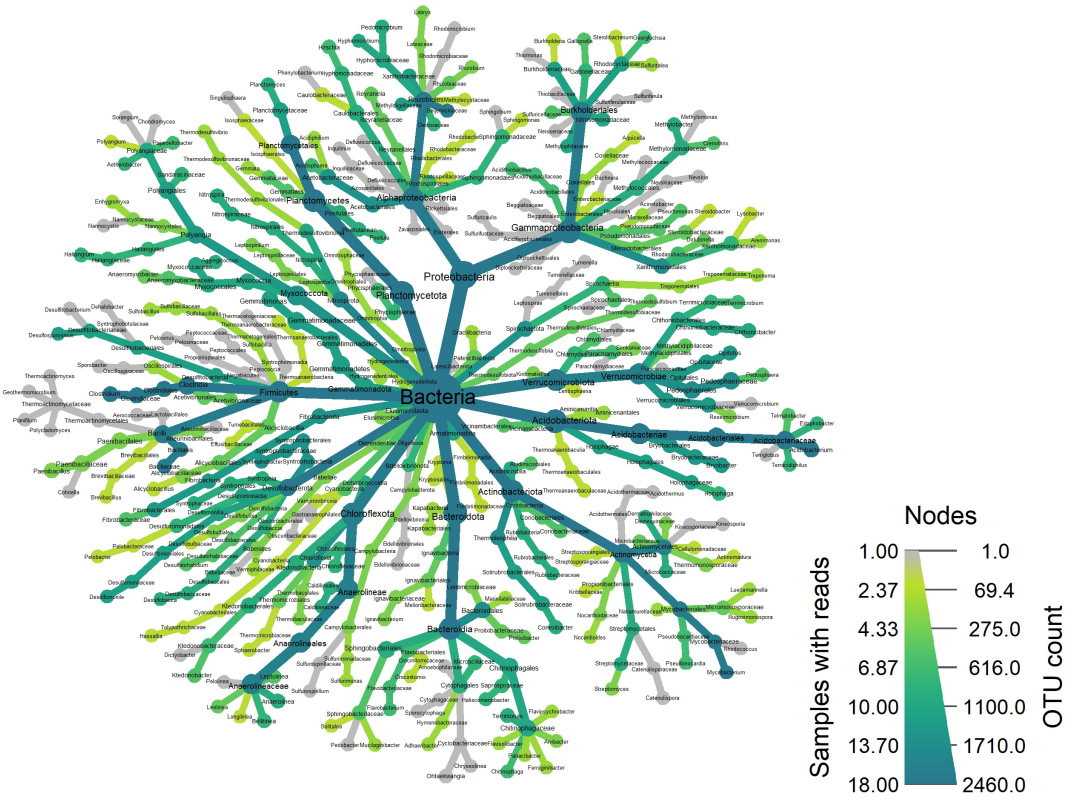

Figure 5. A taxonomic heat tree showing the number of OTUs across all samples for soil bacterial taxa down to the order rank. Each node (the circles) is a taxon and the edges (lines) show hierarchical relationships between taxa. The color scale and the relative width of the node represent the number of taxa at each level.

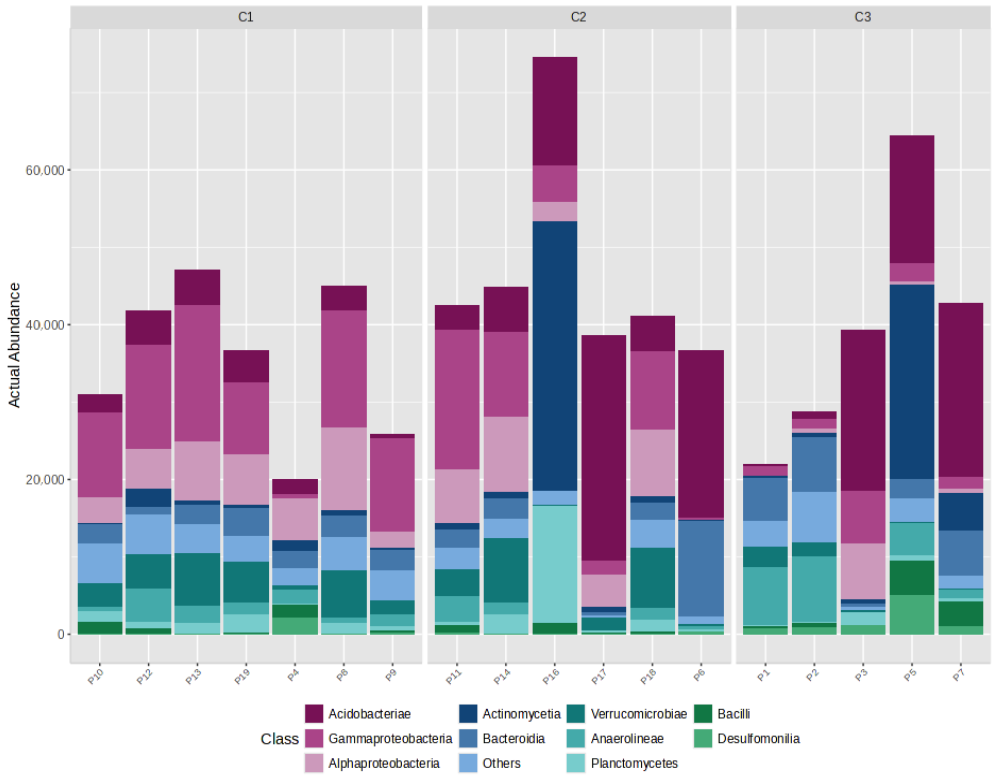

Figure 6. Distribution by class of the bacteria in the three categories C1, C2 and C3 per sample (actual abundance).

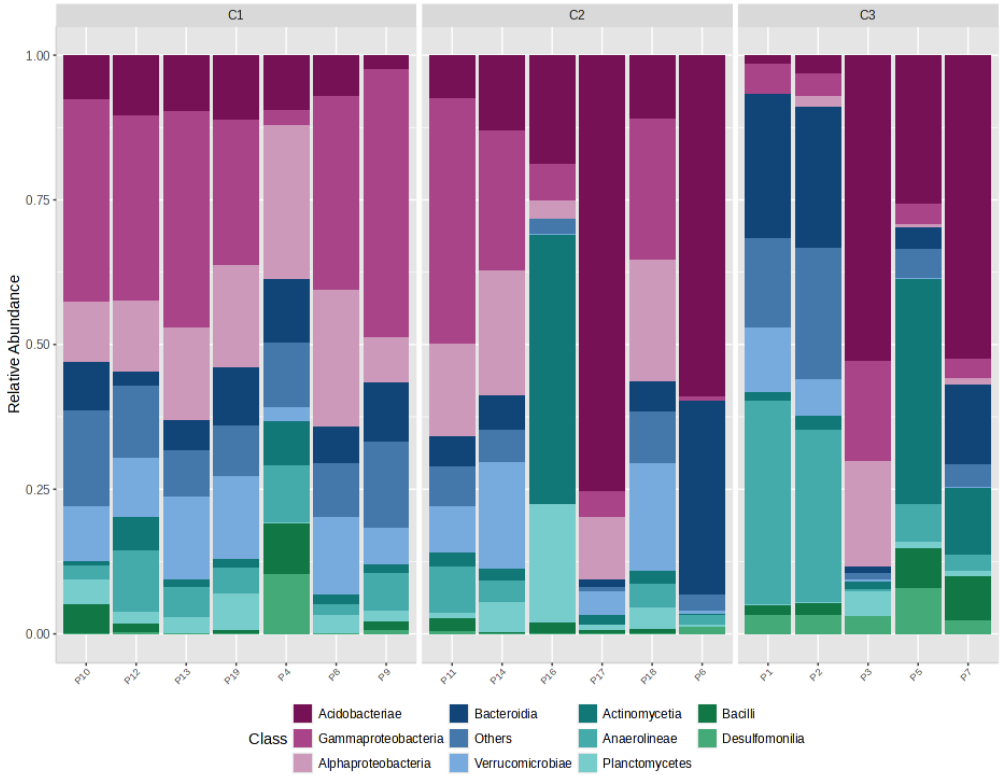

Figure 7. Distribution by class of the bacteria in the three categories C1, C2 and C3 per sample (relative abundance).

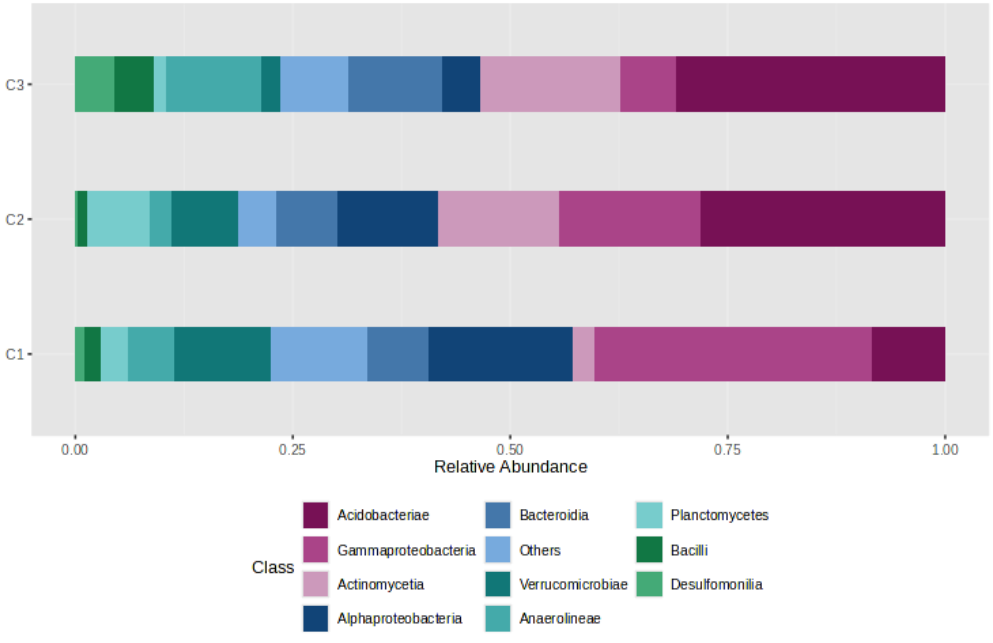

Figure 8. Distribution by class of relative abundance of bacteria in the three categories C1, C2 and C3 with samples taken together.

Alpha diversity profiling was performed using data filtered at the taxonomy level at the trait level, with the three CO_2 concentration groups defined as experimental factors. Chao1 and Fisher diversity indices were used, with statistical comparisons performed using Welch's T/ANOVA test and pairwise post hoc tests. Specifically, samples with CO_2 concentrations below 1000 ppm generally showed higher bacterial diversity, while

samples exceeding 3000 ppm showed significantly lower diversity. Intermediate CO_2 concentrations (1000–3000 ppm) showed a wide range of diversity values, indicating that this CO_2 range may correspond to diverse ecological niches that support varying levels of bacterial diversity (Figures 8–10).

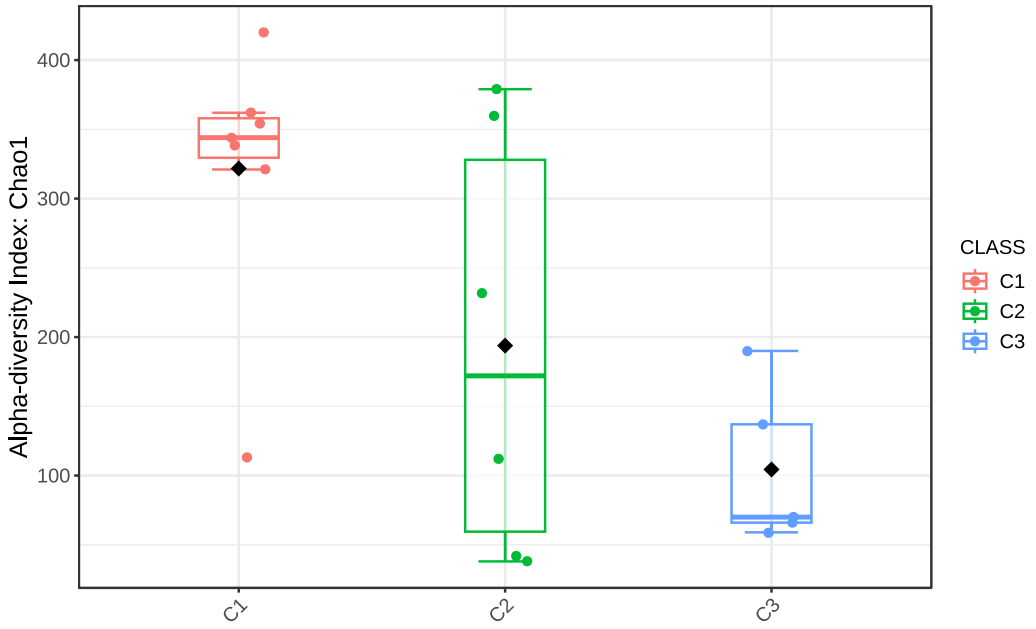

Figure 9. Boxplots of alpha-diversity index Chao1 for the three categories C1, C2 and C3.

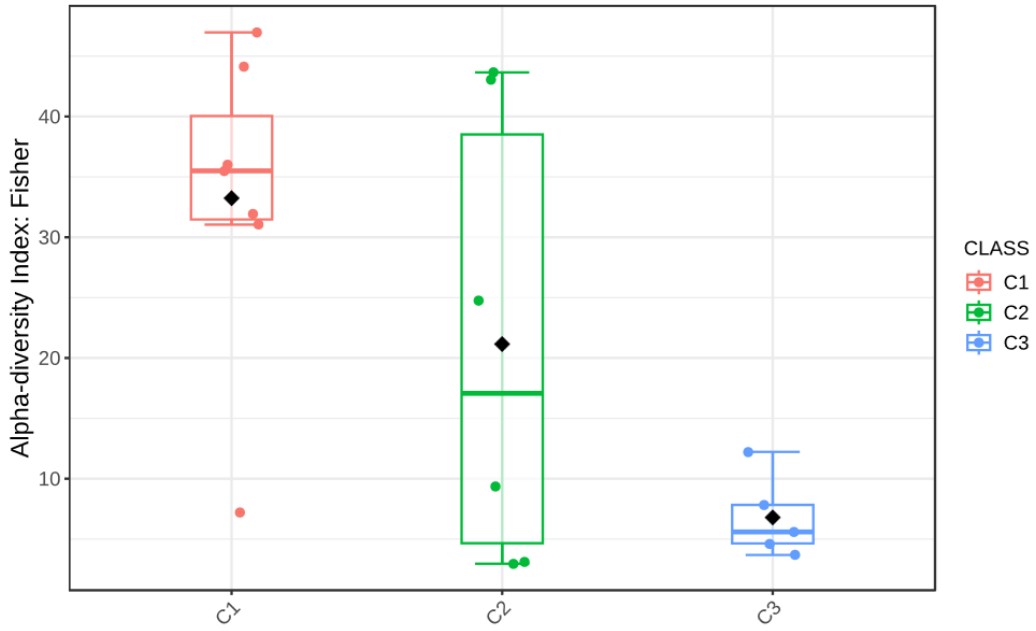

Figure 10. Boxplots of alpha-diversity index Fisher for the three categories C1, C2 and C3.

Beta diversity analyses further supported the influence of gas composition on bacterial communities. A Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarities (Figure 11) showed that high-CO_2 soils (>3000 ppm) tended to cluster separately from low-CO_2 soils (<1000 ppm), while intermediate soils (1000–3000 ppm) displayed heterogeneous positions, consistent with variable ecological niches. Hierarchical clustering analysis (Ward's method, Bray–Curtis distance) confirmed these patterns (Figure 12), with most high-CO_2 samples forming a distinct cluster, while sample P16 (characterized by

the highest CH_4 concentration) appeared as an outlier. These results indicate that CO_2 is the main driver of microbial community structure, with additional local effects of CH_4 contributing to outlier behavior.

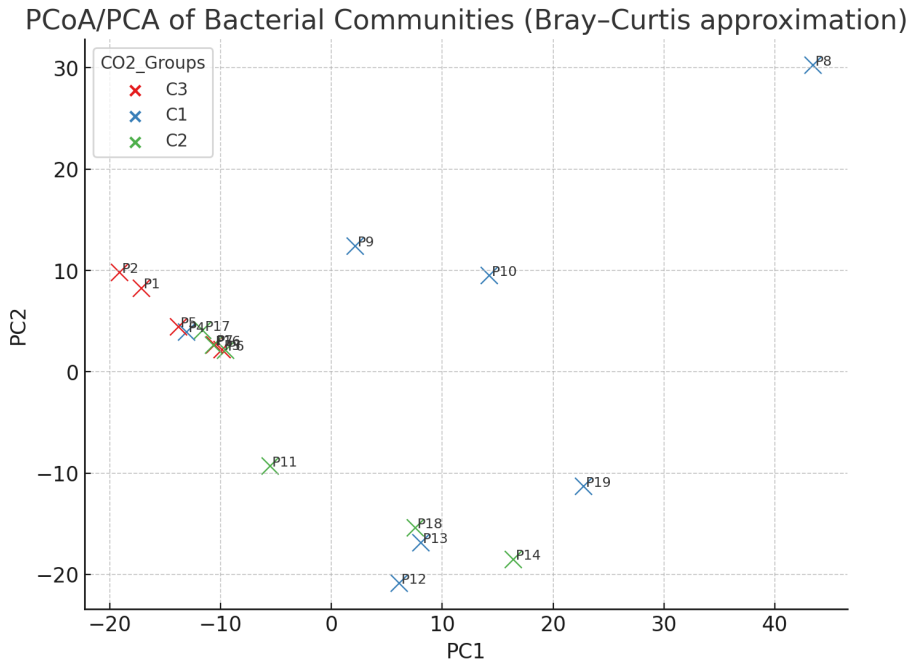

Figure 11. PCoA/PCA of Bacterial Communities (Bray–Curtis approximation).

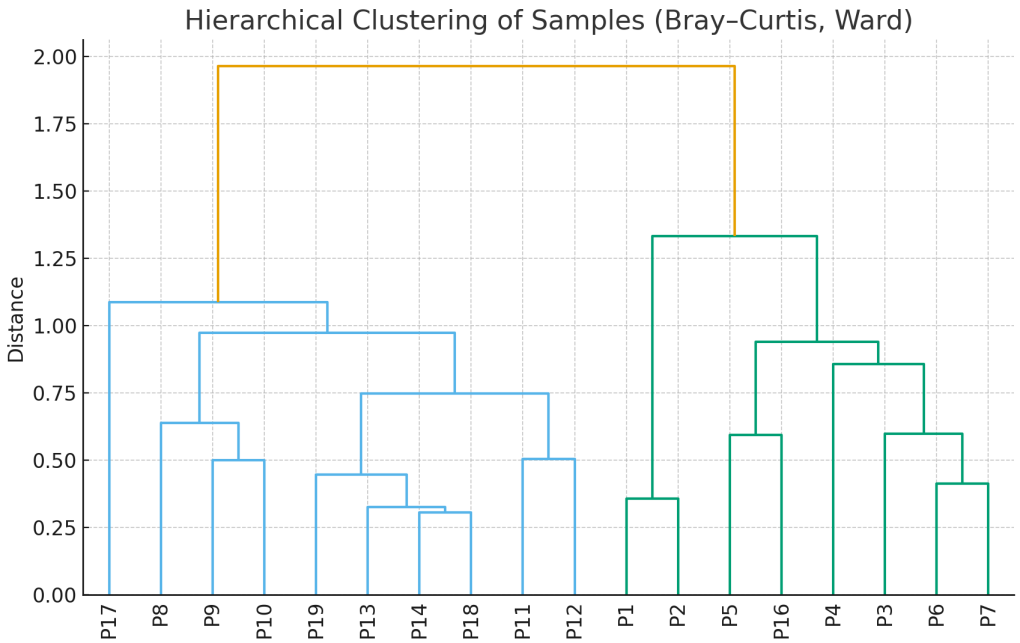

Figure 12. Hierarchical Clustering of Samples (Bray–Curtis, Ward).

Statistical testing confirmed the influence of CO_2 on microbial diversity and community composition. As shown in Table 3, Welch's ANOVA revealed significant differences in bacterial alpha diversity between low- and high-CO_2 groups (Chao1: $p = 0.017$; Fisher: $p = 0.023$). In contrast, fungal alpha diversity differences were not statistically significant (Chao1: $p = 0.11$; Fisher: $p = 0.09$). PERMANOVA analysis further supported these results,

showing a strong effect of CO_2 category on bacterial community composition ($p = 0.001$) and a weaker but significant effect for fungi ($p = 0.045$).

Table 3. Summary of statistical tests (Welch's ANOVA for alpha diversity; PERMANOVA for beta diversity) with corresponding *p*-values across CO_2 categories.

Analysis	Test	Result	*p*-Value
Alpha diversity (Bacteria, Chao1)	Welch's ANOVA + post hoc	Significant	0.017
Alpha diversity (Bacteria, Fisher)	Welch's ANOVA + post hoc	Significant	0.023
Alpha diversity (Fungi, Chao1)	Welch's ANOVA	Not significant	0.11
Alpha diversity (Fungi, Fisher)	Welch's ANOVA	Not significant	0.09
Beta diversity (Bacteria)	PERMANOVA (Bray–Curtis, 999 permutations)	Significant	0.001
Beta diversity (Fungi)	PERMANOVA (Bray–Curtis, 999 permutations)	Significant (weak)	0.045

A preliminary correlation analysis suggests that bacterial diversity decreases under high-CO_2 concentrations (>3000 ppm), while the highest richness values occur in soils with <1000 ppm CO_2. Intermediate CO_2 concentrations (1000–3000 ppm) show large variability in community composition, consistent with the existence of heterogeneous ecological niches. Moreover, sample P16, characterized by the highest CH_4 concentration (~8271 ppm), displayed a distinct community structure with increased relative abundances of Actinomycetia and Planctomycetes, suggesting a potential role of CH_4 in shaping microbial assemblages. Although these results indicate that gas composition (CO_2, CH_4) can influence bacterial diversity and community structure, the limited number of samples does not yet allow for robust statistical correlation, and further work with expanded datasets is needed to confirm these trends.

3.1.2. Fungi

In the fungal dataset, OTUs were detected in six phyla of the kingdom Fungi. The average fungal taxa richness per sample, calculated using rarefied data, was 81, ranging from a low of 3 OTUs (sample LAZ-22p-2p-05) to a high of 208 OTUs (sample LAZ-22p-2p-08). Ascomycota was the dominant fungal phylum in terms of OTU richness, while the fungal OTU with the highest number of reads belonged to the order Thelebolales, detected in 18 out of 19 samples. No single fungal OTU was universally present in all samples, and 268 OTUs (44.6%) occurred in only one sample each (Figure 13).

At the class level, Leotiomycetes and Sordariomycetes were the most abundant classes and did not show a clear association with CO_2 concentrations. However, other abundant classes, such as Dothideomycetes (Ascomycota), Mortierellomycetes (Zygomycota), and Tremellomycetes (Basidiomycota), displayed variable responses to CO_2 levels. Dothideomycetes generally increased in relative abundance with higher CO_2, although their distribution was inconsistent between samples. Mortierellomycetes and Tremellomycetes showed a trend of decreasing abundance as CO_2 levels increased, but exhibited significant variability between samples. Thus, none of these classes demonstrated sufficient consistency in abundance between samples to serve as reliable indicators of CO_2 levels (Figures 14–16).

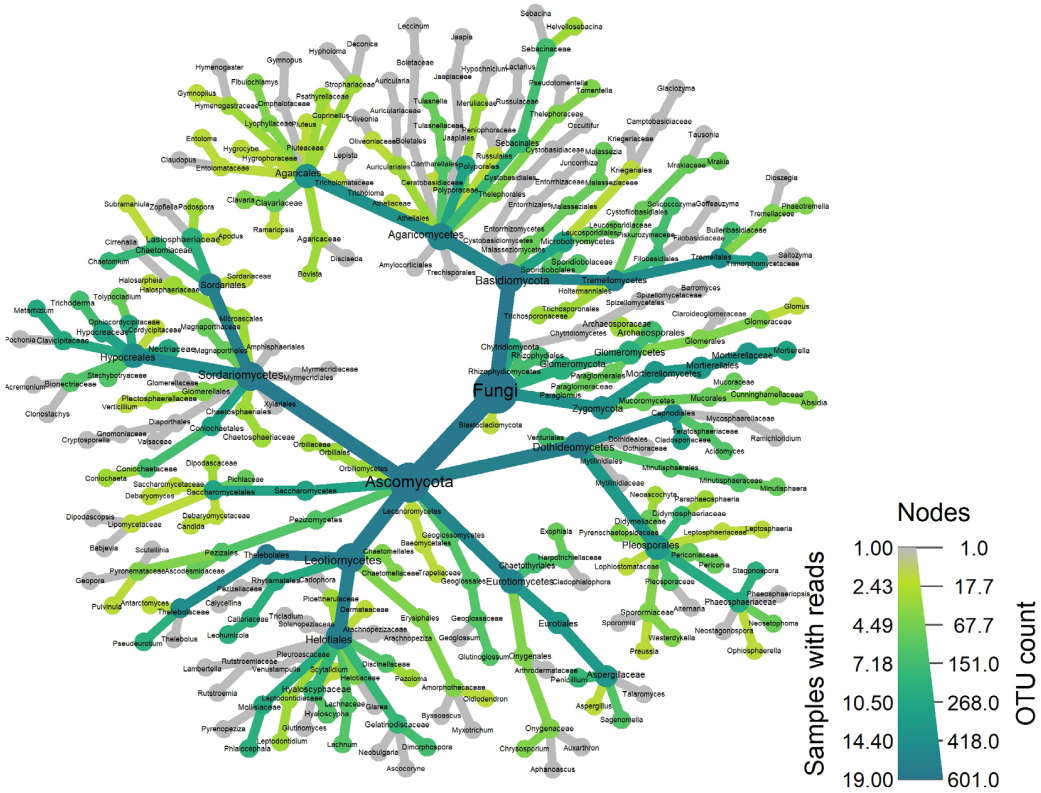

Figure 13. A taxonomic heat tree showing the number of OTUs across all samples for soil fungal taxa down to the order rank. Each node (the circles) is a taxon and the edges (lines) show hierarchical relationships between taxa. The color scale and the relative width of the node represent the number of taxa at each level.

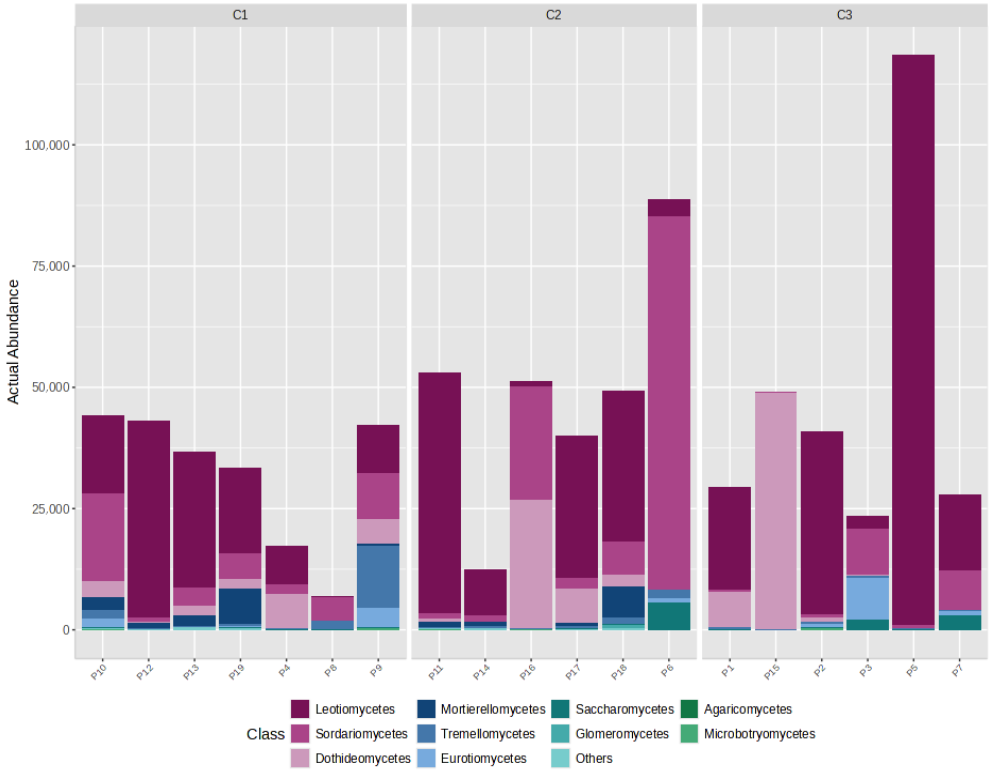

Figure 14. Distribution by class of the fungi in the three categories C1, C2 and C3 per sample (actual abundance).

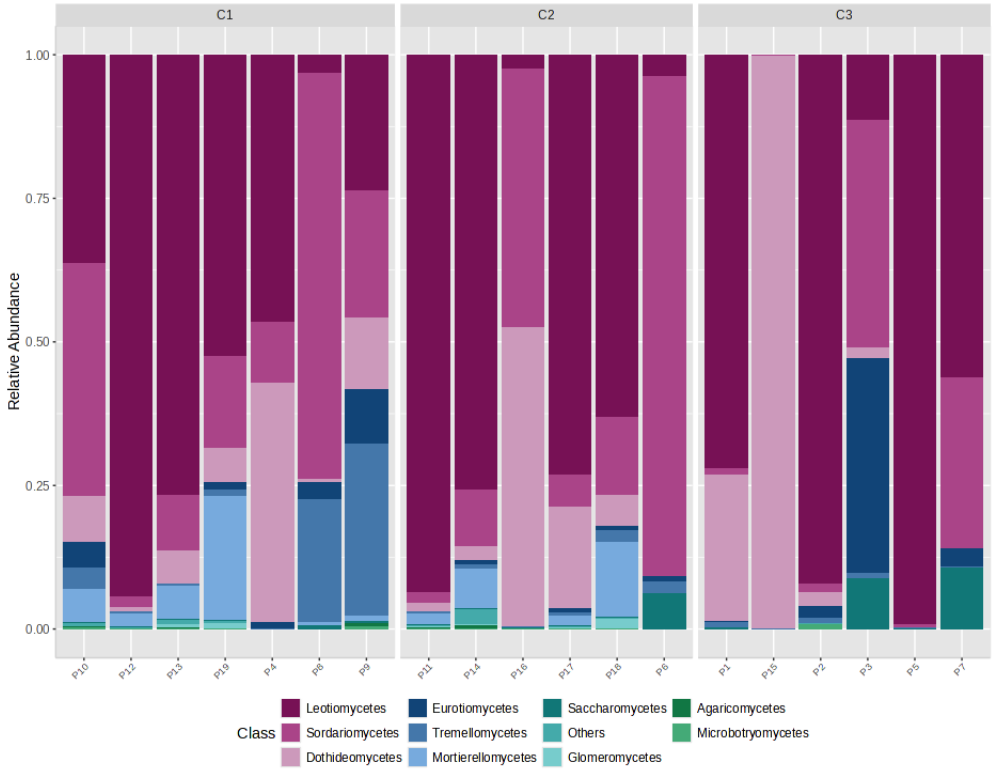

Figure 15. Distribution by class of the fungi in the three categories C1, C2 and C3 per sample (relative abundance).

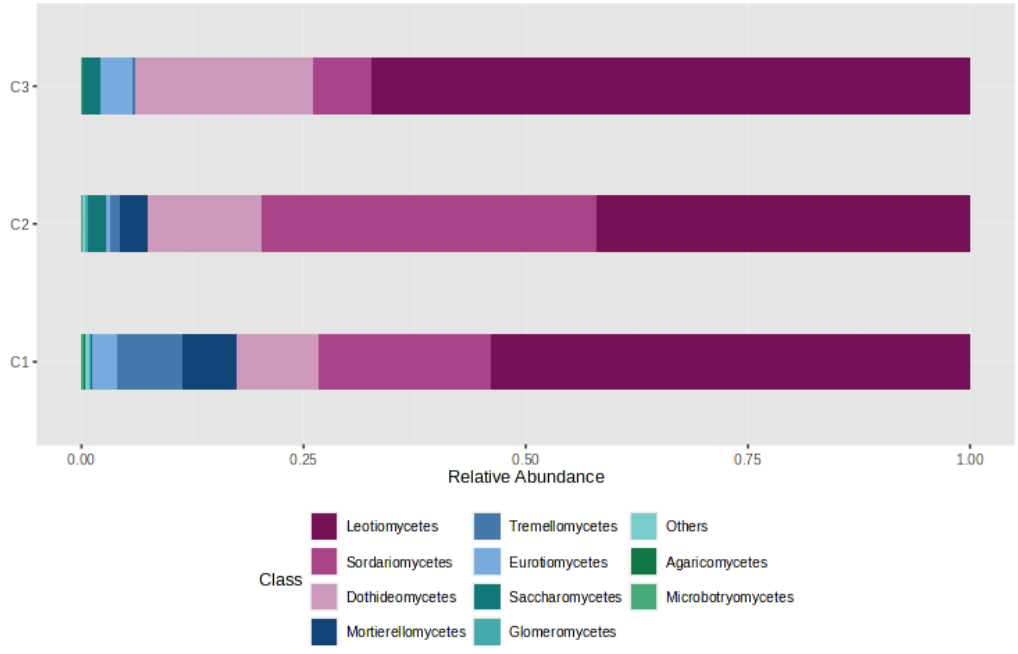

Figure 16. Distribution by class of relative abundance of fungi in the three categories C1, C2 and C3 with samples taken together.

Alpha diversity indices (Chao1 and Fisher) for fungi indicated that samples from high-CO_2 environments (>3000 ppm) generally exhibited lower diversity compared to those with CO_2 levels below 3000 ppm. Samples with lower CO_2 displayed a wide range of fungal diversity values, highlighting that fungal communities can vary significantly even under similar CO_2 conditions (Figures 17 and 18).

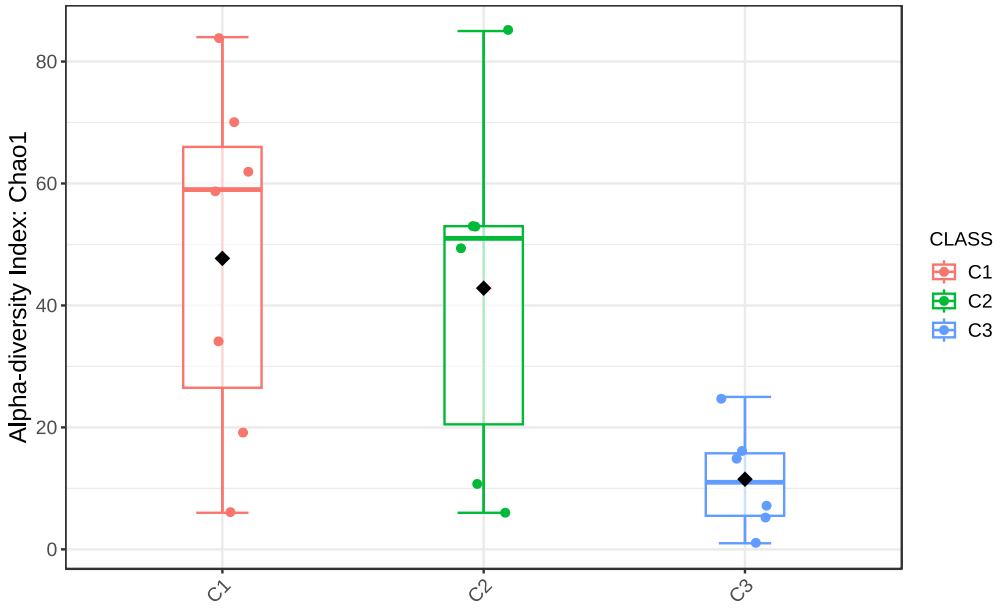

Figure 17. Boxplots of alpha-diversity index Chao1 for the three categories C1, C2 and C3.

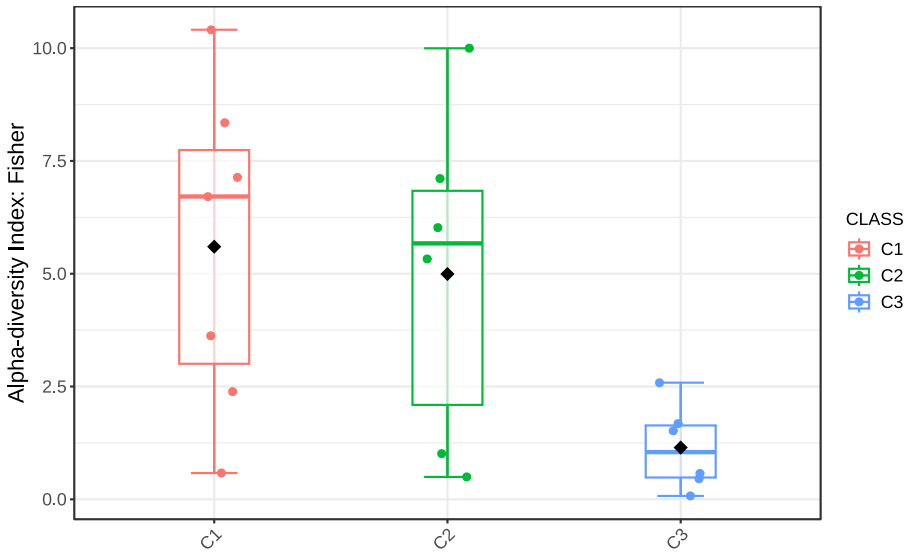

Figure 18. Boxplots of alpha-diversity index Fisher for the three categories C1, C2 and C3.

For fungi, Welch's ANOVA showed a negative trend in diversity with increasing CO_2, though differences between groups were not statistically significant (Chao1: $p = 0.11$; Fisher: $p = 0.09$). However, PERMANOVA detected a weak but significant effect of CO_2 category on fungal community composition (pseudo-F = 1.69, $p = 0.045$).

Beta diversity analyses revealed that fungal communities also varied along the CO_2 gradient. PCoA based on Bray–Curtis dissimilarities (Figure 19) showed that high-CO_2 soils (>3000 ppm) tended to cluster apart from low-CO_2 soils (<1000 ppm), while intermediate soils (1000–3000 ppm) were more scattered. Hierarchical clustering (Figure 20) confirmed this pattern, with most high-CO_2 samples forming a distinct group. However, the separation was less pronounced than for bacteria, consistent with the higher variability observed in fungal responses.

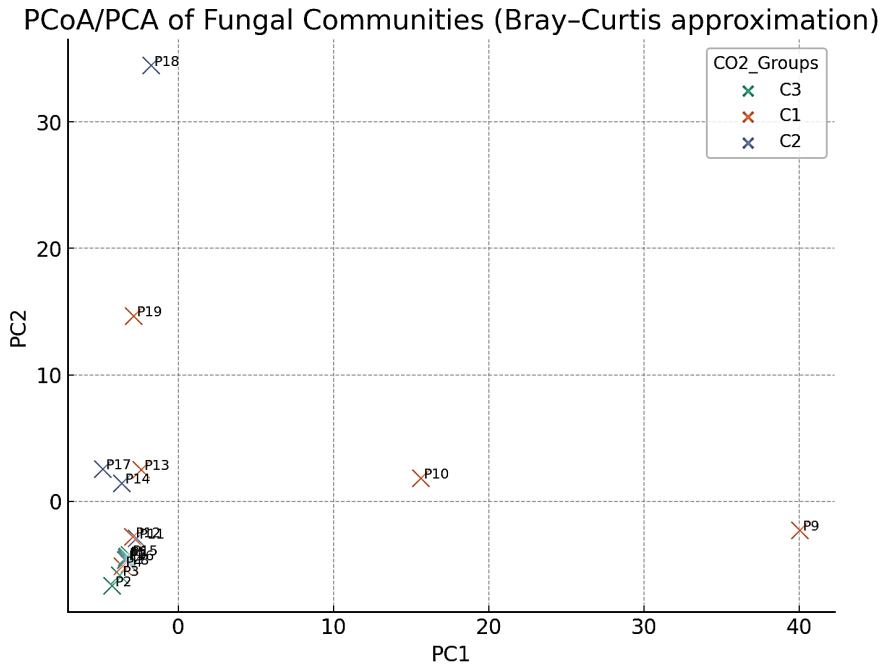

Figure 19. PCoA/PCA of Bacterial Communities (Bray–Curtis approximation).

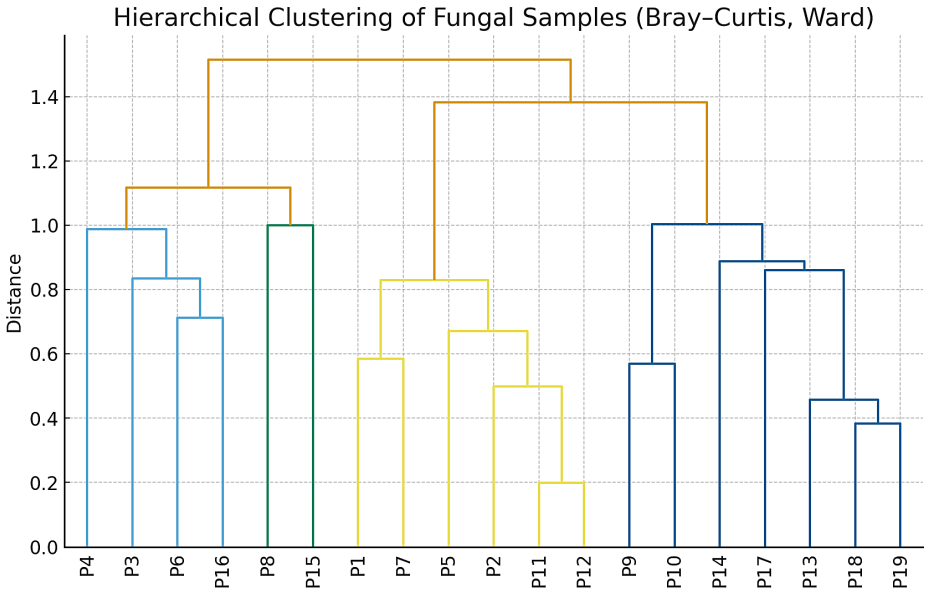

Figure 20. Hierarchical Clustering of Samples (Bray–Curtis, Ward).

4. Discussion

4.1. Quantitative Synthesis of the eDNA Set

It should be noted that our analysis is based on environmental DNA (eDNA), which captures both living and non-living DNA traces in the soil. While this provides a comprehensive view of community composition, it does not directly distinguish between metabolically active and inactive organisms.

Soil DNA analysis identified a total of 3064 OTUs in 19 samples: 2463 bacterial OTUs and 601 fungal OTUs. Species-level identifications were more frequent in fungi than in bacteria, a difference attributed to uneven database coverage and higher frequency of ambiguities in bacteria. Among bacteria, Proteobacteria had the highest OTU richness, while Ascomycota dominated among fungi [31,32]. One bacterial OTU was consistently

detected in all samples, suggesting ubiquity, while fungal OTUs from the order Thelebolales were present in almost all samples. The mean bacterial richness was 418.8 OTUs/sample (range: 79 OTUs at P6—745 OTUs at P9).

The beta diversity analyses indicate that fungal communities also shift along the CO_2 gradient, although the separation between groups was less pronounced than for bacteria. High-CO_2 soils (>3000 ppm) tended to cluster together, while intermediate soils (1000–3000 ppm) displayed high variability, suggesting that CO_2-enriched environments support diverse fungal responses depending on local conditions. This is consistent with observations from natural analog sites, where fungi show both tolerance and sensitivity to CO_2 exposure. For example, Thelebolus species are frequently reported as dominant in high-CO_2 mofettas, reflecting adaptation to acidic, CO_2-rich soils [33,34], whereas genera such as *Mortierella* tend to decline in abundance under elevated CO_2 [31]. The weaker clustering observed for fungi compared to bacteria therefore highlights their greater ecological plasticity, with some taxa persisting under stressful conditions while others are excluded.

4.2. Geochemical Context

Field measurements show that CO_2 is the dominant gas at the site scale, while CH_4 and H_2S appear mainly as local, spatially heterogeneous peaks. According to Table 2, the maxima in the set of 19 samples are: CO_2 = 46,220.71 ppm at P7 (~4.62%), CH_4 = 8271.47 ppm at P16 (~0.83%), H_2S = 10.12 ppm at P7.

Methane concentrations at Băile Lăzărești were generally lower, but local peaks were recorded (e.g., up to ~0.83% in sample P16), a pattern compatible with the influence of local ecological conditions (e.g., peaty/marshy microhabitats), also reported in studies from areas with natural CO_2 degassing, such as those investigated by [31,35]).

Hydrogen sulfide. H_2S levels were relatively low throughout the site (maximum ~10.12 ppm at P7), consistent with observations from other analogous degassing sites where H_2S occurs at much lower concentrations than CO_2 [35,36]. Variability in gas composition is plausible to contribute to differential effects on the structure and diversity of microbial communities.

4.3. Distribution of Gases by CO_2 Category (C1/C2/C3)

The samples were classified into three categories based on CO_2 concentration: C1 < 1000 ppm, C2 = 1000–3000 ppm, C3 > 3000 ppm. Based on the CH_4–H_2S–CO_2 sums in Table 2, the aggregate composition by category is:

- C1 (n = 7): $CO_2 \approx 99.66\%$, $CH_4 \approx 0.27\%$, $H_2S \approx 0.06\%$;
- C2 (n = 6): $CO_2 \approx 54.13\%$, $CH_4 \approx 45.85\%$, $H_2S \approx 0.02\%$;
- C3 (n = 6): $CO_2 \approx 99.34\%$, $CH_4 \approx 0.64\%$, $H_2S \approx 0.02\%$.

This shows that the extremes of the gradient (C1 and C3) are clearly dominated by CO_2, while the intermediate zone (C2) includes CH_4-rich micro-niches, strongly influenced by P16.

The choice of three CO_2 concentration categories was guided by ecological relevance and sample distribution. Concentrations below 1000 ppm (C1) represent near-background conditions, where microbial diversity is expected to be least affected. The 1000–3000 ppm interval (C2) captures intermediate degassing environments, often characterized by high spatial variability and the coexistence of micro-niches. Concentrations above 3000 ppm (C3) correspond to strongly degassing mofetta zones, where gas exposure is sufficiently high to induce stress responses in soil biota. These thresholds also provided a balanced number of samples per category, enabling comparative analyses of microbial community structure along the CO_2 gradient.

4.4. Gas–Bacteria Relationship

At the bacterial level, Proteobacteria remains the most abundant phylum in terms of OTU richness, but community structure reconfigures along the CO_2 gradient: classes such as Acidobacteriae, Actinomycetia and Bacteroidia increase their relative abundance with increasing CO_2, while Gammaproteobacteria, Alphaproteobacteria and Verrucomicrobiae tend to decrease. P16 represents a special case: high CH_4 concentration is associated with a relative increase in Actinomycetia and Planctomycetes taxa, suggesting that electron availability from methane may regulate community composition in anoxic or poorly oxygenated micro-niches.

At the genus level, several taxa showed consistent responses to the CO_2 gradient. *Acidobacterium* and *Granulicella* increased under high CO_2, reflecting their preference for acidic, low-nutrient soils [37,38]. *Streptomyces* and *Nocardia* (Actinomycetia) were enriched, consistent with their resilience and ability to decompose complex organic matter under stress [39]). *Flavobacterium* and *Chitinophaga* (Bacteroidetes) were more abundant in CO_2-rich soils, suggesting adaptation to altered carbon cycling and reduced oxygen availability [40]. In contrast, *Rhizobium* and *Pseudomonas* (Proteobacteria) declined at high CO_2, in line with their sensitivity to low pH and oxygen depletion [33,35].

Alpha diversity profiling (Chao1, Fisher) confirms the decrease in bacterial richness towards high CO_2 values (>3000 ppm), with pronounced variability in the intermediate range (1000–3000 ppm). This variability is plausible in a mosaic of geochemical conditions (diffuse fluxes vs. point emanations, moisture, pH, organic carbon availability), which can support contrasting ecological niches within the same CO_2 class.

Overall, the variability of bacterial composition is accentuated, with P16 (CH_4 peak) as an outlier, where Actinomycetia and Planctomycetes increase relatively. Along the CO_2 gradient, the same general trends are observed: Acidobacteriae, Actinomycetia and Bacteroidia increase with CO_2, while Gammaproteobacteria, Alphaproteobacteria and Verrucomicrobiae decrease (in the context of high intra-group variability and limited sampling). Alpha bacterial diversity is, on average, higher below 1000 ppm CO_2 and lower above 3000 ppm, and the range 1000–3000 ppm shows a wide range of responses (diverse micro-niches).

The observed decrease in bacterial diversity with increasing CO_2 and the distinct taxonomic profile in the CH_4-rich sample (P16) support the idea that gas composition exerts a selective pressure on microbial communities. Elevated CO_2 appears to reduce overall diversity, whereas localized CH_4 enrichment may create ecological niches favoring taxa such as Actinomycetia and Planctomycetes. These findings align with previous studies linking gas flux heterogeneity to shifts in microbial composition in volcanic and post-volcanic environments. While correlations in our dataset remain preliminary, they highlight the importance of integrating geochemical and microbial data to better understand ecosystem responses to gas seepage.

Correlation analysis (Spearman's rank) confirmed a significant negative relationship between CO_2 concentration and bacterial alpha diversity indices (Chao1: $\rho = -0.58$, $p < 0.05$; Fisher: $\rho = -0.62$, $p < 0.05$). This quantitative evidence supports the observed decrease in bacterial richness with increasing CO_2 levels.

4.5. Gas–Fungi Relationship

In the fungal set, Ascomycota dominates at the phylum level, and Thelebolales occurs in most samples (almost ubiquitously), suggesting a broad tolerance to acidic and CO_2-rich conditions. At the class level, Leotiomycetes and Sordariomycetes are not clearly related to CO_2 levels, while Dothideomycetes tend to increase with CO_2, and Mortierellomycetes and

Tremellomycetes tend to decrease; however, these patterns are not consistent enough to serve as robust bioindicators of CO_2 levels.

Fungal responses to CO_2 enrichment were heterogeneous at the genus level. *Thelebolus* (Thelebolales) was nearly ubiquitous, consistent with its adaptation to acidic, CO_2-rich environments [33,34]. *Mortierella* decreased in high-CO_2 soils, reflecting its sensitivity to pH and oxygen limitation [31]. *Cladosporium* showed tolerance and even enrichment under elevated CO_2, likely due to its ecological plasticity [32]. In contrast, yeast genera such as *Cryptococcus* and *Vishniacozyma* declined, reflecting their dependence on aerobic niches [41].

The dominance of Ascomycota and the prevalence of the order Thelebolales, consistent with other volcanic regions [33] (wet skunk), [34] (Hartoušov, Eger Rift)), indicate that these taxa are specifically adapted to high-CO_2 environments. However, fungal diversity was considerably lower than that reported by Zhao et al. [31] in similar areas with high CO_2 flux. This discrepancy may result from local variations in environmental conditions, such as moisture content, temperature regimes, and spatial heterogeneity of gas emissions, all of which influence fungal diversity.

Similarly to bacteria, fungal alpha diversity decreases in the high-CO_2 zone (>3000 ppm), while at low CO_2 a wide range of values is observed, highlighting the sensitivity of fungal communities to fine-spatial environmental heterogeneity. Overall, the mean fungal richness is 81 OTU/sample (range 3–208 OTU).

The CO_2 concentrations recorded at Băile Lăzărești, reaching ~4.62% *v/v* (i.e., 46,220.71 ppm in sample P7) near the wet mofettas, significantly exceed levels typically reported under similar volcanic conditions [42–46]. Such high concentrations substantially influence microbial communities, reducing their diversity and altering community composition, as documented in various studies exploring high-CO_2 environments [31–34,41].

For fungi, the correlation between CO_2 concentration and alpha diversity indices was weaker (Chao1: $\rho = -0.41$, $p = 0.08$; Fisher: $\rho = -0.39$, $p = 0.09$), indicating a negative but less robust trend compared to bacteria. These results suggest that fungal communities are also sensitive to elevated CO_2, though the strength of the relationship appears lower than in bacterial assemblages (Table 4).

Table 4. Examples of bacterial and fungal genera detected in the study and their responses to CO_2 enrichment, with supporting references. Symbols and abbreviations: ↑ = increased/relative enrichment under high CO_2; ↓ = decreased/relative depletion under high CO_2. OM = organic matter. C-rich = carbon-rich. Anoxic = low-oxygen conditions. Mofetta = natural cold CO_2 emission site.

Domain	Genus	Trend Under High CO_2	Reference(s)
Bacteria	*Acidobacterium*, *Granulicella*	↑ Enriched in acidic, CO_2-rich soils	[37,38]
Bacteria	*Streptomyces*, *Nocardia*	↑ Tolerant, degrade complex OM	[39]
Bacteria	*Flavobacterium*, *Chitinophaga*	↑ Adapted to C-rich, anoxic niches	[40]
Bacteria	*Rhizobium*, *Pseudomonas*	↓ Sensitive to acidity/anoxia	[33,35]
Fungi	*Thelebolus*	↑ Ubiquitous in mofettas	[33,34]
Fungi	*Mortierella*	↓ Reduced under high CO_2	[31]
Fungi	*Cladosporium*	↑ Tolerant, stress-adapted	[32]
Fungi	*Cryptococcus*, *Vishniacozyma*	↓ Sensitive, aerobic niches	[41]

4.6. Statistical Significance of Diversity Patterns

The statistical analyses reinforce the interpretation that CO_2 exerts a major influence on microbial communities at Băile Lăzărești. Welch's ANOVA showed significant reductions in bacterial alpha diversity between low- and high-CO_2 soils (Chao1: $p = 0.017$; Fisher: $p = 0.023$), consistent with findings from other natural analog sites where prolonged CO_2 exposure reduces bacterial richness [33,35]. In contrast, fungal alpha diversity did not differ significantly between groups ($p > 0.09$), although a general decreasing trend was observed

at high CO_2, in line with reports from mofetta soils in Italy and China where fungal responses were more variable [31,34]. PERMANOVA results demonstrated a significant effect of CO_2 concentration on bacterial community composition ($p = 0.001$), and a weaker but still significant effect on fungi ($p = 0.045$). This suggests that bacterial communities are more tightly structured by CO_2 gradients, while fungi show greater ecological plasticity and higher variability in their responses.

4.7. Site-Scale Ecological Implications

CO_2 migration along N–S oriented fractures is accompanied by vegetation stress/absence and mortality of small fauna (insects, rodents), signaling hostile conditions that reduce diversity and remodel microbial communities (Figure 21). These observations are consistent with the literature from other volcanic regions and support the use of natural sites as analogs for assessing risks in CO_2 leakage scenarios associated with geological storage [47].

Figure 21. Vegetation exposed to gas emissions.

Vegetation sparseness and plant stress observed at Băile Lăzărești coincide with zones of elevated CO_2 flux (>30,000 ppm), consistent with reports from natural analog sites where soil gas emissions suppress plant growth or cause complete absence of vegetation [6,32,46,48–52]. While gas emissions represent the primary stressor, other factors such as soil characteristics, seasonal humidity, or local hydrology may modulate vegetation responses. This interplay of drivers underscores the importance of integrated monitoring (gas flux, soil chemistry, vegetation surveys) for understanding ecosystem-level effects of CO_2 leakage.

We compared vegetation responses at Băile Lăzărești with those documented at several well-studied natural analog sites. As summarized in Table 5, high CO_2 fluxes (>2000–3000 g m^{-2} d^{-1} or >20% soil CO_2) consistently coincide with vegetation absence or stress, while transition zones with lower CO_2 concentrations often support grasses or mixed plant communities. These cross-site comparisons indicate that although soil properties, humidity, and local ecological factors can modulate vegetation cover, elevated CO_2 remains the dominant driver of vegetation sparseness in degassing areas [6,46,48–51].

Table 5. Summarizing CO_2 fluxes and vegetation responses at several natural analog sites.

Site (Reference)	Max CO_2 Flux/Conc.	Vegetation Response
Latera, Italy [6]	>2000–3000 g m^{-2} d^{-1}, >95% soil CO_2	No vegetation in vent core; transition zone with grasses/clover
Laacher See, Germany [47,48]	Up to 95% soil CO_2	Vegetation absent directly above vents
Florina, Greece [49]	Localized vents with high soil CO_2 (>20%)	Grassland degraded; plant diversity reduced near vents
ASGARD, UK [50]	Injected 2–50% soil CO_2	Crop stress, reduced biomass at >15% CO_2
Australia [51]	Fluxes >10,000 g m^{-2} d^{-1}	Vegetation death in high flux zones
Daepyeong, Korea [46]	29% soil CO_2, in high flux group	Lower pH soils, vegetation stress and reduced cover

4.8. Integration of Tracers

Together, the results paint a coherent picture: (i) CO_2 shapes microbial communities along the entire gradient; (ii) CH_4 gains weight in the intermediate zone (C2), generating distinct micro-niches (e.g., P16) with specific bacterial fingerprints; (iii) H_2S has a limited impact at the study scale; (iv) the decrease in diversity at high CO_2 implies possible effects on soil functions (C and nutrient cycling, organic matter decomposition)—more evident in fungi—relevant both for risk assessment in natural analogs of CO_2 leakage and, by extension, for the context of geological CO_2 storage.

The dominance of CO_2 at the ends of the gradient and the pronounced methane signature in C2 together explain the observed compositional and diversity changes. The results support the combined use of gas monitoring and metabarcoding as complementary tools for the ecological diagnosis of post-volcanic degassing areas and for establishing useful benchmarks in CO_2 leakage monitoring programs associated with geological CO_2 storage. These results align closely with findings from other volcanic regions [35,46], reinforcing the idea that sustained high CO_2 emissions create inhospitable conditions that lead to significant biodiversity losses.

The PCA including CO_2, CH_4, and H_2S concentrations (Figure Q) confirmed the dominant role of CO_2 as the main structuring factor across the study site, explaining 65.7% of the variance (PC1). CH_4 accounted for 33.2% of the variance (PC2) and highlighted local micro-niches such as sample P16, where elevated methane concentrations strongly influenced bacterial community structure. In contrast, H_2S contributed negligibly, consistent with its low absolute concentrations (<10 ppm) and limited spatial distribution. These results reinforce the decision to use CO_2 as the primary grouping variable, while acknowledging the secondary role of CH_4 in shaping outlier community responses. Together, the combined gas analyses confirm that the observed microbial community patterns are primarily controlled by the CO_2 gradient, with localized contributions from CH_4.

Given the large surface extent of the mofetta field (>1 ha, with several hundred square meters of active vents), low-emission soils within the area were used as controls. Selecting an external reference site with no gas influence would have required moving far away from the studied degassing zone, thereby introducing confounding differences in geology, soil type, and ecological conditions. For this reason, our design focused on internal contrasts along the CO_2 gradient, while future studies could expand to include more distant reference sites for comparison.

5. Conclusions

This study provides an integrated assessment of the spatial distribution and ecological impact of post-volcanic gas emissions (CO_2, CH_4, H_2S) at Băile Lăzărești. Gas measurements revealed substantial variability, with CO_2 dominating at the site scale (up to 46,220.71 ppm, ~4.62% *v/v* at P7), while CH_4 reached localized maxima of 8271.47 ppm

(~0.83% *v/v* at P16) and H_2S remained low (10.12 ppm at P7). These spatially heterogeneous peaks reflect the influence of gas migration along fractures and microhabitat conditions. Soil eDNA metabarcoding identified 3064 OTUs in 19 samples (2463 bacterial and 601 fungal). Proteobacteria were the most diverse bacterial phylum, while Ascomycota dominated fungi. Thelebolus (Thelebolales) was nearly ubiquitous, indicating broad tolerance to acidic and CO_2-rich conditions. Species-level identifications were more frequent in fungi than in bacteria, reflecting uneven database coverage. Alpha diversity analyses (Welch's ANOVA) demonstrated that bacterial richness (Chao1, Fisher indices) was significantly lower in high-CO_2 soils (>3000 ppm; $p < 0.05$) compared to low-CO_2 soils (<1000 ppm), with intermediate CO_2 concentrations showing heterogeneous responses. Fungal alpha diversity showed a decreasing trend at high CO_2 but without statistical significance. Beta diversity (PERMANOVA) confirmed significant differences in bacterial ($p = 0.001$) and fungal ($p = 0.045$) community composition between CO_2 categories. Taxonomic patterns revealed that bacterial genera such as *Acidobacterium*, *Granulicella*, *Streptomyces*, *Nocardia*, *Flavobacterium*, and *Chitinophaga* were enriched under high CO_2, whereas *Rhizobium* and *Pseudomonas* declined, reflecting sensitivity to acidity and oxygen limitation. Among fungi, *Thelebolus* and *Cladosporium* tolerated CO_2-rich soils, while *Mortierella*, *Cryptococcus*, and *Vishniacozyma* decreased, highlighting contrasting adaptive strategies. Sample P16, characterized by a methane peak, was an ecological outlier with distinct bacterial signatures (increased Actinomycetia and Planctomycetes). Multivariate analysis (PCA) including CO_2, CH_4, and H_2S concentrations confirmed that CO_2 is the dominant environmental driver (PC1, 65.7% variance), while CH_4 contributed to localized variability (PC2, 33.2%), particularly in sample P16. H_2S had negligible influence.

Overall, our findings show that post-volcanic gas emissions restructure microbial communities, reducing diversity at high CO_2 and establishing distinct ecological niches that can alter soil processes such as nutrient cycling and organic matter decomposition. These results emphasize the ecological risks of sustained CO_2 exposure and highlight the value of natural analog sites for assessing the potential impacts of CO_2 leakage in geological storage scenarios.

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References

1. IPCC. *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Lee, H., Romero, J., Eds.; IPCC: Geneva, Switzerland, 2023; p. 184. [CrossRef]
2. IPCC. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; p. 151.
3. West, J.M.; Pearce, J.M.; Bentham, M.; Maul, P. Environmental issues and the geological storage of CO_2. *Eur. Environ.* **2005**, *15*, 250–259. [CrossRef]
4. West, J.M.; Pearce, J.M.; Bentham, M.; Rochelle, C.; Maul, P.; Lombardi, S. Environmental Issues and the Geological Storage of CO_2—A European Perspective. In Proceedings of the 8th International Conference on Greenhouse Gas Control Technologies, Trondheim, Norway, 19–22 June 2006; Elsevier: Amsterdam, The Netherlands, 2006; pp. 1281–1286. Available online: https://hdl.handle.net/11573/417124 (accessed on 20 September 2025).
5. Jossi, M.; Fromin, N.; Tarnawski, S.; Kohler, F.; Gillet, F.; Aragno, M.; Hamelin, J. How elevated pCO_2 modifies total and metabolically active bacterial communities in the rhizosphere of two perennial grasses grown under field conditions. *FEMS Microbiol. Ecol.* **2006**, *55*, 339–350. [CrossRef] [PubMed]
6. Beaubien, S.E.; Ciotoli, G.; Coombs, P.; Dictor, M.C.; Krüger, M.; Lombardi, S.; Pearce, J.M.; West, J.M. The impact of a naturally occurring CO_2 gas vent on the shallow ecosystem and soil chemistry of a Mediterranean pasture (Latera, Italy). *Int. J. Greenh. Gas Control* **2008**, *2*, 373–387. [CrossRef]
7. Oppermann, B.I.; Michaelis, W.; Blumenberg, M.; Frerichs, J.; Schulz, H.M.; Schippers, A.; Beaubien, S.E.; Krüger, M. Soil microbial community changes as a result of long-term exposure to a natural CO_2 vent. *Geochim. Cosmochim. Acta* **2010**, *74*, 2697–2716. [CrossRef]
8. Stephens, J.C.; Hering, J.G. Factors affecting the dissolution kinetics of volcanic ash soils: Dependencies on pH, CO_2, and oxalate. *Appl. Geochem.* **2004**, *19*, 1217–1232. [CrossRef]
9. Macek, I.; Pfanz, H.; Francetic, V.; Batic, F.; Vodnik, D. Root respiration response to high CO_2 concentrations in plants from natural CO_2 springs. *Environ. Exp. Bot.* **2005**, *54*, 90–99. [CrossRef]
10. Pfanz, H.; Vodnik, D.; Wittmann, C.; Aschan, G.; Batic, F.; Turk, B.; Macek, I. Photosynthetic performance (CO_2-compensation point, carboxylation efficiency, and net photosynthesis) of timothy grass (*Phleum pratense* L.) is affected by elevated carbon dioxide in post-volcanic mofette areas. *Environ. Exp. Bot.* **2007**, *61*, 41–48. [CrossRef]
11. Harvey, O.R.; Qafoku, N.P.; Cantrell, K.J.; Lee, G.; Amonette, J.E.; Brown, C.F. Geochemical implications of gas leakage associated with geologic CO_2 storage—A qualitative review. *Environ. Sci. Technol.* **2013**, *47*, 23–36. [CrossRef]
12. Humez, P.; Négrel, P.; Lagneau, V.; Lions, J.; Kloppmann, W.; Gal, F.; Millot, R.; Guerrot, C.; Flehoc, C.; Widory, D.; et al. CO_2–water–mineral reactions during CO_2 leakage: Geochemical and isotopic monitoring of a CO_2 injection field test. *Chem. Geol.* **2014**, *368*, 11–30. [CrossRef]
13. Lions, J.; Devau, N.; de Lary, L.; Dupraz, S.; Parmentier, M.; Gombert, P.; Dictor, M.C. Potential impacts of leakage from CO_2 geological storage on geochemical processes controlling fresh groundwater quality: A review. *Int. J. Greenh. Gas Control* **2014**, *22*, 165–175. [CrossRef]
14. Kirk, M.F.; Altman, S.J.; Santillan, E.-F.U.; Bennett, P.C. Interplay between microorganisms and geochemistry in geological carbon storage. *Int. J. Greenh. Gas Control* **2016**, *47*, 386–395. [CrossRef]
15. Beulig, F.; Urich, T.; Nowak, M.; Trumbore, S.E.; Gleixner, G.; Gilfillan, G.D.; Küsel, K. Altered carbon turnover processes and microbiomes in soils under long-term extremely high CO_2 exposure. *Nat. Microbiol.* **2016**, *1*, 15025. [CrossRef]
16. Emerson, J.B.; Thomas, B.C.; Alvarez, W.; Banfield, J.F. Metagenomic analysis of a high carbon dioxide subsurface microbial community populated by chemolithoautotrophs and bacteria and archaea from candidate phyla. *Environ. Microbiol.* **2016**, *18*, 1686–1703. [CrossRef] [PubMed]
17. Probst, A.J.; Castelle, C.J.; Singh, A.; Brown, C.T.; Anantharaman, K.; Sharon, I.; Hug, L.A.; Burstein, D.; Emerson, J.B.; Thomas, B.C.; et al. Genomic resolution of a cold subsurface aquifer community provides metabolic insights for novel microbes adapted to high CO_2 concentrations. *Environ. Microbiol.* **2017**, *19*, 459–474. [CrossRef] [PubMed]
18. Pricăjan, A. *Mineral and Thermal Waters from Romania*; Ed. Tehnica: Bucharest, Romania, 1972.
19. Pricăjan, A. *Therapeutic Mineral Substances from Romania*; Scientific and Encyclopedic Publishing House: Bucharest, Romania, 1985.
20. Karátson, D.; Veres, D.; Gertisser, R.; Magyari, E.K.; Jánosi, C.; Hambach, U. *Ciomadul (Csomád), The Youngest Volcano in the Carpathians*; Springer Nature: Cham, Switzerland, 2022.

21. Berner, Z.; Jánosi, C.; Péter, É. *A Kelemen-Görgényi-Hargita vonulat ásványvizei [Mineral Waters of Călimani-Gurghiu-Harghita Mountain Chain]*; Report for Harghita County Council; The Official Gazette of Romania: Harghita County, Romania, 2000.
22. Szakács, A.; Seghedi, I.; Pécskay, Z.; Mirea, V. Eruptive history of a low-frequency and low-output rate Pleistocene volcano, Ciomadul, South Harghita Mts., Romania. *Bull. Volcanol.* **2015**, *77*, 12. [CrossRef]
23. Bálint-Bálint, L.; Magyari-Sáska, Z.; Irimuş, I.A.; Peteley, A.; Niţă, A. The Valuation of the Urban Ecotourism Potential of the Volcanic Geomorphohydrosite Băile Tuşnad in Harghita County, Romania. *Mod. Geograph.* **2024**, *19*, 49–68. [CrossRef]
24. Kis, B.M.; Szalay, R.; Aiuppa, A.; Bitetto, M.; Palcsu, L.; Harangi, S. Compositional measurement of gas emissions in the Eastern Carpathians (Romania) using the multi-GAS instrument: Approach for in situ data gathering at non-volcanic areas. *J. Geochem. Explor.* **2022**, *240*, 107051. [CrossRef]
25. Tămaș, D.M.; Kis, B.M.; Tămaș, A.; Szalay, R. Identifying CO_2 Seeps in a Long-Dormant Volcanic Area Using Uncrewed Aerial Vehicle-Based Infrared Thermometry: A Qualitative Study. *Sensors* **2022**, *22*, 2719. [CrossRef]
26. Molnár, K.; Czuppon, G.; Palcsu, L.; Benkó, Z.; Lukács, R.; Kis, B.-M.; Németh, B.; Harangi, S. Noble gas geochemistry of phenocrysts from the Ciomadul volcanic dome field (Eastern Carpathians). *Lithos* **2021**, *394–395*, 106152. [CrossRef]
27. NOAA. Global Monitoring Laboratory—Annual Greenhouse Gas Index. 2023. Available online: https://gml.noaa.gov/ccgg/ (accessed on 7 September 2025).
28. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E Ltd.: Plymouth, UK, 2008.
29. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **2001**, *4*, 4.
30. Dhariwal, A.; Chong, J.; Habib, S.; King, I.L.; Agellon, L.B.; Xia, J. MicrobiomeAnalyst: A web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res.* **2017**, *45*, W180–W188. [CrossRef]
31. Zhao, X.; Deng, H.; Wang, W.; Han, F.; Li, C.; Zhang, H.; Dai, Z. Impact of naturally leaking carbon dioxide on soil properties and ecosystems in the Qinghai-Tibet Plateau. *Sci. Rep.* **2017**, *7*, 3001. [CrossRef] [PubMed]
32. Ham, B.; Choi, B.-Y.; Chae, G.-T.; Kirk, M.F.; Kwon, M.J. Geochemical influence on microbial communities at CO_2-leakage analog sites. *Front. Microbiol.* **2017**, *8*, 2203. [CrossRef] [PubMed]
33. Beulig, F.; Heuer, V.B.; Akob, D.M.; Viehweger, B.; Elvert, M.; Herrmann, M.; Küsel, K. Carbon flow from volcanic CO_2 into soil microbial communities of a wetland mofette. *ISME J.* **2015**, *9*, 746–759. [CrossRef] [PubMed]
34. Liu, Q.; Kämpf, H.; Bussert, R.; Krauze, P.; Horn, F.; Nickschick, T.; Alawi, M. Influence of CO_2 degassing on the microbial community in a dry mofette field in Hartoušov, Czech Republic (Western Eger Rift). *Front. Microbiol.* **2018**, *9*, 2787. [CrossRef]
35. Zhalnina, K.; Dias, R.; de Quadros, P.D.; Davis-Richardson, A.; Camargo, F.A.; Clark, I.M.; Triplett, E.W. Soil pH determines microbial diversity and composition in the Park Grass Experiment. *Microb. Ecol.* **2015**, *69*, 395–406. [CrossRef]
36. Zhalnina, K.; de Quadros, P.D.; Camargo, F.A.; Triplett, E.W. Drivers of archaeal ammonia-oxidizing communities in soil. *Front. Microbiol.* **2012**, *3*, 210. [CrossRef]
37. Jones, R.T.; Robeson, M.S.; Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* **2009**, *3*, 442–453. [CrossRef]
38. Lee, S.H.; Ka, J.O.; Cho, J.C. Members of the phylum Acidobacteria are dominant and metabolically active in rhizosphere soil. *FEMS Microbiol. Lett.* **2008**, *285*, 263–269. [CrossRef]
39. Bergmann, G.T.; Bates, S.T.; Eilers, K.G.; Lauber, C.L.; Caporaso, J.G.; Walters, W.A.; Knight, R.; Fierer, N. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biol. Biochem.* **2011**, *43*, 1450–1455. [CrossRef]
40. Prosser, J.I.; Bohannan, B.J.M.; Curtis, T.P.; Ellis, R.J.; Firestone, M.K.; Freckleton, R.P.; Green, J.L.; Green, L.E.; Killham, K.; Lennon, J.J.; et al. The role of ecological theory in microbial ecology. *Nat. Rev. Microbiol.* **2007**, *5*, 384–392. [CrossRef]
41. de Miera, L.E.S.; Arroyo, P.; de Luis Calabuig, E.; Falagán, J.; Ansola, G. High-throughput sequencing of 16S rRNA genes of soil bacterial communities from a naturally occurring CO_2 gas vent. *Int. J. Greenh. Gas Control* **2014**, *29*, 176–184. [CrossRef]
42. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [CrossRef] [PubMed]
43. Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **2009**, *75*, 5111–5120. [CrossRef] [PubMed]
44. Power, J.F.; Carere, C.R.; Lee, C.K.; Wakerley, G.L.; Evans, D.W.; Button, M.; Stott, M.B. Microbial biogeography of 925 geothermal springs in New Zealand. *Nat. Commun.* **2018**, *9*, 2876. [CrossRef]
45. Rousk, J.; Bååth, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Caporaso, J.G.; Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **2010**, *4*, 1340–1351. [CrossRef]
46. Park, K.; Kim, C.Y.; Kirk, M.F.; Chae, G.; Kwon, M.J. Effects of natural non-volcanic CO_2 leakage on soil microbial community composition and diversity. *Sci. Total Environ.* **2023**, *862*, 160754. [CrossRef]

47. Krüger, M.; Jones, D.; Frerichs, J.; Oppermann, B.I.; West, J.; Coombs, P.; Green, K.; Barlow, T.; Lister, B.; Shaw, R.; et al. Effects of elevated CO_2 concentrations on the vegetation and microbial populations at a terrestrial CO_2 vent at Laacher See, Germany. *Int. J. Greenh. Gas Control* **2011**, *5*, 1093–1098. [CrossRef]
48. Krüger, M.; West, J.; Frerichs, J.; Oppermann, B.; Dictor, M.-C.; Jouliand, C.; Jones, D.; Coombs, P.; Green, K.; Pearce, J.; et al. Ecosystem effects of elevated CO_2 concentrations on microbial populations at a terrestrial CO_2 vent at Laacher See, Germany. *Energy Procedia* **2009**, *1*, 1933–1939. [CrossRef]
49. Ziogou, F.; Gemeni, V.; Koukouzas, N.; de Angelis, D.; Libertini, S.; Beaubien, S.E.; Lombardi, S.; West, J.M.; Jones, D.G.; Coombs, P.; et al. Potential Environmental Impacts of CO_2 Leakage from the Study of Natural Analogue Sites in Europe. *Energy Procedia* **2013**, *37*, 3521–3528. [CrossRef]
50. Smith, K.L.; Steven, M.D.; Jones, D.G.; West, J.M.; Coombs, P.; Green, K.A.; Barlow, T.S.; Breward, N.; Gwosdz, S.; Krüger, M.; et al. Environmental impacts of CO_2 leakage: Recent results from the ASGARD facility, UK. *Energy Procedia* **2013**, *37*, 791–799. [CrossRef]
51. Feitz, A.; Jenkins, C.; Schacht, U.; McGrath, A.; Berko, H.; Schroder, I.; Noble, R.; Kuske, T.; George, S.; Heath, C.; et al. An assessment of near surface CO_2 leakage detection techniques under Australian conditions. *Energy Procedia* **2014**, *63*, 3891–3906. [CrossRef]
52. Gwosdz, S.; West, J.M.; Jones, D.; Rakoczy, J.; Green, K.; Barlow, T.; Blöthe, M.; Smith, K.; Steven, M.; Krüger, M. Long-term CO_2 injection and its impact on near-surface soil microbiology. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw193. [CrossRef]