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Influence of Desert Microhabitats on the Abundance and Composition of Live and Dead Soil Bacterial Communities

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Abstract

Soil DNA studies often present a misleading view of microbial communities by failing to distinguish between live and dead bacterial cells. There is a critical gap in research on how the composition of dead cells diverges from that of living ones and which ecological factors drive these differences. Understanding the live/dead distinction is essential for gaining insights into soil biological activity and nutrient reservoirs. In this study, we employed flow cytometry with fluorescent labeling, which allowed us to obtain single-cell measurements from large samples and effectively differentiate between live and dead bacteria, focusing on the impact of proximity to desert plants on soil microbial communities. We specifically investigated three desert plants—*Zygophyllum dumosum*, *Hammada scoparia*, and *Atriplex halimus*—and compared their influence to that of soil crust and bare soil. Our findings reveal that Proteobacteria and Actinobacteria phyla are abundant in both live and dead fractions. The diversity of live bacterial communities is significantly higher in samples near *H. scoparia* and *A. halimus* compared to *Z. dumosum* and soil crust. Each plant species forms distinct nutrient islands, which markedly influence bacterial composition and the live-to-dead cell ratio. Importantly, living bacterial cells are less abundant in soil crust and bare ground compared to those beneath *H. scoparia* and *A. halimus*. The nutrient-poor conditions of bare soil favor communities of phyla known for their long-term ecological persistence. Conversely, *Z. dumosum* correlates with a higher proportion of dead cells, underscoring a paradox in its influence on microbial dynamics. In conclusion, the live/dead ratio alone does not provide a complete picture of ecological processes. Our results assert the necessity of looking beyond total bacterial communities to comprehensively understand the key contributors to soil ecology

and function.

Keywords

Rhizosphere, Microbial Community, Live/Dead Bacteria, Plants, Arid System

1. Introduction

Soil is a fascinating and dynamic ecosystem, driven by the diverse microbial community that it hosts. Both when alive and after death, microbes play pivotal roles in maintaining soil health, nutrient cycling, and overall ecosystem resilience. By understanding the significance of both the living and dead components, and the interplay between microbial communities, we gain valuable insights into the processes that uphold soil productivity and environmental stability [1] [2]. The living portion of the microbial community performs crucial functions for ecosystem sustainability, including nutrient cycling, decomposition, soil aggregation, and fostering symbiotic relationships with plants to enhance nutrient uptake and aid soil resilience against environmental challenges like drought [3] [4]. Meanwhile, the dead microbial community, known as necromass, significantly contributes to the creation of stable organic matter, which acts as a carbon sink, helping to combat climate change. The interaction between living and dead microbial components creates feedback loops that bolster ecosystem stability.

The determination of live versus dead bacteria and their viability can be traced back to 1939, when Steinhaous and Birkeland [5] published their study defining this as a phenomenon, using culture media. In their pioneering study, they concluded that "quantitative examination of death and death rate in bacterial culture might be desirable". In their summary [5], they elucidated that cultivable bacteria can be found after two years and even longer, depending on abiotic influences.

Interest in the turnover and death rates of bacterial communities is related to the importance of their effects on human health and broader aspects of the environment [6]. Understanding and managing these processes may potentially have significant implications for agricultural productivity, environmental sustainability, and the global carbon cycle.

Several methods, considered the "gold standard" in this respect, including plate counting, microscopy, and flow cytometry, have proven useful in determining bacterial viability [7] [8]. Estimates are often focused on the potential numbers of colony-forming units, a method that is considered for cultivable bacteria but does not include cryptobiotic, dormant, moribund or latent cells, or those that cannot be determined to species the taxon level [9]-[13], in his study on yeast, showed that plate count, which is known as the "gold standard", detects less than 50% of the total viable community. Considering such limitations, the most advanced existing method that could be beneficial is flow cytometry, which has the ability to swiftly analyze and characterize individual cells within a diverse population. The

LIVE/DEAD® BacLight™ Bacterial Viability and Bacteria Counting Kit enables researchers to accurately differentiate and quantify live and dead bacteria using flow cytometry, even in mixed populations with diverse bacterial types [14]. Flow cytometry (FCM) has long been recognized as a sophisticated technique, particularly in proteomic analysis. However, its application in microbiology is expanding, allowing for the identification of microbial populations with similar characteristics while collecting data at the single-cell level. By this method, samples are stained with nucleic acid-binding dyes, and 6 μm reference beads are added as part of the LIVE/DEAD® BacLight™ kit. The calibration samples are then analyzed using bead-based FCM [15]. This technique provides a rapid and quantitative approach to microbial analysis [16].

A study on bacterial viability assessment by flow cytometry analysis in a sandy desert soil by Shamir *et al.* [17] considered the importance of abiotic factors that can affect the size of the active bacterial community, which ranges between 1.44% to 25.4%, while the rest were determined as non-active or dead cells.

The bacterial community is usually the largest biotic component of the soil, reaching its greatest density in the plant rhizosphere, and the 0 - 10 cm depth soil horizon [18]-[21]. In arid environments, plant rhizospheres play an especially important role in supporting an active and abundant bacterial community, with the soil outside of rhizospheres having a less important role in nutrient cycling [22] [23].

The environmental conditions of arid regions have led to the evolution of ecophysiological adaptation mechanisms that enable soil microbial communities to carry out their life cycle processes [24] [25]. In these environments, soil bacteria often face prolonged periods of dehydration and disruptions in growth and nutrient availability.

The survival of soil bacterial communities in arid environments is governed by adaptations aligned with the "pulse-reserve" model [26]. According to this model, bacteria endure extended phases of low metabolic activity by relying on brief pulses of nutrient availability during short periods of activity.

In previous studies, methodological approaches, including molecular tools, have been used to obtain a better understanding of soil microbial community dynamics linked to seasonal and temporal changes in arid environments [3] [27]-[29]. Sokol *et al.* (2022) [30] elucidated the importance of living and decaying soil microbial communities in the resource-rich habitat of the rhizosphere in promoting organic matter cycling and accumulation, as determined by microbial necromass accumulation.

During the dry season in the Negev Desert, the survival of the soil microbial community under the canopies of dominant shrubs, such as *Zygophyllum dumosum*, *Hammada scoparia*, and *Atriplex hirtum*, as well as the soil crust, can have either a positive or negative effect on the composition of the microbial community compared to the bare soil in the open spaces between these shrubs. The survival of the dominant shrubs in this challenging environment is related to their effective eco-

physiological adaptations, whereby a reduction of water loss is achieved by shedding all their leaves, with photosynthesis undertaken by the cortex of green stems. This is combined with physiological mechanisms for tolerance of high salinity [31] [32].

Perennial plants are among the key factors influencing microbial activity in desert ecosystems. Species such as *Zygophyllum dumosum*, *Hammada scoparia*, and *Atriplex hirtum* play a crucial role in shaping the ecosystem by differentiating between the physical and organic contributions of perennial vegetation. This process leads to the formation of "fertile islands," which sustain biological activity over time [24]. Patchy desert shrubs enhance horizontal heterogeneity in carbon sources and nutrient distribution within arid environments, significantly impacting the abundance and activity of soil microbial communities [33].

In the present study, our goal was to determine the detailed taxonomic composition of the viable and non-viable bacterial community obtained from labeling and flow-cytometric technique applied to different soil samples in the vicinity of three desert perennial plant species, namely *Zygophylum dumosum*, *Hammada scoparia* and *Atriplex hirtum* with dissimilar ecophysiological adaptations, and to evaluate the diversity of the microbial community structure in the soil crust compared to control soil located in the open space.

We hypothesized that:

The taxonomic composition of soil microbial communities near the shrub canopy and within the soil crust will differ from that of the control soil.

These differences are significantly influenced by both the ecophysiological adaptations of plants and the presence of the biological soil crust, compared to control soil collected from open areas.

The ratio between viable (live) and non-viable (dead) bacteria in the soil samples collected from the plant vicinity and soil biological crust will differ significantly from the control open space soil samples.

Our study focused on three plant species: *Zygophyllum dumosum*, *Hammada scoparia*, and *Atriplex hirtum*. These species were selected for their distinct ecophysiological adaptations, despite coexisting within the same ecosystem. We observed that the intershrub areas facilitated the formation of a well-developed biological soil crust, characterized by similar microclimatic conditions and soil pedology.

The novelty in studying the live/dead bacterial ratio in soil near desert plants using flow cytometry lies in its ability to quantitatively assess viable and total bacteria in soil samples from extreme desert environments. This approach enables a precise evaluation of the impact of abiotic factors and spatial scales on the taxonomic composition of the live and dead soil microbial community.

2. Materials and Methods

2.1. Study Site

Complementary randomized soil samples were collected from the 0 - 10 cm upper

soil layer in the vicinity of *Zygophylum dumosum*, *Hammada scoparia* and *Atriplex halimus* L. (Saltbush), soil crust and control samples from the open space at a minimum of 30 m from the plants (n = 5). All 25 soil samples were gathered at the M. Evenari Runoff Research Farm Avdat Negev Desert research station (30˚47', 34˚36' E), Israel. The area has a temperate desert climate, with cool winters (5˚C - 14˚C in January) and hot, dry summers (18 ˚C - 23˚C in June). The multi-annual mean rainfall is 89.5 mm (at Avdat station), ranging from 24.0 mm in an extreme drought year to 183.3 mm in a wet year, with an annual evaporation rate of 2618 mm, radiation can reach 3.14×10^4 kJ·m^{-2}·d^{-1}. The soil at the study site is an aeolian loess deposit, alkaline, and exhibits low salinity in the upper layer [31].

The soil samples were collected (0 - 10 cm soil layer) at the end of the dry, hot summer season in August in the early morning hours. Five soil samples were collected around each plant and open space (soil crust and control) into one polyethylene bag and mixed into one composite sample composed of replicates. The sampling procedure was repeated five times (n = 5) for each one of the 5-sampling locations and placed in an insulated container in order to maintain the relatively low environmental temperature and prevent overheating and transported to the laboratory. Before physicochemical and biological analysis, the soil was sieved through a 2 mm mesh to remove stones, roots and other organic debris and then stored at 4˚C for a maximum of 24 hours. A soil subsample from each of the 25 soil samples was placed in an Eppendorf test tube and stored at −20˚C for DNA microbial community determination.

2.2. Live/Dead Bacteria Staining and Cell Sorting

A BacLight Bacterial Viability kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States) was used to stain the samples for live/dead determination. The method for assessing bacterial viability in soil using flow cytometry affects both the live/dead bacteria ratio due to the utilization of fluorescent labeling techniques, which dyes help to differentiate between the two, combined with flow cytometry that rapidly analyzes the whole bacterial population [17].

The kit consists of a mixture of SYTO9 green-fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, propidium iodide (PI). SYTO9 stain can penetrate healthy and membrane-damaged cells and stain them with green fluorescence. PI can penetrate only into dead cells, causing a reduction in SYTO9 staining. The two dyes were diluted according to the manufacturer's instructions in sterile DDW and mixed into one staining solution. The concentration of each dye in the staining solution is 12 μM SYTO9 stain and 60 μM PI.

From each soil sample (n = 5), 0.5 g was diluted in sterile DDW (0.5 g soil in 4.5 mL DDW), vortexed, and then diluted to a final dilution of 1:1000 in DDW. A 500 μL aliquot of each diluted sample was stained with 100 μL of the staining solution and incubated at RT for 10 min in the dark. The samples were analyzed and sorted using a BD FACSAria™ III flow cytometer (BD Biosciences, San Jose, CA, United States) equipped with 4 lasers (405, 488, 561 and 633 nm). Live cells ex-

hibited green fluorescence (excitation 488 nm, emission 520/50 nm) and dead cells had only red fluorescence (excitation 561 nm, emission 610/20 nm). Live and dead cell populations were sorted to separate Eppendorf tubes (100k cells each). The flow rate was medium (6 of 11) and the sorting precision was set to 4-way purity. Gating strategy and controls are presented in Figure 1. Post-acquisition analysis was carried out using the FlowJo software (ver. 10.9) (Ashland, OR, United States).

Pre-counting of the bacteria in the samples was conducted before the actual study in order to determine the counting reproducibility. The results showed a very high (96% - 99%) duplicability. All cytometric analyses were undertaken on each of the five replicates, yielding a total of 25 samples.

At the end of the sorting process, collected cells were kept at −20˚C for bacterial community molecular taxa determination.

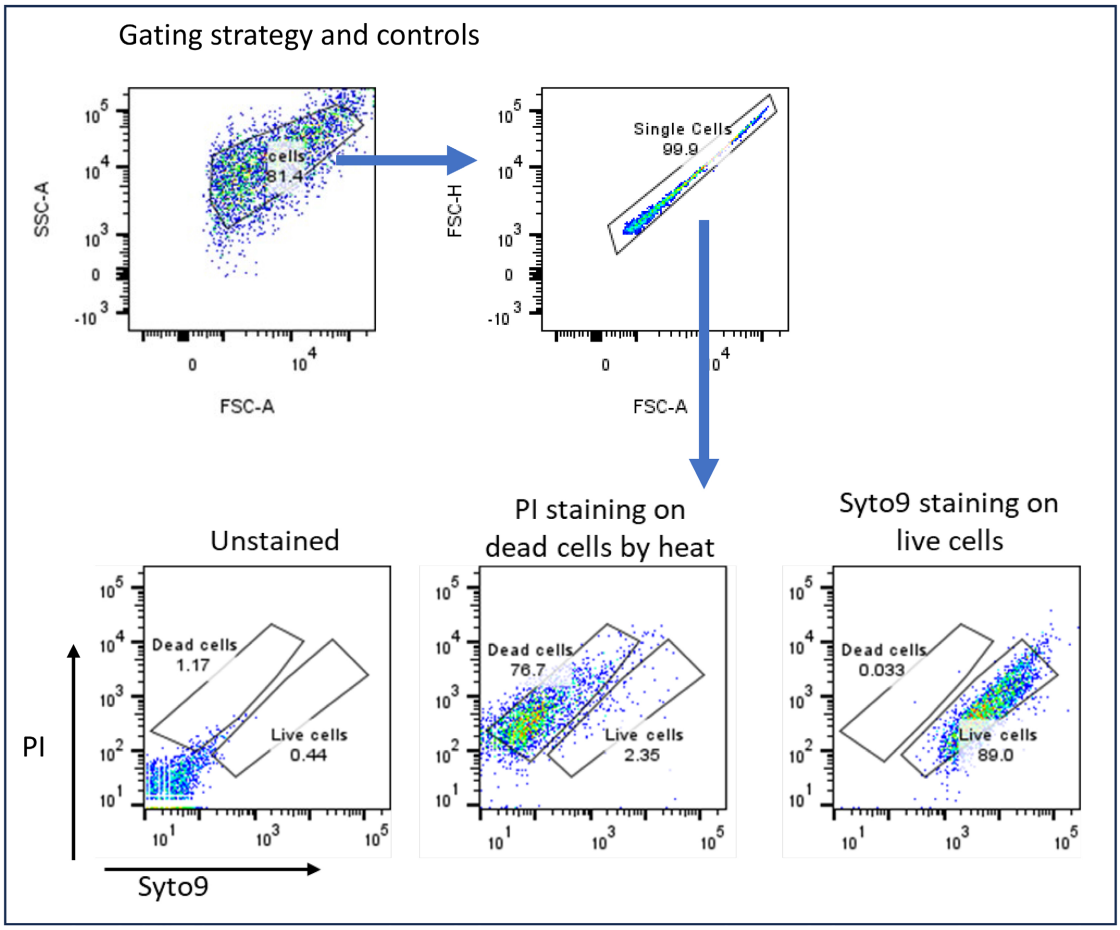

Figure 1. Flow rate was medium (6 of 11) and the sorting precision was set to 4-way purity.

DNA extraction, amplification, and sequencing

DNA was extracted from 1 mL solution obtained from the cell sorter containing live or dead bacterial cells by using an Exgene soil DNA mini kit from GeneAll (Seoul, Korea), according to the producer's protocol, using 50 µL elution buffer in the elution stage and storing the eluted DNA at −20˚C until DNA amplification.

The eluted DNA was amplified twice using a two-step PCR process.

PCR1 was conducted by mixing 12.5 μL PCRBIO HS Taq Mix Red, 9.5 μL ultrapure water, 1 μL of primer CS1_515F (ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGT), 1 μL of primer CS2_806R (TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCT), and 1 μL of the eluted DNA. The thermal cycling program was 95˚C for 3 min, 24 cycles of 98˚C for 10 s, 55˚C for 10 s, 72˚C for 20 s, followed by 72˚C for 1 min.

A 2 μL sample from the PCR1 amplified sample containing CS1/CS2 adaptors was amplified for 10 cycles in 10 μL using the Fluidigm Access Array Barcode library according to the manufacturer's protocol (2 μL barcode per 44 reaction). DNA was purified using Kapa Pure Beads at a ratio of 0.65X and quantified with Qubit using the http://pedosphere.issas.ac.cn/ Denovix DsDNA high sensitivity assay on Quibit. DNA size and integrity were quantified by Tapestation using Agilent DNA screen tape and reagents.

Sequencing—samples were run on a dedicated Miseq (Illumina) machine with 30% PhiX using MiSeq Reagent Kit v2 5 500PE. Demultiplexing was performed using bcl2fastq with default parameters allowing for 0 mismatches. Data were then mapped to PhiX using bowtie2 to remove PhiX control and unmapped reads were quantified, collected, and examined using fastQC.

PRJNA 1196642: Desert plant resource islands determine the abundance of live and dead soil bacterial communities.

Statistical analysis

Statistical analysis was conducted using the XLSTAT [34].

A one-way analysis of variance (ANOVA) was applied to assess the effects of sampling locality (under perennial shrub canopies and open spaces) on a range of response variables, including plant biomass, live/dead bacterial ratios, bacterial community composition, species richness, Shannon diversity, evenness, soil moisture, and organic carbon content. For variables showing significant differences ($p < 0.05$), post hoc comparisons were conducted using **Duncan's Multiple Range Test**. Duncan's test was selected due to its greater sensitivity in detecting pairwise differences among group means, which was suitable given the study's exploratory nature. This approach allows for the identification of subtle ecological differences while accepting a slightly increased risk of Type I error compared to more conservative methods such as Tukey's HSD.

3. Results

3.1. Soil Abiotic Parameters

No significant differences in soil moisture (%) were found between the control samples and samples obtained in the plant's vicinity and soil crust (**Figure 2**). The highest soil organic matter (%) was observed in soil samples collected near *Atriplex halimus* L, with a mean value of 4.56% followed by *Zygophylum dumosum* (3.33%), soil crust (2.58%), *Hammada scoparia* (2.35%), followed by a lower value obtained in the control soil sample with 1.59% (**Figure 2**).

Gating strategy and controls. The **cell** population was gated on FSC-A vs. SSC-A plot from all events. The **single-cell population was gated on FSC-H vs. FSC-A from the cell** population. The **Live and Dead cell** populations were gated from the single cell population on Syto9 (green fluorescence) vs PI (red fluorescence). Unstained cells were used as a negative control. To validate the dead cells' gating, we heated the cells and stained them with PI dye. Live cell gating was validated by staining only with Syto9.

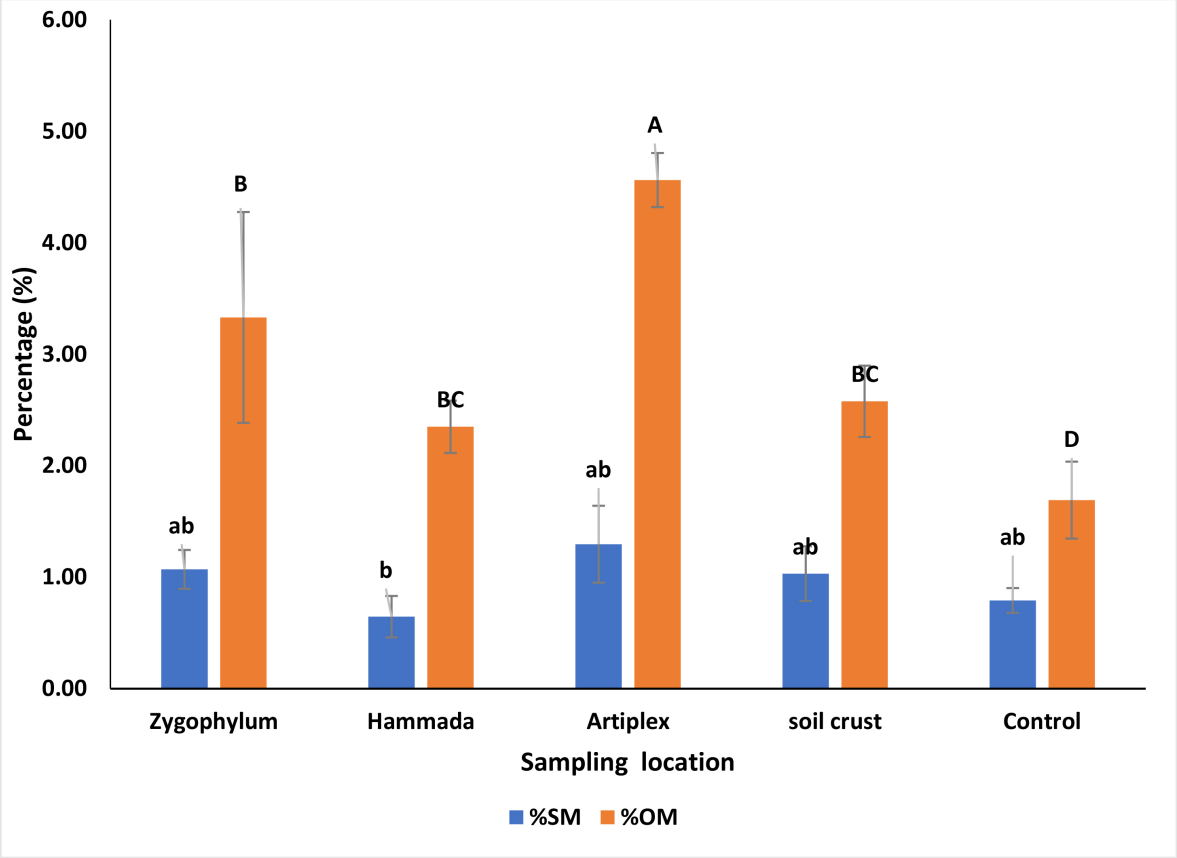

Figure 2. Mean values (±SD) for soil moisture (SM%) and organic matter (OM%) in the soil samples collected beneath the plant canopies, the soil crust, and the open space (control soil samples) (n = 5). Significant differences are indicated by small letters determined by using Duncan's multiple range tests.

Various dyes are available for flow cytometry to assess different aspects of microbial cells. In the present study, the soil samples obtained in the Negev Desert from the rhizosphere of different desert perennial plants were stained with a BacLight Bacterial Viability kit and analyzed in BD FACS AriaIII flow cytometer and compared to an unstained sample. Live cells were positive for green fluorescence (Syto9) and dead cells were only positive for red fluorescence (PI). From each plant soil sample (n = 5), 10^6 live and dead cells were collected by sorting into separate test tubes. Figure 3 shows representative graphs of the five soil groups and unstained control samples. Standard credibility results of over 99% were obtained.

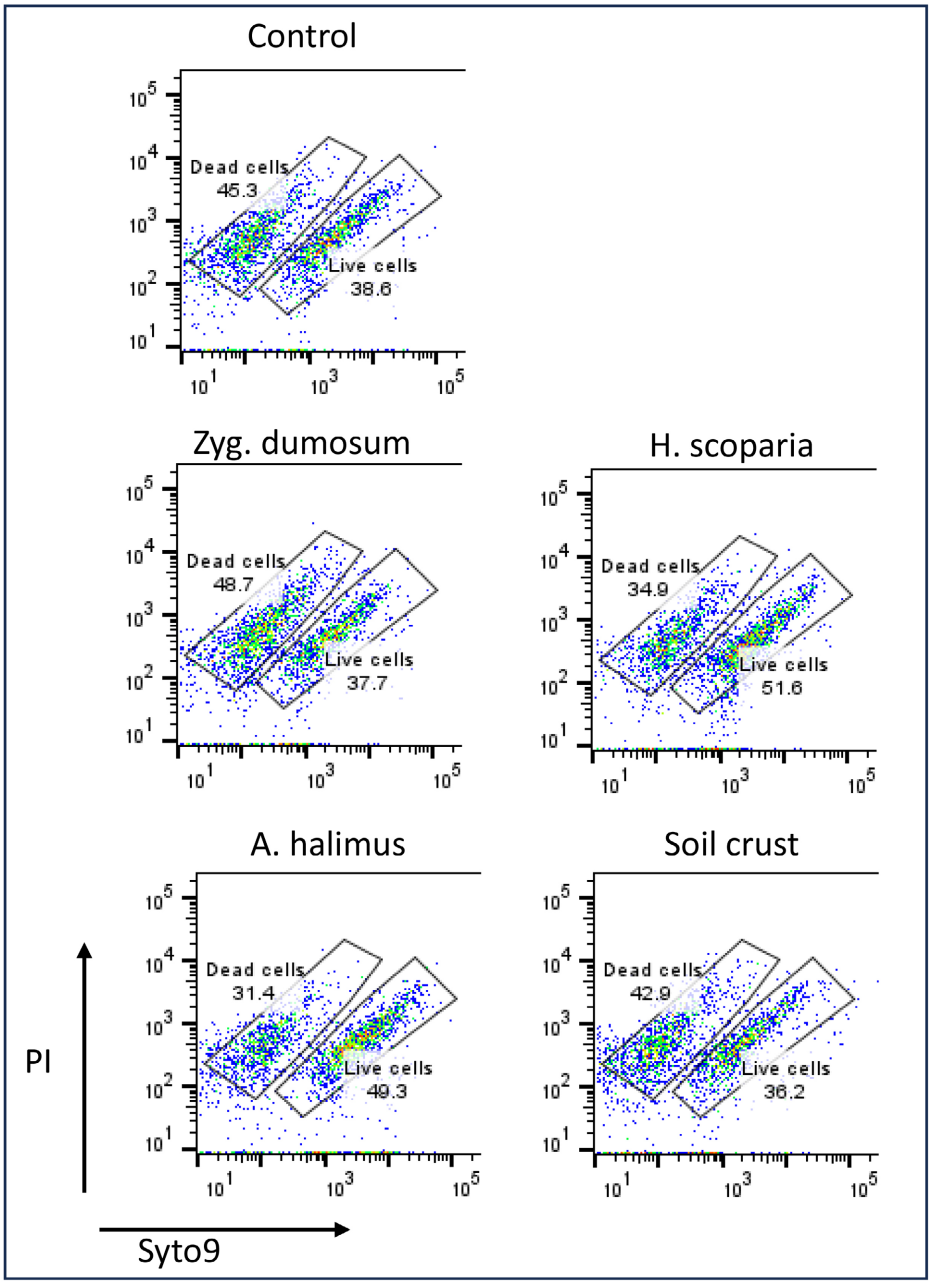

Figure 3. Illustration of representative results of unstained and stained live and dead bacteria obtained from each of the five-soil samples compared to an unstained sample. The soil samples were stained with a BacLight Bacterial Viability kit and analyzed in a BD FACS AriaIII flow cytometer and compared to an unstained sample. Live cells were positive for green fluorescence (Syto9) and dead cells were only positive for red fluorescence (PI). Each population (live and dead cells) was collected by sorting into separate tubes.

The percentage of dead and live cells present in each sample (n = 5) was compared to control samples (**Figure 4**). A significantly higher percentage of live cells was found in the vicinity *H. scoparia* compared to live cells in control soil ($P < 0.05$). Cells in the vicinity of *A. halimus* had significantly fewer dead cells compared to the control sample ($P < 0.05$). At three sampling sites (Z. *dumosum*, soil

crust and control soils), we observed a higher dead cell count compared to live cells (Figure 4).

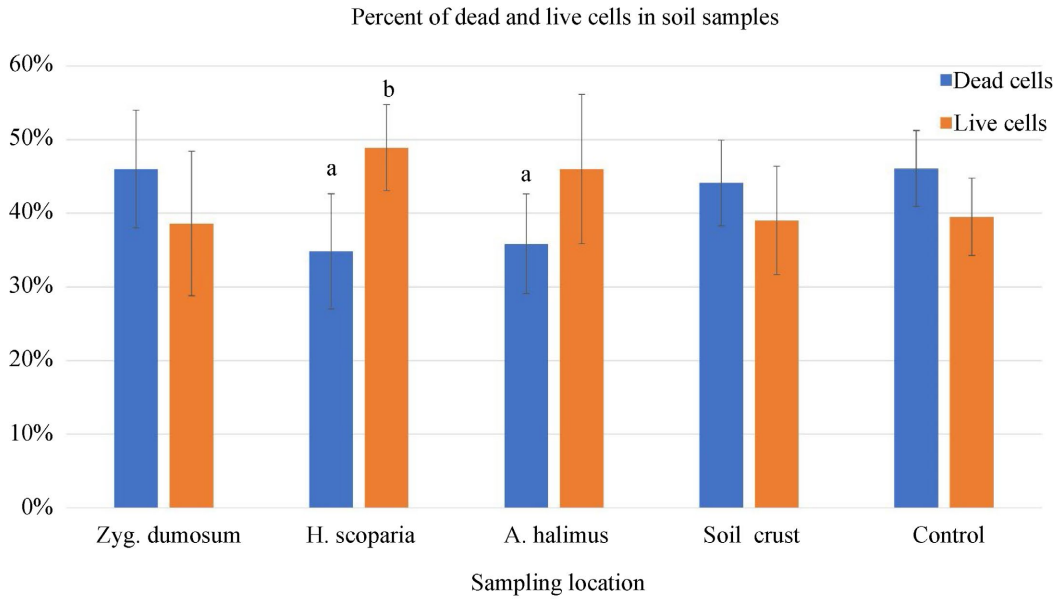

Figure 4. Percent of live and dead bacteria cells in 100,000 individual counts (n = 5) of cells in soil samples collected in the vicinity of three plants: *Z. dumosum*, *H. scoparia*, and *A. halimus* and two soil samples—soil crust and control. Each sample type, dead and live cells were compared to the control dead or live cells, respectively, for significance. (a) P value < 0.05 of dead cells; (b) P value < 0.05 of live cells.

The *Z. dumosum* plants, in comparison to the two others, adapt to desert conditions through water conservation (succulent leaves, drought deciduousness), efficient resource use (deep roots, salt tolerance), and flexible photosynthesis (C3-CAM switching for water efficiency). Such adaptation also influences soil microbial communities, though it paradoxically supports more dead bacterial cells, unlike *H. scoparia* and *A. halimus*, which support more viable bacterial communities. These traits help it thrive in arid, nutrient-poor environments.

3.2. Community Composition

This study aimed to examine the sequencing outcomes of bacterial communities in live and dead samples acquired from cell sorting within various desert soil environments, including plant rhizosphere, soil crust, and open space. The objective was to track the ratio and occurrence of these two groups throughout the analysis. The most dominant phylum was the Proteobacteria, reaching up to 99% of the total phyla in both live and dead samples (Figure 5(A)). The second phylum following the Proteobacteria was the Actinobacteria, with representatives ranging around 1%.

By assessing the ratio of live to dead (L/D) bacteria at the phylum taxonomic level, significant variations in the ratio (Figure 5(B)) can be observed across the samples. In two soil samples collected in the rhizosphere of *H. scoparia* and *A.*

halimus, the most abundant phylum is the Protobacteria, in the soil crust and control samples the most abundant is the group that includes the "other". In the soil crust samples, the relative L/D abundance is represented by *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, with a relatively high level of Firmicutes. In the samples obtained under the canopy of *Z. dumosum*, the main phyla L/D levels were the *Actinobacteria* (23.6), *Proteobacteria* (20.9), *Firmicutes* (20.18) and the TM7 with 18.9, with fewer dead compared to live bacteria. No significant differences in H' (Shannon index) were found between the sampling locations and between the groups of live and dead bacteria, with values between 1.25 and 1.44 for TM7.

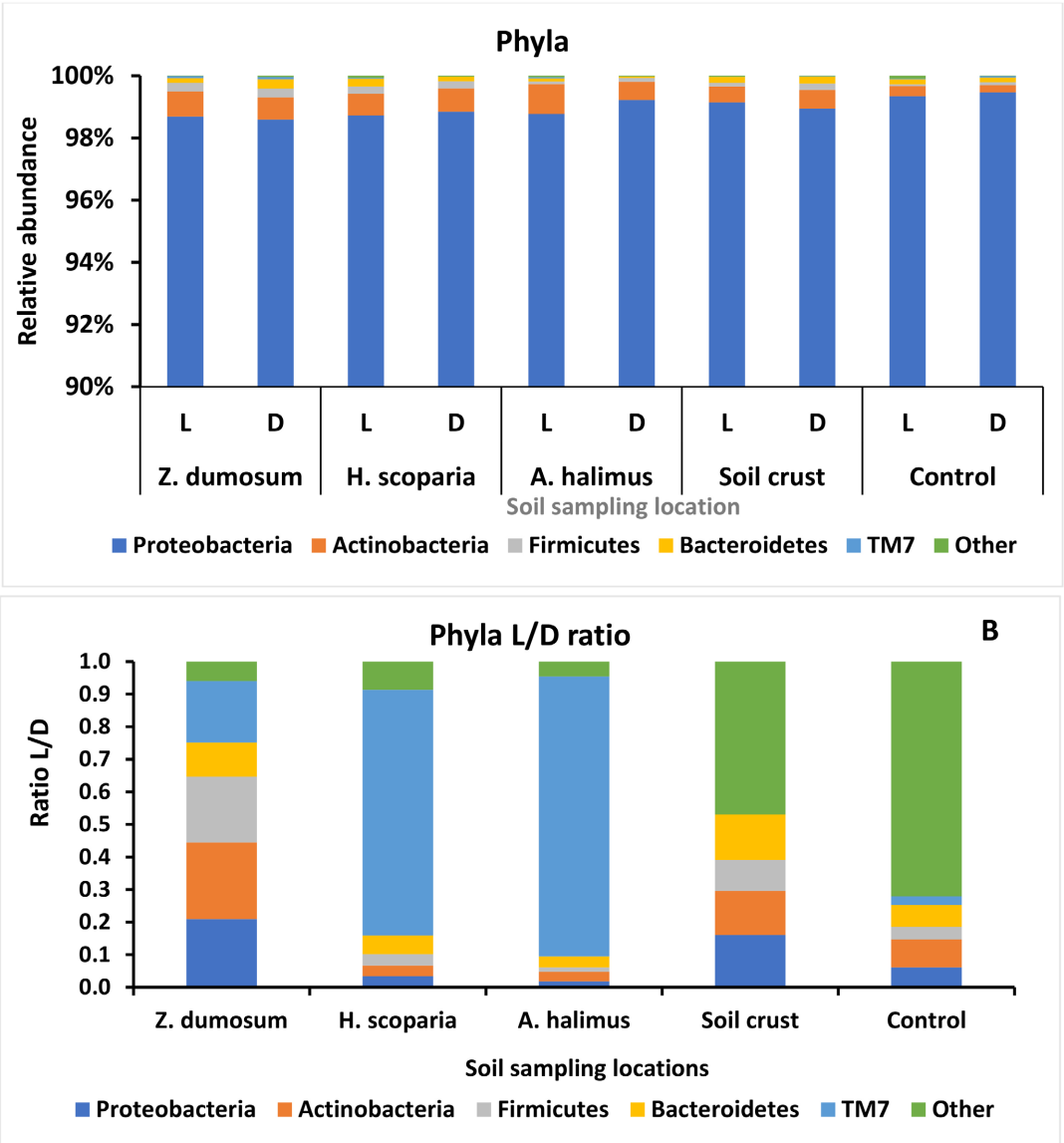

Figure 5. (A, B) The relative abundance of various bacterial phyla (A) and the relative ratio between live and dead (L/D) phyla; (B) classes in each of the five soil samples: plant rhizosphere, soil crust and control open space with the L/D ratio of each phylum in each one of the soil sample.

Determining the ratio between the live and dead (L/D) bacteria at the phylum taxonomic level, we observed large changes in the ratio (Figure 5(B)) between sets of soil samples. In soil samples collected in the rhizosphere of *H. scoparia* and *A. halimus*, the most abundant phylum is the *Proteobacteria*. In the soil crust and control samples, the most abundant is the group of "others". In the soil crust samples, the relative L/D abundance are the Proteobacteria, Actinobacteria, and Bacteroidetes, with a relatively high level of Firmicutes. In the samples obtained from the vicinity of the canopy of *Z. dumosum*, the main phyla L/D ratios are *Actinobacteria* (23.6), *Proteobacteria* (20.9), *Firmicutes* (20.18) and TM7 with 18.9, where the dead bacteria were found at a lower count compared to the live bacteria. No significant differences in H' (Shannon index) were found between the sampling locations and between the live and dead bacteria, where values were between 1.25 and 1.44.

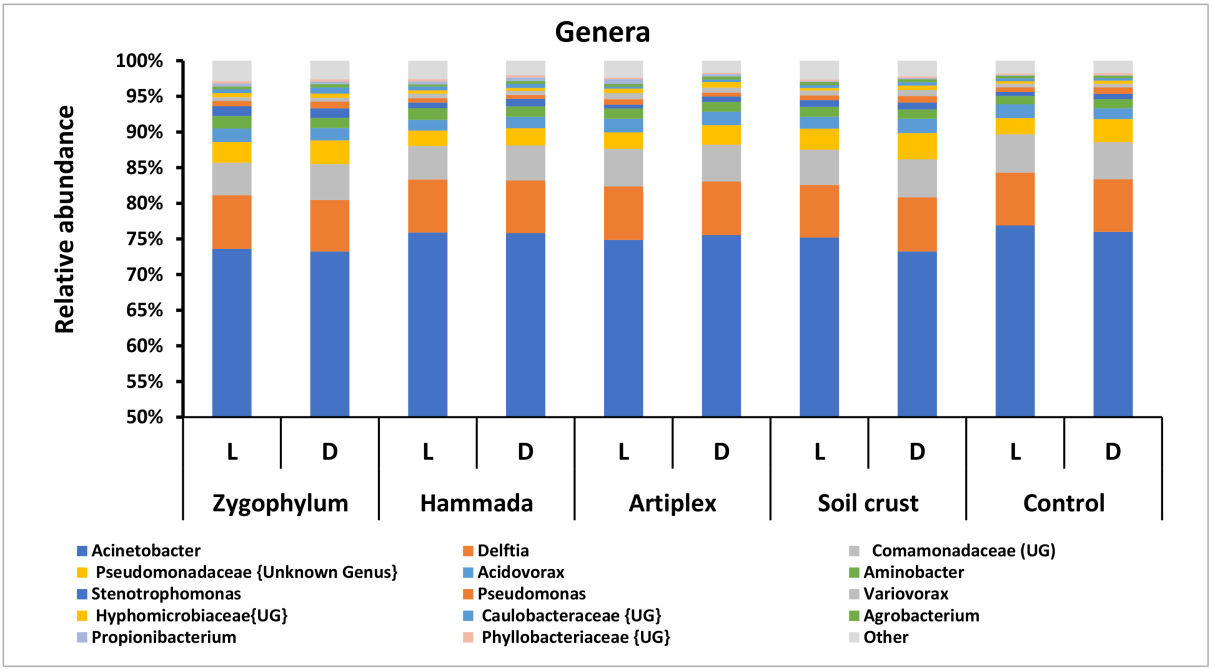

Figure 6. Relative abundance of the fifteen genera live (L) and dead (D) present in the soil samples collected in the vicinity of five sampling sites. Taxons and the relative ratio between live and dead (L/D) phyla; (B)classes in each one of the five soil samples, e.g., plant rhizosphere, soil crust and control open space sampling sites.

A total of 189 genera representatives were obtained, of which 175 genera were represented by less than 1% of each sample. In the fifteen genera, we included one unknown group represented by 10.9% of the total count. The five most abundant genera included Acinetobacter with a relative abundance ranging between 73% - 75%, *Delftia* (5.7% - 8.9%), *Acidovorax* (1.2% - 2.2%), and *Aminobacter* (1.2 - 2.6). The remaining genera ranged around one percent (Figure 6).

Efforts were made to ascertain the relative responses of the main fifteen genera in comparison to the control soil sample. Analyzing the fold change, which measures the relative ratio between live and dead microbial communities compared to the

microbial community obtained from the control soil, can shed light on the influence of activated or delayed biotic components on specific genera in response to the plant rhizosphere effect. This effect reflects the desert plant's ecophysiological adaptation (Figure 7). The *Acinetobacter* and *Delftia* genera were found to have a similar level of activity in all soil sampling locations, with no sampling site effect. By contrast, *Acidovorax*, *Caulobacteraceae*, and *Agrobacterium* were suppressed in all samples (except the soil crust). Such suppression triggered by desert halophytes in the root zone eliminates the above two genera, which are known as one of the major natural vectors in genetic engineering. However, *Pseudomonadaceae*, *Aminobacter*, *Pseudomonas* and *Phyllobacteriaceae* genera were suppressed in all sampling sites. The validation of site effect using the FC model was found to be a useful tool for validating the effect of sampling site on taxa genera, thus helping in the characterization of biological characteristics.

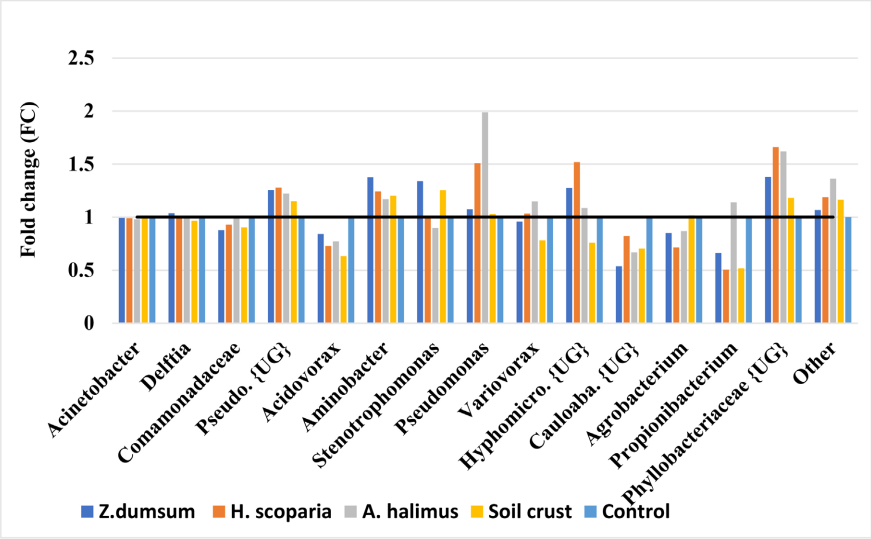

Figure 7. The bar graph illustrates alterations in genera taxa, with fold changes depicted as values below 1 or above, indicating variances relative to control soil samples. The shifts in taxa signify either downgrades or upgrades, representing the impact of plant ecophysiological adaptation on specific genera in the soil microbial community.

4. Discussion

Flow-cytometry methods are well-established methods for monitoring and differentiating between live and dead microbial populations for molecular taxonomic analysis. We compared the effect of desert plant ecophysiological adaptations on the below canopy soil microbial live/dead ratio and its taxonomical composition and diversity in samples collected at the end of the autumn dry season. Understanding the division between the live and dead members of a microbial community underscores the significance of population dynamics within the soil environment (Figure 3). Soil biota diversity in the upper soil surface layer (0 - 10 cm) is highly correlated with the edaphic environmental factors of soil moisture, pH, temperature, and radiation. Plants that live in such an extreme desert environ-

ment with a low nutrient content will promote micro-biome growth in order to improve nutrient levels (Kaplan *et al.*, 2013) [35]. This snapshot illustrates a distinct contrast among plant species related to plant ecophysiological adaptation. In the open (control) sampling sites between plants, which endure a more extreme environment, the relative abundance of dead bacterial communities is notably higher (45%). The similarity between soil crust and *Z. dumosum* contrasts with *H. scoparia* and *A. halimus*, where there is a decrease in the percentage of dead bacteria to approximately 30%, where a thriving community is evident, with around 50% representing live bacterial populations. The significantly higher ratio of dead cells under *Z. dumosum*, compared to *H. scoparia* and *A. halimus* in soil collected at the shrub base, may be linked to phylogenetic aspects of C4 photosynthesis. However, the genus Zygophyllum includes both C3 and C4 species, complicating this interpretation. An alternative explanation for these differences could be the presence and variation of endophytic fungi, which are known for their multifaceted roles in stress mitigation [36]. Despite these considerations, the question of why there is a high proportion of dead bacterial cells under *Z. dumosum* remains unresolved—particularly given the central role bacterial communities play in the nitrogen cycle.

As live soil biota is among the major participants in decomposing organic matter and supplying nutrients, the live and dead components each fulfill a role in the ecosystem. The dead cells serve as an energy source for the live active cells. A dynamic ratio determined by both environmental and plant adaptation factors will determine the nutrient pulse and the cycling frequency of the nutrient supply [37]. The use of cell sorting followed by molecular methods gave different views of the community structure, suggesting a significant effect of plant adaptation on the bacterial community structure.

The fold change, a straightforward ratio indicating quantitative differences between final values compared to the original ones relative to the control, has proven effective in detecting variations in gene taxa levels compared to control samples. Both the genera Acinetobacter and Dlftia, recognized as ubiquitous, are abundant in all the soil types sampled here. Their success stems from their ability to utilize diverse substrates [36]. The genus Acidovorax includes some plant pathogens, while the family Caulobacteraceae plays a crucial role in lignocellulose degradation [38] [39]. To counter their adverse effects on plant growth, all three plant species and the soil biocrust tended to suppress their impact. Agrobacterium, known for its role as a soil-borne pathogen affecting plants, was only suppressed due to the ecophysiological adaptations of the three plants [40]. It is noteworthy as the only cellular organism in natural environments capable of transferring genetic material between different kingdoms of life [41].

Contrary to the above trend, there is an increased abundance in Pseudomonadaceae {UG}, Aminobacter, Pseudomonas, and Phyllobacteriaceae {UG} genera in all soil samples obtained from the plant rhizospheres. This increase contributes to promoting plant growth by enhancing solubility (e.g., chlorides, Cu, P), soil aggregation, nutrient recycling, plant growth regulation (PGR), and controlling phy-

topathogens, as evidenced by various studies [42]-[44].

Our research emphasizes the importance of plant ecophysiological adaptation in influencing the balance within microbial communities. This influence extends beyond the presence of subterranean canopy shrubs, extending to the regulation of bacterial composition and activity. The main bacterial phyla present in soil samples in both living and dead categories were the Proteobacteria, recognized as a diverse phylum that plays a central role in nitrogen cycling, including nitrogen fixation, nitrification, and denitrification [44]. Additionally, Actinobacteria were found in relatively high abundance in all the soil samples, fulfilling important functional roles in decomposing complex organic compounds, such as cellulose and lignin, and producing antibiotics that help regulate soil pathogen populations. The presence of live bacteria in these two phyla exhibited similar behavior, decreasing relative to live bacteria in soil samples beneath *Z. dumosum*, soil crust, and control samples [45]. In arid environments, where the soil remains dry for over 90% of the year, microbial access to soluble resources is severely limited. While it is commonly believed that environmental factors play a crucial role in determining soil microbial communities and their functions, our findings suggest that this composition is primarily influenced on the local scale.

The key takeaway from this study is that the ecophysiological adaptations of plants significantly influence the functional diversity of microbes in the soil beneath their canopy, known as the laimosphere. Additionally, soil properties such as pH, composition, and electrical conductivity collectively determine the structure of microbial communities [46] [47].

The findings highlight the crucial impact of plant ecophysiological adaptations and soil physico-chemical parameters on the distribution, diversity, and live/dead ratio of soil bacteria. Ecologically important biomes support a rich diversity of soil bacteria, underscoring their microbial significance and the need for further exploration [48]. It is essential to conduct additional research to understand the long-term effects of plant ecophysiological adaptations on soil biota. Furthermore, future studies should investigate the impact of various unidentified desert plants on their surrounding environment, especially regarding their ability to transform, produce phytohormones, and adapt to extreme conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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