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Soil Bacterial and Fungal Community Composition in Top- and Subsoil From Irrigated Mediterranean Orchards

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The soil microbiome, crucial for nutrient cycling and soil health, has been extensively studied in topsoil, yet the subsoil microbiome remains relatively unexamined despite its potential contributions to agroecosystem functionality. This research aimed to bridge this knowledge gap by investigating the interconnections between soil properties and the microbial communities residing in the topsoil and the subsoil of irrigated orchards under a semiarid Mediterranean climate. We collected soil samples from two depths, topsoil (0–10 cm) and subsoil (30–50 cm), noting elevated salinity levels in the topsoil due to irrigation practices. Utilizing high-throughput 16S rRNA gene and ITS1 region amplicon sequencing, we characterized the bacterial and fungal communities across these two depths. Our findings indicated that soil pH (higher in subsoil), electrical conductivity (higher in topsoil) and soil organic carbon (higher in topsoil) were the primary physicochemical drivers of microbial community composition shifts between top- and subsoil. Genera such as *Rhizobium, Skermanella, Microvirga* and *Rubrobacter* (bacteria) and *Aspergillus*, *Gibellulopsis, Alternaria, Preussia* and *Monocillium* (fungi) were identified as key genera more abundant in the topsoil, while *MB-A2-108, Streptomyces* and *Bacillus* (bacteria), and *Mortierella, Fusarium, Necosmospora, Chaetomium* and *Emericellopsis* (fungi), were key genera more abundant in the subsoil, associated with key studied soil properties. So they can be considered as key microorganisms contributing to soil processes in the topsoil and the subsoil. Our study gives insights about how soil bacterial and fungal communities respond differently to changes in the soil physicochemical properties across topsoil and subsoil, with salinity as important driver, reflecting the crucial need to develop a better understanding of how environmental changes impact soil properties and the microbiome throughout the soil profile.

Keywords: subsoil, topsoil, soil pH, salinity, soil organic carbon, soil microbiome

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INTRODUCTION

In the last decades there has been an increasing attention on the importance of soil to provide multiple ecosystem services (ES) such as C sequestration, water regulation, biodiversity and soil fertility. However, most attention is given to topsoil (0–30 cm), especially in agriculture and forestry (Button et al., 2022). In this line, only 1.3% of the total number of articles dealing with "soil" in SCOPUS includes the study of the subsoil. In addition, only 3.2% of the total number of articles

dealing with "subsoil" in SCOPUS includes the study of the soil microbiome. Thus, among the low quantity of studies dealing with subsoil, most of them focus on subsoil properties and functions separately, without establishing the proper interconnection between soil properties and soil biological communities (Button et al., 2022; Djemiel et al., 2022). As a consequence, the role of the subsoil (>30 cm depth) in regulating multiple ecosystem functions (EF) and ES is poorly understood, limiting our ability to predict how subsoil processes can affect human wellbeing and agroecosystems sustainability (Button et al., 2022; He et al., 2023). Hence, there is a need to identify and characterize subsoil microbial communities which can be involved in the delivery of multiple ES such as crop productivity, water regulation, nutrient balance, decrease in soilborne disease incidence, or climate change mitigation in agroecosystems, to ensure long-term sustainability and avoiding current unsustainable management (Kautz et al., 2013; Sale et al., 2021).

Inadequate management and climate change are two of the greatest threats to soil biodiversity, with impacts on the soil microbiota which moderates multiple EF and provides ES (Davison et al., 2021; Nielsen et al., 2015). Orchards from Mediterranean and semiarid environments require the use of irrigation water to keep productivity (Espinoza-Meza et al., 2023). Quality of irrigation water is not always adequate, and can have elevated quantity of salts, mostly NaCl, which can accumulate in soils and negatively affect soil structure (Measho et al., 2022), soil microbiota (Rath et al., 2019), and the associated ES in which they are involved (Islam et al., 2023). It has been reported that 245 million ha of land in the Mediterranean countries are devoted to agriculture, out of which only 19.6 million ha are irrigated (Mrabet et al., 2020). Moreover, 25% of the irrigated Mediterranean croplands are salt-affected, with the potential of soil degradation (Brainich et al., 2018). The effects of soil salinity on soil functionality can be aggravated by climate change (Corwin, 2021), associated with socioeconomic problems due to drought and/or loss of yields (Kheyruri et al., 2023). However, the effect of climate change is still unclear, and therefore, a proper characterization and identification of soil microbial communities across the soil profile is essential (Zhang Z. Y. et al., 2023), to create tools to support the inclusion of subsoil processes in conservation and management programs and in the ES reckoning (Thorsøe et al., 2019), with particular attention to carbon credits and biodiversity certification (Buck and Palumbo-Compton, 2022). A global evaluation of the topsoil layer revealed that fungal and bacterial communities inhabit distinct ecological roles and exhibit different responses to rainfall patterns and soil pH, suggesting that climate change would have varying effects on their abundance (Bahram et al., 2018).

Soil is a dynamic system whose functionality is connected to the interactions among its physicochemical properties, soil organisms and environmental factors (Al-Kaisi et al., 2017). Soil microbial diversity is important to many ecosystem functions, including C and nutrient cycling and productivity (Maron et al., 2018), protecting plants from biotic stress (Hashem et al., 2017). Soil depth is an important element in determining several conditions that affect plant growth, such as nutrient and water reserves (Yost and Hartemink, 2020). Environmental factors like pH, water and nutrient availability vary with soil depth, impacting the composition of microbial communities (Wang et al., 2021). Previous studies have reported that soil physicochemical properties shape microbial community structure (Pang and Ryan, 2017; Xun et al., 2015), such as pH, electrical conductivity (EC) and SOC content, which vary with soil depth (Cai et al., 2016; Pang and Ryan, 2017; Wang et al., 2020). The microbial community composition typically shifts in response to variations in soil nutrients and soil organic carbon (SOC) at different soil depths (Hsiao et al., 2018). Soil ionic constituents, including SO_4^{2-}, Ca^{2+}, Na^{+}, and Mg^{2+}, have been found to exert significant influence on bacterial populations (Xia et al., 2023). Despite the resource scarcity, subsoil microbial populations can rapidly respond to organic C and N inputs (Jones et al., 2018). Soil depth and soil salinity has been reported to be important factors influencing the abundance, homogeneity, and diversity of microbial communities (Xin et al., 2023). In this line, soil salinity has emerged as a pivotal factor shaping the composition of microorganisms (Zhang C et al., 2019), with the capacity to diminish microbial production, activity, and modify the microbial community structure (Wichern et al., 2006). Microbial population size, including bacteria and fungi, decreases as soil depth increases (Beule et al., 2022; Yan et al., 2019), and therefore, the loss of biodiversity negatively impacts ES (Isbell et al., 2017). Hence, understanding subsoil microbial communities is vital for ecosystem restoration and environmental management (Yan et al., 2019).

Soil depth is not easily managed in agroecosystems, and it is highly significant for soil health and trees productivity, and still requires further analysis (Bünemann et al., 2018). Hence, understanding the microbiome structure in the top- and subsoil is crucial for determining how the overall microbial populations in soil respond to environmental and management changes, so this knowledge is practically applied to soil health management. The objectives of this study were to: i) understand how soil bacterial and fungal communities change with soil depth in irrigated orchards under Mediterranean semiarid conditions; ii) explore the connections between soil physicochemical properties and the structure of bacterial and fungal communities in response to soil depth; and iii) identify the main soil properties influencing the variations in bacterial and fungal communities in the top- and subsoil. For this purpose, we have performed an observational study in irrigated orchards under semiarid Mediterranean climate, where high soil salinity in the topsoil is attributed to the use of irrigation water with elevated salt content. For this, two different soil depths (topsoil and subsoil) were collected. We hypothesized that fungal and bacterial diversity would be lower in subsoil, with specific taxa disappearing with soil depth, mostly related to decreases in soil organic matter, soil salinity and available nutrient concentrations, and increases in soil pH.

MATERIALS AND METHODS

Study Site, Experimental Design and Soil Sampling

The study site was located in Chaouat, Manouba, Tunisia (36°51′51″N, 9°58′39″E), placed in the basin of the low valley of Medjerda river, an alluvial plain occupied by quaternary sandy-clay to sandy-clay-silt sediments. The climate is semiarid Mediterranean with annual mean temperature of 19°C, annual mean precipitation of 450 mm, and annual mean potential evapotranspiration of 1,370 mm. Three commercial apple orchards (*Malus domestica Borkh* var. Gala) were selected in the area. The orchards had an extension of 3–5 ha, with trees planted in 2016 at a spacing of 3 m between rows and 1.5 m between trees within the same row. The Medjerda river is used to convey water released from the Sidi Salem and El Aroussia dams to irrigate croplands. The average analytical data of the river water used for irrigation is shown in **Supplementary Table S1**. Irrigation was performed by pumping water from the Medjerda river, transporting it through pipes and distributing it to the field through a drip irrigation system. The frequency of irrigation varied according to the evaporative demand, which was once every month in winter, once every 2 weeks in autumn and spring, and two times per week in summer. Soil type is a Halpic Calcisol (IUSS Working Group WRB, 2022), with silt loam texture, soil organic matter (SOM) content of 1.2%, pH of 7.9 and mean electrical conductivity of 1.2 mS cm^{-1} in the Ap horizon (0–30 cm). Standard cultural practices (e.g., fertilization, pruning, fruit thinning and banding) were carried out by the technical team of the commercial orchard. Weed control was managed manually. Fertilizers, such as cow manure and crop residues, were applied two times per year, as provider of nutrients for crop growth.

One soil sampling campaign was conducted in August 2021 to assess differences in soil depth after the management with irrigation water with high salinity since 2016 (5 years period). We collected four random composite samples in each orchard across the entire surface, at two soil depths: topsoil (0–10 cm) and subsoil (30–50 cm), to ensure differences in soil bacterial and fungal communities in top- and subsoil layers. Although we also collected soil at 10–30 cm depth, owing to budget constraints, we decided to use only those. This is because topsoil is considered between 0 and 30 cm (Poeplau et al., 2020; Szatmári et al., 2019), but it has been previously reported that microbial activity and abundance are generally higher within the 0–10 cm range (Zhao et al., 2022). The 30–50 cm depth is already widely considered as subsoil (Poeplau et al., 2020; Szatmári et al., 2019). Sampling was performed in the tree row area, in the middle of two trees (at 70–80 cm distance from the tree). Each composite sample derived from 5 random subsamples collected in the same spot for topsoil and subsoil. Soil samples were divided in the field into two aliquots, one maintained at ambient temperature for physicochemical analyses, while the other was kept in a cool box with ice for biological analyses. The samples were immediately taken to the laboratory. The soil was air-dried for 1 week and sieved at <2 mm for analyses. Soil intended for biological analyses was sieved at <2 mm upon arrival at the lab and stored at −80°C.

Soil Physicochemical Analyses

Particle size distribution was determined using the Mastersizer 2000LF laser diffraction analyzer (Malvern Instruments) following the oxidation of organic matter and dispersion of clays. Soil organic carbon (SOC), inorganic carbon (IC) and total nitrogen (N) were measured using an elemental analyzer (CNHS-O, Leco Soil Standard 502-697). The $CaCO_3$ content was calculated from the analysis of IC. The pH and electrical conductivity (EC) were measured in deionized water (1: 2.5 and 1:5 m/v, respectively) in a pH-meter Crison GLP-21 and a conductivity meter Crison GLP-31. Actual available cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) and anions (Cl^{-}, NO_3^{-}, SO_4^{2-} and NO_2^{-}) were extracted with deionized water (1:5 m/v) and quantified using ion chromatography (Metrohm 861). Soil CO_2 and N_2O emission rates were measured by the quantifying the gases released from 2 g of soil in 25 mL glass vials closed with crimp caps after 24 h incubation at 25°C and 60% of water holding capacity, using a gas chromatograph (AGILENT 6890N).

Soil DNA Extraction and 16S rRNA and Fungal ITS1 amplicon Sequencing

Soil DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). All procedures were done following the instruction manual, except for the bead-beating step, which was conducted using the environmental sample setting on an MP Fastprep-24 instrument (MP-bio, Solon, OH, USA). Purification of the obtained DNA was performed using NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel, Düren, Germany). Qubit® 2.0 Fluorometer combined with Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, California, US) and NanoPhotometer N60 (Implen GmbH, Munich, Germany) were employed for subsequent DNA quantification and purity assessment, respectively. Extracted DNA was stored at −20°C until further analysis (Barrena-González et al., 2025).

For bacterial library preparation, a ~290 bp fragment of the V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F and 806R (modified from Parada et al., 2016, and from Apprill et al., 2015; respectively). Fungal library preparation was constructed targeting a ~300 bp fragment of the internal transcribed spacer (ITS) region 1 employing the primers ITS1f and ITS2 (White et al., 1990). Both primers sets included the Illumina primer adapters attached to their 5′ ends to facilitate downstream sequencing. PCR amplifications were performed in a final volume of 12.5 μL, containing 1.25 μL of template DNA, 0.3 μM of each primer, 3.25 μL of Supreme NZYTaq 2x Green Master Mix (NZYTech, Lisbon, Portugal), and ultrapure nuclease-free water up to 12.5 μL. The thermal cycling conditions for bacterial amplification consisted of an initial denaturation step at 95°C for 5 min, and 25 cycles of 95°C for

30 s, 46°C for 45 s, 72°C for 45 s, followed by a final extension step at 72°C for 7 min (Ollio et al., 2025). For fungal library preparation, PCR cycling conditions included the same initial denaturation step and final extension step as for bacteria, but cycling conditions consisted of 30 cycles of 95°C for 30 s, 49°C for 45 s, 72°C for 45 s. A second PCR was conducted to incorporate dual indices and Illumina sequencing adapters, using the same reaction composition. This indexing PCR was performed under the same cycling conditions but limited to 5 cycles and used a 60°C annealing temperature. Negative controls containing no DNA template (BPCR) were included in every PCR round to assess potential contamination. The libraries were visualized on 2% agarose gels stained with GreenSafe (NZYTech, Lisbon, Portugal), and examined under UV light to confirm the expected library size. Libraries were then purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek, Norcross, GA, USA) and pooled in equimolar amounts based on quantification data obtained by the Qubit dsDNA HS Assay (Thermo Fisher Scientific, Waltham, MA, USA) (Ollio et al., 2025). This pool, which included a testimonial amount (1 µL) of the PCR negative controls, was sequenced on an Illumina NovaSeq 6000 platform (PE250 configuration), generating approximately 25 GB of data (Barrena-González et al., 2025). Amplicon sequencing was carried out by AllGenetics & Biology SL (www.allgenetics.eu). Raw sequencing data are publicly available at: https://github.com/MohamedMdaini8/Mohamed-Mdaini-16S-and-ITS-raw-data.

Bioinformatic Analysis

Illumina paired-end raw reads were sorted by library and their quality scores, indices and sequencing primers were trimmed during the demultiplexing step. FastQC (Andrews, 2010) was employed to check the quality of the FASTQ files while MultiQC (Ewels et al., 2016) was employed to aggregate their content. Bioinformatic analysis was performed with QIIME 2 2021.8 (Bolyen et al., 2019), being raw sequence data quality filtered, denoised, chimera-checked, merged, and clustered into amplicon sequence variants (ASVs) (Callahan et al., 2017) with DADA2 (Callahan et al., 2016) (via q2-dada2). Bacterial ASVs were aligned with mafft (Katoh et al., 2002) (via q2-alignment) and used to construct a phylogeny with fasttree2 (Price et al., 2010) (via q2-phylogeny), employed for the construction of alpha rarefaction curves and phylogenetic alpha and beta diversity indices. Alpha and beta diversity metrics and Principal Coordinate Analysis (PCoA) were estimated using q2-diversity, and one underperformed library was removed from the analysis in the case of fungal sequences. PCoA is an ordination method, which was based on a Bray–Curtis distance matrix in this study, used to visualize the differences in microbial community composition between samples. Each point on the plot represents one soil sample, and the distance between points reflects how dissimilar the microbial communities are. Clustering or separation of points indicates differences in community structure related to soil depth (Seuradge et al., 2017). Taxonomy was assigned to bacterial and fungal ASVs via the q2-feature-classifier plugin (Bokulich et al., 2018) using a pre-trained classifier (Bokulich et al., 2018) of the SILVA reference database (Quast et al., 2013, release 138.1 August 2020), for bacteria, and a pre-trained classifier of the UNITE reference database UNITE (Abarenkov et al., 2021, release May 2021) for fungi.

Statistical Analysis

All data used in this study has been uploaded to the repository Zenodo (https://doi.org/10.5281/zenodo.14517703). The Shapiro test was used to assess whether the data followed a normal distribution. The Student's t-test was performed to assess significant differences for soil physicochemical properties between topsoil and subsoil. A Kruskal-Wallis test was used to assess differences between soil depth of alpha diversity estimates (observed number of ASVs, Chao1, Shannon, Faith and Pielou's evenness). A non-parametric permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), with 999 random permutations was employed on Bray-Curtis distance matrices to evaluate the variance in the community composition across soil depth (beta diversity). Bivariate correlations were performed to assess the relationships between the relative abundance of fungal and bacterial taxa and physicochemical properties using Pearson analysis in the ggcorrplot package in R software. A redundancy analysis (RDA) was conducted on Hellinger-transformed data (Legendre and Gallagher, 2001) using the *vegan* and *ggplot* packages in R to evaluate the extent to which the variation in bacterial and fungal communities (at the genus level) can be attributed to differences in physicochemical properties. Samples with similar bacterial and fungal profiles have similar scores and therefore appear closer to each other in the plot. Soil physicochemical properties are depicted as vectors; those with longer lengths and smaller angles relative to an axis are more strongly associated with that axis (Özbolat et al., 2023).

RESULTS

Soil Physicochemical Properties

Most of the studied properties showed significant differences between topsoil and subsoil (**Table 1**). Soil pH was significantly lower in the topsoil (7.73) compared to the subsoil (8.18), although $CaCO_3$ content was not significantly different between depths (~39.5%). EC showed significantly higher values in topsoil (1.71 mS cm^{-1}) compared to subsoil (0.56 mS cm^{-1}), and there was a strong negative correlation between pH and EC ($R = -0.94$; $P < 0.001$; **Supplementary Figure S1**), and between EC and $CaCO_3$ ($R = -0.68$; $P < 0.001$). SOC was significantly higher in topsoil (1.61%) than in subsoil (0.79%), although N showed no significant differences with depth. SOC was positively correlated with EC ($R = 0.61$; $P < 0.001$), and negatively correlated with pH ($R = -0.82$; $P < 0.001$). The concentrations of SO_4^{2-}, Cl^-, Na^+ and Ca^{2+} were the dominant ions in the topsoil, accounting for 97%, 81%, 74%

TABLE 1 | Main soil characteristics for both soil depths (topsoil and subsoil) in the studied orchards. Values are mean ± standard error (SE), n = 12.

Soil properties	Soil depth (cm)				Statistical test	
	Topsoil (0-10)		Subsoil (30-50)		Student's t-test	
	Average	SE	Average	SE	t-value	*p-value*
pH	7.73	0.08	8.18	0.05	–7.00	**0.000**
EC (mS cm^{-1})	1.71	0.40	0.56	0.04	3.40	**0.013**
Carbonates (%)	39.12	0.62	40.14	0.84	–0.40	0.720
SOC (%)	1.62	0.22	0.79	0.09	3.40	**0.003**
N (%)	0.12	0.02	0.14	0.03	–2.30	0.083
Cl^- (mg kg^{-1})	410.00	120.00	79.00	12.00	2.70	**0.014**
NO_2^- (mg kg^{-1})	2.07	0.67	0.95	0.17	2.00	0.060
NO_3^- (mg kg^{-1})	21.66	7.68	6.75	0.79	2.30	**0.050**
SO_4^{2-} (mg kg^{-1})	228.8	80.80	86.77	14.20	2.00	0.063
Na^+ (mg kg^{-1})	216.60	63.80	76.62	9.60	3.00	**0.012**
K^+ (mg kg^{-1})	20.93	7.48	4.30	0.97	3.70	**0.002**
Ca^{2+} (mg kg^{-1})	107.32	29.32	24.91	2.50	3.10	**0.006**
Mg^{2+} (mg kg^{-1})	27.71	9.68	4.60	0.64	5.00	**0.000**
CO_2 (μg kg^{-1} h^{-1})	879.00	133.00	644.00	112.00	3.30	**0.004**
N_2O (ηg kg^{-1} h^{-1})	2.81	0.41	4.94	0.51	–3.30	**0.004**
NH_4^+ (mg kg^{-1})	1.00	0.57	0.45	0.14	1.10	0.259

EC, electrical conductivity; SOC, soil organic carbon; N, total nitrogen.
Statistically significant value is highlighted in bold (confidence level at 95%, p < 0.05).

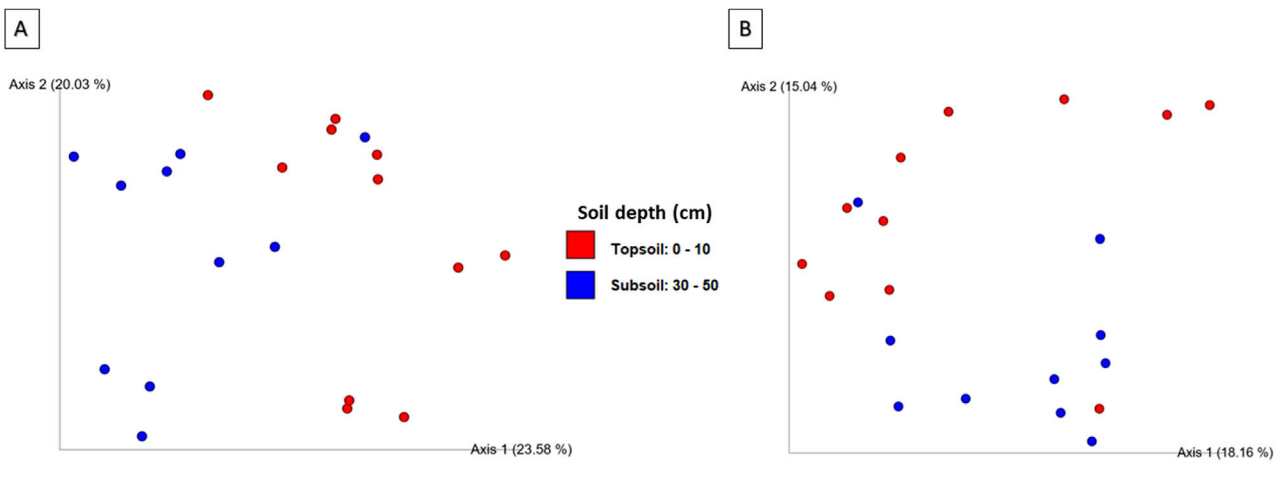

FIGURE 1 | Principal Coordinates Analysis (PCoA) using Bray-Curtis distance matrices for bacterial **(A)** and fungal **(B)** communities in topsoil and subsoil.

and 82%, respectively, of the total ionic content (**Table 1**). These ions, however, accounted for 3%, 19%, 26% and 18% in the subsoil. The concentration of cations and anions was significantly higher in the topsoil than in the subsoil, except for SO_4^{2-}, NO_2^- and NH_4^+, that showed no significant differences with depth. Soil CO_2 emission rates were significantly higher in topsoil (879 μg kg^{-1} h^{-1}) compared to subsoil (644 μg kg^{-1} h^{-1}), while N_2O emission rates were significantly higher in subsoil (4.94 ηg kg^{-1} h^{-1}) than in topsoil (2.81 ηg kg^{-1} h^{-1}). EC showed strong positive correlations with all cations and anions, and a strong negative correlation with N_2O (**Supplementary Figure S1**). Contrarily, soil pH showed strong negative correlations with all the cations and anions, and a strong positive correlation with N_2O. N_2O was also negatively correlated with SOC, NO_2^- and NO_3^-.

Soil Microbial Diversity and Community Structure

After the filtering steps, the 16S rRNA gene sequences ranged from 99,387 to 167,345 sequences per sample (mean: 128,646.47) and 8,830 ASVs were included in the ASV table. For ITS1 region fungal reads ranged from 34,991 to 249,389 sequences per sample (mean: 143,659.2) and a total of 1,648 ASVs were included in the table.

With regards to the alpha diversity indices, there were no significant differences for Chao1, Pielou's evenness, observed

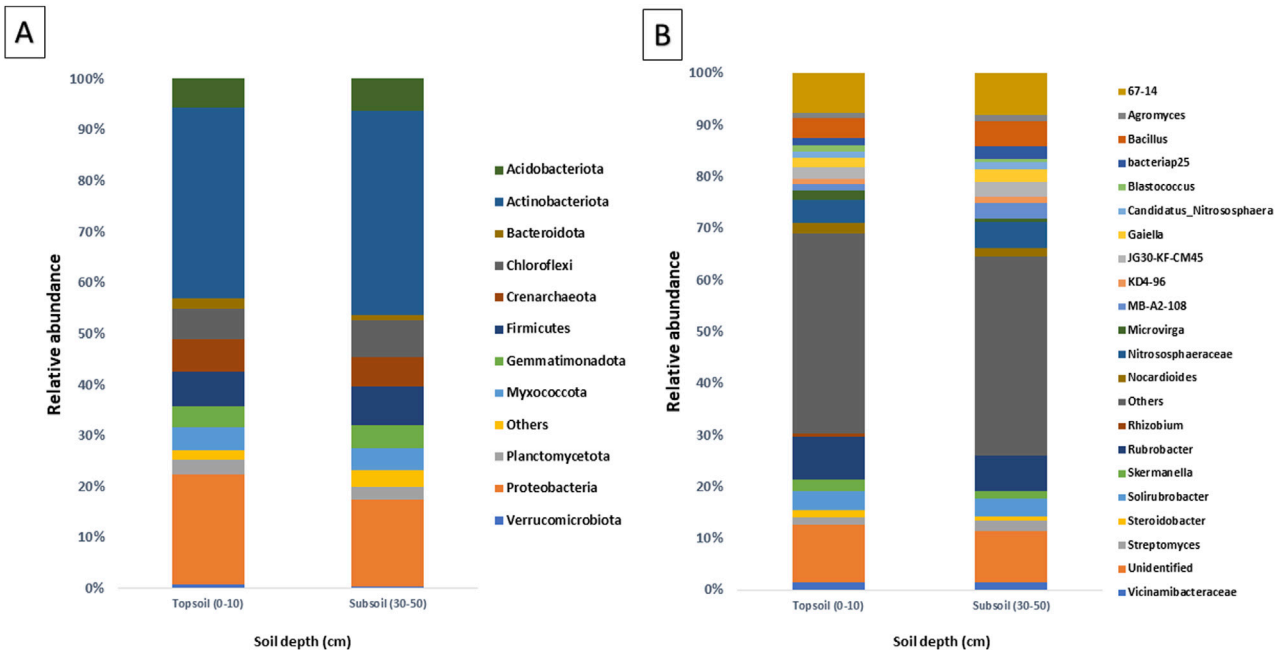

FIGURE 2 | Mean relative abundance (>1%) of bacterial phyla **(A)** and genera **(B)** in terms of soil depth.

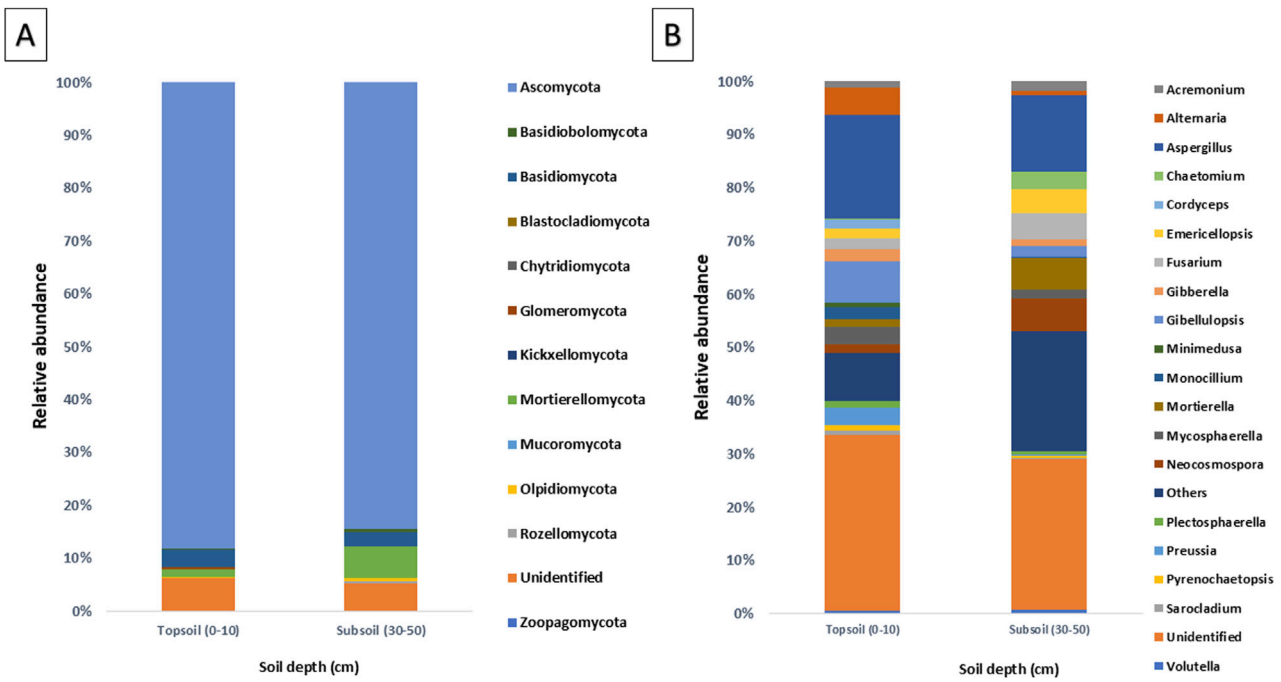

FIGURE 3 | Mean relative abundance (>0.5%) of fungal phyla **(A)** and genera **(B)** in terms of soil depth.

number of ASVs, and Shannon index across the soil depth gradient for bacteria (**Supplementary Table S2**). For fungi, Pielou's evenness and Shannon index showed significant differences between depths, with higher values in the subsoil for Pielou's evenness, and higher values in the topsoil for Shannon index (**Supplementary Table S2**). The beta diversity

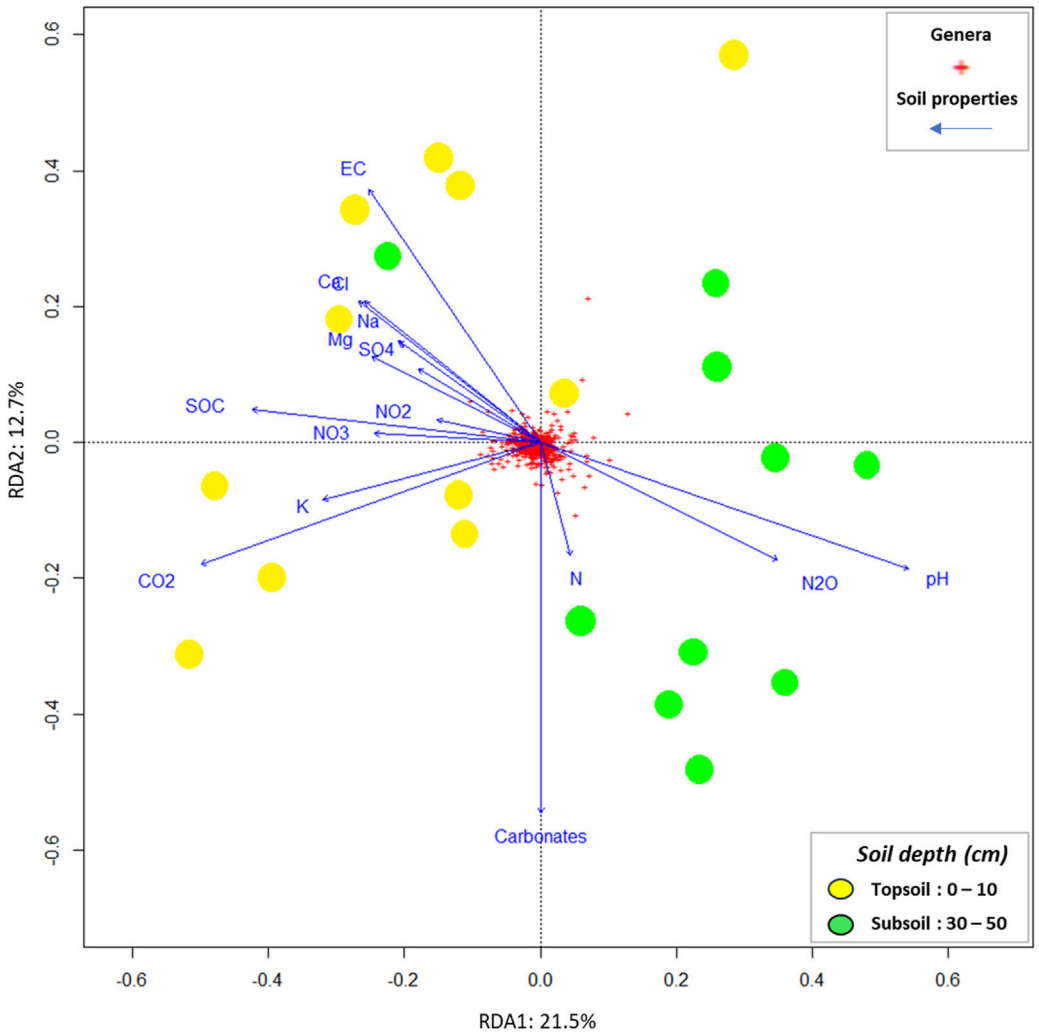

FIGURE 4 | Redundancy analysis between the bacterial microbiome composition (genus level) and soil physicochemical properties. SOC, soil organic carbon; EC, electrical conductivity; N, total nitrogen.

analysis, based on the Bray-Curtis dissimilarity matrix, revealed significant differences in the bacterial ($F = 3.52$; $P < 0.001$), and fungal ($F = 1.70$; $P < 0.05$) communities between the top- and subsoil. The composition of both bacterial and fungal communities was strongly clustered by soil depth, suggesting that the microbiome is shaped by soil properties and characteristics unique to that depth (**Figure 1**).

Soil fungal community composition was more affected by soil depth than bacterial community composition, with most phyla and genera showing significant differences between depths (**Figures 2**, **3**). The ASVs that could not be identified below family level are summarized as "Unidentified", and the genera represented by less than 1% (bacteria) and less than 0.5% (fungi) of total reads were collapsed in "Others". For the soil bacterial community, the most abundant phyla in both topsoil and subsoil, were *Actinobacteriota* (36%–40%) and *Proteobacteria* (20%–16%), followed by *Firmicutes* (5%–6%), *Chloroflexi* (5%–7%), *Crenarchaeota* (5%–3%) and *Acidobacteriota* (4%–6%) (**Figure 2A**). Topsoil exhibited higher relative abundances of *Proteobacteria* and *Bacteroidota*, while subsoil showed higher relative abundances of *Actinobacteriota* and *Chloroflexi*. The most abundant classified bacterial genera for both soil depths were *67–14* (8%–9%), *Rubrobacter* (9%–6%), *Nitrososphaeraceae* (4%–5%), and *Bacillus* (4%–7%) (**Figure 2B**). The genera *Rhizobium, Rubrobacter*, *Skermanella*, and *Microvirga* showed higher relative abundances in topsoil, while *MB-A2-108*, *bacteriap25, Streptomyces* and *Gaiella* showed higher relative abundances in subsoil. For the fungal community, *Ascomycota* (89%–86%), *Mortierellomycota* (3%–9%) and *Basidiomycota* (1%–2%) were the most abundant fungal phyla (**Figure 3A**). Topsoil showed higher relative abundances of *Ascomycota*, *Basidiomycota* and *Glomeromycota*, while subsoil showed higher relative abundances of *Mortierellomycota*, *Rozellomycota*, *Olpidiomycota* and *Basidiobolomycota*. The

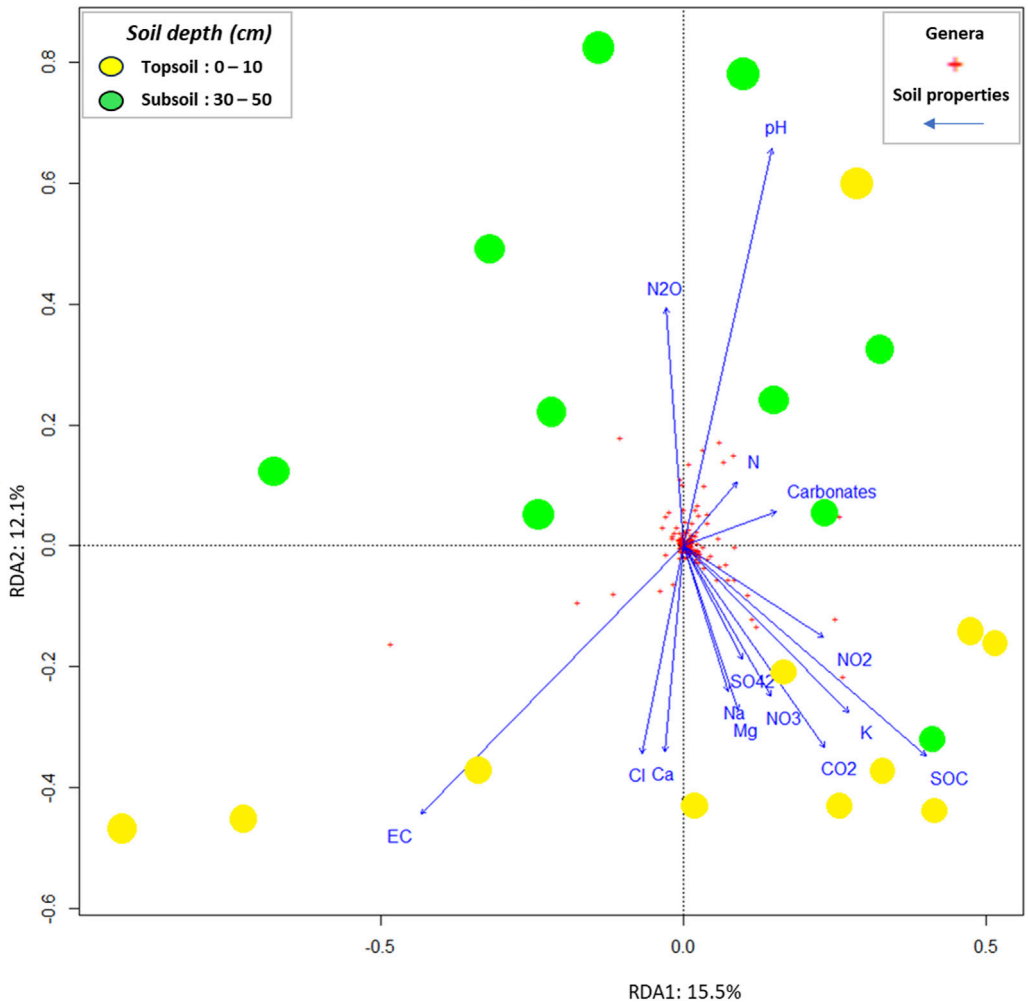

FIGURE 5 | Redundancy analysis between the fungal microbiome composition (genus level) and soil physicochemical properties. SOC, soil organic carbon; EC, electrical conductivity; N, total nitrogen.

genera *Aspergillus* (17%), *Gibellulopsis* (8%), *Alternaria* (5%), *Preussia* (4%), *Mycosphaerella* (3%), *Monocillium* (2%), *Gibberella* (2%), *Cordyceps* (2%), and *Saroclaudium* (1%) were more abundant in the topsoil, while *Fusarium* (6%), *Emericellopsis* (5%), *Neocosmospora* (7%), *Mortierella* (6%), *Chaetomium* (3%) and *Acremonium* (2%) were more abundant in the subsoil (**Figure 3B**).

Soil Physicochemical Properties can Explain Variations in Bacterial and Fungal Communities Across Soil Depth

The RDA showed that the first two axes explained 34.2% of the variance of the bacterial community (**Figure 4**). The first axis explained 21.5% of the variation, and clearly separated topsoil (negative scores) from subsoil (positive scores). The second axis explained 12.7% of the variation and was mostly associated with soil carbonate content. Soil pH, CO_2 emission rates, SOC and EC were the properties that mostly contributed to explaining the variability in the composition of bacterial communities. The RDA performed with the fungal genera showed that the first and second axes explained 27.6% of the total variation, accounting for 15.5% and 12.1%, respectively (**Figure 5**). Topsoil and subsoil samples were clearly separated by axis 2. As for bacteria, soil pH, CO_2 emission rates, SOC and EC were the properties that mostly contributed to explain the variability in the fungal community composition.

Pearson correlation analysis between the relative abundance of the most abundant classified bacterial genera and soil physicochemical properties is shown in **Figure 6**. The relative abundances of *Skermanella*, *Ensifer*, *Geodermatohphilus*, *Microvirga* and *Blastoccocus* showed significant positive correlations with SOC, while were negatively correlated with pH and N. Soil pH was strongly associated with the relative abundances of *Bacteriap25*, *Gaiella*, *MB.A2.108* and *Gitt-GS-136*. CO_2 emissions had positive correlations with the relative

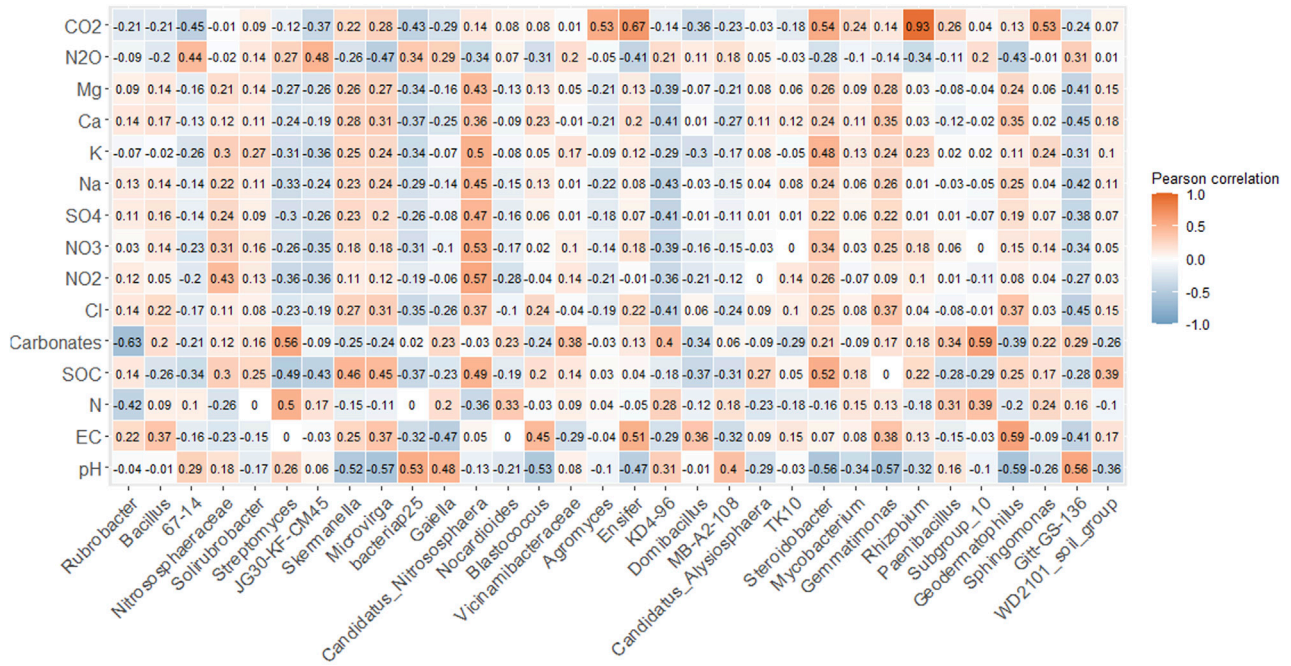

FIGURE 6 | Heatmap showing Pearson correlation analysis between soil physicochemical properties and the relative abundance of topmost abundant identified bacterial genera. EC, electrical conductivity; SOC, soil organic carbon; N, total nitrogen.

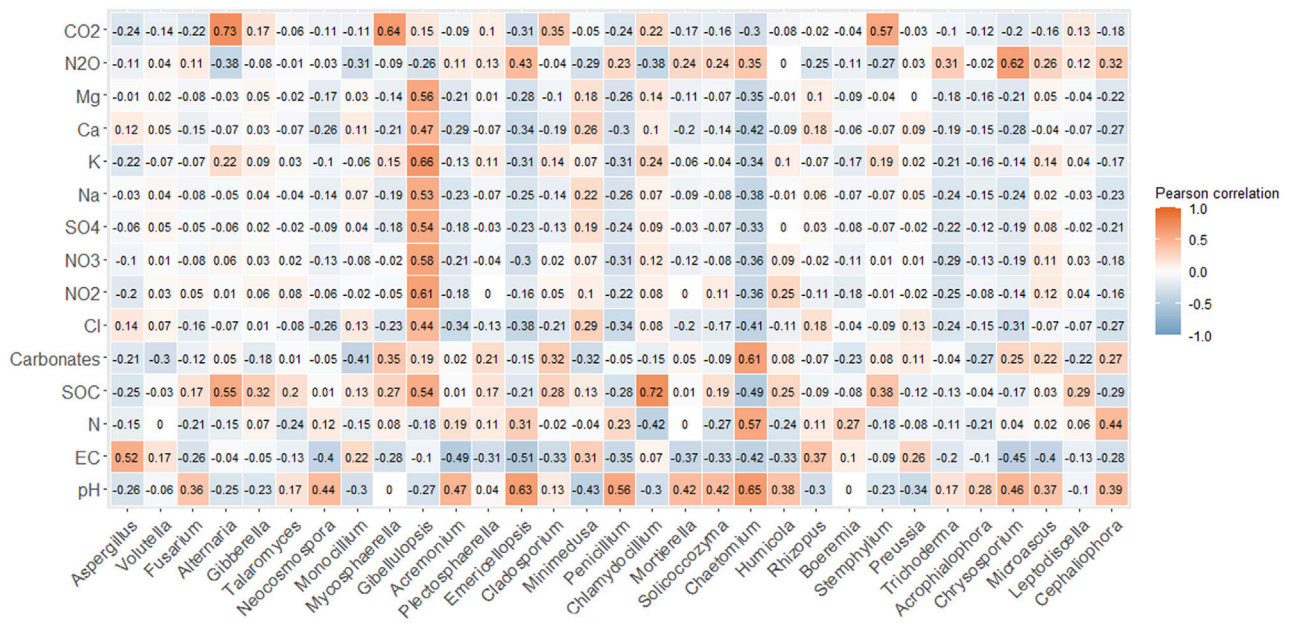

FIGURE 7 | Heatmap showing Pearson correlation analysis between soil physicochemical properties and the relative abundance of topmost abundant identified fungal genera. EC, electrical conductivity; SOC, soil organic carbon, N, total nitrogen.

abundances of *Agromyces*, *Ensifer*, *Steroidobacter* and *Rhizobium* and negative correlations with *JG30-KF-CM45* and *bacteriap25*. EC showed positive correlations with *Microvirga, Ensifer, Blastococcus,* and *Geodermatophilus*, and a negative correlation with *Gaiella*. Soil N_2O emissions showed positive correlation with the abundance of *JG30-KF-CM45* and *X67-14*. For the fungal community (**Figure 7**), soil pH exhibited significant positive correlations with the relative abundances of *Chaetomium,*

Acremonium, *Emericellopsis*, *Neocosmosphora*, *Solicoccozyma*, *Mortierella* and *Penicillium*, while showing a negative correlation with *Aspergillus*. EC showed significant positive correlations with *Aspergillus*, while negatively correlated with *Mortierella, Chaetomium, Acremonium*, *Emericellopsis,* and *Chrysosporium*. The relative abundances of *Alternaria, Stemphyllium* and *Mycosphaerella* had a significant positive correlation with CO_2 emissions. *Chlamydocillium, Alternaria*, *Gibellulopsis,* and *Gibberella* were positively correlated with SOC, while *Chaetomium* showed a negative correlation with SOC. *Gibellulopsis* showed positive correlations with all the cations and anions. N_2O emissions showed positive correlations with *Emerecillopsis*, *Chaetomium*, *Trichoderma*, *Cephaliophora* and *Chrysosporium*, and negative correlations with *Alternaria*, *Monocillium,* and *Gibellulopsis*. N was positively correlated *Emerecillopsis* and *Chaetomium*.

DISCUSSION

Differences in Soil Physicochemical Properties Across a Depth Gradient and Relationships With Soil Microbial Groups

Soil pH and EC showed a significant negative relationship since pH decreased with an increasing salinity in the topsoil. This is contrary to most research, since the increase in the concentration of bases is linked to increases in soil pH (Ge et al., 2023; Hou et al., 2021; Luo et al., 2024). However, several studies reported that soil pH was higher in low salinity levels (Yang et al., 2021; Yang and Sun, 2020). An increase in salinity is typically caused by the input of Na^+ and Cl^- from irrigation water drawn from rivers (Arikan, 2022), and also SO_4^{2-} ions (Srivastava et al., 2019). The high concentrations of SO_4^{2-} within the saline irrigation water proceeded from the river in the study area (Ferchichi et al., 2018), and this is also confirmed by the results of SO_4^{2-} in the soil samples analyzed in this study. The Na^+ ions compete with other cations from attachment sites on soil colloids, releasing to the soil solution previously retained bases such as Ca^{2+}, Mg^{2+} or K^+ (Violante, 2013). This can explain the significant increases in the concentration of these bases in the topsoil compared with subsoil, besides the application of manure that could slightly be contributing to the increase of salinity on the topsoil (Awad et al., 2015). However, soil pH is a key property that influences almost all soil characteristics, and it is impacted by the soil buffering capacity (Hong et al., 2019). Hence, soil carbonates function as a pH buffer and maintain a stable pH (Li A. et al., 2023), given the high content of carbonates across the soil surface. Nevertheless, owing to the high quantity of carbonates and soluble salts on the topsoil, it is more likely that soil pH decreased in topsoil owing to the highest SOC content. Soil pH tends to decrease in the topsoil as a result of SOM accumulation, because the decomposition of organic compounds generates additional organic acids, resulting in the reduction of pH in the topsoil (Macias-Benitez et al., 2020). Thus, the presence of organic acids in the soil can help maintain a lower pH by counteracting the effects of other pH-increasing factors such as bases (Ca, Mg, Na, K). In fact, Rukshana et al. (2014) reported that the addition of organic acids into the soil, such as citrate/citric acid, were successful to decrease soil pH in basic/alkaline soils with problems of nutrient availability. Hossain et al. (2023) explored the effect of organic farming practices in an agricultural soil on soil pH and EC, and exhibited that the application of manure as an organic practice led to the decline in soil pH and the increase of soil salinity. Xue et al. (2020) investigated how increased soil salinity influences the topsoil SOC (0–30 cm) in a salt marsh field using both field and manipulative experiments. They reported a significant positive relationship between SOC and soil salinity in the topsoil, indicating that soil salinity could be a key factor controlling SOC concentrations.

Our study revealed higher N_2O emissions in the subsoil than in the topsoil, strongly associated with higher pH values. These findings suggest that denitrification rates may be higher in the subsoil compared to the topsoil, likely due to lower aeration and higher pH (Fudjoe et al., 2022). Zhang B. et al. (2023) investigated the interactive effects of soil pH and NO_3^- concentration on soil denitrification through a mesocosm experiment. They reported that soil NO_3^- concentration significantly impacted the denitrification-derived N_2O emissions, and soil pH influenced N_2O emissions from denitrification. Qiu et al. (2024) conducted several field experiments with N fertilizers to identify the relationship between soil pH and N_2O emissions, and reported positive correlations between soil pH and N_2O emissions, and negative correlation between SOC and N_2O, as in our study. Moreover, manure addition to the soil, with higher availability of available N and labile organic carbon sources, that can leach through the profile, could significantly enhance N_2O emissions (Shakoor et al., 2021). The genus *JG30-KF-CM45*, that was associated with N_2O emissions, can be affected by the use of organic fertilizers, and has been found to be playing a crucial role in soil functioning, including N cycling and organic decomposition (Guo et al., 2024). With regards to the N content, we observed a high relationship between N and the genus *Streptomyces*. Dahal et al. (2017) investigated and characterized free-living diazotrophs in arid soils of South Dakota (USA) and concluded that *Streptomyces* have the ability to fix N. Several species of *Streptomyces* have been identified with the ability to fix N (Vurukonda et al., 2018). Furthermore, *Streptomyces* are renowned for their capability to produce antimicrobial compounds (Aislabie and Deslippe, 2013). Within the fungal community, the genera *Emericellopsis* and *Chrysosporium*, with higher abundance in the subsoil, were associated with higher soil pH and N_2O emissions. While both *Ascomycota* and *Basidiomycota* are the predominant fungal phyla, *Ascomycota* appears to be a more functionally important phylum for N_2O production based on their greater relative abundance and stronger positive correlations with N_2O fluxes in the soil (Muneer et al., 2021; Peng and Valentine, 2021; Xun et al., 2020). Overall, fungi play a complex and important role in N_2O emissions from saline and semiarid soils, with factors like oxygen availability and SOM being crucial for their activity

(Li X. et al., 2023; Lin et al., 2024). *Emericellopsis* and *Chaetomium* were also significantly correlated with N in our study, as previously reported by Ji et al. (2023), suggesting that they may play an important role in N cycling processes (Yang et al., 2022). *Emericellopsis* species are capable of producing various of bioactive metabolites with antimicrobial properties effective against plant and human pathogens, as well as anticancer activity (Kuvarina et al., 2021, 2022).

The Soil Microbiome Is Shaped by Soil pH, SOC and Salinity Across a Depth Gradient

This study showed that some of the most dominant phyla, such as *Actinobacteria, Proteobacteria* and *Chloroflexi*, have already been identified in other studies as the main bacterial taxa in other saline soils (Ding et al., 2023; Li J. et al., 2023; Lin L. et al., 2023; Xia et al., 2023; Yang et al., 2023). *Ascomycota* and *Basidiomycota,* on the other hand, have been identified as the dominant fungal phyla (Zhang G. et al., 2023b; Zhang Z. Y. et al., 2023). Consistently with our hypothesis, the composition of the soil microbial communities across soil depth, was mostly impacted by soil pH, followed by SOC and soil salinity, with a significant association with CO_2 emissions, highly dependent on SOC content. Some studies have also reported that pH, salinity and SOC were the main drivers modifying the composition and structure of the microbial communities across a soil depth gradient (O'Brien et al., 2019; Xu et al., 2021; Zhao et al., 2021).

The alpha diversity metrics were higher in the topsoil for bacterial communities. Numerous studies also reported this decrease of bacterial diversity across a soil depth gradient (Aguado-Norese et al., 2023; Will et al., 2010; Zhang W. et al., 2019), suggesting that this variation could be related to variations in the soil properties, such as soil pH, between top- and subsoil (He et al., 2023). Soil pH plays a key role in shaping the bacterial composition across soil depth (Yun et al., 2016). Several genera from the *Proteobacteria* phylum, such as *Microvirga*, were negatively correlated with soil pH. The relative abundance of *Microvirga* was higher in the topsoil, suggesting that this genus thrives in nutrient-rich environments with high SOC content (Araujo et al., 2023). Additionally, the optimal growth of many *Microvirga* strains occurs at a pH around 7 (Msaddak et al., 2017). Many studies have reported a negative relationship between soil pH and *Proteobacteria*, as in our study (Muneer et al., 2022; Wang et al., 2019). Soil pH and the genus *MB-A2-108 (Actinobacteria)* were positively correlated (Cheng et al., 2023), being soil pH and the abundance of *MB-A2-108* higher in subsoil, suggesting that *MB-A2-108* has the potential to thrive in low nutrient environments (Megyes et al., 2021). The highest SOC content in the topsoil contributed to higher CO_2 emissions by microbial mineralization (Sosulski et al., 2023; Xin et al., 2023). CO_2 emissions were strongly correlated with several bacterial genera, such as *Rhizobium*, *Ensifer* and *Steroidobacter*, belonging to the phylum *Proteobacteria*, which showed a higher relative abundance in the topsoil compared to the subsoil. *Proteobacteria* are a prevalent group in soil that play a crucial role in soil metabolism and nutrient cycling (Magadlela et al., 2023), making them essential for the global C cycling in soils (Spain et al., 2009). *Proteobacteria* play a crucial role as the primary bacteria responsible for the breakdown and transformation of organic matter (Zhang Z. Y. et al., 2023), and a key role in nutrient cycling under climate warming (Zhou et al., 2023). The relative abundance of *Actinobacteria* was slightly higher in the subsoil, likely due to the relatively high N content in the subsoil, as *Actinobacteria* are involved in nitrogen-fixing processes and nutrient cycling (Ravi Kumar et al., 2023). On the contrary, the *Rubrobacter* genus (*Actinobacteria)* was more abundant in the topsoil, and it has been reported to be a significant part of microbial communities in harsh soil environments, playing a vital role in processes such as nutrient cycling, which are essential for ecosystem functioning in these difficult habitats (Lupwayi et al., 2021; Miralles et al., 2023; Raimi et al., 2023).

Salt-associated microorganisms are promising both as indicators and as tools for improving soil health in saline agricultural systems (Mishra et al., 2023). Their abundance and diversity respond rapidly to changes in soil salinity (Hou et al., 2021), making them valuable for monitoring soil conditions and guiding management practices (Kumawat et al., 2022). The genera *Microvirga* and *Ensifer*, which are members of the Alphaproteobacteria (Rahimlou et al., 2021), correlated positively with salinity. A *meta*-analysis study reported that Alphaproteobacteria showed higher abundance under higher salinity conditions (Chen et al., 2022). In environments under salt stress, certain *Actinobacteria* genera, such as *Solirubrobacter*, tend to maintain a stable population. These bacteria can endure osmotic stress and have the ability to promote plant growth (Jiang et al., 2023), and have genes that help them adapt to harsh environments, playing a key role in nutrient cycling and contributing to the stability of soil EF (Ezeokoli et al., 2020; Jiang et al., 2023; Xun et al., 2021). The genus *Geodermatophilus* (*Actinobacteria)* was associated with EC and SOC, hence their abundances were higher in the topsoil, suggesting their involvement in the decomposition of SOC, owing to a higher organic matter content in the topsoil. The genera *Microvirga, Solirubrobacter* and *Geodermatophilus* are known to flourish in nutrient-rich conditions, and their increased abundance may serve as an indicator of elevated SOM levels after compost application (Araujo et al., 2023). The positive correlation between *Skermanella* genus *(Proteobacteria)* and SOC can be attributed to its involvement in nutrient cycling and the decomposition of organic matter (Liu et al., 2023). Certain *Proteobacteria* have been reported to utilize the reductive tricarboxylic acid cycle for autotrophic CO_2 fixation (Hügler et al., 2005). CO_2 emissions were strongly associated with *Rhizobium* (*Proteobacteria).* Sugawara and Sadowsky (2013) studied the transcriptional responses of *Bradyrhizobium japonicum* cells inhabiting in the rhizoplane of soybean plants exposed to increased atmospheric CO_2 levels. They found that several *B. japonicum* genes related to carbon cycling were upregulated in the soybean rhizoplane under elevated CO_2.

Previous studies reported that soil salinity, soil pH and SOC content were the driving factors in shaping and affecting fungal diversity and structure across soil depth (Estrada et al., 2024; Ma W. et al., 2024). In this study, the fungal phylum *Ascomycota* was

more abundant in topsoil, where soil salinity was higher, and the relative abundance of *Mortierellomycota* was higher in subsoil. These fungal phyla play a crucial role in the C cycle and the SOM decomposition (Muneer et al., 2021). In this study, these two phyla were the most abundant fungi, alongside *Basidiomycota*, as previously reported in agricultural soils (Guo et al., 2020). *Ascomycota* hold a significant role in the saline soils and play a crucial role in the stability of fungal communities (Ji et al., 2023). *Basidiomycota* are considered to be the second largest fungal group in soil (Ko et al., 2017), and are known as an important element in the saprotrophic functional group, and play a key factor in decomposing organic matter (Curlevski et al., 2010). *Aspergillus* was the genus with the higher relative abundance among all *Ascomycota* genera. It has been reported that this genus has developed an increased salt tolerance and could serve as a bioindicator for salt-tolerant communities (Cao et al., 2021). The genera *Chaetomium (Ascomycota)* and *Mortierella* (*Mortierellomycota*) were linked to higher pH and lower salinity in the subsoil. Guo et al. (2020) explored the effects of irrigation water salinity on fungal communities and reported a negative relationship between salinity and *Mortierella*, suggesting that the abundance of *Mortierellomycota* would be reduced with high salinity. This genus, which was more abundant in the subsoil, has the potential to decompose organic compounds and improve soil health (Li et al., 2018), and it is also known as plant growth-promoting fungi (Ozimek and Hanaka, 2021). Adding organic amendments along with *Ascomycota*-rich inoculants (*Chaetomium* and *Aspergillus* strains) can enhance root development and nutrient absorption in crops cultivated on saline soils (Ma Y. N. et al., 2024).

Alpha diversity Pielou's evenness index was significantly higher in the subsoil, suggesting that fungal communities tend to become more evenly distributed with soil depth, although drivers should be still elucidated with further research. One of the drivers of the evenness in fungal distribution may be soil pH, identified as the most important factor linked to variations in fungal community composition (Huang et al., 2023; Kang et al., 2021). *Aspergillus* and pH were negatively correlated, suggesting that *Aspergillus* may not prefer to grow under basic conditions (Murphy et al., 2011). Contrarily, *Acremonium* (*Ascomycota*) was positively associated with pH, suggesting that this genus would prefer basic soils. Pereira et al. (2013) investigated the yields and characteristics of lipase enzyme from *Acremonium alcalophilium*, and reported that the enzyme has properties that could be useful for several industrial sectors. Several fungal genera belonging to *Ascomycota* phylum were strongly related with CO_2 emissions and SOC, such as *Alternaria* and *Mycosphaerella*, with the highest relative abundances in the topsoil. These genera seemed not to be negatively affected by the high content of salts in the soil (Ji et al., 2023). In fact, previous studies have shown that *Alternaria* and *Mycosphaerella* are resilient to projected climate change scenarios (Wahdan et al., 2020), and they are also important decomposers that help break down complex organic matter, contributing to soil CO_2 emissions (Campbell et al., 2022; García-Díaz et al., 2018). Despite *Alternaria* is considered an important fungal pathogen, this genus has some species that could be used in the industry, such as *Alternaria citri*, which is a fungi enzyme producer (e.g., Pectinase) and it is known for its potential application in the pre-treatment of pectin rich wastewater from vegetable food processing (Mostafa & Abd El Aty, 2013; Ma et al., 2019).

The findings of this study highlight the importance of adopting soil management strategies that not only mitigate soil salinity but also preserve microbial diversity. However, effective strategies are essential to sustain productivity and mitigate soil salinity, such as crop selection emphasizing salinity-resistant species (Tedeschi et al., 2023), and applying organic amendments (e.g., biochar) to enhance soil quality under saline water conditions (Chen et al., 2024). Maintaining microbial diversity involves different practices such as the introduction of beneficial microbes like PGPR into the rhizosphere, which can reduce plant susceptibility to salinity stress (Khan et al., 2022), and the incorporation of cover crops which can help counteract the negative effects of salinity on soil microbial communities and promote sustainable productivity in saline agroecosystems (Dasgupta et al., 2023; Souza et al., 2025).

As a final remark, it is important to highlight that climate change is supposed to increase soil salinity due to the accumulation of salts in the topsoil owing to higher evaporation and lower quality of irrigation water (Akça et al., 2020). Thus, in this study, we provide insights about several bacterial and fungal taxonomic groups associated with soil salinity that could be enhanced in the future to counteract the effects of climate change. This will be also associated to changes in soil functionality associated to these microbial groups, such as *Proteobacteria* and *Actinobacteria* in the bacterial communities, and *Ascomycota, Basidiomycota* and *Mortierellomycota* in the fungal communities. Moreover, although the 10–30 cm layer was not included due to budget constraints, the selected depth (0-10 and 30–50 cm) represent ecologically distinct zones, a biologically active topsoil and a less altered subsoil. These contrasting layers are widely used to assess vertical patterns and are potentially sufficient to capture meaningful depth-related differences in microbial communities and soil properties (Poeplau et al., 2020; Szatmári et al., 2019; Zhao et al., 2022). Additionally, we have to comment that our experimental approach of a single sampling date prevents understanding the temporal variability of microbial communities with depth. It has been previously reported that seasonality is important at shaping soil bacterial and fungal community composition (Lin Y. et al., 2023; Zhang G. et al., 2023a). Thus, seasonality is a factor that should be included in further research to properly understand the links between soil salinity, soil depth and microbial community structure (Kramer et al., 2025; Wang et al., 2025).

CONCLUSION

This study showed that the composition of microbial communities significantly shifted between topsoil (0–10 cm) and subsoil (30–50 cm) layers. Soil pH, electrical conductivity and SOC were identified as the primary driving factors of these shifts. Bacterial diversity in the topsoil was linked to salinity and SOC, while fungal diversity in the subsoil was more strongly linked to increases in soil pH. Increases of SOC and salinity in the topsoil were associated with a higher abundance of various microbial taxa, such *as Proteobacteria* and *Ascomycota*.

Meanwhile, rising soil pH levels in the subsoil was associated with increased abundances of *Actinobacteria* and *Mortierellomycota*. Some key genera within these phyla, such as *Microvirga*, *Ensifer* and *Skermanella* (bacteria) and *Aspergillus*, *Acremonium* and *Chaetomium* (fungi), may play important roles in enhancing the soil functioning and microbiome belowground. These findings underscore the importance of deepening our understanding of the environmental changes that influence the soil properties and microbiome through the soil profile, which is essential for improving soil health.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

The study was conceptualized and designed by RZ, MM, and EL. Material preparation, data collection, and analysis were conducted by MM, RZ, EL, and NB. RZ, EL, NB, and NS contributed to the experimental design. MM prepared the initial manuscript draft, with RZ and EL providing feedback on earlier versions. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/sjss.2025.14537/full#supplementary-material

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