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ORIGINAL PAPER

Combined Compost and Biochar Application Maximizes Soil Microbial Resilience of Barley Crop Grown Under Drought Stress Conditions

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Abstract

Drought stress impairs plant growth soil fertility. Organic amendments like compost and biochar are increasingly recognized for their potential to mitigate environmental stresses in soil ecosystems. This study investigates their effects on the soil microbiome of a barley (*Hordeum vulgare* L.) field under drought stress. Eight treatments were established under controlled conditions: CK (control), B (biochar), C (compost), CB (compost + biochar), D (drought stress), DB (drought + biochar), DC (drought + compost), and DCB (drought + compost + biochar). High-throughput MiSeq sequencing was employed to assess changes in microbial diversity and taxonomic composition across treatments. Our study showed that the bacteria-to-fungi ratio remained stable, while alpha and beta diversity varied across treatments. Proteobacteriota and Actinobacteriota dominated the prokaryotes communities, whereas Ascomycota and Basidiomycota were the main fungal phyla. Drought (D) significantly reduced microbial diversity. DB-treatment slightly enhanced key decomposers such as Penicillium and some Proteobacteriota and Actinobacteriota but had a limited overall effect. DC-treatment produced a more pronounced impact, promoting beneficial microbes such as Firmicuteota, Talaromyces, and Sordariales, while also stimulating opportunistic taxa like Alternaria, indicating a partially beneficial yet suboptimal outcome. In contrast, DCB treatment elicited the most favorable shifts in taxonomic composition, enriching microbial groups potentially associated with stress resistance, antibiotic production, and phosphorus solubilization. It stimulated beneficial fungi that improve soil structure, water retention, and organic matter decomposition. Overall, DCB provided synergistic and balanced stimulation of beneficial soil microbiota under drought, offering a promising strategy for sustaining soil fertility and plant health under environmental stress.

Keywords Barley · Biochar · Compost · Drought Stress · Soil Microbiome

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1 Introduction

The soil microbiome, composed of a vast diversity of living organisms such as bacteria, fungi, archaea, protozoa, and viruses, plays a central role in maintaining soil and plant health (Yadav et al. 2021). Although often invisible to the naked eye, these microbial communities are responsible for numerous ecological processes essential to soil fertility and agricultural productivity (Dubey et al. 2019). Among their crucial functions are the degradation of organic matter, nutrient cycling, the formation and stabilization of soil aggregates, and the regulation of pathogens (Ghouili et al. 2023a; Kaundal et al. 2024). Furthermore, the soil biota facilitates nutrients absorption by plants by enhancing the availability of vital elements such as nitrogen, phosphorus, sulfur, and potassium (Yadav et al. 2021).

Soil microorganisms are also involved in symbiotic interactions with plants, such as biological nitrogen fixation by prokaryota of the genera *Rhizobium*, *Bradyrhizobium* and *Azotobacter* (Ibrahim and El-Sawah 2022), and mycorrhization, where arbuscular mycorrhizal fungi colonize plant root cortical cells forming arbuscules and vesicles that enhance plant access to water and nutrients, particularly phosphorus (Rostami and Maleki Farahani 2024). Additionally, these microbial communities act as a biological shield against pathogens by producing antibiotics and competing for resources, thereby reducing the incidence of root infections and other plant diseases (Yadav et al. 2021). The soil microbiome is, therefore, essential for soil health, maintaining a dynamic balance that regulates biogeochemical and biological processes and supports the resilience of agricultural ecosystems. In this study, microbial resilience is defined as the capacity of soil microbial communities to maintain diversity, taxonomic composition, and key functional groups under drought stress and organic amendment application.

Healthy soil plays a crucial role in agricultural productivity. According to the FAO (2015), soils contribute to 95% of global food production, and the sustainable management of the microbiome directly influences food security. However, the pressures of climate change are increasingly threatening the stability and effectiveness of this vital resource. Rising temperatures and shifting precipitation patterns disrupt agricultural ecosystems, complicating soil management (Muluneh 2021). Water stress has thus emerged as a critical limiting factor for soil productivity. According to the FAO (2023), the Middle East and North Africa region, including Tunisia, is particularly susceptible to water stress, which is intensified by climate change. Drought conditions not only impede plant growth and crop yields but also significantly alter the composition and diversity of the soil microorganisms (Liao et al. 2025). Prolonged drought can lead to the reduction or disruption of microbial communities, ultimately affecting nutrient availability and plant resilience.

Several studies reported that soils harboring a rich and functionally diverse microbiome exhibit enhanced resistance to environmental stresses. Such microbial communities can rapidly reorganize and adapt to abiotic fluctuations, including variations in temperature and moisture that are increasingly intensified by climate change (Bender et al. 2016). Recent evidence further highlights this adaptive capacity: soil prokaryotes consortia contain functionally redundant and preadapted taxa that can become active under shifting thermal regimes, thereby stabilizing ecosystem processes (Smith et al. 2022). Moreover, extreme drought events have been shown to disrupt microbial diversity and network stability more severely than gradual climatic changes, underscoring the pivotal role of community structure in determining resilience under global change scenarios (Philipp et al. 2025). In this context, the edaphic microbial community plays an even more critical role in helping soils withstand water stress and maintain high agricultural productivity. By improving microbiome health and management, it is possible to enhance the soil's ability to retain moisture, regulate nutrients, and support robust vegetation even under challenging climatic conditions.

Organic amendments such as compost and biochar have attracted increasing interest for their ability to mitigate the effects of abiotic and biotic stresses. Proteomic analyses revealed that compost application altered the abundance of several proteins associated with abiotic stress and plant defense in barley leaves (Ghouili et al. 2022a). Additionally, compost treatment activated enzymes involved in redox homeostasis and stress response regulation, suggesting a protective role in barley roots (Ghouili et al. 2023b). These changes contribute to improved barley growth and stress acclimation while also reducing the environmental impact of agricultural practices. Several studies have demonstrated that incorporating compost improves soil physical, chemical, and biological properties, enhancing soil organic matter (SOM) and nutrient status (Adugna 2018; Ghouili et al. 2023a). Adugna (2018) also reported that compost stabilizes water, air, and heat balance in soils, increases nutrient availability, and boosts crop productivity and quality, especially when combined with other fertilization methods. Our previous study showed that compost application can enhance the proliferation of beneficial microorganisms, leading to increased bioavailability of essential nutrients to barley plants (Ghouili et al. 2023a). Saison et al. (2006) showed that compost affects the activity, size, and composition of the soil microbial community, owing to the physicochemical characteristics of the compost matrix. However, there is limited research specifically examining the combined effects

of compost and biochar on both prokaryotic and fungal community composition and diversity under drought stress in cereal crop systems. In particular, the taxonomic and functional restructuring of the soil microbiome in response to co-application of these amendments during water deficit remains poorly characterized.

Biochar, a carbon-rich material obtained through biomass pyrolysis, is generally known to improve plant growth under normal conditions as well as the physicochemical properties of soil (Allohverdi et al. 2021; Edeh et al. 2020). Moreover, recent studies have highlighted the positive impact of biochar on boosting plant growth and resilience under abiotic stresses such as salinity and drought (Bolhassani et al. 2024; Ghouili et al. 2025a, b; Liu et al. 2021a, b a). Our recent studies revealed that compost and/or biochar applications improve barley growth and antioxidant responses under drought and salt stress (Ghouili et al. 2025a, b). Liu et al. (2021a, b) reported that compost and biochar improved soil aggregation, total porosity, and microbial biomass under salt stress. Under drought stress, biochar application enhances plant growth and improves the physical and biological properties of soils, including water-holding capacity, nutrient concentrations (NPK), organic carbon content, and soil respiration (Ali et al. 2017; Zaheer et al. 2021).

At the level of soil microbiology, Allohverdi et al. (2021) reported that biochar addition favored prokaryotes populations over fungal populations, with no significant increase in microbial diversity but an increase in microbial biomass. This shift is attributed to the higher sensitivity of prokaryotes groups to biochar, while fungal populations are less affected. Nonetheless, few studies have investigated the combined impact of compost and biochar on soil microbial communities specifically under drought stress, and most existing work does not simultaneously profile both prokaryotic and fungal communities at the ASV level.

Based on previous research and the properties of compost and biochar, these amendments, when applied separately or in combination, may improve soil fertility under drought stress conditions. They, therefore, hold potential to improve soil microbiome health, increase plant resilience, and contribute to the sustainability of agricultural systems in the face of climate change (Hasnain et al. 2023). This study thus aims to analyze the impact of organic amendments, compost and biochar, applied separately or in combination on the microbial community under drought stress conditions. The study relies on prokaryotes 16 S rRNA and fungal ITS rDNA amplicons MiSeq sequencing to evaluate variations in microbial diversity and the taxonomic responses to the different treatments. We hypothesized that the combined application of compost and biochar would synergistically enhance soil microbial diversity and community stability under drought stress, more effectively than either amendment applied alone, by providing complementary benefits of nutrient enrichment (compost) and improved soil physical properties (biochar).

2 Materials and Methods

2.1 Growth Conditions and Experimental Treatments

This experiment was conducted under controlled conditions in a glasshouse at the Experimental Station of the Biotechnology Center of Borj Cedria (Tunisia). The temperature was maintained at 23 ± 2 °C, with relative humidity ranging from 65% to 70%, light intensity of 270 µmol photons $m^{-2} \cdot s^{-1}$ of photosynthetically active radiation, and a 14/10-hour day/night photoperiod.

Ten barley seeds (*Hordeum vulgare* L., *Sahli* cultivar) were sown at a depth of 0.5–1 cm in 5 L plastic pots containing either unamended soil or soil amended with biochar and/or compost. Plants were irrigated every two days with tap water to maintain 80% of the pots' water-holding capacity throughout the initial growth phase. At approximately 45 days after sowing, corresponding to the tillering stage, irrigation was withheld for 12 consecutive days in the drought treatments (D, DB, DC, DCB), while the regular irrigation regime (every two days at 80% water-holding capacity) was maintained for the well-watered treatments (CK, B, C, CB).

Eight treatments were thus established: CK (control, unamended soil), B (soil + biochar at 6% w/w or 300 g/pot), C (soil + compost at 2% w/w or 96 g/pot), CB (soil + compost + biochar), D (drought stress), DB (drought stress + biochar), DC (drought stress + compost), and DCB (drought stress + compost + biochar). This factorial design (with/without biochar × with/without compost × with/without drought) was chosen to assess the individual and combined effects of organic amendments under both well-watered and water-deficit conditions, enabling detection of synergistic interactions between amendments and drought. Each treatment consisted of ten pots randomly positioned in the greenhouse to minimize environmental variation.

Soil samples were collected once, at the end of the 12-day drought period, by taking three cores from each pot and pooling them to form composite samples. There were four replicates (four composite samples) per treatment. Visible root fragments and stones were removed, and a portion of each sample was stored at -80 °C for DNA extraction and subsequent molecular analyses.

3 Soil Composition, Biochar, and Compost Specifications

The soil was collected from the 0–20 cm layer of a paddy field in the Oasis of Chenini, Gabes, Tunisia. Its texture comprised 84.4% sand, 8.3% silt, and 5.5% clay. Detailed soil characteristics were previously described by Ghouili et al. (2024).

Biochar was provided by the Biofire Society (Manouba, Tunisia) and produced by pyrolysis of forestry wood composed of pine (90%) and olive (10%) under anaerobic conditions at 450 °C for 10 h (Rajhi et al. 2024). The material was subsequently sieved to obtain a particle size ranging between 0.5 and 2 mm. The resulting biochar contained 81.2% organic matter, had a pH of 7.63, an electrical conductivity of 1.3 dS cm^{-1}, and a cation exchange capacity of 54.6 meq 100 g^{-1} (Rajhi et al. 2024).

Compost was supplied by the Association for Saving the Oasis of Chenini (Gabes, Tunisia) and produced from date palm waste and sheep manure following the turned windrow method with aerobic composting. The mixture was manually aerated daily during the active (thermophilic) stage, which lasted 72 days, and once a week during the curing stage of 60 days, for a total composting duration of approximately 132 days. The finished compost contained 18.58% total organic carbon, 1.21% total nitrogen, a C: N ratio of 15.36, 0.54% phosphorus, and 0.95% potassium. Concentrations of heavy metals and micronutrients were below national limits, and the compost was free of harmful pathogens (Ghouili et al. 2022b).

4 DNA Extraction

DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. Briefly, 0.5 g of soil was placed in a 2 ml tube with 2 ml of Lysing Matrix E, 978 µl Sodium Phosphate buffer (SPB), and 122 µl MT buffer. The mixture was vortexed and processed on a FastPrep instrument for 40 s, then centrifuged and 900 µl of the supernatant were transferred to a 1.5 ml tube, mixed with 250 µl PPS, and centrifuged again. The supernatant was moved to a 15 ml Falcon tube, combined with 1 ml Binding Matrix, and incubated. After discarding 700 µl of the supernatant, the matrix was transferred to a SPIN Filter and centrifuged twice. The matrix was washed with SEWS-M (Salt Ethanol Wash Medium), dried, resuspended in DES (DNA Elution Solution), and incubated at 55 °C to elute DNA. DNA extracts were stored at -20 °C. DNA concentration and purity were assessed by UV spectrophotometry (Biophotometer, Eppendorf, Mississauga, ON, Canada), measuring absorbance at 260, 280, 230, and 320 nm.

5 Amplicon Library Preparation and Sequencing

DNA extracts were diluted 1:100 in DES. PCR amplification was performed using the Q5 High-Fidelity DNA Polymerase kit and Illumina primers in 25 µl reactions, following the manufacturer's instructions. The V4 hypervariable region of the 16 S rRNA gene was targeted for procaryotes, and the ITS1 region of the rDNA for fungi (primer sets in Supplementary Table S1) (Bokulich et al. 2013; Parada et al. 2015). The amplification reaction was carried out under the following conditions: an initial 30-second denaturation step at 98 °C, followed by 35 cycles of 10 s at 98 °C, 10 s at 55 °C for prokaryotes and 50 °C for fungi, and 30 s at 72 °C, with a final extension step of 2 min at 72 °C. PCR products were purified with AxyPrep Mag PCR CleanUp beads and quantified on an Epoch microplate reader (Agilent, Santa Clara, CA, USA). Products were standardized to 2 µg/µl. A second PCR for indexing used dual-indexed primers (unique combinations per sample specifically designed for Illumina instruments by the Genomic Analysis Platform (IBIS, Université Laval, Québec City, QC, Canada) under the same cycling conditions, but with 13 cycles. Indexed amplicons were purified, quantified, normalized to 20 ng/µl, and sequenced on an AVITI instrument at the Genomic Analysis Platform IBIS.

6 Quantitative Real-Time PCR (qPCR) Analysis

To assess the abundances of total bacteria and fungi in the bulk soil, qPCR was performed following the protocol detailed by (Ghouili et al. 2023a). For the detection of bacteria, the primers Eub338: 5-ACTCCTACGGGAGGCAGCAG and Eub518: 5-ATTACCGCGGCTGCTGG were employed, while for fungi, the primers FF390: 5-CGATAACGAACGAGACCT and FR1: 5-AICCATTCAATCGGTAIT were used. qPCR was conducted on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using a SYBR Green qPCR mix (Qiagen, Toronto, ON, Canada). Briefly, the qPCR reactions were conducted with an initial preheating at 95 °C for 15 min, followed by 40 cycles of: 95 °C for 1 min, an annealing step at 53 °C for bacteria and 51 °C for fungi for 30 s, and a final extension at 72 °C for 1 min. Standard curves were prepared with 10-fold serial dilutions of M13 (pBluescript II). Gene copy

numbers were normalized to dry soil weight (copies per gram). All reactions were performed in triplicate. Means were compared using Duncan's test where a significant F-value ($p < 0.05$) was observed.

6.1 Bioinformatics and Statistical Analysis

Prokaryotes 16 S rRNA and fungal ITS rDNA reads were analyzed in QIIME2 (Bolyen et al. 2019). Amplicon sequence variants (ASVs) were inferred via DADA2 (Callahan et al. 2016). For fungi, adapter sequences were trimmed using Cutadapt (Martin 2011). Weighted UniFrac distances were computed to assess phylogenetic dissimilarities, followed by Principal Coordinate Analysis (PCoA) with the vegan package in R.

A one-way permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was used to evaluate treatment effects on microbial communities, along with pairwise comparisons (Callahan et al. 2017). Non-parametric tests (Kruskal–Wallis and Wilcoxon) assessed group differences via ggpubr in R (version 4.1.1). Alpha-diversity indices (Chao1 and Shannon) were calculated in QIIME2 (Anderson 2001; Chao and Shen 2003), with Chao1 reflecting species richness and Shannon accounting for both richness and evenness of microbial communities. Genus-level data were represented as heatmaps using the ampvis2 package. Differential abundance of ASVs between treatments (e.g., drought stress vs. controls, and drought stress vs. drought + amendments) was determined using Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) (Lin and Peddada 2020).

7 Results

7.1 Changes in Soil Prokaryotes and Fungal Abundances

The boxplot in Fig. 1 presents the abundances of total bacteria (Fig. 1.A), total fungi (Fig. 1.C), and their ratio (Fig. 1.B) in soils under the different treatments. The bacteria-to-fungal ratio remained statistically unchanged across all treatments, indicating that neither drought stress nor the application of biochar, compost, or their combination significantly altered the balance between bacteria and fungal populations. Notably, a lower variability in bacteria and fungal abundances was observed under DB and DCB compared with B and CB, suggesting that biochar application may have enhanced water retention capacity under drought conditions, thereby contributing to greater stability of microbial populations.

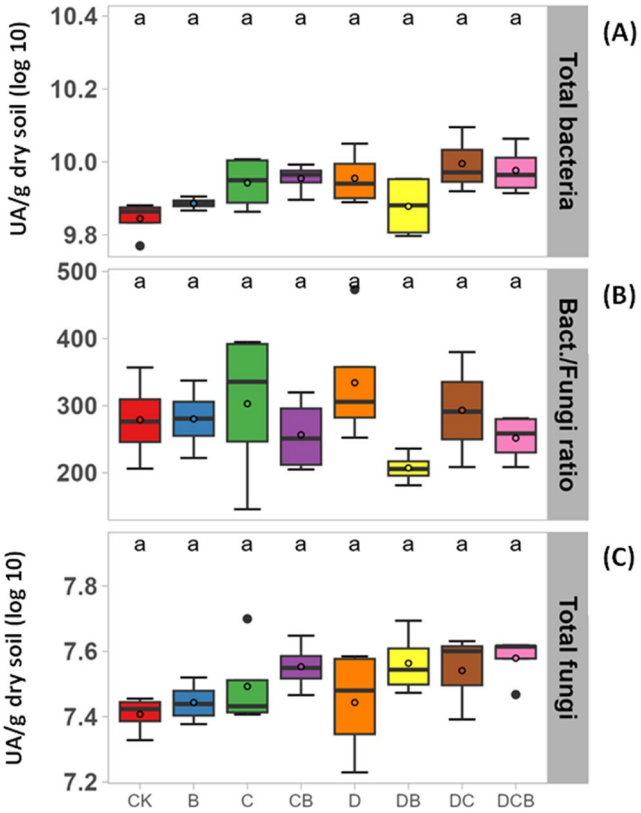


Fig. 1 Quantitative PCR (qPCR) measurements of bacterial (**A**) and fungal communities (**C**) and their ratio (**B**) in soils subjected to different treatments under drought conditions. Columns sharing the same letter are not significantly different ($P < 0.05$, Duncan's test). Error bars represent ± standard deviation (SD); $n = 4$. **CK**: control; **B**: soil + Biochar; **C**: soil + Compost; **CB**: soil + Compost + Biochar; **D**: Drought stress; **DB**: Drought + Biochar; **DC**: Drought + Compost; **DCB**: Drought + Compost + Biochar

7.2 Effect of Treatments on Prokaryota and Fungal Diversity in Soil

Figure 2 presents the alpha diversity of prokaryotic and fungal communities across treatments under drought conditions, based on Shannon and Chao1 diversity indices. Overall, prokaryotic communities (Fig. 2.C) exhibited higher Shannon diversity and richness (Chao1) than fungal communities (Fig. 2.D), a difference that was expected but remains ecologically relevant in the context of the applied treatments and drought stress. This pattern reflects the generally greater ecological plasticity and metabolic versatility of prokaryotes, which enables them to better withstand environmental fluctuations such as drought. For prokaryotes (Fig. 2.A), the Shannon index indicated that diversity remained generally stable across treatments, but a more pronounced reduction was observed under the DCB treatment compared with CB, highlighting the impact of drought stress. In contrast, fungal diversity (Fig. 2.B) varied among treatments: the highest

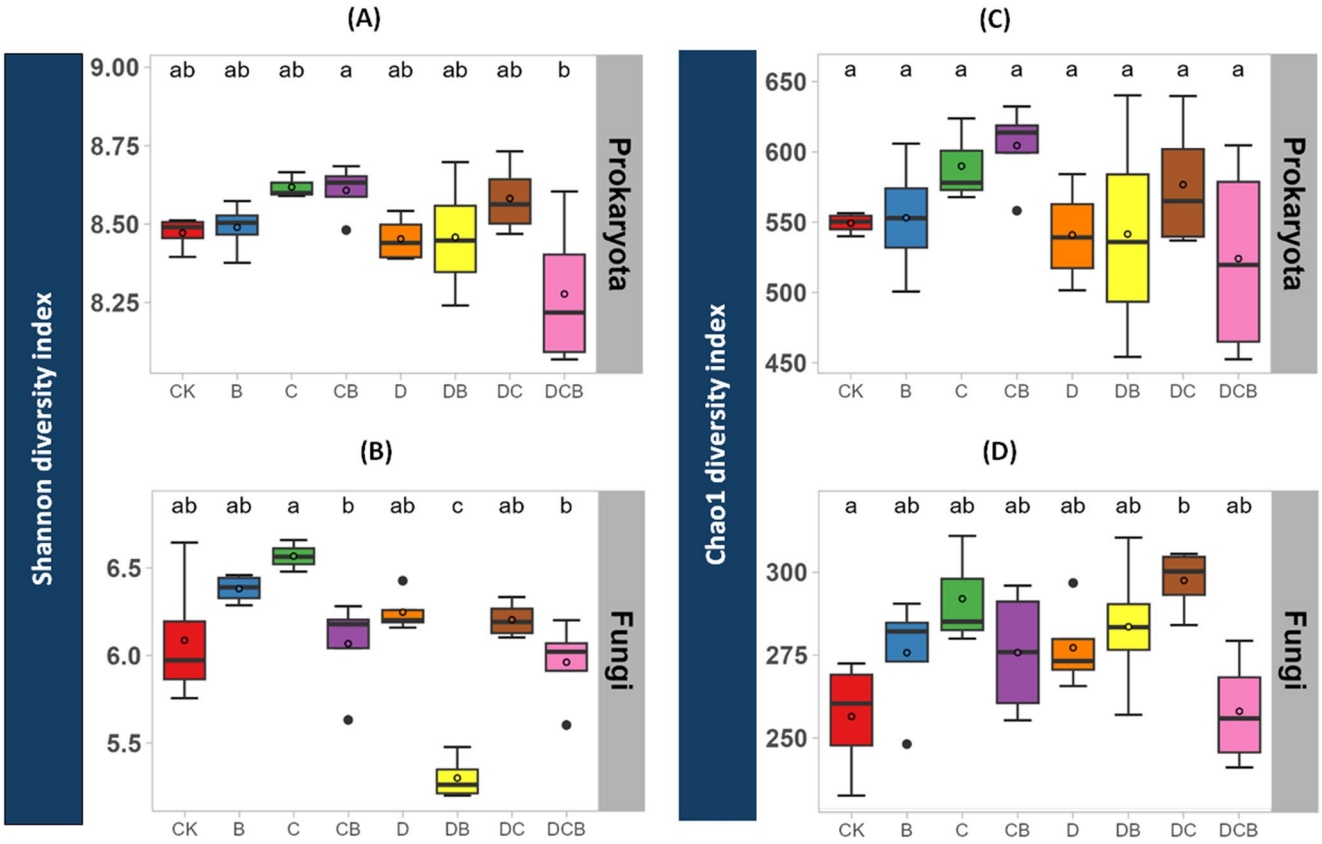


Fig. 2 Microbial alpha diversity analysis. Boxplots showing Shannon (**A** and **B**) and (**C** and **D**) Chao1 diversity indices of prokaryotes and fungal communities in soil under different treatments during drought conditions. Columns sharing the same letter are not significantly different ($P<0.05$, Duncan's test). **CK**: control; **B**: soil + Biochar; **C**: soil + Compost; **CB**: soil + Compost + Biochar; **D**: Drought stress; **DB**: Drought + Biochar; **DC**: Drought + Compost; **DCB**: Drought + Compost + Biochar

diversity was observed in soils amended with compost (C), while drought-stressed soils amended with biochar (DB) exhibited the lowest fungal diversity. Moreover, the combined application of compost and biochar (CB and DCB) slightly decreased fungal diversity compared with C and DC treatments, respectively. Regarding richness, the Chao1 index showed no overall significant difference between treatments for prokaryotes, with a more distinct effect observed in treatment CB compared to the other treatments CK, B, and C, despite increasing variability particularly in samples C and B. This explains the lack of a significant difference. Although fungi richness remained generally stable, the fungi illustrate a similarity in the arrangement of boxplots between the treatments subjected to and unsubjected to drought stress, and the stress appears to have had less impact on the variability of Chao1 values compared to those of prokaryotes.

Figure 3 presents the Principal Coordinate Analysis (PCoA) results that assess beta diversity, revealing clear differences in microbial community composition across treatments under drought stress. For prokaryota (Fig. 3.A), the first two PCoA axes explained 37.5% and 18.9% of the total variation, respectively. Prokaryotic communities in control (CK) and drought-stressed (D) soils clustered separately, confirming that drought significantly altered prokaryotic composition. Notably, DC treatment grouped closer to CK, whereas DB formed a distinct cluster, indicating a unique microbial response. The combination of compost and biochar under drought (DCB) exhibited among the drought treatments the most distinct prokaryota composition overall.

For fungi (Fig. 2.B), PCoA axes explained 22.1% and 17.1% of the variation, respectively. Treatments involving B, C, and CB formed distinct clusters, confirming that these amendments strongly influenced fungal community structure. The D, DB, and DC treatments were clearly separated, and DCB stood out as the most distinct fungal composition among all treatments.

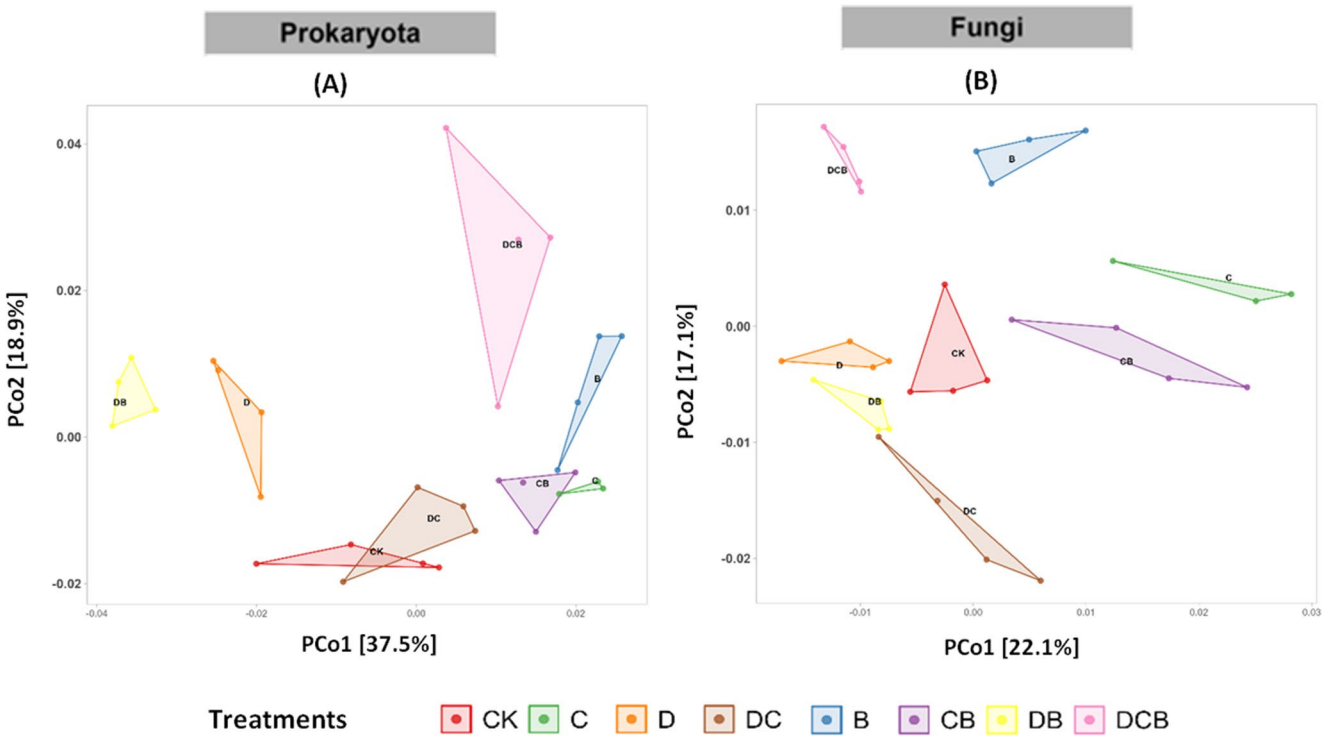


Fig. 3 Beta diversity analysis. Principal Coordinates Analysis (PCoA) of prokaryota (**A**) and fungal (**B**) communities in soils subjected to various treatments under drought conditions

7.3 Taxonomic Changes in Prokariota and Fungal Populations in Soil

Figure 4 displays heatmaps of prokaryotic phyla relative abundances under drought stress. *Proteobacteriota* dominated across treatments (28.6% in C to 38% in DB), with the highest abundance recorded in DB. *Actinobacteriota* increased in soils amended under non-stress conditions (notably B: 30.7%) but decreased under drought (D: 24.1%; DB: 23.2%), although DC and DCB showed relative increases compared to CK and D. *Firmicuteota* were highest in CK and CB treatments (14.3% and 14.5%) and declined in B, C, and most drought treatments, except DC, which remained stable (14%).

Gemmatimonadota maintained stable abundance under normal conditions but declined under drought (D, DB, DC). However, CB and DCB treatments increased *Gemmatimonadota* even under water-limited conditions. *Chloroflexota* decreased slightly under drought but increased with compost under both normal and stressful conditions. *Bacteroidota* increased with amendments in all conditions, with DCB reaching the highest abundance (6.3%).

Conversely, *Crenarchaeota*, *Planctomycetota*, *Acidobacteriota*, and *Myxococcota* generally declined when amendments were added under normal conditions, with further reductions under drought treatments, especially DCB. The last five phyla, *Entotheonellaeota*, *Patescibacteriota*, *Deinococcota*, *Nitrospirota*, and *Methylomirabilota*, showed limited variation (0.1–0.7%), except for *Deinococcota*, which increased under C treatment (1.1%).

Figure 5 shows fungal taxonomic changes. *Ascomycota* was the most dominant fungal phylum, followed by *Basidiomycota*, *Mortierellomycota*, and *Glomeromycota*. Within *Ascomycota*, *Eurotiomycetes* (primarily Eurotiales and Onygenales) were most abundant, ranging from 19.88% in D to 45.94% in DB. Amendments (B, C, CB) decreased their abundance compared to CK, while drought alone also reduced them. However, organic amendments under drought (DB, DC, DCB) significantly increased *Eurotiomycetes* compared to D. *Sordariomycetes* increased under B and C but declined when amendments were combined. Drought increased their abundance, but DB and DC reduced it, while DCB raised it again compared to D. Unclassified *Ascomycota* increased under most treatments (B, C, CB, D, DC) but declined under DB and DCB. *Dothideomycetes* increased under B, C, DB, and DC but decreased with drought alone (D) or combined amendments (CB, DCB).

Unidentified fungal phyla generally decreased, except for C and DC treatments. *Agaricomycetes* increased under

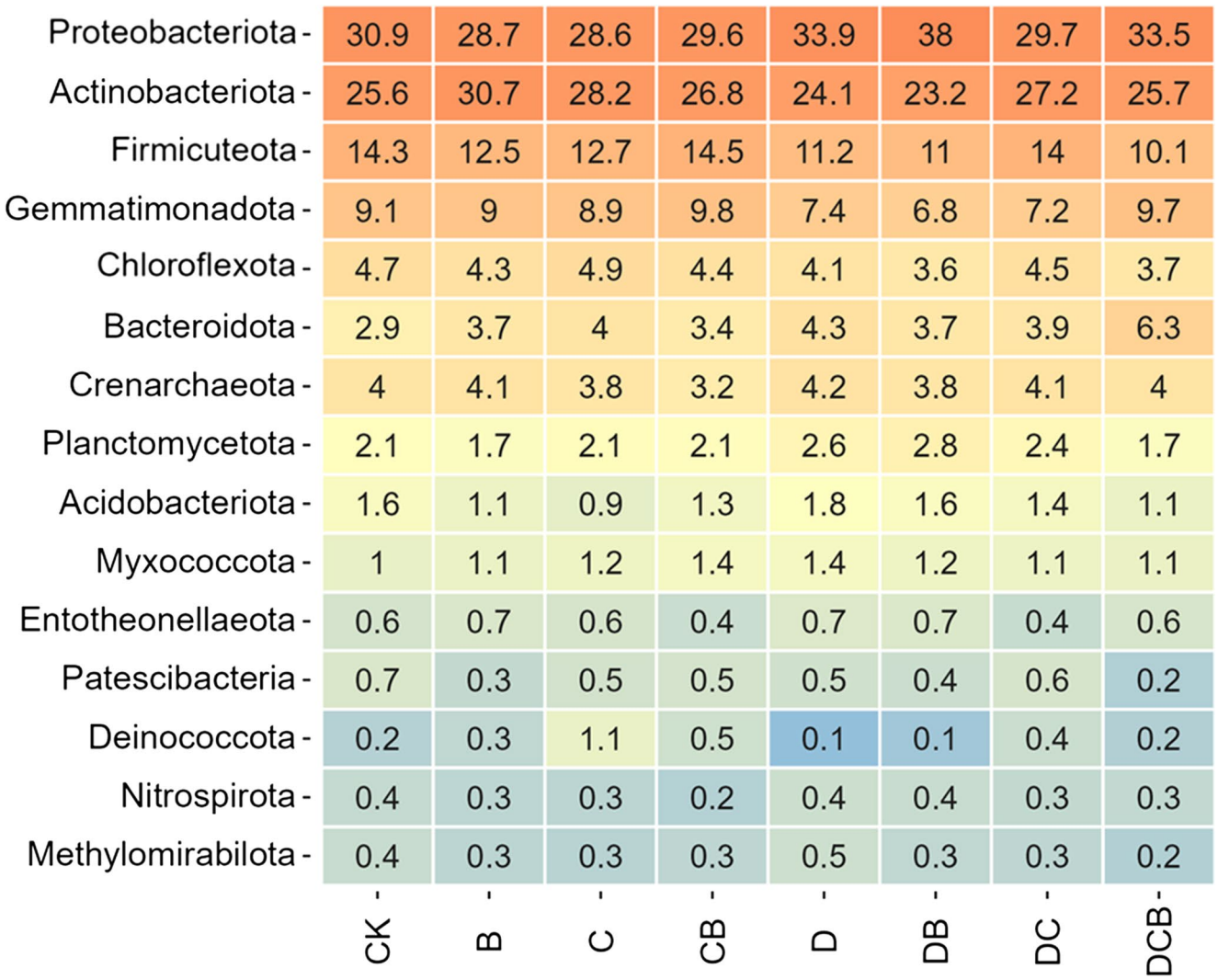


Fig. 4 Heatmap showing the correlation between prokaryota phyla and different treatments under drought conditions. **CK**: control; **B**: soil + Biochar; **C**: soil + Compost; **CB**: soil + Compost + Biochar; **D**: Drought stress; **DB**: Drought + Biochar; **DC**: Drought + Compost; **DCB**: Drought + Compost + Biochar

normal conditions but declined under drought (D, DB, DC), except for DCB, which restored their abundance. *Pezizomycetes* increased under most treatments, except DC, which fell below control levels. *Mortierellomycetes* declined across treatments, except B and DCB, where they increased. *Leotiomycetes* and *Glomeromycetes* were absent in CK but appeared under B and CB (normal) and in D, DB, and DC under drought. *Leotiomycetes* were most abundant in DC (0.11%), while *Glomeromycetes* appeared only in DC (0.011%).

7.4 Major ASVs Within Barley Soil Microbial Communities

Figure 6 presents the differential abundance plot (ANCOM-BC analysis) of microbial taxa comparing drought (D) to the control (CK), showing that drought induced significant shifts. Only taxa with significant differences ($p < 0.01$) between control (CK) and drought conditions are shown. Drought induces significant changes in soil microbial composition. Indeed, abundances of 7 prokaryotic phyla (15 ASVs) were reduced, however 5 other phyla (10 ASVs) were enriched (Fig. 6.A). On the one hand, abundances most of the *Proteobacteriota* ASVs (9 ASVs) were reduced under drought conditions, along with 2 ASVs belonging to each of *Thermoplasmatota* and *Myxococcota*. Additionally, reduced abundance of only one ASV was detected from each of the following phyla: *Nitrospiraota*, *Crenarchaeota*, *Bacteroidota*, and *Actinobacteriota*. On the other hand, abundances of ASVs from taxa like *Gemmatimonadota*, *Acidobacteriota*, *Bacteroidota*, and *Chloroflexota* were enriched under drought. As shown in Fig. 6.B, drought conditions caused

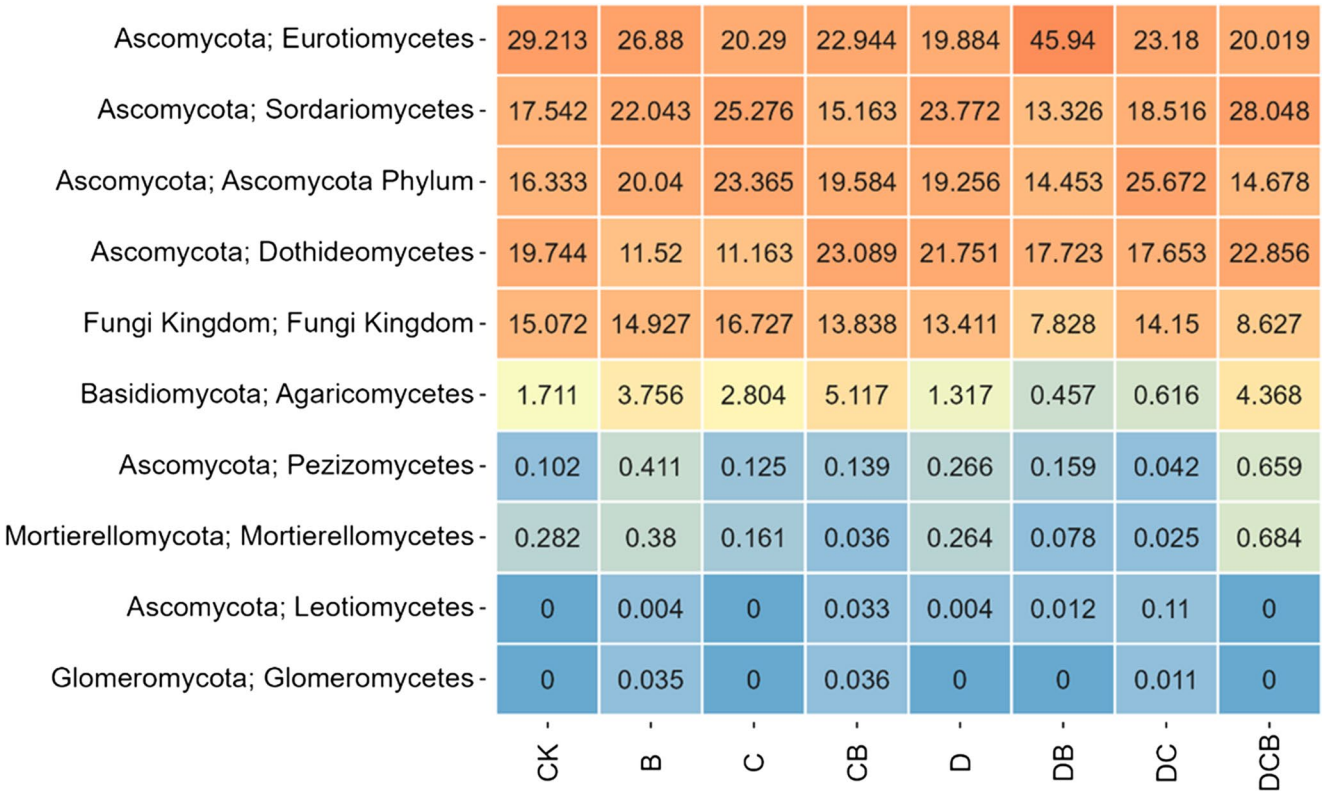
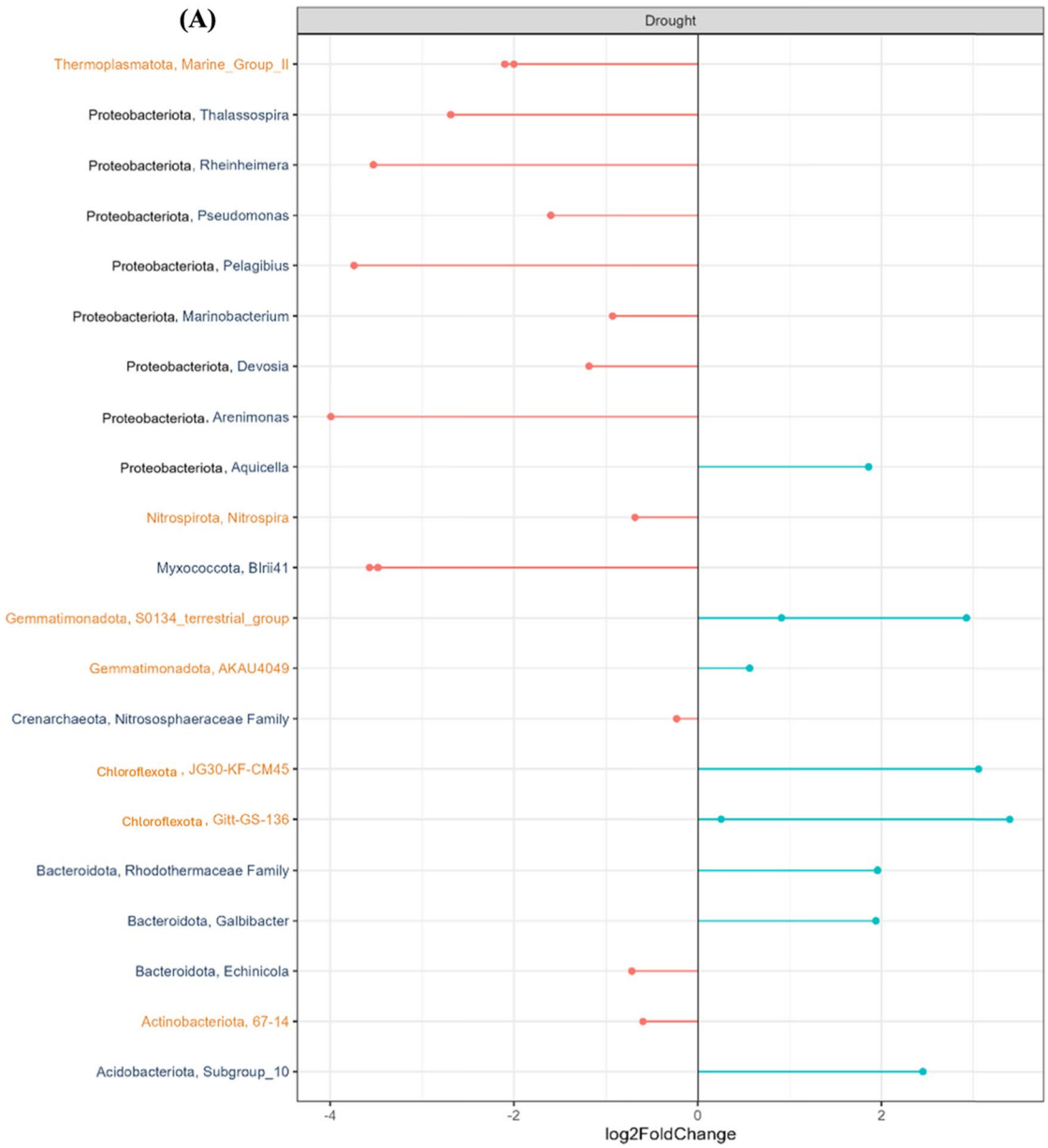


Fig. 5 Heatmap showing the correlation between fungal phyla and different treatments under drought conditions. **CK**: control; **B**: soil + Biochar; **C**: soil + Compost; **CB**: soil + Compost + Biochar; **D**: Drought stress; **DB**: Drought + Biochar; **DC**: Drought + Compost; **DCB**: Drought + Compost + Biochar

a significant reduction in the abundance of fungi belonging to the phylum *Ascomycota* (total of 4 ASVs), in particular from *Penicillium genera* and Sordariomycetes Class.

The ANCOM-BC analysis of soil prokaryotic community under drought and organic amendments (DB, DC, and DCB) revealed distinct taxonomic responses depending on the treatment applied compared to drought conditions (D) treatment (Fig. 7.A). Under drought conditions combined with biochar application (DB), only a limited number of microbial taxa exhibited significant shifts in relative abundance. Specifically, reductions were observed in *Thermoplasmatota* (1 ASV), *Actinobacteriota* (1 ASV), and *Proteobacteriota* (2 ASVs), indicating a partial abundance reduction of these groups. In contrast, increases were recorded in nine *Proteobacteriota* ASVs, including genera such as *Methylophaga* and *Pseudomonas*. Additionally, an enrichment of *Bacteroidota* (1 ASV) and *Actinobacteriota* (2 ASVs) was noted. In contrast, the application of compost under drought (DC) had a much more pronounced impact on the microbial community structure. In total, 21 ASVs from 9 phyla decreased, while 21 ASVs from 8 phyla increased in abundance. Notably, several copiotrophic prokaryotes taxa were significantly enriched, including members of *Proteobacteriota* (4 ASVs), *Firmicuteota* (4 ASVs), *Bacteroidota* (3 ASVs), as well as *Actinobacteriota* (5 ASVs) and *Gemmatimonadota* (2 ASVs). Interestingly, the treatment also resulted in a decline of several oligotrophic and drought-adapted taxa, such as representatives of *Thermoplasmatota*, *Actinobacteriota*, *Planctomycetota*, *Nitrospiraota*, *Myxococcota*, *Chloroflexota*, and *Entotheonellaeota*. The most profound changes in microbial community structure were observed under the combined treatment of compost and biochar (DCB), which led to both increases (6 phyla comprising 23 ASVs) and decreases (11 phyla comprising 39 ASVs) across a broad spectrum of microbial groups. Notably, this treatment significantly enhanced the relative abundance of *Gemmatimonadota* (8 ASVs), *Bacteroidota* (6 ASVs), *Proteobacteriota* (4 ASVs), and *Actinobacteriota* (3 ASVs). Conversely, other taxa, including *Proteobacteriota* (16 ASVs), *Bacteroidota* (6 ASVs), *Acidobacteriota* (3 ASVs), *Gemmatimonadota* (3 ASVs), *Planctomycetota* (2 ASVs), *Chloroflexota* (2 ASVs), and *Actinobacteriota* (2 ASVs), were significantly reduced. Additionally, archaeal groups such as *Halobacterota* and *Thermoplasmatota* displayed notable responses.

The ANCOM-BC analysis of soil fungal community structure responded differentially to the application of biochar, compost, and their combination under drought conditions as


(A)
Drought
Thermoplasmatota, Marine_Group_II
Proteobacteriota, Thalassospira
Proteobacteriota, Rheinheimera
Proteobacteriota, Pseudomonas
Proteobacteriota, Pelagibius
Proteobacteriota, Marinobacterium
Proteobacteriota, Devosia
Proteobacteriota, Arenimonas
Proteobacteriota, Aquicella
Nitrospirota, Nitrospira
Myxococcota, Blrii41
Gemmatimonadota, S0134_terrestrial_group
Gemmatimonadota, AKAU4049
Crenarchaeota, Nitrososphaeraceae Family
Chloroflexota , JG30-KF-CM45
Chloroflexota , Gitt-GS-136
Bacteroidota, Rhodothermaceae Family
Bacteroidota, Galbibacter
Bacteroidota, Echinicola
Actinobacteriota, 67-14
Acidobacteriota, Subgroup_10
-4
-2
0
2
log2FoldChange

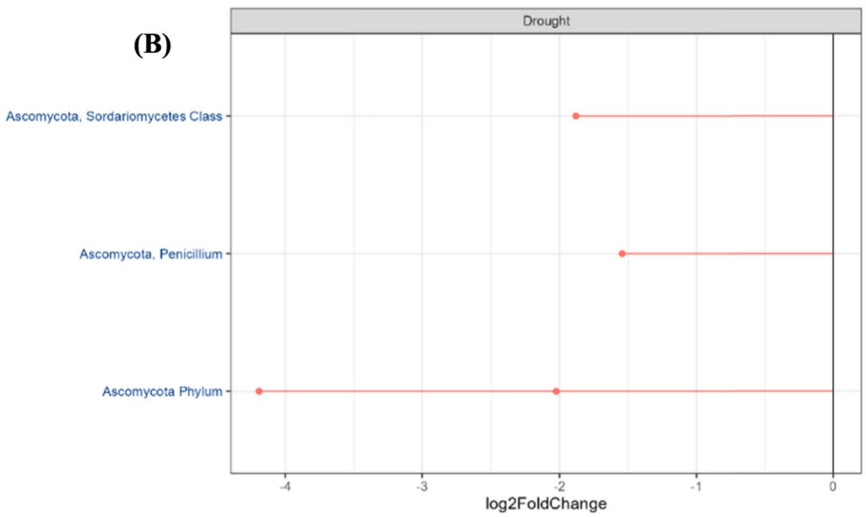

(B)
Drought
Ascomycota, Sordariomycetes Class
Ascomycota, Penicillium
Ascomycota Phylum
-4
-3
-2
-1
0
log2FoldChange

Fig. 6 Differential abundance analysis of microbial amplicon sequence variants (ASVs). (**A**) Prokaryotes and (**B**) Fungal ASVs showing significant differences in soils under drought stress compared to normal (non-stressed) conditions

shown in Fig. 7.B. Under the DB treatment, changes were relatively limited (total of 9 ASV), with notable increases observed in several *Ascomycota* taxa, including *Hamigera*, *Alternaria*, and *Penicillium*. Conversely, declines were observed in this taxon including *Sordariomycetes* Class and *Mycothermus*. In the DC treatment, significant enrichment of specific fungal taxa was detected. Among these, *Talaromyces*, *Myceliophthora*, *Microascaceae*, *Chaetomiaceae*, and *Sordariales*. Conversely, *Hyalosphaerella* showed a significant decrease. The DCB treatment elicited the most complex response within the fungal community, characterized by both significant enrichments (12 ASVs) and declines (9 ASVs). Notably, taxa affiliated with the *Agaricomycetes* class (within the *Basidiomycota* phylum), as well as several *Ascomycota* members (including *Preussia*, *Penicillium*, *Ascobolus*, and representatives of the *Chaetomiaceae* family) were significantly enriched. Conversely, a substantial reduction was observed among other *Ascomycota* taxa, including *Penicillium*, *Chrysosporium*, *Aspergillus*, and members of the *Eurotiomycetes* class.

8 Discussion

Amendments such as compost and biochar are increasingly recognized for their potential to improve soil fertility, stimulate plant growth, and enhance crop yields, particularly under drought stress (Akmal et al. 2019; Shaaban et al. 2024 dère et al. 2023). These organic amendments can modulate the diversity, composition, and resilience of soil microbial communities in response to water deficits. In this context, the present study aimed to elucidate the effects of compost and biochar applications on the structure of prokaryota and fungal communities in barley-cultivated soils subjected to drought stress.

The first objective was to determine how compost and biochar, applied individually or in combination, influenced soil microbial communities under well-watered barley cultivation. Although the bacteria-to-fungi ratio remained unchanged across amendment treatments, indicating that the overall balance between these major microbial groups was maintained, clear shifts occurred in community diversity and composition. The stable bacteria-to-fungi ratio suggests that the indigenous soil microbiome was resilient to amendment-induced changes, consistent with previous studies reporting no significant effects of compost or biochar on prokaryotic 16 S rRNA or fungal ITS gene abundances (Azeem et al. 2020) or on functional genes involved in nitrogen cycling (Wu et al. 2016). This stability likely reflects the capacity of native microbial communities to preserve their overall structure despite changes in resource availability. Despite the stable microbial abundance, amendments influenced community diversity, particularly in response to compost. Compared with our previous field study, where compost significantly altered prokaryotic richness and diversity while increasing fungal diversity (Ghouili et al. 2023a), the present pot experiment showed only a slight increase in prokaryotic Shannon diversity with compost alone or combined with biochar, whereas richness remained unchanged. This pattern suggests that amendments primarily redistributed the relative abundance of existing taxa rather than introducing new ones. Fungal communities generally responded more strongly to compost, reflecting the greater availability of organic substrates that support saprotrophic fungi. However, the lower Shannon and Chao1 indices observed under the combined compost–biochar treatment indicate that a limited number of well-adapted fungal taxa became dominant, probably because amendment-induced changes in soil physicochemical properties, such as pH, nutrient availability, and habitat structure, favored specific ecological groups. The observed changes in α-diversity were accompanied by clear shifts in β-diversity, as revealed by the PCoA, demonstrating that both individual and combined amendments reshaped microbial community assembly relative to the unamended soil. These compositional changes are consistent with the creation of new ecological niches through increased organic carbon availability, improved nutrient retention, and the porous microhabitats provided by biochar, which collectively modify microbial colonization and competitive interactions.

At the taxonomic level, *Proteobacteriota*, *Actinobacteriota*, and *Firmicuteota* remained the dominant prokaryotic phyla across all treatments, in agreement with previous reports showing the predominance of these groups in organically amended soils due to their efficient utilization of labile carbon sources (Ghouili et al. 2023a). Nevertheless, because the experiment was conducted using oasis soil with its well-established native microbiota, the pedoclimatic characteristics of the soil likely exerted a stronger influence on community composition than the microbial populations introduced with compost or biochar. Many members of these dominant bacterial phyla contribute to nutrient cycling, osmolyte production, and plant growth promotion, thereby enhancing soil functioning (Cui et al. 2019; 2022; Zhang et al. 2021; Yang et al. 2021). Similarly, *Ascomycota* and *Basidiomycota* dominated the fungal communities across amended treatments, consistent with previous observations in compost-amended barley soils (Ghouili et al. 2023a). Their predominance can be explained by their strong capacity to decompose organic matter and exploit the carbon-rich

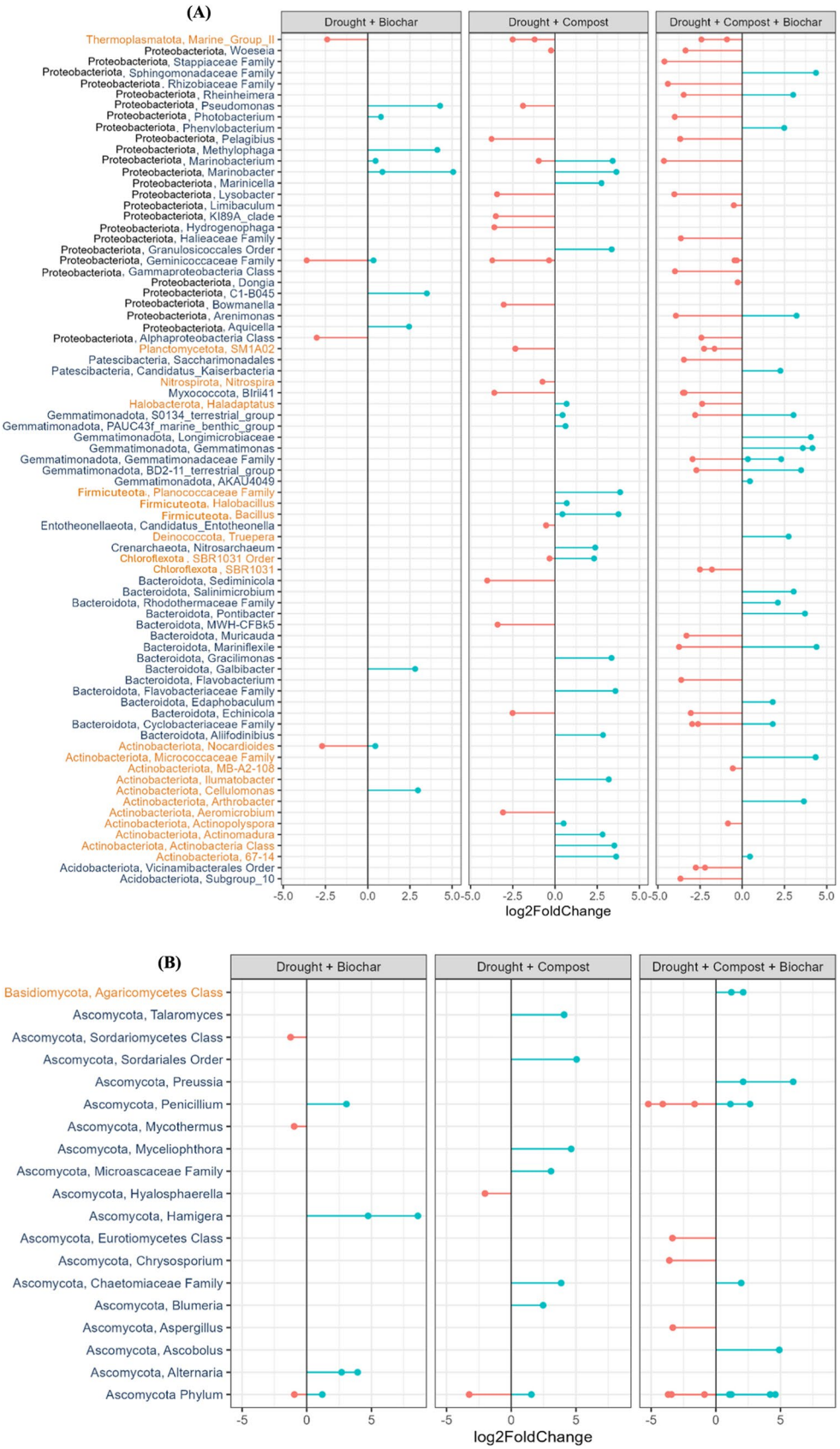


Fig. 7 Differential abundance analysis of microbial amplicon sequence variants (ASVs). (**A**) Prokaryotes and (**B**) Fungal ASVs showing significant differences in soils under drought stress compared to those amended with biochar and/or compost

environment created by the amendments (Agerer 2006; Boddy and Jones 2008; Yadav et al. 2024). Within these phyla, Eurotiomycetes, Sordariomycetes, Dothideomycetes, and Agaricomycetes include taxa involved in organic matter decomposition, nutrient mobilization, soil aggregation, and beneficial plant-microbe interactions, all of which contribute to maintaining soil fertility (Frąc et al. 2022; Gadd et al. 2005; Liu et al. 2020; Ritz and Young 2004). Overall, these findings indicate that compost and biochar did not disrupt the overall microbial balance but selectively reorganized microbial communities toward taxa associated with improved soil functioning, providing a favorable microbial baseline before exposure to drought stress.

Drought is a major ecological filter that reshapes soil microbial communities by altering water availability, nutrient diffusion, and microbial interactions. In the present study, drought did not significantly affect the bacteria-to-fungi ratio, although a trend toward lower values was observed in DB and DC soils, suggesting that the overall balance between these microbial groups remained relatively stable despite water limitation. Likewise, drought did not significantly alter the Shannon diversity of either prokaryotic or fungal communities, while a slight increase in fungal richness (Chao1) was observed. Similar patterns have been reported by Bian et al. (2025), who showed that fungal diversity is generally less affected by drought than prokaryotic diversity. This greater fungal resilience has been attributed to their filamentous growth, resistant spores, and broad ecological strategies that allow survival and activity under low soil moisture (Preece et al. 2019). Moreover, drought may activate dormant or rare fungal taxa and alter root exudation patterns, thereby recruiting stress-adapted saprotrophic and mycorrhizal fungi and increasing the observed richness (de Oliveira et al. 2020). In contrast, drought commonly reduces prokaryotic diversity in grassland and agricultural soils because bacterial activity is more strongly constrained by reduced water films and substrate diffusion (Bian et al. 2025; Preece et al. 2019; Qu et al. 2025). Overall, these findings indicate that fungal communities were inherently more resistant than prokaryotic communities to drought stress. The effects of drought were more evident at the community composition level than at the diversity level. Principal coordinates analysis clearly separated drought-stressed and well-watered soils, demonstrating that water limitation substantially altered microbial community assembly. Prokaryotic communities were more responsive to drought than fungal communities, as indicated by the higher proportion of variation explained by the first PCoA axis (37.5% vs. 22.1%), whereas fungal communities exhibited greater dispersion across treatments, reflecting higher compositional heterogeneity. This broader variability likely results from the greater functional diversity and adaptive strategies of fungi, enabling them to exploit heterogeneous microhabitats under water-limited conditions.

At the taxonomic level, drought selectively favored microbial groups adapted to water limitation while reducing taxa that are more sensitive to moisture depletion. In prokaryotic communities, drought increased the relative abundance of *Proteobacteriota*, *Bacteroidota*, *Crenarchaeota*, *Planctomycetota*, and *Acidobacteriota*, whereas *Actinobacteriota*, *Firmicuteota*, *Gemmatimonadota*, and *Chloroflexota* declined. Previous studies frequently reported increased *Actinobacteriota* and *Firmicuteota* and decreased *Gemmatimonadota*, *Proteobacteriota* and *Acidobacteriota* under drought (Babić et al. 2024; Peng et al. 2021; Preece et al. 2019). These discrepancies may be attributed to differences in soil type, vegetation, climate, and experimental conditions. Likewise, *Ascomycota* remained the dominant fungal phylum, followed by *Basidiomycota*, despite reports that drought often promotes *Basidiomycota* while reducing *Ascomycota* (Preece et al. 2019). The persistence of *Ascomycota* may be explained by their strong capacity for organic matter decomposition, carbon and nitrogen cycling, soil stabilization, production of plant growth regulators, and endophytic associations that improve nutrient acquisition and drought tolerance (Challacombe et al. 2019; Devi et al. 2020; Yadav et al. 2022, 2024; Manici et al. 2024). These contrasting taxonomic responses highlight the influence of local soil and environmental conditions on microbial adaptation to drought.

The ANCOM-BC analysis further demonstrated that drought altered the abundance of several microbial taxa with key ecological functions. Water deficit reduced beneficial prokaryotic groups such as *Pseudomonas*, *Devosia*, Actinobacteriota, and *Nitrospira*, organisms involved in plant growth promotion, biological control, nitrogen cycling, nutrient availability, and drought tolerance (Pandey et al. 2018; Zhu et al. 2020; Al-Surhanee et al. 2021; Zhang et al. 2023). Conversely, drought enriched *Gemmatiomonadota*, AKAU4049, S0134_terrestrial_group, and *Chloroflexota* taxa including Gitt-GS-136 and JG30-KF-CM45, whose ecological traits suggest greater adaptation to varying environmental conditions through efficient nutrient cycling and organic matter degradation (Hemmat-Jou et al. 2018; Xian et al. 2020). Among fungi, drought reduced the abundance of *Penicillium* and Sordariomycetes, despite previous reports describing an increase in *Penicillium* under drought owing to its ability to decompose organic matter, facilitate nutrient cycling, and produce gibberellins that enhance plant stress tolerance (Leitão and Enguita 2016; Yue et al. 2021; Wei et al. 2024). The decline of Sordariomycetes agrees with earlier studies and may reflect their comparatively lower drought tolerance (Tian et al. 2024; Yue et al. 2021). Collectively, these results show that drought reshaped microbial

communities primarily through taxonomic replacement rather than major losses in diversity, favoring stress-adapted microorganisms while reducing taxa that support nutrient cycling and plant growth.

Organic amendments substantially modified the response of soil microbial communities to drought by buffering drought-induced disturbances and reshaping community assembly. Although biochar application under drought reduced fungal Shannon diversity compared with drought alone, compost increased fungal richness (Chao1), while the combined compost-biochar treatment decreased the Shannon diversity of both fungal and prokaryotic communities. At first glance, these reductions in diversity may appear contradictory to the beneficial effects of organic amendments. However, Shannon diversity reflects both richness and evenness, and the observed decline likely resulted from the selective enrichment of drought-adapted microorganisms rather than a loss of ecological function. By altering soil pH, nutrient availability, organic carbon content, and habitat heterogeneity, the amendments created environmental conditions that favored specialized microbial taxa capable of thriving under water-limited conditions. Similar observations have been reported for biochar and compost applied individually, although studies investigating their combined effects under drought remain limited (Ali and Elshaikh 2022; Abideen et al. 2024). Compost, in particular, provides labile organic matter, nutrients, and microbial *inocula* that stimulate microbial biomass and enzyme activities under drought (Bastida et al. 2017; Dang et al. 2021; Ghouili et al. 2023a; Zarezadeh et al. 2025), whereas biochar improves soil physical properties by increasing porosity, water-holding capacity, and aggregate stability, thereby creating protected microsites for microbial colonization.

The influence of the amendments was even more evident at the community level than at the diversity level. Principal coordinates analysis showed that compost partially maintained the prokaryotic community structure close to that of the well-watered control, indicating its capacity to mitigate drought-induced shifts, whereas the combined application of compost and biochar produced the greatest restructuring of both prokaryotic and fungal communities. These compositional changes likely reflect the complementary mechanisms of the two amendments: compost continuously supplies readily available carbon and nutrients that sustain microbial metabolism, while biochar improves habitat quality through enhanced moisture retention, aeration, and colonizable pore networks. Together, these complementary effects modify microbial interactions and niche availability, promoting the establishment of microbial communities better adapted to drought. Similar improvements in microbial activity, community composition, and soil functioning following the application of compost and biochar have been reported previously under water-limited conditions (Akmal et al. 2019; Luis Moreno et al. 2022; Zarezadeh et al. 2025). Overall, these findings suggest that organic amendments did not simply preserve microbial diversity under drought but actively reorganized microbial communities toward a composition potentially better suited to withstand prolonged water limitation.

The restructuring of microbial communities by organic amendments was reflected in marked shifts in the dominant bacterial and fungal taxa, highlighting the complementary ecological roles of compost and biochar under drought. Overall, the amendments favored microbial groups associated with nutrient cycling, plant growth promotion, and stress adaptation, while reducing taxa that were more sensitive to water limitation. These changes suggest that amendment-induced improvements in soil moisture, organic carbon availability, and habitat quality acted as the primary drivers of microbial community reassembly (Bello et al. 2023; He et al. 2023; Wang et al. 2020).

Among the bacterial phyla, *Proteobacteriota* remained dominant across all treatments, with the highest relative abundance observed under DB followed by DCB, suggesting an important role in microbial adaptation to drought. Members of this phylum rapidly exploit labile carbon sources and contribute to nutrient cycling and beneficial plant-microbe interactions, thereby supporting soil functioning under environmental stress (Cui et al. 2022; Yang et al. 2021). Their abundance is strongly influenced by soil water availability and management practices (Chen et al. 2024). Although drought has often been reported to reduce *Proteobacteriota* (Moreno-Espíndola et al. 2018), biochar can counteract this decline by improving water retention and providing protected colonization sites, while compost supplies readily available carbon that supports their metabolic activity (Palansooriya et al. 2022; Ghouili et al. 2023a). Likewise, compost promoted *Actinobacteriota* under DC and DCB treatments, consistent with previous findings (Ghouili et al. 2023a). This response is ecologically relevant because *Actinobacteriota* maintain nutrient cycling under drought through extracellular enzymes such as leucine aminopeptidase and chitinase and enhance plant tolerance by regulating phytohormones, antioxidant defenses, and nutrient acquisition (Zhang et al. 2021; Ebrahimi-Zarandi et al. 2023). *Firmicuteota* showed a contrasting response, declining under DB and DCB but increasing with compost under drought, indicating that nutrient availability rather than water status alone regulated their abundance. Their contribution to carbon and phosphorus cycling through β-1,4-glucosidase and phosphatase activities further highlights their importance for maintaining soil fertility under stress (Cui et al. 2019; Liu et al. 2021a, b b).

Other bacterial groups exhibited more treatment-specific responses. *Gemmatimonadota* declined under DB and DC but increased markedly following the combined application of compost and biochar, in agreement with reports describing their enrichment under multiple abiotic stresses (Yuan et al. 2024). Their involvement in nutrient cycling, energy metabolism, degradation of complex organic compounds, and antagonism against plant pathogenic fungi suggests an important contribution to drought adaptation (Du et al. 2024; Mujakić et al. 2022; Ghouili et al. 2023a). Similarly, *Bacteroidota* were enriched under combined amendments, probably because the increased availability of organic substrates favored taxa capable of degrading complex carbohydrates through diverse carbohydrate-active enzymes while maintaining activity under stress through biofilm formation and protective enzymes (Dheeman et al. 2024; Larsbrink and McKee 2020; Babić et al. 2024). In contrast, *Chloroflexota*, *Crenarchaeota*, *Planctomycetota*, *Acidobacteriota*, and *Myxococcota* generally declined under drought despite amendment application. These taxa are closely associated with organic matter turnover, nitrogen cycling, methane dynamics, and microbial food-web interactions, and their responses are strongly influenced by soil moisture, pH, calcium availability, and organic carbon content (Buckley et al. 2006; Clarke et al. 2009; Nicol et al. 2006; Wang et al. 2014; Kang et al. 2022; Lage et al. 2019; Li et al. 2021; Zou et al. 2024). Their decline may therefore reflect competitive replacement by faster-growing or more drought-adapted microorganisms in the amended soils.

Fungal communities exhibited comparable functional reorganization. Biochar increased the relative abundance of Eurotiomycetes, likely because improved soil porosity, lower bulk density, and enhanced water infiltration created favorable habitats for these drought-adapted fungi (Guo 2015). Conversely, Dothideomycetes declined following biochar or compost application, a potentially beneficial response given that this class includes numerous phytopathogenic taxa (Xu et al. 2020). The combined amendment further promoted Agaricomycetes and *Mortierellomycota*, both recognized for their contributions to organic matter decomposition, nutrient mobilization, and plant drought tolerance (Sánchez-García et al. 2020; Devi et al. 2020; Xu et al. 2022; Ning et al. 2022). Compost also stimulated Glomeromycetes, whose arbuscular mycorrhizal associations enhance water and nutrient uptake while improving soil aggregation and plant performance under drought (Devi et al. 2020; Yadav et al. 2024). Collectively, these taxonomic shifts indicate that organic amendments favored microbial groups supporting nutrient cycling, beneficial plant-microbe interactions, and drought adaptation, thereby establishing the functional basis for the differential responses of individual microbial taxa discussed below.

Differential abundance analysis (ANCOM-BC) further demonstrated that biochar and compost selected distinct but complementary microbial taxa, reflecting their contrasting modes of action under drought. Biochar primarily modified the soil physical environment by increasing water retention, porosity, aeration, aggregate stability, and the availability of protected microsites, thereby favoring microorganisms adapted to these improved habitats (Bolhassani et al. 2024; Ghouili et al. 2025a, b; Liu et al. 2021a). Accordingly, taxa belonging to *Proteobacteriota*, *Bacteroidota*, and *Actinobacteriota* were enriched under biochar-amended soils, including the plant growth-promoting genus *Pseudomonas*, whose ability to enhance nutrient acquisition, phytohormone production, and stress tolerance likely contributed to microbial adaptation under drought (Cherni et al. 2019). Biochar also favored *Photobacterium*, *Methylophaga*, *Marinobacterium*, *Marinobacter*, and *Aquicella*, suggesting that its porous structure generated localized moisture-retaining microenvironments suitable for these taxa. Likewise, *Nocardioides*, recognized for degrading complex organic compounds and promoting plant growth under abiotic stress, responded positively to biochar application (Meena et al. 2023). In contrast, *Marine_Group_II*, *Geminicoccaceae*, *Alphaproteobacteria*, and, in some treatments, *Nocardioides* declined, indicating that environmental filtering and microbial competition also shaped community assembly. Biochar similarly exerted selective effects on fungi by enriching *Hamigera* and *Penicillium*, taxa associated with decomposition and stress tolerance, while reducing *Sordariomycetes* and *Mycothermus*, probably because changes in soil pH, pore architecture, and carbon availability favored fungi with specific ecological strategies.

Compost, in contrast, exerted a stronger biological influence by supplying readily available organic substrates, nutrients, and microbial *inocula* that stimulated copiotrophic microorganisms. This effect was reflected in the enrichment of *Proteobacteriota*, *Firmicuteota*, *Bacteroidota*, *Actinobacteriota*, and *Gemmatimonadota*, together with beneficial genera such as *Bacillus*, which promotes nitrogen fixation, phosphate solubilization, iron acquisition, and plant tolerance to abiotic stress (Poulaki and Tjamos 2023; Vasques et al. 2024). Compost also favored saprotrophic fungal taxa including *Talaromyces*, *Myceliophthora*, *Microascaceae*, *Chaetomiaceae*, and Sordariales, indicating enhanced decomposition of complex organic matter and nutrient mineralization, whereas *Hyalosphaerella* declined, likely because of competitive exclusion by faster-growing decomposers. These responses agree with previous studies showing that compost-derived organic matter and resident microbial populations improve soil structure, moisture retention, and microbial proliferation under drought (Ghouili et al. 2022b, 2023a).

The combined application of compost and biochar produced the most pronounced taxonomic shifts, supporting the hypothesis that their interaction generates a more favorable microbial habitat than either amendment alone. The enrichment of *Gemmatimonadota*, *Bacteroidota*, *Proteobacteriota*, and *Actinobacteriota* reflected the complementary supply of labile carbon and nutrients from compost together with the stable colonization surfaces and moisture-retaining properties provided by biochar. Beneficial genera such as *Sphingomonas*, *Arthrobacter*, *Truepera*, and *Salinimicrobium* increased under the combined treatment, reinforcing functions related to phytohormone production, nutrient cycling, bioremediation, and carbon, nitrogen, and sulfur turnover (Asaf et al. 2020; Roy and Kumar 2020; Li et al. 2015; He et al. 2024). Fungal communities exhibited a similar synergistic response, with increased abundance of Agaricomycetes and other taxa within *Basidiomycota* and *Ascomycota* that contribute to ectomycorrhizal associations, saprotrophic activity, nutrient mobilization, and improved plant water acquisition under drought (Baruah and Sahu 2019; Singh et al. 2022). Collectively, these taxonomic shifts indicate that compost and biochar reshaped the soil microbiome through complementary biological and physicochemical mechanisms, providing the basis to evaluate whether these compositional changes translated into greater microbial resilience under drought stress.

The compositional changes induced by compost and biochar suggest that these amendments not only reshaped the soil microbiome but also enhanced its capacity to withstand drought disturbance. Microbial resilience is reflected by the ability of microbial communities to maintain their diversity, structure, and ecological functions despite environmental stress. In the present study, prokaryotic communities exhibited greater resilience than fungal communities, as indicated by the relatively stable Shannon and Chao1 indices across treatments, whereas fungal communities showed greater sensitivity to drought, particularly in terms of diversity. Nevertheless, the enrichment of beneficial bacterial and fungal taxa by compost and biochar, applied individually or in combination, indicates that drought-induced community shifts were directed toward microbial assemblages with greater functional potential rather than representing a generalized loss of diversity. These findings suggest that resilience under organic amendment management is achieved primarily through functional reorganization of the microbiome rather than through complete conservation of its original taxonomic composition.

Our results further indicate that the mechanisms underlying this resilience differ between the two amendments but become complementary when they are combined. As discussed above, compost enriches the soil with labile organic matter, nutrients, and diverse microbial inocula, whereas biochar improves the physical habitat by enhancing water retention, aeration, and pore connectivity. Their combined application therefore promotes complementary microbial interactions, restructures ecological networks, and favors taxa associated with nutrient cycling, plant growth promotion, stress tolerance, and suppression of potential pathogens. Similar amendment-dependent changes in microbial composition and function have been reported previously (Ghouili et al. 2023a; Wang et al. 2020; Zarezadeh et al. 2025; Peng et al. 2024; Preece et al. 2019; Zhang et al. 2025). Consequently, the stronger response observed under DCB indicates that the interaction between compost and biochar provides a more effective ecological buffering capacity against drought than either amendment applied individually.

These microbiome findings are complemented and contextualized by a companion study conducted under the identical experimental framework, same eight treatments, cultivar, and glasshouse conditions (Ghouili et al. 2025). That study documented the morphophysiological, biochemical, and molecular responses of barley plants and demonstrated that the combined application of compost and biochar (DCB) most effectively alleviated drought-induced growth reduction, reduced oxidative damage (modulating SOD, APX, and GPOX activities), and regulated key stress-responsive transcription factors (*HvAP2/ERF*, *HvDREB*) and functional genes involved in proline biosynthesis (*HvP5CS*) and water transport (*HvPIP*). Although the present study did not directly establish causal relationships between microbial taxa and plant responses, the enrichment under DCB of microorganisms associated with nutrient mobilization, plant growth promotion, and stress adaptation provides a plausible biological explanation for the superior physiological performance observed in barley. The improved soil microbial community likely enhanced nutrient availability, strengthened soil biological functioning, and contributed to a more favorable rhizosphere environment, thereby reducing the metabolic cost of drought acclimation. Together, these complementary findings support a broader mechanistic framework in which compost and biochar jointly improve soil biological quality, promote a functionally resilient microbiome, and ultimately enhance barley tolerance to drought stress.

8.1 Limitations and Future Research

Although this study provides new insights into the effects of compost and biochar on the soil microbiome under drought stress, several limitations should be considered when interpreting the results. First, the experiment was conducted in pots under controlled glasshouse conditions, which may not fully represent the complexity of field environments where soil heterogeneity, climatic fluctuations, and plant–soil

interactions are more variable. In addition, the 12-day drought period represents an acute rather than chronic stress scenario, and therefore may not capture microbial responses to prolonged or recurrent drought events.

A second limitation concerns the temporal scope of the microbial analyses. Soil microbial communities were sampled only once, at the end of the drought period, preventing assessment of microbial succession, recovery dynamics, or resilience over time. Furthermore, the experiment covered a single growing season, precluding conclusions regarding the long-term persistence of the observed effects. Multi-season and field-based studies are therefore needed to determine whether the beneficial effects of the compost–biochar combination remain stable under repeated drought cycles and changing environmental conditions.

Moreover, the mechanisms underlying the observed microbial responses could not be fully resolved. Key soil physicochemical properties, including pH, available nitrogen and phosphorus, dissolved organic carbon, and soil water content, were not monitored throughout the experiment, limiting mechanistic attribution of microbial community shifts to specific changes in soil properties. Finally, functional interpretation relied exclusively on taxonomic information obtained from amplicon sequencing, whereas metagenomic, metatranscriptomic, metabolomic, or enzyme activity analyses were not performed. Consequently, the functional roles discussed throughout this study should be considered potential ecological functions inferred from published literature rather than direct evidence. Integrating high-resolution functional omics, soil physicochemical monitoring, and repeated temporal sampling in future field-scale experiments will provide a more comprehensive understanding of how compost and biochar jointly regulate microbial community assembly, ecosystem functioning, and long-term drought resilience.

9 Conclusion

This study demonstrates that compost and biochar differentially influenced the soil microbial community of barley cultivated under drought stress, with their combined application producing the most consistent positive effects. Although drought modified microbial community composition, the co-application of compost and biochar more effectively promoted beneficial bacterial and fungal taxa associated with key soil ecological functions than either amendment applied alone. These microbial responses indicate an improved capacity of the soil microbiome to cope with water limitation while maintaining processes essential for soil functioning. Overall, our findings provide evidence that integrating compost and biochar is a promising strategy for enhancing the biological quality of drought-affected soils. By promoting a more stable and functionally relevant soil microbiome, this combined amendment has the potential to support sustainable barley production and improve the resilience of agricultural systems facing increasingly frequent drought events.

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Authors' Contributions Emna Ghouili, Ghassen Abid, Richard Hogue and Moez Jebara conceptualized and designed the study. Emna Ghouili and Fatma Souissi conducted the work. Laboratory analysis was performed by Emna Ghouili. Data analysis was performed by Joël D'Astous-Pagé. Emna Ghouili Writing - original draft. Emna Ghouili, Ghassen Abid, Richard Hogue and Yordan Muhovski revised the manuscript. Emna Ghouili, Ghassen Abid, Richard Hogue and Yordan Muhovski contributed to editing the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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