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RESEARCH ARTICLE

Impact of Mycorrhizal Symbiosis on Nutrient Use Efficiency, Plant Growth, and Soil Microbiome Dynamics in Sugar Beet

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Abstract

Sugar beet (*Beta vulgaris* L.) is a significant sugar crop worldwide. However, environmental stress and excessive use of synthetic fertilizers, which compromise yield and soil health, are increasingly affecting it. Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with most plants, enhancing nutrient uptake, growth, and stress resistance. Therefore, this work aimed to investigate the effects of AMF on sugar beet physiology, defense gene expression, and rhizosphere microbiome. Plants were inoculated with a mix of spores from the AMF consortium. Plants treated with AMF showed significant improvements in root development and quality, with root diameter reaching 4.2 cm and root weight 64 g, compared to the control plants, 2.4 cm and 26.8 g, respectively. Nutrient uptake of the roots increased significantly (phosphorus by 37% and nitrogen by 24%), accompanied by a 17% rise in sugar yield. The expression of defense-related genes (*BvJac, BvProt, BvJI,* and *BvPR10*) was assessed via qPCR and showed a 1.5–2.4-fold increase, suggesting a possible role for AMF in enhancing plant stress adaptation and strengthening plant defense responses. Analysis of the rhizosphere microbiome via 16S rRNA and ITS amplicon sequencing, utilizing MOTHUR, CosmosID, and MicrobiomeAnalyst, revealed distinct shifts in bacterial and fungal communities, including an increase in the potentially beneficial phylum Patescibacteria (12.5% vs. 7.1% in controls), along with a ~ 50% reduction in *Fusarium*. These findings highlight the potential of AMF to enhance sugar beet performance while promoting soil health and beneficial plant–microbe interactions, providing a sustainable strategy for crop production and reducing reliance on chemical inputs.

Keywords Sugar beet · AMF · Soil microbiome · Defense gene expression · Soil health · Sustainable agriculture

Introduction

Sugar beet (*Beta vulgaris* L.) is the second most significant sugar crop worldwide. Egypt is heavily dependent on sugar beet cultivation for its sugar industry due to its exceptional adaptability to saline conditions and desert climates (El-Zayat 2022).

Microorganisms and plants communicate in complex ways as plants exude carbohydrates, flavonoids, amino acids, and organic compounds into the soil, with chemical signals from these molecules attracting beneficial microorganisms and repelling harmful ones. This balance of soil microbes is crucial for plant development (Jamil et al. 2022).

Microbial-based fertilizers, or biofertilizers, including plant growth-promoting microorganisms (PGPMs) and arbuscular mycorrhizal fungi (AMF), improve soil health and yield sustainable crops. (Samantaray et al. 2024). PGPMs application enhances nutrient use efficiency, enabling reduced fertilizer inputs and translating into superior economic returns and profitability in crop production systems (Youssef et al. 2025). In particular, AMF are crucial in symbiosis with plants, boosting plant immunity and

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increasing the bioavailability of nutrients from natural sources such as rock phosphate (Jaitieng et al. 2021).

During mycorrhizal colonization, host plants activate their defense mechanisms in response to fungal interaction. These interactions are accompanied by changes in plant hormone levels and photosynthetic metabolism. Exogenous elicitors are degraded, and defense signaling molecules, including hydrogen peroxide and jasmonic acid. Understanding plant–microbe interactions and the role of contemporary multi-omics methods, including those still being developed, is crucial for elucidating the complex network between plant hosts and microorganisms (Diwan et al. 2022).

Previous studies have highlighted the broader role of AMF in regulating physiological and biochemical processes in sugar beet and demonstrated their ability to enhance plant performance under abiotic stress conditions (Cui et al. 2024). The interaction between arbuscular mycorrhizal fungi (AMF) and sugar beet still requires further investigation.

This study aimed to clarify the AMF–sugar beet interaction, focusing on the underlying molecular and physiological mechanisms, by analyzing the microbial community to assess AMF's influence on soil microorganisms through sequencing of the 16S rRNA and the ITS regions. This study's goal is to support ecologically friendly farming practices by investigating the relationships between plants and their microbial communities.

Materials and Methods

AMF and Sugar Beet Seeds

Sugar beet seeds, a variety of Faralada originating from Germany, were kindly provided by the Sugar Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt. The mix of three genera (*Glomus, Gigaspora*, and *Acaulospora*) mycorrhizal fungi mix was obtained from the Microbiology Department at the Soil, Water, and Environmental Research Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt.

Seeds Germination and AMF Materials and Mycorrhizal Inoculation

The experiments were initiated at the greenhouse. Sugar beet seeds were germinated in 30 cm turbid pots containing a mixed soil with a 3:1:1 (v/v/v) ratio of peat moss, perlite, and sand supplemented with 10% rock phosphate as a phosphorus source (Yadav et al. 2017). AMF were inoculated using Arabic gum as a sticking agent, uniformly coating the seeds with about 30 AMF spores per seed, and then air-dried for two hours before sowing (Abd El-Monium and Massoud 2009). 30 days after germination, the plants were transplanted into wooden boxes measuring 80 cm in height and 50 cm in width. The experiment consisted of two main groups: (1) control group, (untreated with AMF) and (2) treated group, sugar beet plants treated with AMF. No plant control group (no plants, no AMF) was used as a negative control for metagenomic analysis. All pots were watered regularly and received the required elements according to the plants' developmental stage.

AMF–Root Colonization Detection

To assess the sugar beet AMF-inoculated roots, the plant roots were washed and sectioned into 1 cm fragments after one month of inoculation. The samples were then stained with 0.05% trypan blue (Brundrett et al., 1996). The root colonization was determined using a 40 × magnification microscope (Giovannetti and Mosse 1980), with the presence of AMF in roots confirmed by the clear visibility of vesicles or hyphae of AMF.

Morphological and Physiological Parameters

To evaluate the AMF effect on sugar beet, different morphological and physiological parameters were observed at two intervals: 30 days post-germination and at harvest (120 days). Morphological characteristics, including plant height (cm), leaf count, leaf fresh weight (g/plant), root diameter (cm), and root fresh weight (g/plant), were assessed for both control and AMF-treated plants (Chukwuneme et al. 2021). Four spectral vegetation indicators were examined: total chlorophyll content (CHLT) (μg/cm^2), Soil Plant Analysis Development (SPAD), Flavonoid Reflectance Index (FRI), and Normalized Phaeophytinization Index (NPQI), which were computed based on the data collected (Gai et al. 2024). A portable leaf spectrometer (CI-710) was utilized. Measurements were taken from healthy, fully expanded leaves of the same physiological age. Seven replicate readings were collected near the main vein for each treatment.

Plant Chemical Analysis

After 60 days of germination, the 4th–6th youngest fully expanded leaves were collected from the middle of sugar beet plants of each treatment, and leaf nitrogen and phosphorus content were determined. The leaf phosphorus content was assessed using the vanadomolybdophosphoric acid colorimetric method; 2 g of oven-dried leaf material was digested using a mixture of nitric acid and perchloric acid (5:1 v/v). After digestion, the vanadomolybdate reagent was added to develop color, then phosphorus concentration was measured using a UV–Vis spectrophotometer at 410 nm wavelength (Ratti et al. 2001). To evaluate the leaf nitrogen content, the Kjeldahl method was utilized (Garcia-Servin

et al. 2021). After harvest, the total soluble sugar content of the roots was quantified according to the phenol–sulfuric acid method, according to (Dubois et al. 1956).

Plant Defense Genes Expression Analysis

One month after AMF treatment, the expression levels of four sugar beet defense-related genes; *BvJac, BvProt, BvJI,* and *BvPR10*, were evaluated to investigate the influence of AMF on the regulation of host plant defense gene expression. Total RNA was extracted from three biological replicates, each containing three technical replicates of sugar beet plant samples utilizing the SV Total RNA Isolation System (Promega, cat. no. Z3100, USA). Thereafter, cDNA synthesis was conducted using the Thermo Scientific Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, cat. no. K1651, USA). PCR reactions were performed in triplicate utilizing an Applied Biosystems qPCR system. Each 15 µL reaction mixture contained 7.5 µL of iTaq Universal SYBR® Green Supermix (Bio-Rad, cat. no. 1725121, USA), 1 µL of each primer (10 pmol) (Desoignies et al. 2014), and cDNA template adjusted based on beta-tubulin housekeeping gene normalization. The remaining volume was completed with nuclease-free water. Amplification was accomplished under the subsequent PCR conditions: initial denaturation at 95 °C for 5 min and succeeded by 40 cycles of denaturation at 95 °C for 5 s and annealing at 62 °C for 1 min (Youssef et al. 2018). Relative gene expression was determined with the $2^{-\Delta\Delta Ct}$ approach, employing appropriate reference genes for normalization (Livak and Schmittgen 2001).

Soil Microbiome Sequencing

Seventy days post-seed planting, rhizospheric soil samples were obtained from each experimental group; three replicates for each; the roots were shaken to collect the rhizosphere soil. The microbial DNA was extracted from one gram of soil samples utilizing the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, cat. no. D5625-01, USA). The extracted DNA quality was evaluated using a NanoDrop 2000 UV–Visible Spectrophotometer to confirm that the DNA concentration, the extracted DNA samples were sent to Macrogen Inc. (Seoul, Republic of Korea) for NGS amplicon sequencing, targeting the 16S rRNA gene for bacterial identification and ITS for fungal identification utilizing the Illumina paired-end (PE) sequencing platform. Primers targeting the V3–V4 region of the 16S rRNA gene were used (Ye et al. 2016), generating amplicons of approximately 453 bp. Additionally, ITS primers ITS1F 5'CTTGGTCAT TTAGAGGAAGTAA3' and ITS2R5'GCTGCGTTCTTC ATCGATGC3' (Mbareche et al. 2020) were employed.

Bioinformatics Analysis

The 16S data were analyzed using mothur pipeline (https://mothur.org/wiki/miseq_sop/) (Schloss et al. 2009). For the mothur pipeline, this workflow involved initial quality control using screen.seqs command to screen contigs and eliminate sequences more than 490 bp. This is followed by denoising and chimera removal with the chimera. uchime command. For annotation of 16S classification, the acquired clean data were aligned against the SILVA (version 138.1) database (https://www.arb-silva.de/) to generate OTUs clustering. Sequences were clustered into Operational Taxonomic Units (OTUs) based on a 97% sequence similarity threshold using the pre.cluster command. Subsequent analyses, including alpha diversity metrics, were conducted using the Microbiome Analyst (2.0) platform (https://www.microbiomeanalyst.ca/) (Lu et al. 2023). Alpha diversity was calculated using six metrics. Differences in alpha diversity among groups were statistically evaluated using the non-parametric Kruskal–Wallis test with a significance threshold set at $p < 0.05$. Beta diversity was assessed using Bray–Curtis dissimilarity and visualized through principal coordinates analysis (PCoA). To identify differentially abundant taxa across treatments, Linear Discriminant Analysis Effect Size (LEfSe) was applied using default Microbiome Analyst parameters, with a significance cutoff of $p < 0.05$ and an LDA score threshold of 2.0.

FASTQ files generated from ITS amplicon sequencing with a 250–300 bp average were analyzed by CosmosID online platform (https://www.cosmosid.com/), where automated quality control, taxonomic classification, and diversity analyses were performed using the UNITE database, metagenomic analysis performed using CosmosID's pipeline to determine and the most abundant phyla. Venn diagrams were generated using InteractiVenn online tool (https://www.interactivenn.net/) to visualize shared and unique OTUs across groups (Heberle et al. 2015).

Statistical analysis

The one-way ANOVA was utilized for qPCR data analysis, while unpaired t-test statistical analysis was conducted on the remaining data using GraphPad software version 9.0.

Results

AMF–Root Colonization

The stained roots showed vesicles and hyphae, confirming the presence of AMF. Root colonization was detected as a blue spot under the microscope in the AMF-treated plants (Fig. 1).

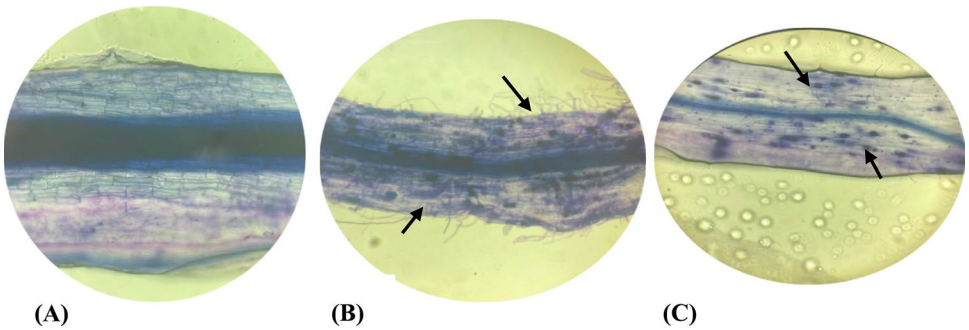


Fig. 1 Sugar beet root–AMF micrographs. (A) Control and (B–C) roots inoculated with arbuscular mycorrhizal fungi (AMF). Arrows indicate hyphae and vesicles stained by trypan blue, after one-month AMF inoculation

Morphological Parameters

The visual assessment indicated an obvious difference in root biomass between the control and AMF-treated groups (Supplementary file Fig. 1). At the two time points, this difference was reflected in vegetative growth, with the AMF-treated group exhibiting superior vegetative development compared to the control group. The results revealed no significant differences in the average number of leaves/plant between the control and AMF-treated groups at transplantation (control: 4.5 ± 0.53 versus treated: 4.6 ± 0.70; $p > 0.05$) or at harvest time (control: 22.29 ± 5.74 versus treated: 22.57 ± 3.95; $p > 0.05$) (Fig. 2A).

In contrast, significant differences were observed for plant height and leaf fresh weight. At the transplantation time, the average plant height of AMF-treated plants was 14.16 ± 2.11 cm, substantially greater than that of control plants (11.35 ± 1.36 cm; $p < 0.01$). This difference increased markedly by harvest, with treated plants reaching 53.86 ± 8.67 cm compared to 34.86 ± 5.81 cm

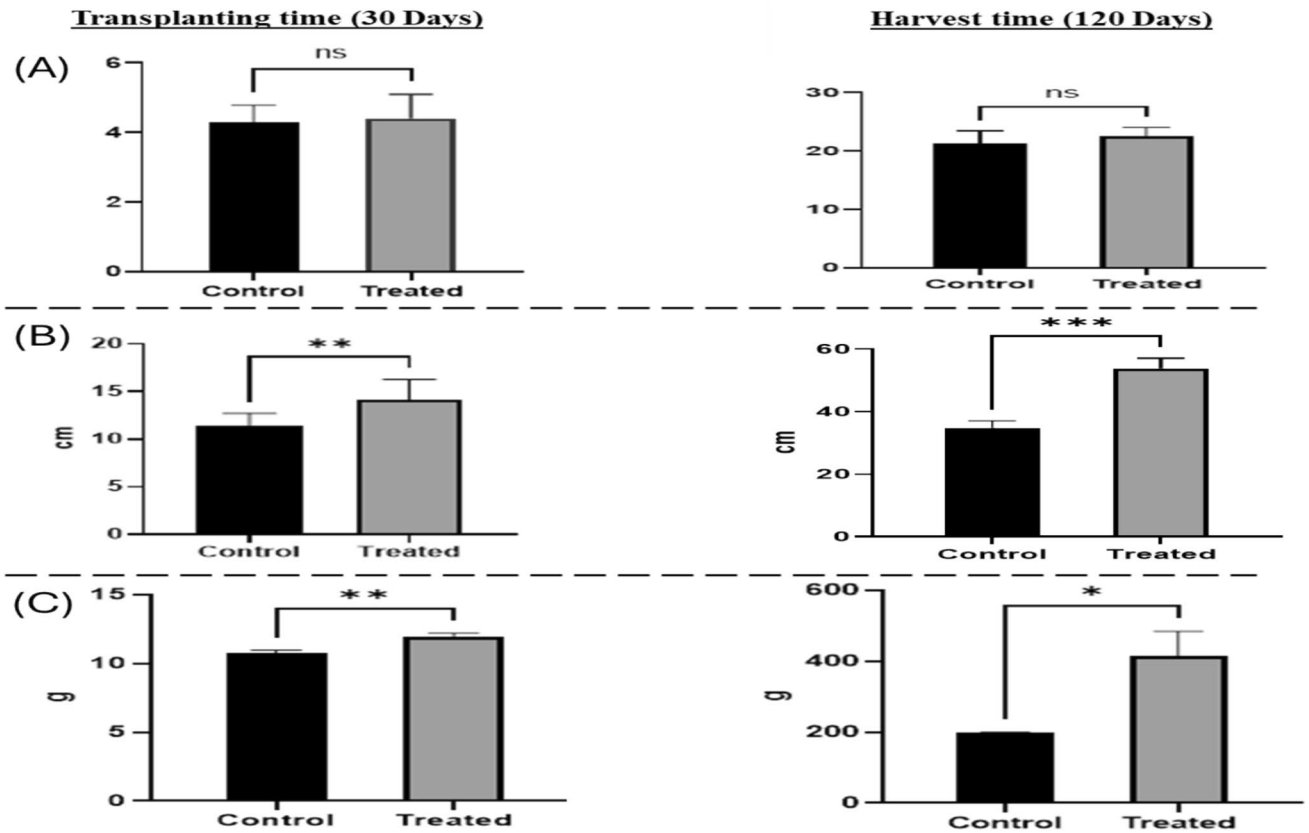


Fig. 2 Effects of AMF inoculation on plant growth parameters at two different time points: transplanting and harvest. (A) Leaves number, (B) plant height, and (C) leaf fresh weight in control and AMF-treated groups. Results are expressed as mean ± standard deviation. Asterisks (*) denote statistically significant differences between the control and treated groups, $p < 0.05$ as *, and $p < 0.01$ as ** and $p < 0.001$ as ***

for controls ($p < 0.001$) (Fig. 2B). Similarly, a significant enhancement was recorded for leaf fresh weight. At transplanting, the average weight was 11.96 ± 0.73 g for treated plants versus 10.74 ± 0.74 g for controls ($p < 0.01$). By harvest, the fresh weight of leaves from AMF-treated plants (413.4 ± 158.53 g) was more than double that of the control plants (197.66 ± 6.08 g; $p < 0.05$) (Fig. 2C).

At the harvest time (120 days), the yield and quality of sugar beet roots were assessed. The beneficial effects of AMF treatment on root diameter and weight were observed. The average root diameter of treated plants was 4.11 ± 1.19 cm, more than 1.7 times that of control plants (2.49 ± 1.02 cm; $p < 0.05$). Likewise, the average root weight was 64.0 ± 23.48 g in the AMF-treated group, compared to 26.8 ± 15.22 g in the control group, representing a 2.4-fold increase ($p < 0.05$) (Fig. 3).

Physiological Parameters

Physiological analysis revealed significant disparities between control and AMF-treated plants at 60 days post-treatment. AMF inoculation significantly increased total chlorophyll content (CHLT) and Soil Plant Analysis Development (SPAD) values ($p < 0.05$ and $p < 0.01$, respectively), while it lowered the Flavonoid Reflectance Index (FRI) compared to controls ($p < 0.05$) (Fig. 4). Also, the Normalized Phaeophytinization Index (NPQI) was reduced in AMF-treated plants, although this was not statistically significant. Collectively, these shifts indicate that AMF treatment enhanced photosynthetic capacity, improved nitrogen status, and reduced chlorophyll degradation in sugar beet leaves.

Plant Chemical Analysis

AMF inoculation of sugar beets resulted in a significant enhancement in nitrogen and phosphorus content, relative to the control group (Fig. 5). This illustrates the favorable impact of AMF on plant nutrient acquisition and metabolic processes. AMF-inoculated roots exhibited a notable increase in total soluble sugar content in treated plants (14.7%) when compared to the control (12.2%) (Fig. 5).

Gene Expression

qPCR was carried out on the leaves of control and AMF-treated plants after one month of germination. The qPCR illustrated significant variability among the four tested genes, *BvJac*, *BvProt*, *BvJI*, and *BvPR10*, revealing significant differences ($F = 13.33$, $p < 0.0009$, $R^2 = 0.7272$). The data demonstrate a significant fold change with values ranging from 1.5 to 2.4 (Fig. 6). *BvJac* and *BvProt* showed similar behavior with a substantial increase in fold change of about twofold, but *BvJI* had a lower response of 1.5-fold relative to the other genes assessed.

Soil Microbial Community Analysis

The obtained 16S rRNA amplicon metagenomics data were processed by the mothur workflow employing the SILVA database.

The mothur pipeline 16S rRNA analysis was annotated with the microbial diversity linked to 16S rRNA gene amplicons, yielding 560,667 high-quality *16S rRNA* gene sequences, averaging 453 bases in length. These sequences were aligned with the SILVA reference database (version 138.1) and trimmed to encompass the V4 region. After the removal of chimeras and dereplication,



Fig. 3 Morphological features of the roots. After harvest roots analysis: (A) root diameter and (B) root weight. Roots of plants after harvest: (C) control and (D) AMF-treated plants. Scale bar 10 cm. Asterisks (*) denote statistically significant differences between the control and treated groups, $p < 0.05$ as *

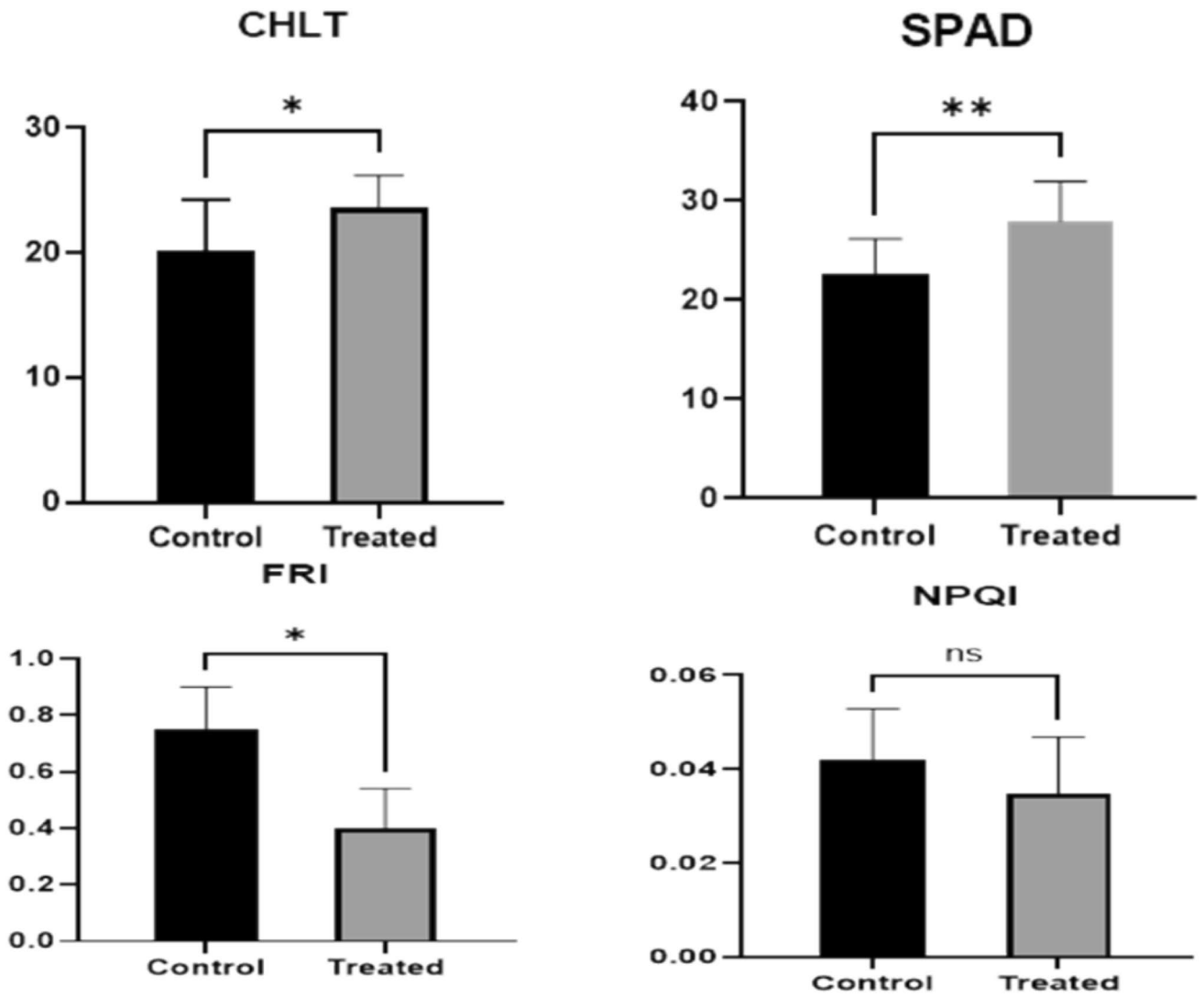


Fig. 4 CHLT, SPAD, FRI, and NPQI in the control and treated groups. Results are expressed as mean ± standard deviation. Asterisks (*) denote statistically significant differences between the groups, $p < 0.05$ as *, and $p < 0.01$ as **

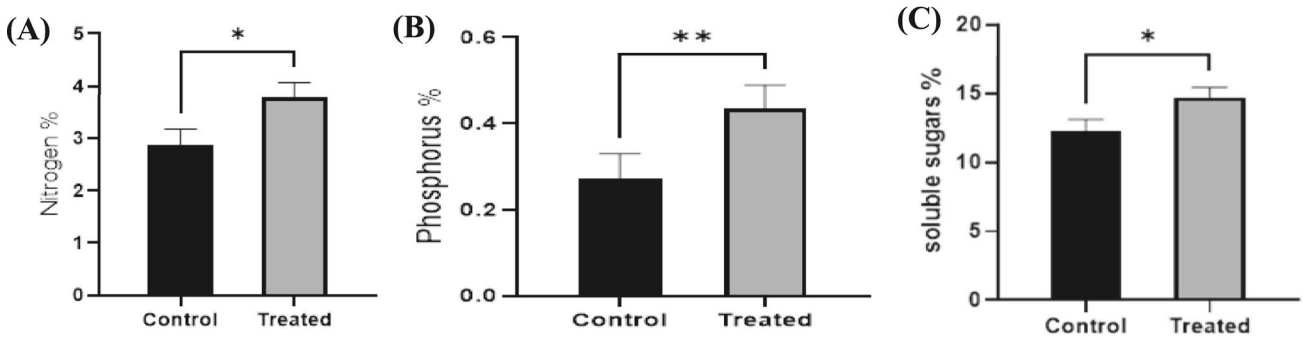


Fig. 5 Effects of AMF inoculation on the nutritional content of plants. Levels of **A** nitrogen, **B** phosphorus, and **C** sucrose in roots. Asterisks (*) denote statistically significant differences between the groups, $p < 0.05$ as *, and $p < 0.01$ as **

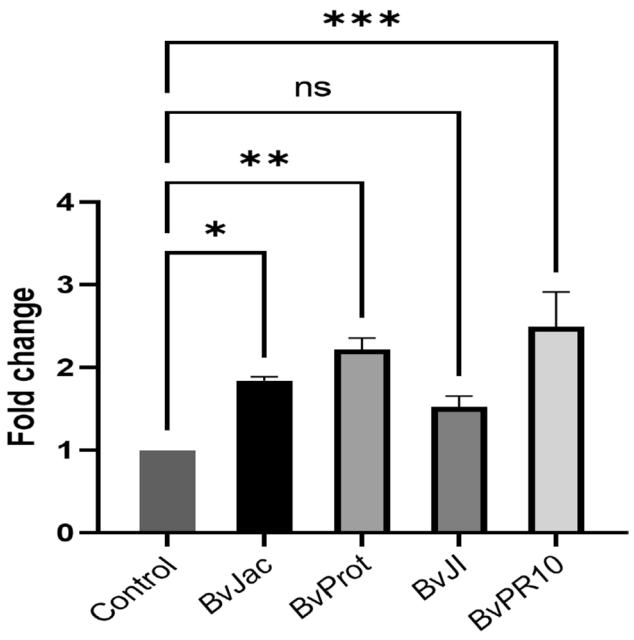


Fig. 6 Fold change in gene expression for BvJac, BvProt, BvJI, and BvPR10 associated with AMF inoculation. Asterisks (*) denote statistically significant differences between the groups, $p < 0.05$ as *, $p < 0.01$ as **, and $p < 0.001$ as ***

a final dataset of 376,131 unique sequences was classified according to the SILVA taxonomy. Alpha diversity analysis using the six indices demonstrated a significant difference among the three experimental groups (*p-value* < 0.05, ANOVA F-value = 6.9401). Beta diversity analysis using Bray–Curtis dissimilarity and PCoA revealed clear separation among groups (Supplementary file Fig. 2). Treated samples clustered tightly, indicating a more uniform and distinct microbial community structure.

The 16S rRNA gene analysis revealed some distinct microbial community structure among the three experimental groups. The relative abundance analysis done through mothur pipeline against SILVA reference database found that Patescibacteria represented 12.52% of the bacterial community in the AMF-treated group, compared to 7.14% in the control and 5.85% in the no-plant control, with a predominance of the phyla Proteobacteria, Acidobacteriota, and Bacteroidia for the three groups (Fig. 7).

Linear Discriminant Analysis Effect Size (LEfSe) identified several differentially abundant taxa among the control, no-plant control, and treated groups. At the phylum level, Acidobacteriota, Bdellovibrionota, Elusimicrobiota, Armatimonadota, and RCP2_54 were significantly enriched in the Control group. The no-plant control group showed enrichment of Actinobacteriota, Chloroflexi, Myxococcota, Gemmatimonadota, and Deinococcota, while the treated group was characterized by higher relative abundance of Patescibacteria, Planctomycetota, Hydrogenedntes, Nitrospirota, and Nanoarchaeota LEfSe confirmed that Patescibacteria, the phylum to which ABY1 belongs, was significantly enriched in the AMF-treated group (Fig. 8).

Fungal community analysis

Venn diagram analysis revealed distinct differences in fungal community composition among the treatment groups (Supplementary file Fig. 3). Only 166 OTUs were shared across all three groups, representing a potential core fungal community. The treated group (AMF-inoculated) exhibited the highest number of unique OTUs (981). In comparison, the

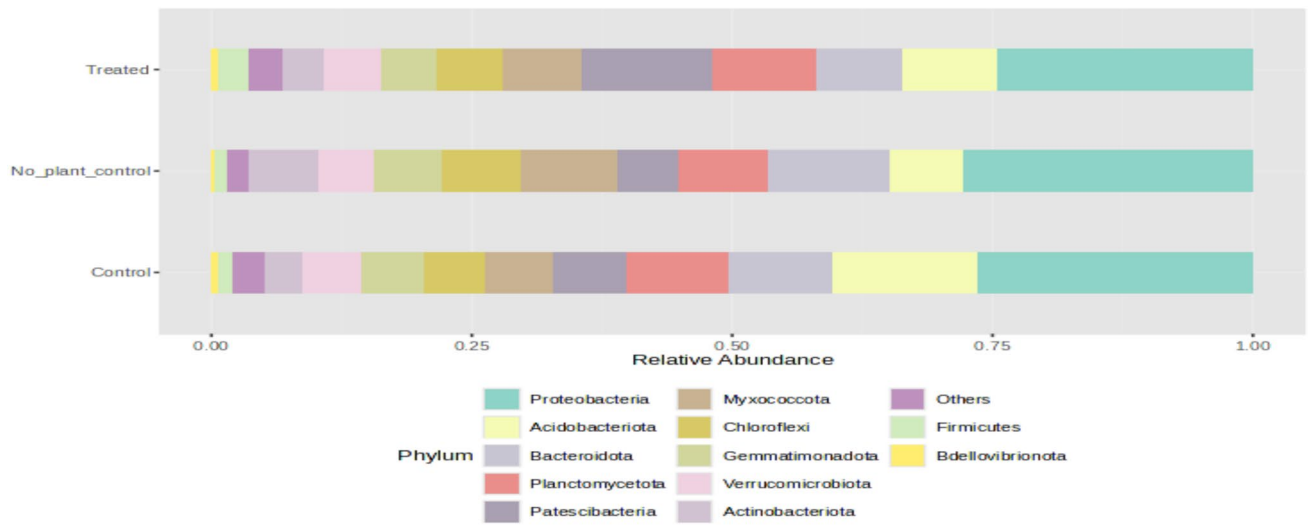


Fig. 7 Relative abundance of dominant microbial class in soil samples from control, treated, and no-plant groups against the SILVA database

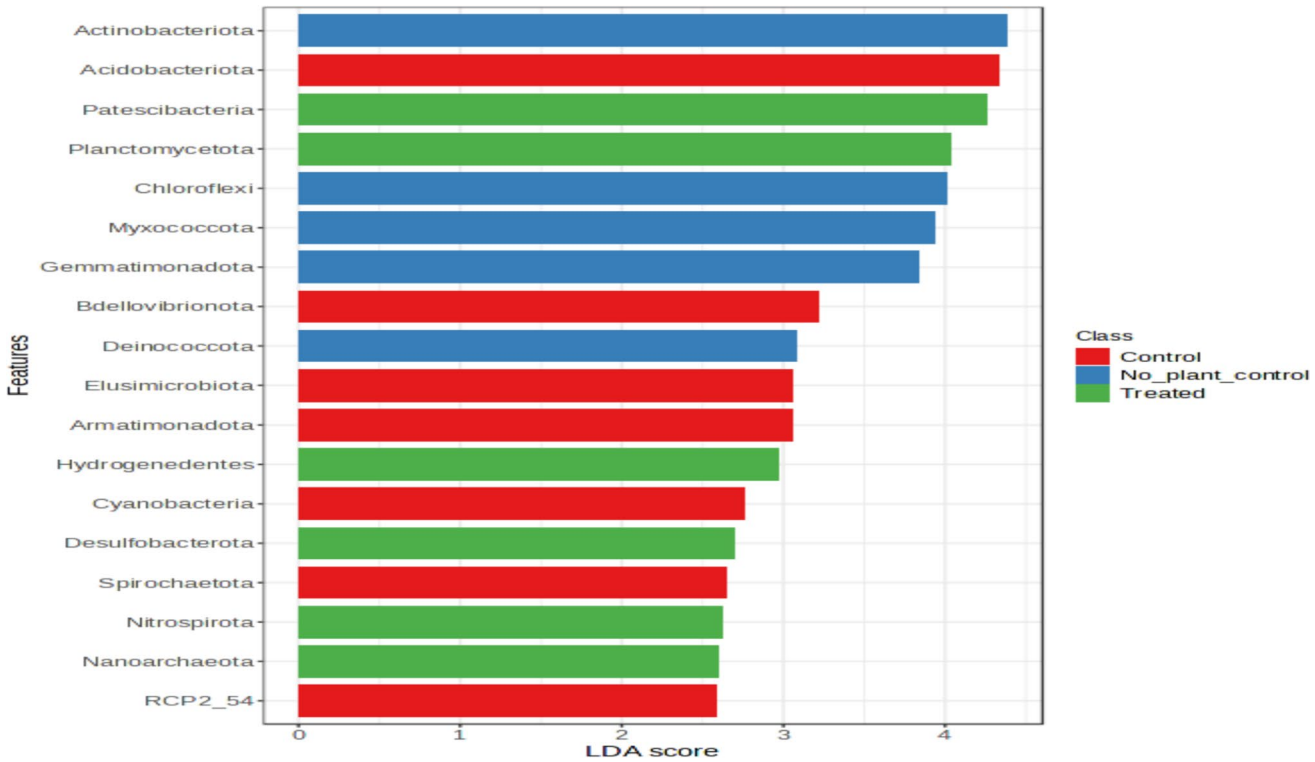


Fig. 8 LEfSe analysis to identify most microbial class associated with different treatment groups

Control and no-plant control groups contained 917 and 887 unique OTUs, respectively.

The ITS amplicon data obtained were analyzed using the CosmosID platform. The Ascomycota phylum, encompassing multiple classes, was consistently detected in all samples, followed by an unidentified phylum. The phylum Basidiomycota is also more abundant in control and no-plant samples than in soil samples treated with AMF.

Despite that, ITS data revealed that AMF affected the composition of some fungal communities. Such as, the control group exhibited a relative abundance of fungal taxa dominated by *Trichoderma* (21%), followed by *Fusarium* (15.44%), *Aspergillus* (9.32%), and *Penicillium* (3.13%). In the AMF-treated group, Trichoderma was almost the same as the control at 20.03%, while *Aspergillus* and *Penicillium* showed considerable increases to 13.30% and 7.10%, respectively. *Fusarium* demonstrated high changes to become 6.05% (Fig. 9).

Discussion

The results of this study showed the potential positive effects of AMF inoculation on soil microbial community structure, plant productivity, and nutritional bioavailability. These results aligned with much research highlighting the role of AMF as one of the promising strategies for improving soil health, plant growth, and productivity (Zhou et al. 2025); they indicated that AMF can establish a mutually symbiotic relationship with most plants and in currently most commonly used microbial-based bio-stimulants.

On the morphological level, the impact of AMF on sugar beet plants has been observed on a vegetative level after 120 days, the increase in plant height and leaf fresh weight in the AMF-treated plants compared to the control group. These findings align with various studies showing the impact of the colonization of AMF on improving plant vegetative growth (Ortas 2003). This is evident by the observed increases in chlorophyll content and SPAD values, coupled with a reduction in the flavonol reflectance index (FRI). Flavonoids, a type of carbon-based secondary metabolite and polyphenol, serve as indicators of nitrogen levels in crops. The FRI, which measures flavonol concentration, inversely correlates with chlorophyll content and increases as nitrogen availability declines. These results are in agreement with previous studies demonstrating the beneficial role of AMF in enhancing plant growth and physiological performance (Agati et al. 2016). AMF colonization has been widely reported to improve key plant physiological processes, including photosynthetic efficiency and tolerance to biotic and abiotic stresses (Ye et al. 2022). In the present study, the observed enhancement in vegetative growth and

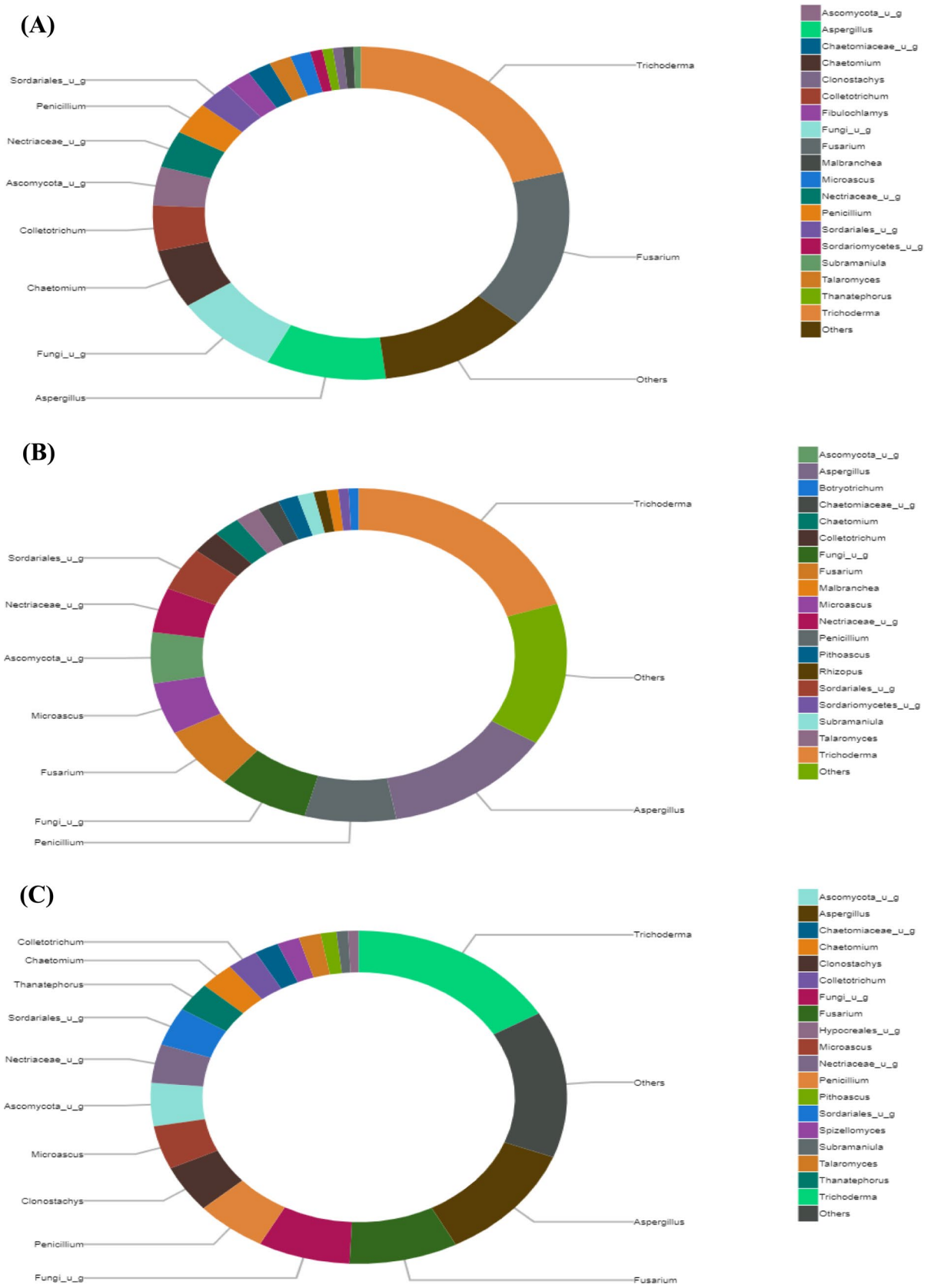


Fig. 9 Comparison of ITS markers for fungal community composition in **A** control, **B** AMF-treated, and **C** no-plant control soil samples by using online platform cosmosID

root soluble sugar content aligns with results reported by Aly and Hussein 2009. Their work assessed the efficiency of mycorrhizal fungi in combination with *Triticum spp.* as biocontrol against *Rhizoctonia* and *Fusarium* in sugar beet. The authors documented a significant increase in soluble sugar content in mycorrhizal treated plants, accompanied by nitrogen, phosphorus, and potassium content in treated plant tissues (Aly and Hussein 2009).

Furthermore, AMF inoculation led to a substantial elevation in nitrogen, phosphorus, plant weight, and sugar content, highlighting the positive impact on plant nutrient uptake. These results are confirmed by Ingraffia et al. 2019, who demonstrated that inoculation of wheat with AMF treatment significantly enhanced root growth traits, including plant biomass, specific root length, and root density. Their study also revealed improved nutrient uptake, particularly of phosphorus (P), iron (Fe), and zinc (Zn), in addition to an approximately 20% increase in nitrogen (N) content (Ingraffia et al. 2019). In agreement with this observation, the present results in sugar beet indicate that AMF colonization stimulates root development and nutrient accumulation, highlighting the efficiency of AMF symbiosis across different crop species. Notably, the increase in leaf phosphorus content in this study, upon using rock phosphate as a source of phosphorus and soil inoculated with AMF, aligned with previous research showing that AMF and rock phosphate applications in robusta coffee enhanced phosphorus uptake by up to 40% and improved plant immunity (Jaitieng et al. 2021).

López-Ráez et al. 2010, reported that AMF can activate plant defense mechanisms in direct-seeded rice against the root-knot nematodes Meloidogyne graminicola. Inoculation with mycorrhiza stimulated multiple defense pathways. This activation resulted in enhanced antioxidant enzyme activities, increasing phenolic compounds and greater lignin deposition. Moreover, these defense responses were observed not only at nematode infection sites but also in distant plant tissues, indicating systemic resistance (López-Ráez et al. 2010).

AMF inoculation has positively influenced the expression of three of the studied genes (*BvJac, BvProt,* and *BvPR10)* associated with various plant functions, including nutrient uptake and stress responses. The qPCR data demonstrated a 1.5–2.4-fold change of the examined genes, reflecting a notable impact of the experimental treatment on gene expression. During symbiosis process the expression of *BvProt* is important in maintaining cell function through the selective degradation and removal of non-targeted proteins by interacting with bi-ubiquitin (Song et al. 2015). *BvJac* and *BvPR10*, displayed moderate upregulation, with fold changes of 1.85 and 2.2, respectively. The *BvPR10* had a moderate 2.2-fold upregulation in AMF-treated sugar beet plants. This aligns with previous findings that PR-10 genes are frequently induced under biotic stress. PR-10 genes are widely responsive to biotic stress; according to a published meta-analysis its expression is typically stronger under pathogenic attack. The upregulation of the PR-10 has been observed in resistant varieties and during the early stages of infection (Song et al. 2015).

BvJI gene corresponding to jasmonate-induced (JA) protein showed a fold change of 1.5 with no significant difference. A study observed a significant upregulation of two JA-induced proteins containing jacalin-like domains in plants colonized by AM fungi. Moreover, numerous jacalin-like lectin domains were strongly associated with AMF–plant interactions, especially under biotic and abiotic stress conditions (Fiorilli et al. 2015). The AMF successfully activated defense-related genes in the host plant, even in the absence of pathogen attack. Including genes involved in signal transduction and calcium-mediated signaling pathways. These findings support the role of AMF symbiosis in enhancing resistance to pathogens through the activation of defense regulatory genes, also reflected in this study (Zhu et al. 2022). Furthermore, soil microbial communities can positively impact AMF by synthesizing phytohormones, improving nutrient availability, promoting fungal propagule germination and growth, and regulating plant growth.

The analysis of the microbial community composition indicated a predominance of the following phyla: Proteobacteria, Acidobacteriota, and Bacteroidia in the treatment group. There was a shift in the microbial community, with Patescibacteria being more abundant in the AMF-treated samples compared to the control samples. The prevalence of Patescibacteria in AMF-treated soil exceeded that of control soil, matching the findings of Hao et al. (Hao et al. 2021). ABY1, a class of the Patescibacteria phylum, is known for its metabolic versatility and ability to degrade complex organic matter (Zhao et al. 2022). The detected increase in ABY1 abundance within the treated group may indicate its involvement in the degradation of introduced compounds or in adapting to the altered environmental conditions. The phylum Patescibacteria, or OD1, was initially recognized through phylogenetic analysis of 16S rRNA genes obtained from environmental samples. These bacteria possess both respiratory and fermentative capabilities, as well as the ability to metabolize nitrogen and fatty acids. Likewise, Li et al. (2023) carried out a soil study on long-term continuous cropping of tobacco and reported that the abundance of Patescibacteria declined over successive years. Patescibacteria may be functionally important in element cycling. Herrmann et al. (2019) indicated that Patescibacteria have been identified as potentially the largest autotrophic taxa involved in the nitrogen, sulfur, and iron cycles (Herrmann et al. 2019), so changes in their abundance could affect the broader microbial community and nutrient availability. Nitrogen bioavailability is affected by microbial biomass in

the soil, with substantial correlations observed between the abundance of Patescibacteria and soil nitrogen mineralization (Bashan and De-Bashan 2005). A study on maize demonstrated that AMF significantly modified the concentrations of 35 compounds in the root exudates. Also, it confirmed that AMF enhanced enzymatic activity and increased bacterial diversity in the maize rhizosphere, indicating a beneficial shift in the microbial community and overall improvement in the soil microenvironment (Ma et al. 2022).

ITS analysis showed that Ascomycota was consistently detected across all samples, confirming its dominance and functional diversity in soil fungal communities. However, many sequences remained unclassified, and this may reflect the limited coverage of current fungal databases for soil fungi (Tedersoo et al. 2018). These results are consistence with previous studies identifying Ascomycota as a major fungal group associated with AMF colonization (Chen et al. 2025). AMF treatment may have played a role in the increase of *Sordariales* when compared to the no-plant control. *Sordariales* have both soil-related functions and plant-root related functions. In terms of soil-related function, *Sordariales* are known as decomposers of organic matter and nutrient recycling, improving soil health. They are responsible for nitrogen-fixing in the soil (Du et al. 2024). In addition, *Sordariales* interact with plant roots by facilitating nutrients uptake, while utilizing plant derived carbohydrates and they may also contribute to plant growth promoting and pathogens suppression (Wulandari et al. 2025).

The AMF treatment influenced the fungal communities by promoting beneficial fungi such as *Aspergillus* and *Penicillium* while reducing pathogenic fungi like *Fusarium*. In this experiment's results, *Fusarium* decreased from 15.44% in the control soil to 6.05% in the AMF-treated soil. These data confirm that AMF inoculation substantially modifies the fungal community structure, potentially diminishing the pathogenic fungi such as *Fusarium*. This rise in advantageous fungi is supported by other studies. Oulad and his team affirm that mycorrhizal fungi have a reduction effect on pathogenic fungi, such as *Fusarium* (Oulad Ziane et al. 2023). It has also been confirmed the impact of AMF on promoting beneficial fungi such as *Aspergillus*, *Sordariales*, and *Penicillium*, which may collectively exert a synergistic positive effect on plant health and soil functionality (Jia et al. 2020; Daigham et al. 2023). A study conducted on wheat plants to investigate the effect of AMF against *Fusarium* showed that the AMF reduces the *Fusarium* population density by 75.7% relative to the control group lacking AMF inoculation (Spagnoletti et al. 2021).

The relative abundance of *Aspergillus* and *Penicillium* increased in the AMF-treated rhizosphere. Members of these genera are commonly detected in agricultural soils and encompass both saprophytic and pathogenic species (Heo et al. 2019). While *Aspergillus* and *Penicillium* include known plant pathogens, their impact depends on the specific species and the surrounding microbial community. A limitation of this study is the lack of species-level identification of the detected *Aspergillus* and *Penicillium*. However, the absence of visible disease symptoms or adverse physiological effects in AMF-treated plants, along with the upregulation of defense-related genes observed via qPCR, suggests that the enriched *Aspergillus* and *Penicillium* in AMF-treated soils are likely non-pathogenic species. These fungi may have contributed positively to the improved morphological and physiological traits observed in the AMF-treated sugar beet. In diverse and balanced soils, many strains of *Aspergillus* and *Penicillium* can act beneficially by solubilizing phosphorus, producing growth-promoting hormones, promoting plant growth through improved root development, and associating with beneficial rhizosphere interactions (Nafaa et al. 2023). Therefore, their presence in AMF-treated soils may reflect beneficial functional, cooperative microbial networks rather than pathogenic behavior (Hussain et al. 2024). LEfSe analysis showed microbial shifts as AMF-treated soils were enriched with Patescibacteria, Planctomycetota, and Cyanobacteria, which are involved in nutrient cycling. This aligns with the phytobiome concept, as plants actively engage in social interactions with beneficial microbes, empowering plants to enhance their resilience against biotic and abiotic challenges (Park and Ryu 2021).

This study emphasized the positive effects of AMF on sugar beet growth, defense response, and rhizosphere microbial dynamics, but has limitations. The experiment was conducted in greenhouse conditions, on a single cultivar, which may limit field extrapolation. Additionally, AMF's obligate symbiotic nature restricts mass production requiring advanced techniques, such as root organ culture systems, or genetic engineering for in vitro propagation (Ghorui et al. 2023). Moreover, fungal ecological studies, using DNA sequencing, also face challenges due to the lack of comprehensive reference databases, leading to uncertain identification of many soil fungi.

Conclusion

This study demonstrates that AMF positively influences sugar beet growth, nutrient uptake, defense-related gene expression, and rhizosphere microbial dynamics. AMF-treated plants exhibited enhanced physiological traits, higher nutrient content, upregulated defense genes, along with alterations in some soil bacterial and fungal populations, including a reduction in potential pathogens. These findings highlight the potential of AMF to improve sugar beet performance and soil health. Further field-based trials are recommended to validate these effects under diverse agronomic conditions and to elucidate the precise mechanisms underlying these plant–microbe–soil interactions.

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Data availability The 16 s and ITS sequences generated and analyzed during the current study are available in the NCBI SRA.

The 16S rRNA was submitted under accession number PRJNA1178642. The ITS were submitted under accession number PRJNA1198073.

Declarations

Conflict of interest The authors declare no conflict of interest.

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References

Abd El-Monium, M. M., and O. N. M. Massoud. 2009. Effect of compost, (AM) mycorrhizae and N2-fixing bacteria on growth and yield of wheat. *Journal of Soil Sciences and Agricultural Engineering* 34 (2): 1495–1512.

Agati, G., L. Tuccio, B. Kusznierewicz, T. Chmiel, A. Bartoszek, A. Kowalski, M. Grzegorzewska, R. Kosson, and S. Kaniszewski. 2016. Nondestructive optical sensing of flavonols and chlorophyll in white head cabbage (*Brassica oleracea* L. var. capitata subvar. alba) grown under different nitrogen regimens. *Journal of Agricultural and Food Chemistry* 64 (1): 85–94.

Aly, M. H., and M. Y. Hussein. 2009. Vesicular-arbuscular mycorrhiza and *Trichoderma virdi* as deterrents against soil-borne root rot disease of sugar beet. *Sugar Tech* 11:387–391.

Bashan, Y., and L. E. De-Bashan. 2005. Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: A critical examination. *Soil Biology & Biochemistry* 37 (10): 1795–1804.

Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. *Working with mycorrhizas in forestry and agriculture*. Canberra: Australian Centre for International Agricultural Research.

Chen, Y., C. Sun, Y. Yan, D. Jiang, S. Huangfu, and L. Tian. 2025. Impact of arbuscular mycorrhizal fungi on maize rhizosphere microbiome stability under moderate drought conditions. *Microbiological Research* 290 : 127957.

Chukwuneme, C. F., A. S. Ayangbenro, and O. O. Babalola. 2021. Metagenomic analyses of plant growth-promoting and carbon-cycling genes in maize rhizosphere soils with distinct land-use and management histories. *Genes* 12 (9) : 1431.

Cui, Z., R. Chen, T. Li, B. Zou, G. Geng, Y. Xu, P. Stevanato, L. Yu, V. N. Nurminsky, J. Liu, and Y. Wang. 2024. Arbuscular mycorrhizal fungi enhance tolerance to drought stress by altering the physiological and biochemical characteristics of sugar beet. *Sugar Tech* 26:1377–1392.

Daigham, G. E., A. Y. Mahfouz, A. M. Abdelaziz, M. M. Nofel, and M. S. Attia. 2023. Protective role of plant growth-promoting fungi *Aspergillus chevalieri* OP593083 and *Aspergillus egyptiacus* OP593080 as biocontrol approach against *Alternaria* leaf spot disease of *Vicia faba* plant. *Biomass Conversion and Biorefinery* 14:23073–23089.

Desoignies, N., J. Carbonell, J. S. Moreau, A. Conesa, J. Dopazo, and A. Legrève. 2014. Molecular interactions between sugar beet and *Polymyxa betae* during its life cycle. *Annals of Applied Biology* 164 (2): 244–256.

Diwan, D., M. M. Rashid, and A. Vaishnav. 2022. Current understanding of plant-microbe interaction through the lenses of multi-omics approaches and their benefits in sustainable agriculture. *Microbiological Research* 265 : 127180.

Du, J., Y. Yu, C. Tang, K. Zong, S. Zhang, Q. Zhang, L. Fang, and Y. Li. 2024. Organic fertilizers increase the proportion of saprotrophs favoring soil nitrification under medicinal plants *Fritillaria thunbergii*. *Industrial Crops and Products* 219 : 119129.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28 (3): 350–356.

El-Zayat, H. 2022. Agriculture study on sugar beet in Egypt. *Acta Scientific Agriculture* 6 (1): 17–26.

Fiorilli, V., M. Vallino, C. Biselli, A. Faccio, P. Bagnaresi, and P. Bonfante. 2015. Host and non-host roots in rice: Cellular and molecular approaches reveal differential responses to arbuscular mycorrhizal fungi. *Frontiers in Plant Science* 6 : 636.

Gai, J., J. Wang, S. Xie, L. Xiang, and Z. Wang. 2024. Spectroscopic determination of chlorophyll content in sugarcane leaves for drought stress detection. *Precision Agriculture* 25 (2): 543–569.

Garcia-Servin, M., L. M. Contreras-Medina, I. Torres-Pacheco, and R. G. Guevara-González. 2021. Estimation of nitrogen status in plants. In *Nitrate Handbook: Environmental, Agricultural, and Health Effects*, ed. Christos Tsadilas, 163–181. Boca Raton: CRC Press.

Ghorui, M., S. Chowdhury, K. Das, K. Sunar, and B. Prakash. 2023. Optimizing factors for large-scale production of arbuscular mycorrhizal fungi consortia using root organ cultures. *Journal of Biological Methods* 10 : jbm-10-e99010006.

Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84 (3): 489–500.

Hao, L., Z. Zhang, B. Hao, F. Diao, J. Zhang, Z. Bao, and W. Guo. 2021. Arbuscular mycorrhizal fungi alter microbiome structure of rhizosphere soil to enhance maize tolerance to La. *Ecotoxicology and Environmental Safety* 212 : 111996.

Heberle, H., V. G. Meirelles, F. R. da Silva, G. P. Telles, and R. Minghim. 2015. InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics* 16 (1) : 169.

Heo, I., K. Hong, H. Yang, H. B. Lee, Y. J. Choi, and S. B. Hong. 2019. Diversity of *Aspergillus*, *Penicillium*, and *Talaromyces* species isolated from freshwater environments in Korea. *Microbiology* 47 (1): 12–19.

Herrmann, M., C. E. Wegner, M. Taubert, P. Geesink, K. Lehmann, L. Yan, R. Lehmann, K. U. Totsche, and K. Küsel. 2019. Predominance of *Cand.* Patescibacteria in groundwater is caused by their preferential mobilization from soils and flourishing under oligotrophic conditions. *Frontiers in Microbiology* 10 : 1407.

Hussain, I., M. Irshad, A. Hussain, M. Qadir, A. Mehmood, M. Rahman, A. F. Alrefaei, M. H. Almutairi, S. Ali, and M. Hamayun. 2024. Phosphate solubilizing *Aspergillus Niger* PH1 ameliorates

growth and alleviates lead stress in maize through improved photosynthetic and antioxidant response. *BMC Plant Biology* 24 : 642.

Ingraffia, R., G. Amato, A. S. Frenda, and D. Giambalvo. 2019. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. *PLoS ONE* 14 (3) : e0213672.

Jaitieng, S., K. Sinma, P. Rungcharoenthong, and S. Amkha. 2021. Arbuscular mycorrhiza fungi applications and rock phosphate fertilizers enhance available phosphorus in soil and promote plant immunity in robusta coffee. *Soil Science and Plant Nutrition* 67 (1): 97–101.

Jamil, F., H. Mukhtar, M. Fouillaud, and L. Dufossé. 2022. Rhizosphere signaling: Insights into plant–rhizomicrobiome interactions for sustainable agronomy. *Microorganisms* 10 (5) : 899.

Jia, T., T. Y. Guo, R. H. Wang, and B. F. Chai. 2020. Effects of heavy metal contents on phyllosphere and rhizosphere fungal communities for *Bothriochloa ischaemum* in copper tailings area. *Huan Jing Ke Xue* 41 (11): 5193–5200.

Li, G., P. Gong, J. Zhou, L. Wang, X. Song, P. Ding, Y. Jin, Y. Zhang, X. Zhou, J. Yang, Z. Wang, L. Yun, and J. Ren. 2023. The succession of rhizosphere microbial community in the continuous cropping soil of tobacco. *Frontiers in Environmental Science* 11 : 1251938.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* 25 (4): 402–408.

López-Ráez, J. A., A. Verhage, I. Fernández, J. M. García, C. Azcón-Aguilar, V. Flors, and M. J. Pozo. 2010. Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. *Journal of Experimental Botany* 61 (10): 2589–2601.

Lu, Y., G. Zhou, J. Ewald, Z. Pang, T. Shiri, and J. Xia. 2023. MicrobiomeAnalyst 2.0: Comprehensive statistical, functional and integrative analysis of microbiome data. *Nucleic Acids Research* 51 (W1): W310–W318.

Ma, J., Y. Xie, Y. Yang, C. Jing, X. You, J. Yang, C. Sun, S. Qin, J. Chen, K. Cao, J. Huang, and Y. Li. 2022. AMF colonization affects allelopathic effects of *Zea mays* L. root exudates and community structure of rhizosphere bacteria. *Frontiers in Plant Science* 13 : 1050104.

Mbareche, H., M. Veillette, G. Bilodeau, and C. Duchaine. 2020. Comparison of the performance of ITS1 and ITS2 as barcodes in amplicon-based sequencing of bioaerosols. *PeerJ* 2020 (2) : e8523.

Nafaa, M., S. M. Rizk, T. A. G. A. Aly, M. A. S. Rashed, D. Abd El-Moneim, A. Ben Bacha, M. Alonazi, and M. Magdy. 2023. Screening and identification of the rhizosphere fungal communities associated with land reclamation in Egypt. *Agriculture* 13 (1): 215.

Ortas, I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in South Anatolia. *Journal of Plant Nutrition* 26 (1): 1–17.

Oulad Ziane, S., Z. El Alaoui Talibi, S. Ibnsouda koraichi, A. Douira, S. Amir, A. Meddich, and C. El Modafar. 2023. Synergistic effects of arbuscular mycorrhizal fungi associated to plant growth-promoting rhizobacteria in suppression of soil-borne Fusarium wilt of date palm. *Biocatalysis and Agricultural Biotechnology* 51 : 102753.

Park, Y. S., and C. M. Ryu. 2021. Understanding plant social networking system: Avoiding deleterious microbiota but calling beneficials. *International Journal of Molecular Sciences* 22 (7) : 3319.

Ratti, N., S. Kumar, H. N. Verma, and S. P. Gautam. 2001. Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martinii* var. motia by rhizobacteria, AMF and Azospirillum inoculation. *Microbiological Research* 156 (2): 145–149.

Samantaray, A., S. Chattaraj, D. Mitra, A. Ganguly, R. Kumar, A. Gaur, P. K. D. Mohapatra, S. de los Santos-Villalobos, A. Rani, and H. Thatoi. 2024. Advances in microbial based bio-inoculum for amelioration of soil health and sustainable crop production. *Current Research in Microbial Sciences* 7 : 100251.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75 (23): 7537–7541.

Song, F., D. Qi, X. Liu, X. Kong, Y. Gao, Z. Zhou, and Q. Wu. 2015. Proteomic analysis of symbiotic proteins of *Glomus mosseae* and *Amorpha fruticosa*. *Scientific Reports* 5 : 18031.

Spagnoletti, F. N., M. Carmona, K. Balestrasse, V. Chiocchio, R. Giacometti, and R. S. Lavado. 2021. The arbuscular mycorrhizal fungus *Rhizophagus intraradices* reduces the root rot caused by *Fusarium pseudograminearum* in wheat. *Rhizosphere* 19 : 100369.

Tedersoo, L., S. Sánchez-Ramírez, U. Kõljalg, M. Bahram, M. Döring, D. Schigel, T. May, M. Ryberg, and K. Abarenkov. 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity* 90:135–159.

Wulandari, N. F., A. R. Syam, L. Alhamd, B. Gunadi, Y. Yosmaniar, E. Widjaja, B. N. Utomo, S. Wahyuwardani, R. Widiastuti, T. Kadarini, and S. Dody. 2025. A bibliometric study on Sordariales fungi covering genetic diversity, ecological interactions and biotechnological applications. *Global Journal of Environmental Science and Management* 11 (3): 1319–1342.

Yadav, H., R. Fatima, A. Sharma, and S. Mathur. 2017. Enhancement of applicability of rock phosphate in alkaline soils by organic compost. *Applied Soil Ecology* 113:80–85.

Ye, F., H. Shen, Z. Li, F. Meng, L. Li, J. Yang, Y. Chen, X. Bo, X. Zhang, and M. Ni. 2016. Influence of the biliary system on biliary bacteria revealed by bacterial communities of the human biliary and upper digestive tracts. *PLoS ONE* 11 (3) : e0150519.

Ye, Q., H. Wang, and H. Li. 2022. Arbuscular mycorrhizal fungi improve growth, photosynthetic activity, and chlorophyll fluorescence of *Vitis vinifera* L. cv. Ecolly under drought stress. *Agronomy* 12 (7) : 1563.

Youssef, Abo Bakr, R. Ismail, S. EL-Assal, and N. Abdallah. 2018. Expression of alpha amylase and osmotin-like protein under drought stress in sugar beet. *Journal of Agricultural Chemistry and Biotechnology* 9 (2): 51–55.

Youssef, Abo Bakr., A. Badr, and W. Fouad. 2025. Agronomic impact and cost analysis of natural rocks and biological inoculants in potato production. *Sustainability* 17 (19) : 8648.

Zhao, R., I. F. Farag, S. L. Jørgensen, and J. F. Biddle. 2022. Occurrence, diversity, and genomes of "*Candidatus* Patescibacteria" along the early diagenesis of marine sediments. *Applied and Environmental Microbiology* 88 (24) : e0140922.

Zhou, R., A. Raza, J. Song, S. Janiad, Q. Li, M. Huang, and M. A. Hassan. 2025. Growth-promoting effects of arbuscular mycorrhizal fungus *Funneliformis mosseae* in rice, sesame, sorghum, Egyptian pea and Mexican hat plant. *Frontiers in Microbiology* 16 : 1549006.

Zhu, L., J. Huang, X. Lu, and C. Zhou. 2022. Development of plant systemic resistance by beneficial rhizobacteria: Recognition, initiation, elicitation and regulation. *Frontiers in Plant Science* 13 : 952397.

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