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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Taxonomical and functional bacterial community profiling in disease-resistant and disease-susceptible soybean cultivars

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Abstract

Highly varied bacterial communities inhabiting the soybean rhizosphere perform important roles in its growth and production; nevertheless, little is known about the changes that occur in these communities under disease-stress conditions. The present study investigated the bacterial diversity and their metabolic profile in the rhizosphere of disease-resistant (JS-20–34) and disease-susceptible (JS-335) soybean (*Glycine max* (L.) Merr.) cultivars using 16S rRNA amplicon sequencing and community-level physiological profiling (CLPP). In disease-resistant soybean (AKADR) samples, the most dominating phyla were Actinobacteria (40%) followed by Chloroflexi (24%), Proteobacteria (20%), and Firmicutes (12%), while in the disease-susceptible (AKADS) sample, the most dominating phyla were Proteobacteria (35%) followed by Actinobacteria (27%) and Bacteroidetes (17%). Functional profiling of bacterial communities was done using the METAGENassist, and PICRUSt2 software, which shows that AKADR samples have more ammonifying, chitin degrading, nitrogen-fixing, and nitrite reducing bacteria compared to AKADS rhizosphere samples. The bacterial communities present in disease-resistant samples were significantly enriched with genes involved in nitrogen fixation, carbon fixation, ammonification, denitrification, and antibiotic production. Furthermore, the CLPP results show that carbohydrates and carboxylic acids were the most frequently utilized nutrients by the microbes. The principal component analysis (PCA) revealed that the AKADR soils had higher functional activity (strong association with the Shannon–Wiener index, richness index, and hydrocarbon consumption) than AKADS rhizospheric soils. Overall, our findings suggested that the rhizosphere of resistant varieties of soybean comprises of beneficial bacterial population over susceptible varieties.

Keywords Soybean · Disease-resistant · Disease-susceptible · DNA sequencing · Biofertilizers

Introduction

Plants live in an intimate relationship with microbes residing in the rhizosphere area. This so-called rhizosphere microbiome is a microbial hotspot and plays an essential role in maintaining plant health and productivity via the acquisition of nutrients, stress tolerance, and providing resistance against plant pathogens [1, 2]. Rhizosphere microbial communities form the part of the complex food web that utilizes the nutrients (exudates, border cells, mucilage) released by the plant. These nutrients function as the major driving forces in the recruitment and regulation of bacterial diversity and function in the rhizosphere [3]. Reports suggest that plants supply carbohydrates-derived rhizodeposition to their microbial counterparts [4, 5]. It has been well documented that around 17% of photo-assimilates are released into the rhizosphere via rhizodeposition, resulting in the selection of helpful or pathogenic soil bacteria from bulk soil [6, 7]. Rhizobacteria play a crucial role in maintaining plant performance and enhancing plant growth in different types of stress conditions. Despite this, changes in the bacterial community in the rhizosphere under stressed and non-stressed conditions are poorly studied. Advanced next-generation and high throughput sequencing (HTS) allow us for an in-depth categorization of the functions associated with these 'soil probiotics' [8–10].

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Soybean has an important place in the world's oilseed production due to its high productivity, profitability, and vital contribution towards maintaining soil fertility [11]. Soybean also has a prominent place as the world's most important seed legume, which contributes 25% to the global vegetable oil production, about two-thirds of the world's protein concentrate for livestock feeding, and is a valuable ingredient in formulated feeds for poultry and fish [12]. The major soybean-producing nations are the USA, Brazil, and Argentina [13]. The three countries dominate global production, accounting for 80% of the world's soybean supply [12, 13]. It flourished in various agro-climatic situations and became a major commercial crop in several countries [11]. Soybean production is constantly challenged by the repetitive occurrence of biotic and abiotic stresses [14, 15]. Various diseases, pests, and insects create biotic constraints for soybean plants, whereas the abiotic constraints include drought, salt, alkalinity, cold, and heat are detrimental to soybean production [16]. Environmental conditions, cultivar selection, previous crop, disease history, and different crop management practices are significant factors that impact the incidence of soybean diseases [17]. Soybean is susceptible to different diseases, including root, stem, and leaf blight caused by *Phytophthora*, *Cercospora* leaf blight, brown spot, frog eye leaf spot, and downy mildew root rot caused by *Macrophomina phaseolina* [18]. Unexpected heavy rainfalls and deviation in temperature create a favorable atmosphere for attack by dormant *Rhizoctonia* (aerial blight) and anthracnose (pod blight), one of the most devastating diseases that cause significant yield loss in soybean reported in Madhya Pradesh, India [19]. Consequently, addressing these concerns is significant for soybean productivity while safeguarding global food security. Previously several soybean varieties have been released, some of them are resistant towards *Rhizoctonia* Aerial Blight (RAB) disease (JS 20–69, JS 20–34, JS 20–53, JS 21–17, DS 3109, JS 20–79, EC 251,358, MACS 1620, VP 1164) [18] and some are susceptible (AMS-99–24, AMS-92–32, JS-335, NRC-64, TAMS-38, AMS-353) for root rot, collar rot, wilt, and RAB disease in soybean [19, 20]. Soybean variety JS 20–34 have shown multiple disease resistance against major diseases like charcoal rot, collar rot, *Rhizoctonia* aerial blight, *Alternaria* leaf spot, bacterial pustules, pod blight, and insect pest whereas, soybean variety JS 335 was found to be susceptible towards these diseases [19, 20].

Several studies have been conducted in recent years to unravel the role of plant species in shaping the rhizosphere Microbiome [6, 8, 21]. These studies include soybean [4, 22–24], Arabidopsis [25–27], rice [28–30], wheat [31–33], tomato [34], fox millet [35], ginger [36], strawberry [37], and sorghum [38]. The study conducted by Sugiyama et al. [39] reported changes in the bacterial community of soybean rhizospheres during different growth stages in the field condition. A similar study was conducted by Mendes et al., [40] wherein they reported the selection of the rhizospheric microbial community under agricultural management of soybean in Amazon forest soils. Still, it is not well stated to what degree plants can select a persistent rhizosphere microbial community from extremely distinct pools of microbial communities present in the bulk soil, especially under stress conditions. Microbial diversity was found to be directly correlated with disease-resistance [41]. Occasionally, host plants also apprentice specific beneficial microbiota after phytopathogen infections, which helps the plants to resist and withstand the diseases caused by these pathogens [1]. In animal science, dysbiosis of the defensive microbiome has been linked with disease prevalence however, in plants, the effect of rhizobacteria disruption in disease suppression is largely unidentified [21, 42–45].

Soil bacteria, in general, play an important role in healthy soil functioning, plant production, and soil health [46]. Culturable and unculturable bacterial and fungal species contribute to rhizosphere diversity, and both are important for agriculture. The following bacterial genera represent well-known rhizosphere dominants: *Acetobacter*, *Bacillus*, *Burkholderia*, *Arthrobacter*, *Serratia*, *Klebsiella*, *Alcaligenes*, *Acinetobacter*, *Azotobacter*, *Rhodococcus*, *Stenotrophomonas*, *Pseudomonas*, and *Enterobacter* [47, 48]. The bacteria belonging to *Bacillus* and *Pseudomonas* are known as plant growth-promoting bacteria (PGPB). Other bacterial representatives with a PGP effect present in the rhizosphere soil include *Pantoea*, *Flavobacterium*, *Mesorhizobium*, *Methylobacterium*, *Paenibacillus*, *Chromobacterium*, *Erwinia*, *Caulobacter*, *Bradyrhizobium*, *Micrococcus*, *Micromonospora*, and *Streptomyces* [2, 49–51].

Few studies reported the changes in bacterial community composition under disease stress conditions, for example, the study conducted by Lee et al. [34] reported that disruption of Actinobacteria and Firmicutes in the rhizosphere causes the incidence of bacterial wilt disease in tomato plants. The study conducted by Zhou et al. [9], and Kaushal et al. [1] compared the root-associated with *Fusarium* wilt-diseased and disease-free banana rhizosphere soil. They reported the abundance of Flavobacteriales was positively correlated with symptom development. The present study profile the changes in the rhizospheric bacterial community composition under diseased and healthy conditions. The comparative basic information of microbial diversity present in the disease-resistant and disease-susceptible soybean rhizosphere soil will help reveal the soybean-microbe interactions and potentially select suitable plant growth-promoting rhizobacteria and bio-control agents for increasing crop production and development of disease-resistant crop varieties.

We hypothesized that the diversity of rhizospheric bacteria and their metabolic activity depend on the soybean cultivars and soil biochemistry. The study's main aim was to understand the distribution and composition of the rhizobacterial community in disease-resistant and disease-susceptible soybean cultivars via 16S rRNA amplicon sequencing. We also aimed to analyze the functional and metabolic capabilities of these rhizobacterial communities colonizing disease-resistant (JS-30–34) and disease-susceptible (JS-335) soybean cultivars (Fig. 1). The novelty of this article is that it combines two approaches (NGS and CLPP) to provide a comprehensive picture of bacterial diversity in the rhizosphere soils of two soybean varieties.

Materials and methods

Sample collection and material processing

Rhizosphere soil samples of two soybean cultivars (disease-resistant JS-20–34) and (disease-susceptible JS-335) were randomly collected from 5 different locations from Krishi Vigyan Kendra, Bamhori Seed Farm Bhopal Road, Sagar (M.P.), India. This sampling method is consistent with those proposed by Gałązka et al. [52] and Praeg et al. [53]. All the collected samples were labelled correctly, sealed in plastic bags, and were immediately transported to the laboratory and stored at 4 °C until DNA extraction and community level physiological profiling (CLPP) analysis. Rhizosphere

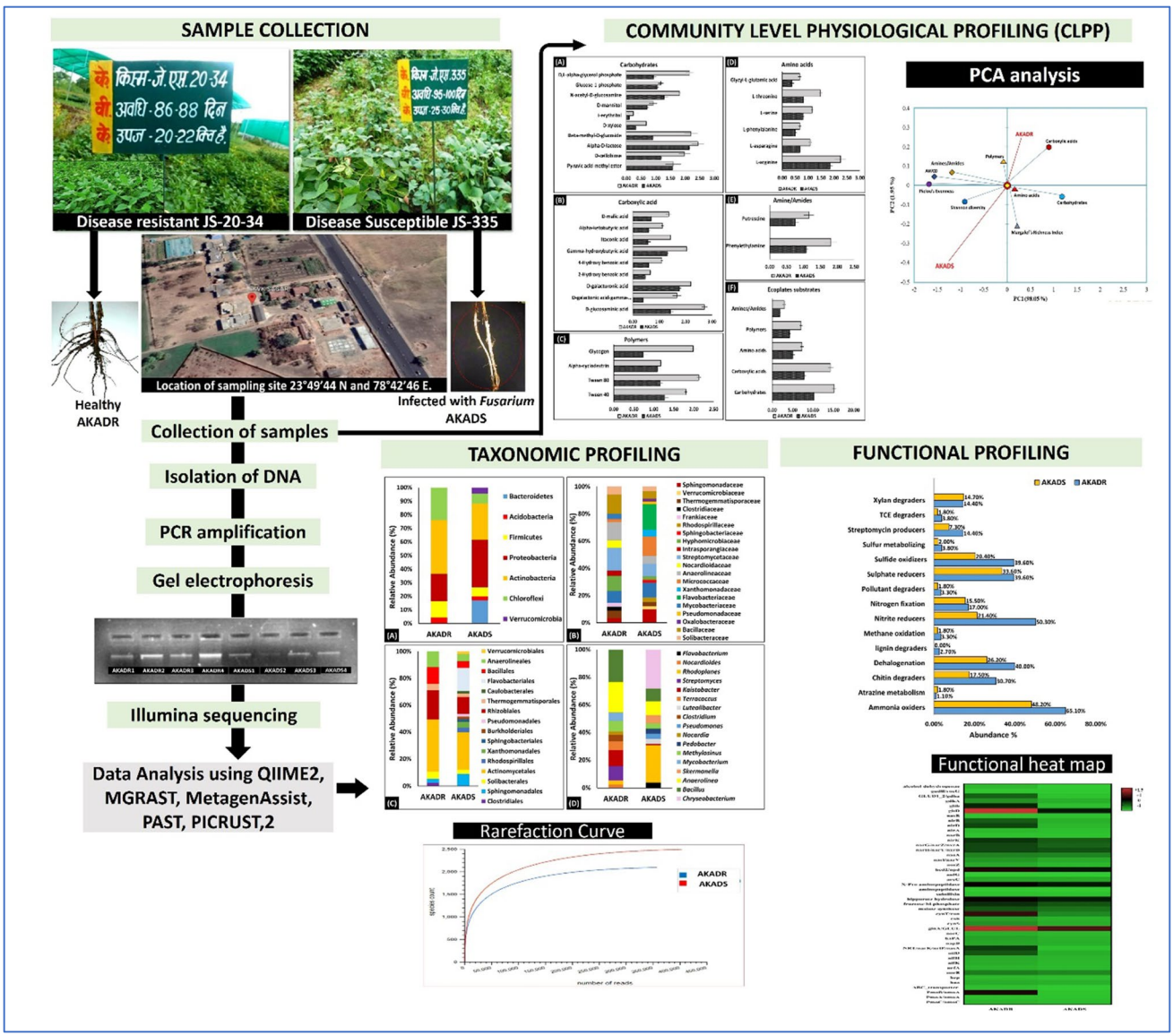

Fig. 1 Graphical representation of the present study

soil samples were collected by digging soybean plants, and the roots with attached soils were gently shaken to remove loose soil until only firmly attached soil remained. This attached soil was collected as the rhizosphere soil using sterilized brushes. Also, the rhizosphere soil samples were subjected to a more precise method for collecting rhizosphere soils through centrifugation [25, 27]. To get the most representative soil material for each site, single samples were mixed and homogenized into one sample. Soil acidity (pH) and electrical conductivity (EC) were evaluated in triplicate from a soil suspension (2:1) in distilled water using pH and EC meter, respectively [54]. All the other chemical properties of the soil like organic carbon (OC) were analyzed by following the standard protocol of Walkley and Black [55], available nitrogen (N) by Subbiah and Asija [56], phosphorus (P) by Dickman and Bray [57], and potassium (K) by Barnes et al. [58].

Soil sampling and community-level physiological profiling

Community-level physiological profiles (CLPP) for each soil sample were assessed using the Biolog EcoPlate™ (BIOLOG. Inc., CA, USA) assay. Each of the 96 biolog well plate contains 31 sole carbon sources and a blank. The different carbon sources are as: 10 different carbohydrates, 9 carboxylic or acetic acids, 6 amino acids, 4 polymers, and 2 amides/amines [59]. Estimation of CLPP for each soil sample was conducted as follows: 1 g of fresh soil was suspended in 10 ml sterile 0.85% saline solution (NaCl), and the mixture was shaken vigorously for about 60 min, at 4 °C and 150 rpm and then allowed to stand for 30 min to allow the soil particles to settle down [60]. Each of the samples was processed in triplicate. One hundred fifty microliters of this soil suspension was poured into each well, and then, EcoPlates were incubated for 5 days at 27 °C. The substrate utilization rate is indicated by the reduction of tetrazolium dyes that reduce from colorless to purple. A well containing no carbon source was inoculated as a blank on each plate. The development of the purple color in each well was measured as a change in optical density (OD). During the cultivation, the OD was recorded every 24 h continuously for 5 days by measuring the absorbance at 595 nm using an automated microplate plate reader (Synergy Microplate Readers (BioTek, US)).

According to Sala et al. [61], the substrate categories were divided into different six groups representing different substrate guilds, as follows: amino acids (L-arginine, L-asparagine, L-phenylalanine, L-serine, glycyl-L-glutamic acid, L-threonine), amines (phenylethylamine, putrescine), carbohydrates (glucose-1-phosphate, D-mannitol, D,L-alpha-glycerol phosphate, D-galactonic acid-gamma-lactone, D-xylose, D-cellobiose, *N*-acetyl-D-glucosamine, beta-methyl-D-glucoside, i-erythritol, alpha-D-lactose), carboxylic acids (alpha-ketobutiryc acid, D-galacturonic acid, pyruvic acid methyl ester, D-glucosaminic acid, D-malic acid, gamma-hydroxybutyric acid, itaconic acid.

Microbial activity in each microplate was expressed as an average well-color development (AWCD) according to Eq. 1:

$$AWCD = \sum \boldsymbol{ODi}/31 \quad (1)$$

ODi is the optical density value from each well after correcting by subtracting the OD value of the blank well [60].

After normalization, CLPP data based on 120-h reading was used for the analysis of substrate diversity (*H′*), richness (*RI*), and evenness (*J′*). *H*′ was calculated based on Shannon–Wiener index [H' = where Pi is OD reading of well (i)/ sum of all wells]. *RI* was based on Margalef's richness index [$RI = (S\text{-}1)/\ln(n)$, where *S* is the total number of substrates utilized and *n* is the total OD reading]. *J′* was based on Pielou's evenness index [$J' = H'/\ln S$] [62].

Principal component analysis (PCA) was used to investigate the relationship between the carbon sources, biodiversity indices, and rhizosphere soils derived from two different soybean varieties was carried out by using Canoco (v 5.12) [63].

Extraction of genomic DNA and PCR purification

To profile the diversity and the predictive metabolic potential, the total metagenomic DNA was isolated from 250 mg rhizosphere soil sample using the MoBio Power soil DNA isolation kit (MoBio Laboratories, Inc. CA, USA) as per the manufacturer's instructions with some modifications. The purity and integrity of the extracted genomic DNA were estimated through DNA gel electrophoresis (1%), and the concentration of DNA was quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific) by measuring the absorbance at 260/280 nm. The samples were stored at − 20 °C until needed for further analysis. Primer set of 341F (5′-CCTACGGGNGGCWGCAG-30) and 805R (5′-GACTACHVGGGTATCTAATCC-30) was used to amplify the V3-V4 dual region. 16S rRNA amplicon sequencing of total genomic DNA was done by using Illumina MiSeq system [64].

Taxonomic and functional profiling of disease-resistant and disease-susceptible rhizosphere microbiome using MG-RAST and METAGENassist sever

Illumina-generated sequence reads were analyzed through an open-source online server Metagenome Rapid Annotation using Subsystem Technology (MG-RAST) version v 4.0.3 (http://metagenomics.anl.gov/) [65]. Briefly, the raw and

unassembled reads generated were merged by using mate-merge pairs. The low-quality reads were trimmed by SolexaQA (http://solexaqa.sourceforge.net/) [66]. Moreover, the artificial and duplicated reads were removed by using k-mer based approach. Annotations were made against the RDP database [67], with default parameters, unless otherwise stated. Taxonomic datasets obtained from MG-RAST were further processed and analyzed by METAGENassist (http://www.metagenassist.ca/METAGENassist/faces/Home.jsp) [68], to provide an overview of functional profiles between the two cultivars. More in-depth details of various genes encoding important enzymes involved in metabolic pathways were obtained from PICRUSt2 v2.3.0 (https://github.com/picrust/picrust2/releases/tag/v2.3.0-b) [69], which predicts the functional potential on the basis of marker gene sequencing profiles. The PICRUSt 2 obtained, gene copy number were summarized with KEGG Orthology (KO) and KEGG reference database (https://www.genome.jp/kegg/).

Diversity analysis

The statistical analyses were performed using the SPSS software version 21 (SPSS Inc. /IBM Corp., Chicago, IL, USA). The collected data were subjected to analysis of variance (ANOVA) for comparison of means, and significant differences were calculated using Duncan's multiple range test (DMRT) at a $p < 0.05$ significance level. A range of alpha diversity parameters was estimated using statistical software Paleontological Statistics (PAST) ver. 2.17c [70] using the Bray–Curtis distance measure method, ACE index, which is a measure of microbial richness, was estimated along with Shannon–Wiener index and Chao-1 indices to determine the taxonomic distribution and diversification within the two rhizosphere microbiomes.

Results

The chemical properties of AKADR and AKADS rhizosphere soil samples are presented in Table 1. Chemical analysis of the AKADR and AKADS rhizosphere soil samples showed a change in pH, which determines the nutrient availability to plants. Soil pH was close to neutral in the AKADR (7.5 ± 0.5), whereas acidic in AKADS (5.8 ± 0.7) rhizospheric samples. Results showed that the EC values of AKADS (0.53 ± 0.05) were higher than AKADR (0.23 ± 0.075). Similarly, the two sampled soils observed a significant difference in all other fertility parameters (organic carbon percentage, nitrogen, phosphorous, potassium, sulfur, boron, zinc, copper, iron, and manganese).

Table 1 Physiochemical characterization of rhizosphere soils of disease-resistant (AKADR) and disease-susceptible (AKADS) soybean cultivars

Measured soil parameters	AKADR	AKADS
pH (KCl)	7.5 ± 0.5^{b}	5.8 ± 0.7^{a}
Electrical conductivity (dS/m)	0.23 ± 0.075^{a}	0.53 ± 0.05^{b}
Organic carbon (kg/ha)	0.84 ± 0.03^{b}	0.33 ± 0.045^{a}
Nitrogen (kg/ha)	270 ± 1.4^{b}	200 ± 1.3^{a}
Phosphorus (kg/ha)	38.32 ± 0.05^{b}	21.3 ± 0.04^{a}
Potassium (kg/ha)	327 ± 1.56^{b}	265 ± 1.2^{a}
Sulfur (ppm)	28.8 ± 0.04^{b}	27.9 ± 0.01^{a}
Boron (ppm)	1.61 ± 0.056^{b}	1.53 ± 0.05^{a}
Zinc (ppm)	2.15 ± 0.045^{b}	2.01 ± 0.034^{a}
Copper (ppm)	2.19 ± 0.08^{b}	2.12 ± 0.09^{a}
Iron (ppm)	4.24 ± 0.03^{a}	4.88 ± 0.071^{b}
Manganese (ppm)	7.41 ± 0.3^{b}	6.85 ± 0.1^{a}

Values are the mean of three replicates ± SD ($n = 3$). The same letter within each rows indicates no significant difference between the treatments ($p < 0.05$) as determined by Duncan's multiple range test

Community-level physiological profiling analysis

Soil functional diversity indices were determined using 31 carbon sources in the Biolog EcoPlate technique after 120 h of incubation. We focused on presenting data from this time point since the maximum consumption of different carbon compounds in all examined soils was reported after 120 h of incubation. The functional diversity of the microbial population as measured as substrate utilization in the Biolog Ecoplate™.

Carbohydrates and carboxylic acids were the most readily consumed compounds among the 31 carbon sources in all rhizosphere soils, but amino acids, polymers, amines, and amides were the least consumed. The greatest utilization rates were found in the AKADR rhizospheric soil for L-arginine, beta-methyl-D-glucoside, alpha-D-lactose, gamma-hydroxybutyric acid, and phenylethylamine compared to AKADS samples (Fig. 2). The Biolog assay was used to identify microbial isolates based on their substrate utilization profiles, with the degree of oxidation being proportional to the metabolic capability of the corresponding microbial communities characterized by AWCD [71–73]. Color intensity was determined by calculating the average well color development (AWCD) on each plate. The AWCD of the carbon sources for the AKADR and AKADS rhizosphere soil samples using the Biolog EcoPlates™ usually followed a sigmoidal curve with the incubation time (120 h). Overall, most of the substrates were highly metabolized by the AKADR sample (Table 2). The AWCD of the microbial community from AKADR was significantly higher ($p < 0.05$) than that of AKADS, indicating that the tested metabolic

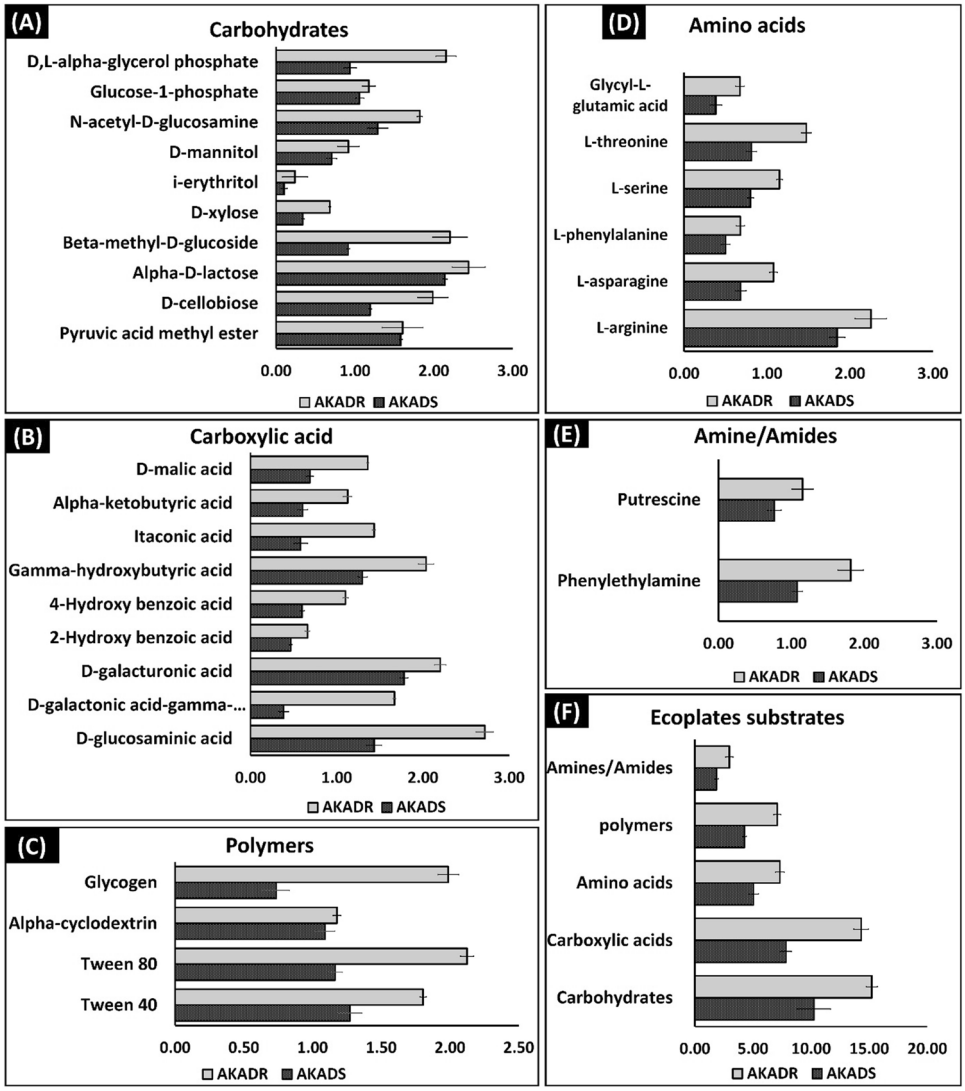

Fig. 2 Community-level physiological profiles (CLPP) of disease-resistant and disease-susceptible soybean rhizosphere soil samples. The error bars indicate the standard error of the mean of three replicates ($n = 3$). Substrates were classified under **A** carbohydrates, **B** carboxylic acids, **C** polymers, **D** amino acids, **E** amines/amides, and **F** overall utilization of the above five categories of substrates, where AKADR represents disease-resistant and AKADS represents disease-susceptible cultivars

capabilities of AKADR samples were higher than that of AKADS (Fig. 2). Based on the Shannon–Wiener diversity calculated from the community-level physiological profiling (CLPP) dataset, there was a significant difference in population diversity due to the soybean genotype. The highest and most significant utilization of amino acids was found in the AKADR compared to AKADS rhizosphere soil (Fig. 2). Finally, bacteria found in disease-resistant rhizospheric soils were shown to have the most remarkable rate of polymer usage.

CLPP result depicted that microbial communities colonizing the AKADR samples are metabolically more

Table 2 Bacterial community average well color development (AWCD), Shannon–Wiener index, evenness, and richness index as indicated from Community-level physiological profiling (CLPP) in the rhizosphere of disease-resistant (AKADR) and disease-susceptible (AKADS) soybean cultivars

Samples	AWCD OD 48 h^{-1}	Shanon diversity index (*H*)	Margalef's richness index (*RI*)	Pielou's evenness (*J*)
AKADR	1.568 ± 0.04^{b}	3.379 ± 0.8^{b}	8.82 ± 0.45^{b}	0.98 ± 0.034^{b}
AKADS	0.967 ± 0.033^{a}	2.780 ± 0.09^{a}	7.89 ± 0.30^{a}	0.809 ± 0.045^{a}

Values are the mean of three replicates ± SD ($n = 3$). The same letter within each column indicates no significant difference between the treatments ($p = 0.05$) as determined by Duncan's multiple range test, where *SD*, standard deviation; *AWCD*, average well color development; *OD*, optical density

active than the microbes present in the AKADS sample. The AWCD of the microbial communities from AKADR (1.568) was significantly higher ($p < 0.05$) than the AKADS (0.967) soil sample. The greatest Shannon–Wiener functional diversity index values were found in AKADR soils ($H' = 3.379$), in contrast to the AKADS rhizosphere, which had the lowest functional diversity index ($H' = 2.780$). Based on the Shannon–Wiener diversity, Margalef's Richness, and Pielou's evenness calculated from the CLPP dataset, there was a significant difference in population diversity, evenness, and richness in AKADR and AKADS rhizosphere soil. Richness (*RI*) index attained maximum levels of 8.82 and 7.89, in AKADR and AKADS samples, respectively. The Pielou's evenness index (*J*) was the highest in the rhizospheres of AKADR ($J = 0.984$) and AKADS ($J = 0.809$) (Table 2).

The primary component of the PCA analysis revealed a significant correlation between the specified Biolog Eco-Plates variables (carboxylic acids, polymers, hydrocarbons, amino acids, amines, and amides, AWCD, Shannon–Wiener index, evenness, and richness index) (Fig. 3). Carbon sources with statistically significant relationships might represent biochemical indicators specific to the rhizospheric soil of different soybean cultivars. The PCA analysis revealed that soils obtained from the rhizosphere of AKADR had higher physiological function (strong connection with the Shannon–Wiener index, the richness index, amino acids, and carbohydrate consumption) compared to that of AKADS samples (Fig. 3).

Comparative taxonomic profiling of bacterial communities colonizing the disease-resistant (AKADR) and disease-susceptible (AKADS) rhizosphere soil

Total high quality of 403,891 sequences with 121,571,191 bps (base pairs) and 356,824 sequences, with 107,404,024 bps were obtained following sequencing through Illumina sequencing of soybean disease-susceptible (AKADS) and disease-resistant (AKADR) rhizosphere soil sample, respectively. All the calculated diversity indices were found significantly higher in AKADR compared to AKADS (Fig. 4). A high ACE value in AKADR indicates the predominance of bacterial communities in AKADR than AKADS (Fig. 4). Similarly, Shannon–Wiener index, which includes both aspects of diversity, i.e. richness and evenness, revealed a significantly ($p < 0.01$) higher diversity in AKADR than in AKADS. Again, the values of the Chao-1 index were significantly ($p < 0.01$) higher in AKADR than in AKADS. Highly diverse communities of bacteria inhabiting the rhizosphere play pivotal roles in plant growth and crop production; however, little is known about the changes in these bacterial communities during stress conditions [1, 39]. This study analyzed bacterial communities from disease-resistant and disease-susceptible varieties of soybean rhizosphere by 16S rRNA amplicon sequencing. This total metagenomic DNA was extracted from the soybean rhizosphere and sequenced using 16S rRNA amplicon sequencing. The taxonomic profiling of the soybean rhizosphere was performed against the

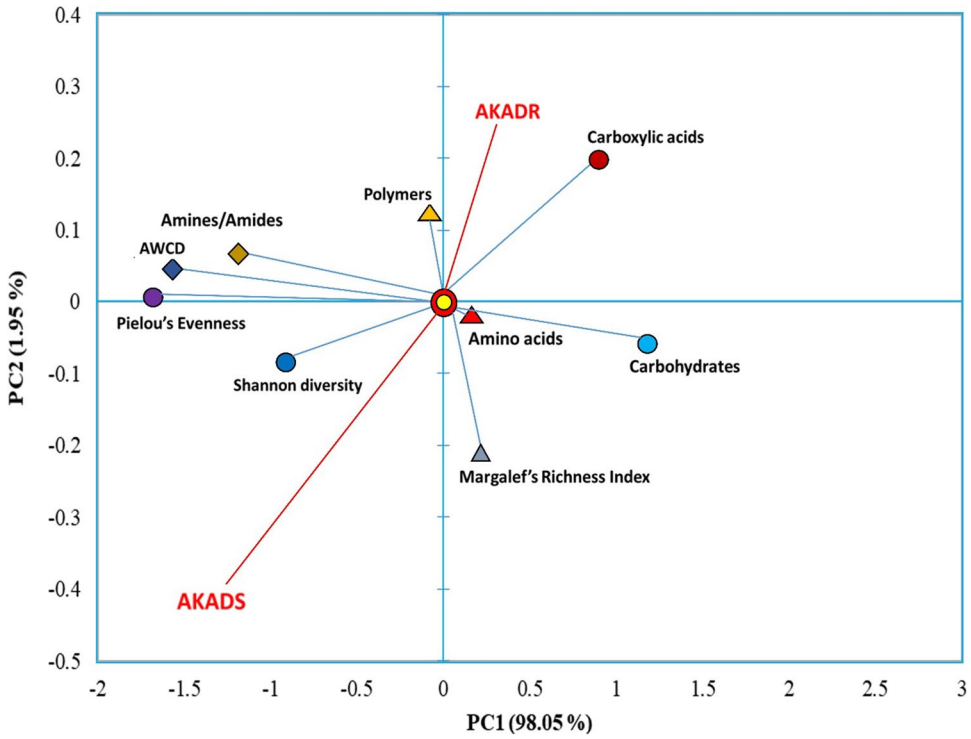

Fig. 3 Principal component analysis (PCA) of the community level physiological profiling (CLPP) at a significance of ($p < 0.05$), where AKADR represents disease-resistant and AKADS represents disease-susceptible cultivars

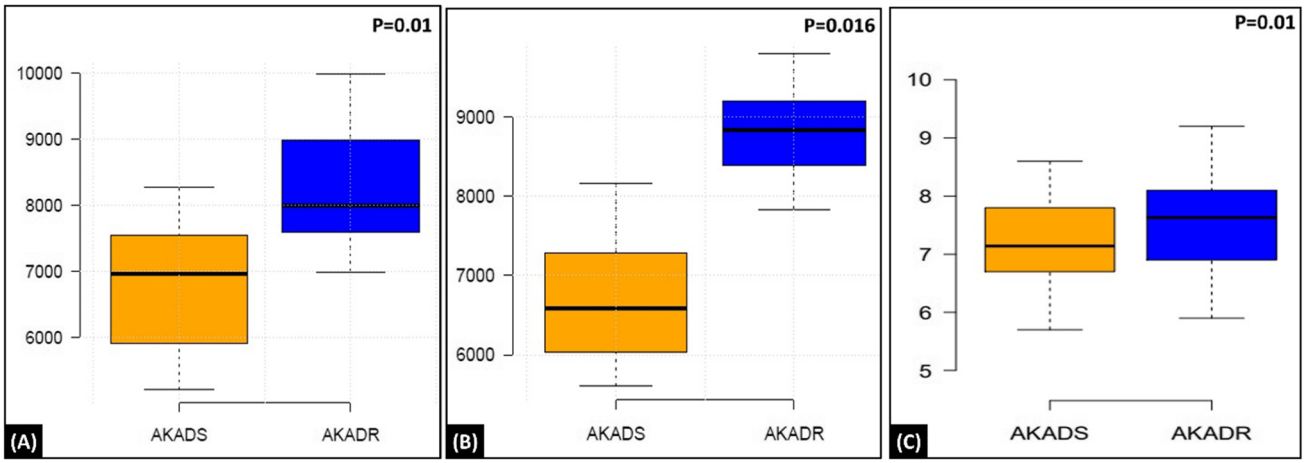

Fig. 4 Microbial diversity and richness. **A** ACE, **B** Chao-1, **C** Shannon–Wiener index, where AKADR represents disease-resistant and AKADS represents disease-susceptible cultivars

RDP database using default parameters, and the phylotypes were analyzed up to the genus level.

For each sample examined, the rarefaction curves plateaued, showing that the sequencing depth was sufficient to acquire the whole bacterial genome (Fig. 1). In AKADR, at the phylum level, Actinobacteria (40%) predominated followed by Chloroflexi (24%), Proteobacteria (20%), Firmicutes (12%), and Acidobacteria (4%), while in the case of AKADS, the most dominating phyla observed were Proteobacteria (35%) followed by Actinobacteria (27%) Bacteroidetes (17%), Firmicutes (7%), Chloroflexi (7%), and Verrucomicrobia (4%) (Fig. 5A). In our study, we found more abundance of Actinobacteria in disease-resistant AKADR samples, one possible reason for such specific recruitment may be due to colonization of more plant growth-promoting bacteria in the rhizosphere of disease-resistant soybean varieties. Phylum to species level taxonomic distribution is presented in Fig. 5 (A–D).

At the genus level, *Bacillus* was predominant, with a relative abundance of 21% in the AKADR sample, which has been used in many studies as a potential PGPR and bio-control agent, whereas in the case of AKADS, *Chryseobacterium* dominated with a relative abundance of (30%) (Fig. 5D) [2, 36]. Similarly, the most abundant bacteria at the species level were *Bacillus* sp. (16%) in the AKADR and *Kaistobacter* (26%) in the AKADS (Fig. 6). In this study, at the genus level, *Streptomyces, Bacillus, Kaistobacter, Rhodoplanes, Clostridium, Verrucomicrobia, Nocardioides, Dehalogenimonas, and Frankia* were predominant in the AKADR sample (Fig. 6).

Functional profiling of bacterial communities presents in the disease-resistant (AKADR) and disease-susceptible (AKADS) rhizosphere soil

The comparative predictive functional profiling of the bacterial communities from the two soybean samples AKADR and AKADS was performed using the METAGENassist software. These bacterial communities were classified based on grams' test, energy source, temperature, habitat, motility, oxygen requirement, spore formation, and shape of the bacteria. More host-associated, gram-positive, motile, spore-forming, thermophiles, mesophiles, aerobic and anaerobic microbes were found in the AKADR sample. In contrast, AKADS consists of soil-associated, non-motile, non-sporulating, and psychrophilic microbes. The functional mapping was carried out by taking into consideration the metabolic compositions. The functional profiling of the sample illustrated that the soybean rhizosphere could be observed as a micro-ecological environment, serving as an environment for several biogeochemical cycles. Functional profiling result shows AKADR sample has more ammonifying (65.10%), nitrite reducing (50.30%), dehalogenation (40%), sulfate reducers and sulfide oxidizers (39.60%), chitin degrading (30.7%), nitrogen-fixing (17%), and streptomycin (14.40%) producing microbes compared to that of microbial species present in AKADS sample (Fig. 7).

Although several genes were identified, we were especially interested in some microbial genes vital to microbial interactions, nutrient cycling, and antibiotic genes. The *nifH* gene in nitrogen-fixing microbes was abundantly present in the AKADR compared to the AKADS rhizosphere sample.

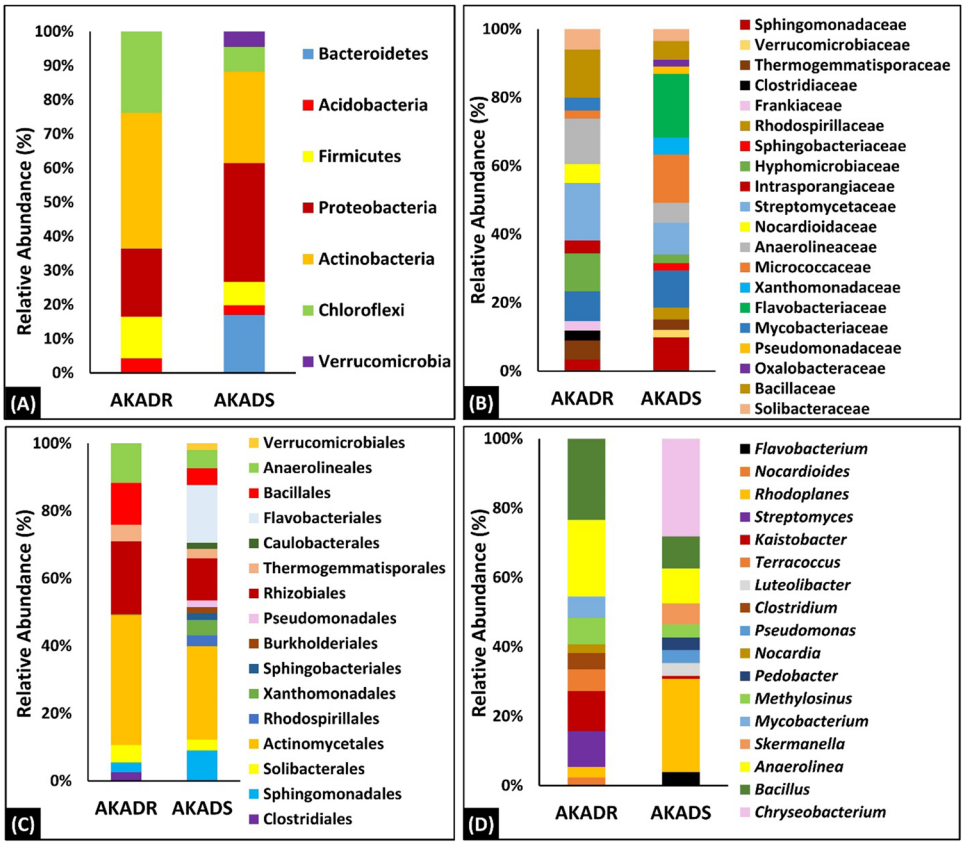

Fig. 5 Comparative taxonomic profiles of the bacterial amplicons from AKADR (disease-resistant) and AKADS (disease-susceptible) at **A** phylum level, **B** class, **C** family, **D** genus

Two genes encoding enzymes responsible for ammonification are urease (*ureC*), and glutamate dehydrogenase (*gdh*) were found more in the AKADR sample than the AKADS sample. *Gdh* genes are mainly found in the bacterial phylum Actinobacteria, abundantly present in AKADR samples, whereas *ureC* genes were mainly distributed in the bacterial phyla Actinobacteria and Proteobacteria. Two genes encoding enzymes hydroxylamine oxidoreductase (*hao*) and ammonia monooxygenase (*amoA*) responsible for nitrification were more in the AKADR sample. These *hao* genes are primarily derived from Rhodobacteraceae, present only in the AKADR samples (Fig. 8). Three genes *nirA*, *nirB*, and *nasA* required to reduce nitrogen to ammonium were highly enriched in the AKADR samples compared to AKADS samples. The *nirA* gene was mainly found in the uncultured archaea, *nirB* gene found in Actinomycetales, Verrucomicrobiae, and genus *Pseudomonas*.

In contrast, *nasA* gene was mainly found in uncultured bacteria. Additionally, five important genes involved in the process of denitrification (*nirS*, *nirK*, *norB*, *nosZ*, and *narG*) were higher in the AKADR compared to AKADS. Antibiotic resistance, including ABC antibiotic transporters, and β-lactamase genes, were abundantly present in the AKADR sample. The ABC antibiotic transporter was abundantly present in the rhizosphere.

Discussions

The present study investigated the richness and metabolic profile of the bacterial community found in the rhizosphere of disease-resistant (JS-20–34) soybean varieties and disease-susceptible (JS-335). Initially, the chemical properties of the soil were assessed to see if there were any differences in soil chemistry between the rhizospheric soils of two soybean cultivars. Soil chemical conditions have long been important drivers of soil microbial composition [71, 74–76]. The pH of AKADR was more or less neutral, while that of AKADS was slightly acidic (Table 1), leading to phosphorus deficiencies. The pH of the soil should be more or less towards the neutral side to avoid nutrient deficiencies, which in turn will weaken the plants and make them more susceptible to disease and pest attacks [8]. The electrical conductivity (EC) of soil is a measure of salinity and is considered an important indicator of soil health [77, 78]. An increase in soil EC may disrupt the microbial population present in soil and may impact vital soil processes such as nitrification, denitrification, respiration, and decomposition, as suggested by Corwin et al. [77]. In addition, the AKADS rhizosphere had the least functional diversity index (Table 2), which might be related to the low pH. This study discovered that pH has a substantial impact on the structure

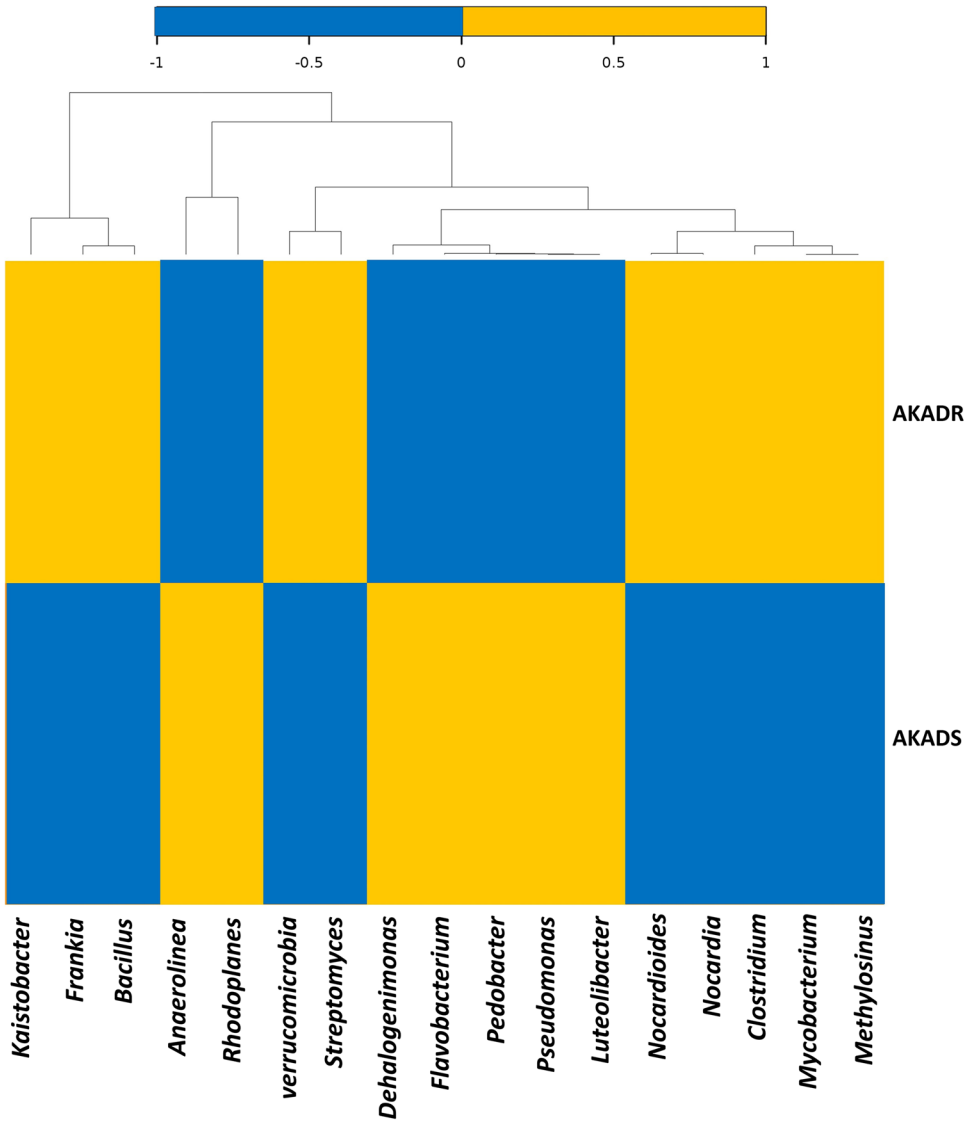

Fig. 6 Heat map showing the taxonomic profiles of the bacterial amplicons from AKADR (disease-resistant) and AKADS (disease-susceptible) at the genus level. The dendrogram depicts the weighted Euclidean distance analysis of bacterial community similarity between the two cultivars

of the rhizosphere Microbiome. Phosphorus (P) is a life-sustaining component commonly used in fertilizers, and its replenishment in the soil is critical for increasing agricultural productivity [71, 79]. In the AKADR rhizosphere, the concentration of P in the soils ranged around 38.32 kg/hc (Table 1). However, the considerable effect of P on rhizospheric soil was exclusively connected to the abundance of Actinobacteria in AKADR samples.

The community-level physiological profile (CLPP), a quick screening method for finding differences between treatments, was used to characterize the bacterial diversity in the two different soybean rhizospheric environments [35, 59, 80]. The selection of these bacterial communities in the rhizosphere is due to the supply of various nutrients and the platform supplied by host plants [3, 38, 59]. Carbohydrates, carboxylic, and acetic acid sources were utilized significantly faster than other substrates such as amino acids, amides/amines, and polymers. Carbohydrate sources are essential in the culture media; several reports showed that the optimum antimicrobial agent production depends upon the type and concentration of carbon sources used in culture media such as glycerol, maltose, fructose, and glucose [81]. This illuminated that pathogenicity has a noticeable effect on the metabolic activity of microorganisms inhabiting the rhizosphere. Biolog substrate utilization assays community-level physiological profiles (CLPP) were used in this study to test the metabolic capabilities of the microbes present in the rhizosphere of disease-resistant soybean were higher than that of microbes colonizing the disease-susceptible one (Fig. 3). We found a high number of bacteria flourishes in the disease-resistant variety compared to disease-susceptible ones. Higher diversity indices in AKADR may be due to

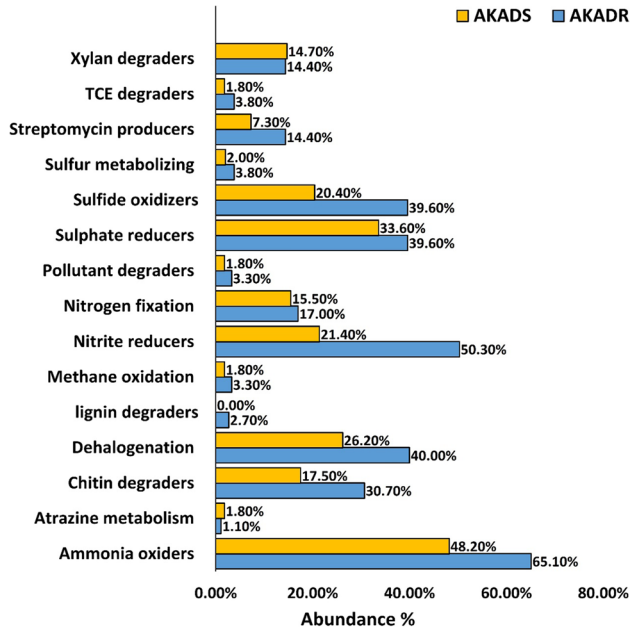

Fig. 7 Expression of functional genes in the rhizosphere soils of AKADR (disease-resistant) and AKADS (disease-susceptible) based on cluster of orthologous groups

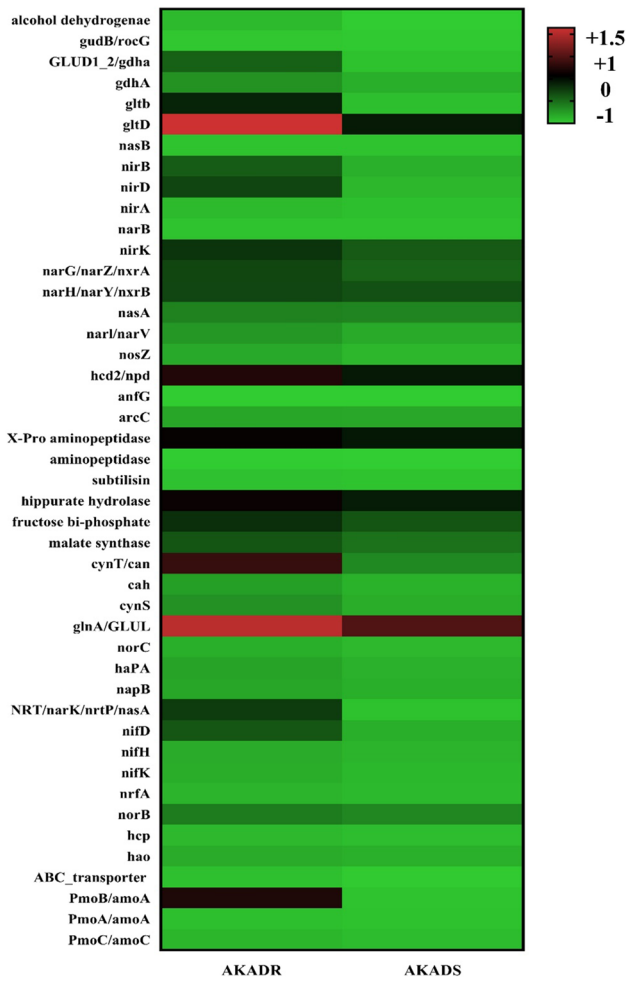

Fig. 8 Heatmap of unigenes identified in the rhizosphere soils of AKADR (disease-resistant) and AKADS (disease-susceptible) calculated using relative abundances of KEGG orthologies (KOs) predicted by PICRUSt 2

higher nutrient availability in AKADR as shown in (Fig. 4). Similar findings were reported by Zhou et al. [82]. The study conducted by Donn et al. [83] and Rascovan et al. [84] demonstrated the maximum metabolic activity in terms of glucose consumption in rhizospheric soils of four different wheat varieties [85]. It is often thought that agricultural management techniques and seasons influence soil microbial populations. Furthermore, wheat cultivars alter the microbial structure and the catabolic activity in the rhizosphere [85, 86].

NGS methods were used to determine genetic fingerprinting in AKADR and AKADS rhizospheric soil samples of soybean. The NGS approach revealed new information on a rhizospheric bacterial group known as viable but not cultivable (VBNC), defined as those with extremely low metabolic activity and are not dividing but are alive and can become culturable if revived [87]. However, it should be noted that, in addition to its benefits, NGS has several drawbacks, including the fact that certain culturable bacteria cannot be identified solely on primer mismatches, and diversity is sometimes exaggerated. This investigation employed well-known universal and suggested primers for bacterial identification in rhizosphere soil [88]. However, the NGS data reported the Actinobacteria, Firmicutes, and Proteobacteria were consistently enriched in the rhizosphere of both healthy and diseased samples, regardless of different cultivars, as observed by other researchers in banana, tomato, maize, rice, and wheat which are commonly known for their good response to labile carbon sources and plant growth promotion [1, 9, 34, 39, 89, 90].

The abundance of Actinobacteria can change carbon utilization and rhizodeposition, affecting carbon sequestration and storage. Considering their role in biogeochemical cycling, any change in their abundance will affect microbial function and structure, and consequently, plant growth. Results from the present study are supported with that of Alvarez et al. [91] and Rizzatti et al. [92], where the authors describe Actinobacteria as a potential candidate for promoting plant growth promoters [91, 93]. The members belonging to *Bacillus* sp. include potential PGPRs and biocontrol agents. For example, various species of *Bacillus* have been associated with phosphorus solubilization [89, 94–96], systemic resistance to pathogens, and by producing antifungal compounds [97–102] and antibiotics [103, 104]. Among

all *Kaistobacter* and *Rhodoplanes* were identified atrazine degraders and helpful in atrazine bioremediation [105]. Other highly abundant genera in AKADR samples include potential PGPRs and biocontrol agents. The existence of these bacterial networks gives clues to the operation of the nitrogen and sulfur cycles within this micro-environment. The dominance of Acidobacteria at the phylum level includes the group of nitrogen-fixing bacteria like *Frankia* that fixes about 15% of the world's total nitrogen [91, 93, 106]. Members of the phylum Actinobacteria are also recognized for producing enzymes like chitinase, urease, catalase [107].

Moreover, these bacterial groups can degrade a broad range of pesticides and hydrocarbons, and their metabolic potential offers a substantial area for future research [81, 106]. The functional analysis results showed that most genes were abundantly present in the AKADR samples than AKADS rhizosphere soil samples (Fig. 8). Some microbes release antimicrobial compounds harmful to other microbes, allowing them to colonize and grow on plant surfaces when other microbial populations are present. Our study reported that more antibiotic resistance, including ABC antibiotic transporters, and β-lactamase genes, were abundantly present in the AKADR sample. The results of our study have collaborated with the study conducted by Li et al., [108] and Yu et al. [109] on maize rhizosphere. The increased incidence of antibiotic resistance genes also serves as the first line of defence for root system attacks by soil-borne microbes [110]. Microbes that degrade or detoxify these compounds via particular functional genes have a competitive advantage [3].

Conclusions

The combination of the two approaches, NGS and CLPP, enabled the identification of bacterial diversity in the rhizospheric soil of the two soybean cultivars in a complete (genetic and catabolic) manner. The new knowledge gained in this study might help in improving soil health, agricultural practices, food production, and food security. The alterations seen in the soil microbial community were demonstrated to result from a combined effect of both the soybean cultivar and rhizospheric soil biochemistry. This study unravels the changes in the selection of bacterial communities by the disease-resistant and disease-susceptible soybeans rhizosphere. However, at the genus level *Streptomyces*, *Bacillus*, *Kaistobacter*, *Rhodoplanes*, *Clostridium*, *Verrucomicrobia*, *Nocardioides*, *Dehalogenimonas*, and *Frankia* were predominant in the AKADR sample. The members belonging to these genera are recognized as potential PGPRs and biocontrol agents. The results obtained from this study showed that the microbiomes of plants that both survived infection and remained healthy were linked to host-specific plant growth-promoting pathogen-suppressing *Bacillus* and antibiotic-producing *Streptomyces* bacterial species. By promoting helpful bacteria in the field soil, it may be possible to enhance plant resistance to specific diseases by utilizing eco-friendly tools like biofertilizers. This, in turn, will reduce the requirement for intensive chemical fertilizers treatments to control disease outbreaks as the damaging effects of the plant pathogens present in the soil would be reduced.

The physiological profile studied at the community level revealed microbial preferences for carbon substrate utilization (catabolic fingerprinting). CLPP demonstrated that metabolic activity was affected by the type of soybean cultivars and the substrate utilized. The most easily metabolized group of substrates for all rhizospheric soils was carbohydrates > carboxylic acids > amino acids > polymers > amines and amides. The metabolic capabilities of disease-resistant (AKADR) were 2–threefold higher than that of disease–susceptible (AKADS) soybean rhizosphere soil. Additional studies on both metabolic activities of soybean, such as root exudation and the physiological functions of these rhizobacteria on plant growth, are necessary to explain the mutual interactions between rhizosphere microbes and their host plants in the fields for better utilization of rhizosphere bacteria for sustainable agriculture production.

Functional profiling of the disease-resistant (AKADR) soybean rhizosphere showed a higher amount of antibiotic resistance genes in the disease-resistant samples. This provides evidence that these bacterial communities in AKADR samples can provide the frontline defence against soil-borne pathogens. These bacterial genera that can detoxify or degrade these metabolites via definite functional genes gain a competitive advantage. The data support the concept that the disease-resistant soybean rhizosphere is a hotspot of functional genes for converting labile and recalcitrant organic compounds like carbon, nitrogen, phosphorus, and sulfur. Future research will focus on exploring microbial communities associated with root pathogenesis, including functions and actions of the microbiome, for understanding intricate microbe-plant-pathogen dealings. This will offer new prospects to recognize how the microbiome maintains plant health and open new avenues to increase crop production. To summarize, combining the CLPP approach with the 16S rRNA amplicon sequencing revealed new information on the taxonomic and physiological bacterial fingerprinting of rhizospheric soils of selected soybean cultivars.

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Author contribution AD conducted the experiments and prepared the manuscript. AD and MM performed the data analysis under the guidance and supervision of AK.

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Data availability The sequence data of Illumina MiSeq Sequencer has been deposited at SRA under the bio project PRJNA589888 disease-resistant (SAMN13293567) and disease-susceptible (SAMN13293568).

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Kaushal M, Mahuku G, Swennen R (2020) Metagenomic insights of the root colonizing microbiome associated with symptomatic and non-symptomatic bananas in Fusarium wilt infected fields. Plants 9(2). https://doi.org/10.3390/plants9020263
2. Ruzzi M, Aroca R, Lee S-WS-H et al (2016) Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants. Commun Soil Sci Plant Anal. https://doi.org/10.1007/s10341-016-0278-6
3. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663. https://doi.org/10.1111/1574-6976.12028
4. Sugiyama A (2019) The soybean rhizosphere: Metabolites, microbes, and beyond—A review. J Adv Res. https://doi.org/10.1016/j.jare.2019.03.005
5. Musilova L, Ridl J, Polivkova M, Macek T, Uhlik O (2016) Effects of secondary plant metabolites on microbial populations: Changes in community structure and metabolic activity in contaminated environments. Int J Mol Sci 17(8). https://doi.org/10.3390/ijms17081205
6. Dubey A, Kumar A, Malla MA et al (2021) Approaches for the amelioration of adverse effects of drought stress on crop plants. Front Biosci - Landmark 26(10):928–947. https://doi.org/10.52586/4998
7. Kumar A, Dubey A (2020) Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. J Adv Res 24:337–352. https://doi.org/10.1016/j.jare.2020.04.014
8. Dubey A, Saiyam D, Kumar A, Hashem A, Abdullah EF, Khan ML (2021) Bacterial root endophytes: Characterization of their competence and plant growth promotion in soybean (glycine max (L.) merr.) under drought stress. Int J Environ Res Public Health 18(3):1–20. https://doi.org/10.3390/ijerph18030931
9. Zhou D, Jing T, Chen Y et al (2019) Deciphering microbial diversity associated with Fusarium wilt-diseased and disease-free banana rhizosphere soil. BMC Microbiol 19(1). https://doi.org/10.1186/s12866-019-1531-6
10. Kwak M-J, Kong HG, Choi K et al (2018) Rhizosphere microbiome structure alters to enable wilt resistance in tomato. Nat Biotechnol 36(11):1100–1109. https://doi.org/10.1038/nbt.4232
11. Dubey A, Kumar A, Abd Allah EF, Hashem A, Khan ML (2019) Growing more with less: Breeding and developing drought resilient soybean to improve food security. Ecol Indic 105:425–437. https://doi.org/10.1016/j.ecolind.2018.03.003
12. Marks BB, Megías M, Nogueira MA, Hungria M (2013) Biotechnological potential of rhizobial metabolites to enhance the performance of Bradyrhizobium spp. and Azospirillum brasilense inoculants with soybean and maize. AMB Express 3(1):21. https://doi.org/10.1186/2191-0855-3-21
13. Banaszkiewicz T (2011) Nutritional Value of Soybean Meal. In: Soybean and Nutrition. https://doi.org/10.5772/23306
14. Miransari M (2016) Abiotic and Biotic Stresses in Soybean Production. https://doi.org/10.1016/c2014-0-00087-1
15. Deshmukh R, Sonah H, Patil G, et al. (2014) Integrating omic approaches for abiotic stress tolerance in soybean. 5(June):1-12. https://doi.org/10.3389/fpls.2014.00244
16. Miransari M, Smith DL (2009) Alleviating salt stress on soybean (Glycine max (L.) Merr.) - Bradyrhizobium japonicum symbiosis, using signal molecule genistein. Eur J Soil Biol 45(2):146–152. https://doi.org/10.1016/j.ejsobi.2008.11.002
17. Bandara AY, Weerasooriya DK, Bradley CA, Allen TW, Esker PD (2020) Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS On. https://doi.org/10.1371/journal.pone.0231141
18. Chattopadhyay C, Kolte SJ, Waliyar F, Chattopadhyay C, Kolte SJ, Waliyar F (2015) Soybean Diseases. In: Diseases of Edible Oilseed Crops.. https://doi.org/10.1201/b19302-9
19. Barpete RD, Verma VK (2019) Management of rhizoctonia root rot disease in soybean in Betul district of Madhya Pradesh. Plant Arch 19(2):2376–2378
20. Amrate PK, Shrivastava MK, Singh G (2020) Screening of genotypes to identify the resistance source against major diseases of soybean under high disease pressure conditions. Int J Curr Microbiol App Sci 9(5):1739–1745. https://doi.org/10.20546/ijcmas.2020.905.195
21. Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd-Allah EF (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.01132
22. Fonseca JP, Hoffmann L, Cabral BCA et al (2018) Contrasting the microbiomes from forest rhizosphere and deeper bulk soil from an Amazon rainforest reserve. Gene. 642:389–397. https://doi.org/10.1016/j.gene.2017.11.039
23. Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J. https://doi.org/10.1038/ismej.2014.17
24. Xu Y, Wang G, Jin J, Liu J, Zhang Q, Liu X (2009) Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2008.10.027
25. Bulgarelli D, Garrido-Oter R, Münch PC et al (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17(3):392–403. https://doi.org/10.1016/j.chom.2015.01.011
26. Lundberg DS, Lebeis SL, Paredes SH et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature. 488(7409):86–90. https://doi.org/10.1038/nature11237
27. Bulgarelli D, Rott M, Schlaeppi K et al (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature. https://doi.org/10.1038/nature11336
28. Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front. Plant Sci 5(MAY). https://doi.org/10.3389/fpls.2014.00216
29. Knief C, Delmotte N, Chaffron S et al (2012) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6(7):1378–1390. https://doi.org/10.1038/ismej.2011.192
30. Edwards J, Johnson C, Santos-Medellín C et al (2015) Structure, variation, and assembly of the root-associated microbiomes of

rice. Proc Natl Acad Sci U S A 112(8):E911–E920. https://doi.org/10.1073/pnas.1414592112
31. Kuźniar A, Włodarczyk K, Grządziel J, Goraj W, Gałązka A, Wolińska A (2020) Culture-independent analysis of an endophytic core microbiome in two species of wheat: Triticum aestivum L. (cv. 'Hondia') and the first report of microbiota in Triticum spelta L. (cv. 'Rokosz'). Syst Appl Microbiol. https://doi.org/10.1016/j.syapm.2019.126025
32. Chen S, Waghmode TR, Sun R, Kuramae EE, Hu C, Liu B (2019) Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. Microbiome. Published online https://doi.org/10.1186/s40168-019-0750-2
33. Solanki MK, Abdelfattah A, Britzi M et al (2019) Shifts in the composition of the microbiota of stored wheat grains in response to fumigation. Front Microbiol 10(MAY). https://doi.org/10.3389/fmicb.2019.01098
34. Lee SM, Kong HG, Song GC, Ryu CM. Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. ISME J 2021;15(1):330-347. doi:https://doi.org/10.1038/s41396-020-00785-x
35. Jin T, Wang Y, Huang Y et al (2017) Taxonomic structure and functional association of foxtail millet root microbiome. Gigascience. 6(10):1–12. https://doi.org/10.1093/gigascience/gix089
36. Liu Y, Wu L, Wu X et al (2017) Analysis of microbial diversity in soil under ginger cultivation. Scientifica (Cairo) 2017. https://doi.org/10.1155/2017/8256865
37. Lazcano C, Boyd E, Holmes G, Hewavitharana S, Pasulka A, Ivors K (2021) The rhizosphere microbiome plays a role in the resistance to soil-borne pathogens and nutrient uptake of strawberry cultivars under field conditions. Sci Rep. https://doi.org/10.1038/s41598-021-82768-2
38. Kumar A, Dubey A, Malla MA, Dames J (2021) Pyrosequencing and phenotypic microarray to decipher bacterial community variation in Sorghum bicolor (L.) Moench rhizosphere. Curr Res Microb Sci. https://doi.org/10.1016/j.crmicr.2021.100025
39. Sugiyama A, Ueda Y, Zushi T, Takase H, Yazaki K (2014) Changes in the bacterial community of soybean rhizospheres during growth in the field. PLoS One 9(6):1–9. https://doi.org/10.1371/journal.pone.0100709
40. Mendes R, Kruijt M, De Bruijn I et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science (80-) 332(6033):1097–1100. https://doi.org/10.1126/science.1203980
41. Mallon CA, Poly F, Le Roux X, Marring I, Van Elsas JD, Salles JF (2015) Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. Ecology 96(4):915–926. https://doi.org/10.1890/14-1001.1
42. Krishna SBN, Dubey A, Malla MA et al (2020) Integrating microbiome network: establishing linkages between plants, microbes and human health. Open Microbiol J. https://doi.org/10.2174/1874285801913020330
43. Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd-Allah EF (2019) Exploring the human microbiome: the potential future role of next-generation sequencing in disease diagnosis and treatment. Front Immunol 9(19):1–23. https://doi.org/10.3389/fimmu.2018.02868
44. Malla MA, Dubey A, Raj A, Kumar A, Upadhyay N, Yadav S (2022) Emerging frontiers in microbe-mediated pesticide remediation: Unveiling role of omics and In silico approaches in engineered environment. Environ Pollut 299(January):118851. https://doi.org/10.1016/j.envpol.2022.118851
45. Dubey A, Kumar A, Khan ML, Payasi DK (2022) Plant growth-promoting and bio-control activity of Micrococcus luteus strain AKAD 3-5 isolated from the soybean (Glycine max (L.) Merr.) rhizosphere. Open Microbiol J 15(1):188–197. https://doi.org/10.2174/1874285802115010188
46. Dubey A, Malla MA, Khan F et al (2019) Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28(8-9):2405–2429. https://doi.org/10.1007/s10531-019-01760-5
47. Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32(11):1559–1570. https://doi.org/10.1007/s10529-010-0347-0
48. Zhang X, Zhang R, Gao J, et al. (2017) Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. Soil Biol Biochem. Published online https://doi.org/10.1016/j.soilbio.2016.10.023
49. Singh VK, Singh AK, Singh PP, Kumar A (2018) Interaction of plant growth promoting bacteria with tomato under abiotic stress: a review. Agric Ecosyst Environ. https://doi.org/10.1016/j.agee.2018.08.020
50. Angayarkanni T (2013) Biomanagement of root rot and leaf spot disease of stevia rebaudiana using plant growth promoting rhizobacteria. University.
51. Kumawat KC, Sharma P, Sirari A et al (2019) Synergism of Pseudomonas aeruginosa (LSE-2) nodule endophyte with Bradyrhizobium sp. (LSBR-3) for improving plant growth, nutrient acquisition and soil health in soybean. World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-019-2622-0
52. Gałązka A, Grzęda E, Jończyk K (2019) Changes of microbial diversity in rhizosphere soils of new quality varieties of winter wheat cultivation in organic farming. Sustain. https://doi.org/10.3390/SU11154057
53. Praeg N, Pauli H, Illmer P (2019) Microbial diversity in bulk and rhizosphere soil of ranunculus glacialis along a high-alpine altitudinal gradient. Front Microbiol. https://doi.org/10.3389/fmicb.2019.01429
54. Wolińska A, Kuźniar A, Zielenkiewicz U et al (2017) Metagenomic Analysis of some potential nitrogen-fixing bacteria in arable soils at different formation processes. Microb Ecol. https://doi.org/10.1007/s00248-016-0837-2
55. Walkley A, Black IA (1934) An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38. https://doi.org/10.1097/00010694-193401000-00003
56. Subbiah BV, Asija GL (1956) A rapid procedure for the estimation of available nitrogen in soils. Curr Sci 25(8):259–260
57. Dickman SR, Bray RH (1940) Colorimetric determination of phosphate. Ind Eng Chem Anal Ed. https://doi.org/10.1021/ac50151a013
58. Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59(1):39–46. https://doi.org/10.1097/00010694-194501000-00006
59. Frac M, Oszust K, Lipiec J (2012) Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors. 12(3):3253–3268. https://doi.org/10.3390/s120303253
60. Siles JA, Cajthaml T, Minerbi S, Margesin R (2016) Effect of altitude and season on microbial activity, abundance and community structure in Alpine forest soils. FEMS Microbiol Ecol 92(3). https://doi.org/10.1093/femsec/fiw008
61. Sala MM, Arrieta JM, Boras JA, Duarte CM, Vaqué D (2010) The impact of ice melting on bacterioplankton in the Arctic Ocean. Polar Biol 33(12):1683–1694. https://doi.org/10.1007/s00300-010-0808-x
62. Suda W, Nagasaki A, Shishido M (2009) Powdery mildew-infection changes bacterial community composition in the phyllosphere. Microbes Environ. Published online https://doi.org/10.1264/jsme2.ME09114

63. ter Braak CJF. Canoco for visualization of multivariate data. 2014;(January):1-10. http://www.wageningenur.nl/en/show/Canoco-for-visualization-of-multivariate-data.htm
64. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl Environ Microbiol 79(17):5112–5120. https://doi.org/10.1128/AEM.01043-13
65. Wilke A, Glass EM, Bischof J, et al. (2015) MG-RAST Manual for version 3.6, revision 3. :130.
66. Cox MP, Peterson DA, Biggs PJ (2010) SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. BMC Bioinformatics 11(1):485. https://doi.org/10.1186/1471-2105-11-485
67. Cole JR, Wang Q, Fish JA et al (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 42(Database issue):D633–D642. https://doi.org/10.1093/nar/gkt1244
68. Arndt D, Xia J, Liu Y et al (2012) METAGENassist: A comprehensive web server for comparative metagenomics. Nucleic Acids Res 40(W1). https://doi.org/10.1093/nar/gks497
69. Douglas GM, Maffei VJ, Zaneveld JR et al (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38(6):685–688. https://doi.org/10.1038/s41587-020-0548-6
70. Hammer Ø, Harper DAT, Ryan PD (2001) Past: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):9
71. Wolinska A, Kuzniar A, Galazka A (2020) Biodiversity in the Rhizosphere of selected winter wheat (Triticum aestivum L.) cultivars-genetic and catabolic fingerprinting. Agronomy. https://doi.org/10.3390/agronomy10070953
72. Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57(8):2351–2359
73. Garland JL (1997) Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol Ecol. https://doi.org/10.1016/S0168-6496(97)00061-5
74. Mendes LW, de Chaves MG, Fonseca M de C, Mendes R, Raaijmakers JM, Tsai SM. Resistance Breeding of Common Bean Shapes the Physiology of the Rhizosphere Microbiome. Front Microbiol 2019. doi:https://doi.org/10.3389/fmicb.2019.02252
75. Natividad AA, Timoneda J, Batlle-Sales J, Bordas V, Murgui A (1997) New Method for MEaduring Dehydrogenase Activity in Soils
76. Wolińska A, Kuźniar A, Zielenkiewicz U et al (2017) Bacteroidetes as a sensitive biological indicator of agricultural soil usage revealed by a culture-independent approach. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2017.06.009
77. Corwin DL, Lesch SM (2003) Application of soil electrical conductivity to precision agriculture: Theory, principles, and guidelines. Agron J 95:455–471. https://doi.org/10.2134/agronj2003.4550
78. Sanches GM, Magalhães PSG, Remacre AZ, Franco HCJ. Potential of apparent soil electrical conductivity to describe the soil pH and improve lime application in a clayey soil. Soil Tillage Res 2018;175:217-225. doi:https://doi.org/https://doi.org/10.1016/j.still.2017.09.010
79. (2021) Influence of phosphate fertilizer on cadmium in agricultural soils and crops. In: Phosphate in soils. https://doi.org/10.1201/9781351228909-9
80. Si P, Shao W, Yu H et al (2018) Rhizosphere microenvironments of eight common deciduous fruit trees were shaped by microbes in northern China. Front Microbiol. https://doi.org/10.3389/fmicb.2018.03147
81. Amin DH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EMH (2020) Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. Bull Natl Res Cent. https://doi.org/10.1186/s42269-019-0266-8
82. Zhou Z, Wang C, Luo Y (2020) Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat Commun 11(1):3072. https://doi.org/10.1038/s41467-020-16881-7
83. Donn S, Kirkegaard JA, Perera G, Richardson AE, Watt M (2015) Evolution of bacterial communities in the wheat crop rhizosphere. Environ Microbiol 17(3):610–621. https://doi.org/10.1111/1462-2920.12452
84. Rascovan N, Carbonetto B, Perrig D et al (2016) Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. Sci Rep. https://doi.org/10.1038/srep28084
85. Gałązka A, Gawryjołek K, Grządziel J, Frąc M, Księżak J (2017) Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant Soil Environ. https://doi.org/10.17221/171/2017-PSE
86. Grządziel J, Gałązka A (2018) Microplot long-term experiment reveals strong soil type influence on bacteria composition and its functional diversity. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2017.10.033
87. Robben C, Fister S, Witte AK, Schoder D, Rossmanith P, Mester P (2018) Induction of the viable but non-culturable state in bacterial pathogens by household cleaners and inorganic salts. Sci Rep. https://doi.org/10.1038/s41598-018-33595-5
88. Thijs S, De Beeck MO, Beckers B, et al. (2017) Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. Front Microbiol. Published online https://doi.org/10.3389/fmicb.2017.00494
89. Chen L, Shi H, Heng J, Wang D, Bian K. Antimicrobial, plant growth-promoting and genomic properties of the Chen L, Shi H, Heng J, Wang D, Bian K. Antimicrobial, plant growth-promoting and genomic properties of the https://doi.org/10.1016/j.micres.2018.10.002
90. Benitez MS, Osborne SL, Lehman RM (2017) Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. Sci Rep 7(1). https://doi.org/10.1038/s41598-017-15955-9
91. Alvarez A, Saez JM, Davila Costa JS et al (2017) Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere. 166:41–62. https://doi.org/10.1016/j.chemosphere.2016.09.070
92. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A (2017) Proteobacteria: A common factor in human diseases. Biomed Res Int. https://doi.org/10.1155/2017/9351507
93. Hayat S, Ashraf A, Aslam B, et al. (2021) Actinobacteria: potential candidate as plant growth promoters. In: Hossain A, ed. Plant stress physiology. IntechOpen. https://doi.org/10.5772/intechopen.93272
94. Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S (2012) Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. Recent Res Sci Technol 4:01–05
95. Chakraborty U, Chakraborty BN, Chakraborty AP, Sunar K, Dey PL (2013) Plant growth promoting rhizobacteria mediated improvement of health status of tea plants. Indian J Biotechnol 12(1):20–31
96. Wani PA, Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. J Plant Nutr Soil Sci 170(2):283–287. https://doi.org/10.1002/jpln.200620602
97. Hashem A, Abd Allah EF, Alqarawi AA, Radhakrishnan R, Kumar A (2017) Plant defense approach of Bacillus subtilis

(BERA 71) against Macrophomina phaseolina (Tassi) Goid in mung bean. J Plant Interact 12(1):390–401. https://doi.org/10.1080/17429145.2017.1373871
98. Gond SK, Bergen MS, Torres MS, White JF (2015) Endophytic Bacillus spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiol Res 172:79–87. https://doi.org/10.1016/j.micres.2014.11.004
99. Etchegaray A, De Castro Bueno C, De Melo IS et al (2008) Effect of a highly concentrated lipopeptide extract of Bacillus subtilis on fungal and bacterial cells. Arch Microbiol 190(6):611–622. https://doi.org/10.1007/s00203-008-0409-z
100. Zouari I, Jlaiel L, Tounsi S, Trigui M (2016) Biocontrol activity of the endophytic Bacillus amyloliquefaciens strain CEIZ-11 against Pythium aphanidermatum and purification of its bioactive compounds. Biol Control 100:54–62. https://doi.org/10.1016/j.biocontrol.2016.05.012
101. Chitarra GS, Breeuwer P, Nout MJR, Van Aelst AC, Rombouts FM, Abee T (2003) An antifungal compound produced by Bacillus subtilis YM 10-20 inhibits germination of Penicillium roqueforti conidiospores. J Appl Microbiol 94(2):159–166. https://doi.org/10.1046/j.1365-2672.2003.01819.x
102. Arrebola E, Jacobs R, Korsten L (2010) Iturin A is the principal inhibitor in the biocontrol activity of Bacillus amyloliquefaciens PPCB004 against postharvest fungal pathogens. J Appl Microbiol 108(2):386–395. https://doi.org/10.1111/j.1365-2672.2009.04438.x
103. Kumar S, Aharwal RP, Shukla H, Rajak RC, Sandhu SS (2014) Endophytic fungi : as a source of antimicrobials bioactive compounds. World J Pharm Pharm Sci 3(2):1179–1197
104. Dubey A, Malla MA, Kumar A, Dayanandan S, Khan ML (2020) Plants endophytes: unveiling hidden agenda for bioprospecting toward sustainable agriculture. Crit Rev Biotechnol 40(8):1210–1231. https://doi.org/10.1080/07388551.2020.1808584
105. Lodha TD, Srinivas A, Sasikala C, Ramana CV (2015) Hopanoid inventory of Rhodoplanes spp. Arch Microbiol 197(6):861–867. https://doi.org/10.1007/s00203-015-1112-5
106. Hopwood D (2007) An introduction to the actinobacteria. Microbiol Today 34(2):60–62
107. Ek-Ramos MJ, Gomez-Flores R, Orozco-Flores AA, Rodríguez-Padilla C, González- Ochoa G, Tamez-Guerra P (2019) Bioactive products from plant-endophytic Gram-positive bacteria. Front Microbiol. https://doi.org/10.3389/fmicb.2019.00463
108. Li X, Rui J, Xiong J et al (2014) Functional potential of soil microbial communities in the maize rhizosphere. PLoS One. https://doi.org/10.1371/journal.pone.0112609
109. Yu K, Liu Y, Tichelaar R et al (2019) Rhizosphere-associated pseudomonas suppress local root immune responses by gluconic acid-mediated lowering of environmental pH. Curr Biol. https://doi.org/10.1016/j.cub.2019.09.015
110. Cook RJ, Thomashow LS, Weller DM et al (1995) Molecular mechanisms of defense by rhizobacteria against root disease. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.92.10.4197