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Soil microbiomes from the groundnut basin of Senegal contain plant growth-promoting bacteria with potential for crop improvement in arid soils

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Abstract

The principal methods to maintain soil fertility in Sahel soils are largely allowing fields to go fallow and manure addition. These methods are not currently sufficient to improve soil fertility. To promote biological amendments, we aimed to understand the plant-growth promoting traits of various soil microbial isolates. The soils collected in different areas in Senegal exhibited a similar eDNA profile of bacteria; the dominant microbes were Firmicutes, followed by Proteobacteria and Actinobacteria. Of 17 isolates identified and tested, the vast majority solubilized rock phosphate and a large number grew on culture medium containing 6% salt, but very few degraded starches or hydrolysed carboxymethyl cellulose or produced siderophores. Upon single inoculation, *Peribacillus asahii* RC16 and *Dietzia cinnamea* 55 significantly increased pearl millet growth and yield parameters. For cowpea, plant shoot length was significantly increased by *Pseudarthrobacter phenanthrenivorans* MKAG7 co-inoculated with *Bradyrhizobium elkanii* 20TpCR5, and nearly all rhizobacteria tested significantly improved cowpea dry weight and pod weight. Additionally, the double inoculation of *Dietzia cinnamea* 55 and MKAG7 significantly increased shoot length, dry weight, and seed head weight of pearl millet. These isolates are promising inoculants because they are ecologically-friendly, cost-effective, sustainable, and have fewer negative effects on the soil and its inhabitants.

Key words: pearl millet, PGPR, eDNA, single inoculation, double inoculation

Introduction

In Senegal, agriculture is subsistence, low-input, and significantly less mechanized than many other nations in Africa, and is also highly dependent on soil, climate, and water. Food crops take up to 46% of the total land and make up 15% of the Gross Domestic Product, ensuring 70%–75% employment (CIAT/BFS/USAID 2016; World Data Atlas 2016). Pearl millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L. Moench), and groundnut/peanut (*Arachis hypogaea* L.) are the major crops grown in Senegal (Leippert et al. 2020).

Temperatures in Senegal range from very warm to hot, with an annual average temperature of 35 °C. At least 4 months of the year are tropical and frequently sultry with temperatures above 35 °C. The distribution of crop types grown in Senegal is correlated with the timing of seasonal rainfall (IFPRI 2013). However, according to a recent study, both seasonal rainfall and temperature are predicted to increase by the mid-century (2040–2069) because of climate change (Araya et al. 2022), the effects of which have become more apparent worldwide since the 1980s. Moreover, some of the practices of the Green Revolution, especially the use of modern crop varieties and the addition of synthetic fertilizers and pesticides/herbicides are not sustainable practices (Evenson and Gollin 2003), especially under climate change conditions.

Pearl millet, an important coarse grain cereal, is the most cultivated cereal crop in Senegal. However, its yield recently decreased by 10%–20% (Sultan et al. 2019) in part because of a decline in the soil quality of the pearl millet-growing regions. It is highly likely that there will be a continued downturn in pearl millet cultivation in northern Senegal as the climate continues to change (Olodo et al. 2020). For example, the crop was grown on 922 008 ha in 2015, which represents 58.9% of the land used for cereal cultivation (DAPSA 2016). It has been the most produced cereal crop and second in production, lagging only behind peanuts, except in 2015 when rice cultivation increased. Total millet production was calculated to be 749 874 tonnes in 2015 (DAPSA 2016). The main production

areas are the peanut basin, Eastern Senegal, and the Haute Casamance. Most of the crop residues are transported from farms for feeding domestic animals, but overexploitation of these resources often results in reduced soil fertility (Henao and Baanante 1999), which exacerbates soil erosion, leaching, and degradation (Roose 1981). The average annual nutrient deficit due to the loss of exported nutrients is estimated at 12 kg N, 2 kg P, and 10 kg K per hectare (Stoorvogel et al. 1993).

Pearl millet (*Pennisetum glaucum* L. R. Br.) is cultivated in drought-prone areas for grain and fodder and is planted on about 30 million hectares worldwide (Yadav et al. 2012). It is the sole economic crop for approximately 20 million Sahelian people who depend on it for their survival (Breman and Kessler 2012). In arid soils, millet yield is correlated not only with reduced water levels but also with soil fertility status. Senegal-grown millet yield was measured as 683 kg/ha (MA/DAPSA 2014), but under optimal conditions, yield can be as much as 2000 kg/ha. Although fertility is maintained largely by allowing fields to go fallow, manure addition remains the principal source of fertilizer for Senegalese soils (Buerkert and Hiernaux 1998). Because of the expense, very few farmers use synthetic fertilizers (McIntire et al. 1989; Shapiro and Sanders 1998), but the current methods of improving soil fertility are not sufficient to ensure good millet yields in the Sahel, in part because of the reduction of fallow time necessary for replenishing soil nutrients. However, soil microorganisms represent another way to enhance soil fertility and to improve millet growth and yield as well as nutrient availability. Beneficial soil microbes restore the delicate interrelationship among microbes, plants, and the environment especially in degraded or overused soils. To pursue this type of biological approach is an important step towards achieving sustainability and fighting climate change in Senegal and other arid nations.

Among the vast array of potential PGP bacteria, numerous species are known to enhance plant growth (Glick 1995) via several mechanisms such as phyto-stimulation, biofertilization, and biocontrol (Gaiero et al. 2013). PGP bacteria produce phytohormones including auxins, cytokinins, and gibberellins (Vessey 2003) and interfere with the production of ethylene, an inhibitor of plant growth (Schaller and Binder 2017). Microbe-mediated biofertilization improves plant mineral nutrition (Pérez-Montaño et al. 2014) via numerous mechanisms; for example, by solubilizing mineral-bound phosphate, fixing atmospheric nitrogen into ammonia, and producing siderophores for iron sequestration (Gamalero and Glick 2011). PGP bacteria also play a phytoprotective role against pathogenic agents. Although their potential to increase plant nutrition and growth is well known, their use in improving millet production is under-studied.

The mechanisms whereby the microbiota improves this cereal's yield need to be further explored because pearl millet yield dropped during the mid-century (2040–2069) even though high levels of N fertilizer (more than 90 kg/ha) were added (Araya et al. 2022). The objective of this study was to understand the plant-growth promoting traits of various soil microbial isolates. That is a crucial stage before beginning developping bacterial inoculants to increase millet production in rural areas of Senegal. Enhancing the plant and soil microbiomes will also sequester carbon, regenerate as well as stabilize soils, and promote plant growth.

Materials and methods

Soil collection

Soils used in this experimentation were collected from three sites, namely (i) Kebemer in the north, (ii) Diohine from the middle of the peanut basin, and (iii) Paoskoto, located in the south peanut basin (Figs. 1 and 2). Soil temperature and rainfall data of the regions are shown in Table S1.

Soil from both a far-from-houses (bush field) and a near-to-houses (house field) were sampled for Diohine and Paoskoto. From Kebemer, a far-from-houses (bush field) was collected. All soil samples were obtained from a depth of 0–25 cm. The near-to-houses and far-from-houses soils for each collection site were eventually combined to prepare a composite soil for analysis to obtain an overall view of the properties that are characteristic of the total soil profiles rather than just the subsets separated by location.

Diohine

Soil type of the near-to-houses is Dior soil, previous crop: millet and cowpea, present crop: millet and cowpea, collection period: July 2016, coordinates: 14°29′52.9″N 16°30′46.3″W. The far-from-houses soil type is Dior soil, previous crop: cowpea, present crop: millet and cowpea, collection period: July 2016, coordinates: 14°30′05.9″N 16°31′37.6″W.

Kebemer

The soil from Kebemer was collected far-from-houses and is similar overall to Diohine soil. The soil type is Dior soil, previous crop: millet, collection period: April 2017, coordinates: 15°20′52.24″N 16°28′23.08″W.

Paoskoto

Two soil samples from Paoskoto were also obtained near-to-houses and far-from-houses. Both are Deck soils that had been used for maize cultivation. They were collected in April 2017. The near-to-houses coordinates are 13°48′11.8″N 15°48′53.2″W, and the far-from-houses coordinates are 13°48′34.3″N 15°49′08.0″W.

Soil properties

Soils collected from Kebemer, Diohine, and Paoskoto as described above were analyzed by Waypoint Analytical (Anaheim, CA 92807) for elemental analysis and other properties.

DNA extraction

DNA was directly extracted from soil samples collected from three different sites using a DNAeasy PowerSoil Pro kit (Qiagen) according to the manufacturer's protocol.

Fig. 1. A map of Africa showing the location of Senegal on the continent. The map has been designed using QGIS Desktop 3.36.2.

Illumina sequencing

Environmental DNA was sequenced by MR (Molecular Research) DNA (Shallowater, TX). Bacterial 16S rRNA gene amplicons were generated using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) flanking the V4 hypervariable region (Caporaso et al. 2011). Bacterial tag-encoded FLX 16S rDNA amplicon pyrosequencing (bTEFAP), a method for barcoded amplicon sequencing and bacterial diversity determination, was adapted for the Illumina MiSeq platform (Illumina), and sequence data were processed using the bTEFAP sequence processing pipeline (Dowd et al. 2008).

Cultivation-dependent analysis

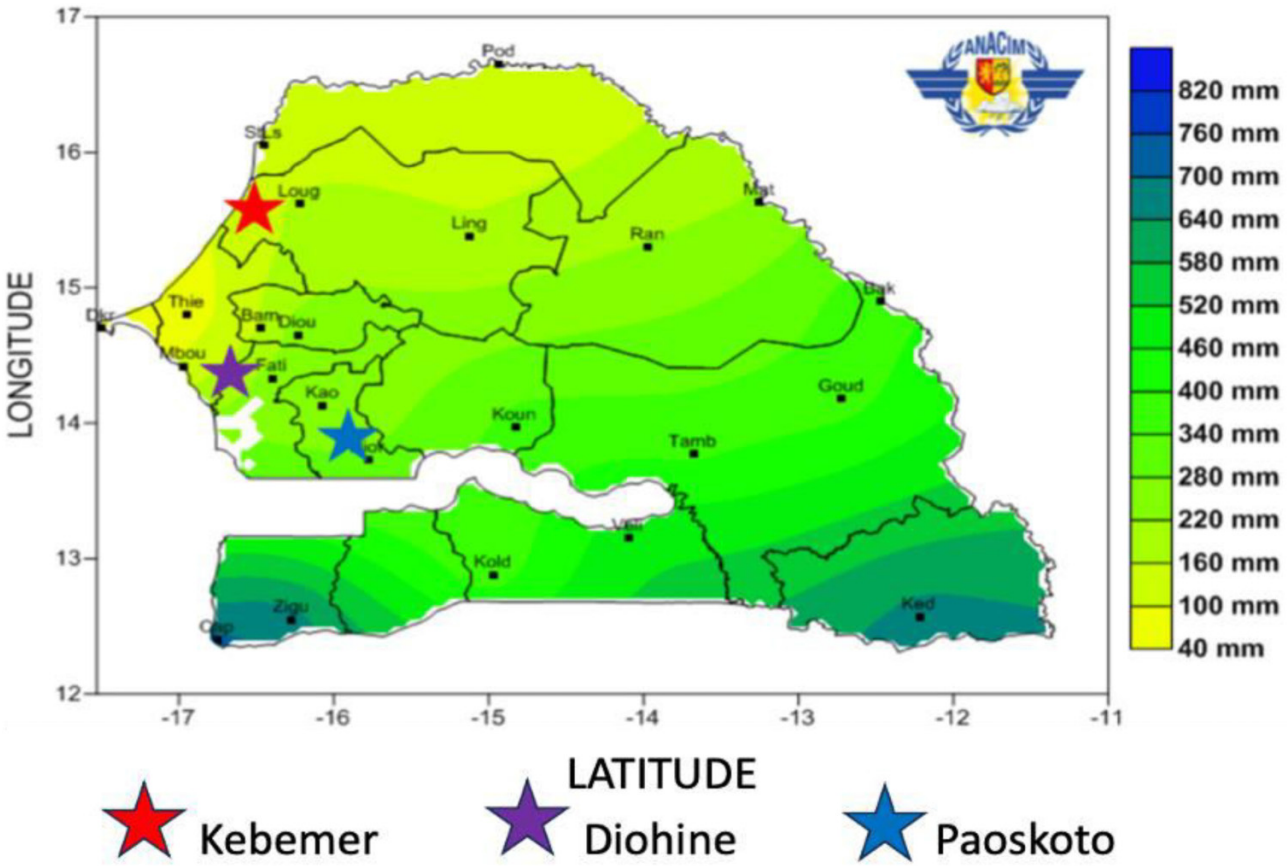
Bacterial isolation

Ten grams of soil from each site were added to 90 mL of 9% NaCl. From this original suspension, dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were prepared. One-hundred-[l of dilution was sampled from the original 10^{-2}, 10^{-4}, and 10^{-6} tubes and plated onto solidified LB (Luria-Bertani; Miller 1972), Tryptone Yeast Extract (TY; Beringer 1974) or Arabinose-Glucose (AG; Tong and Sadowsky 1994) media. Bacteria were dispersed over the plates using sterile glass beads. The plates were dried in a laminar hood and then incubated at 30° C for 3–5 days. Observations were recorded after the colonies became apparent. The colonies were picked carefully using a sterile loop and maintained, followed by their identification and characterization. The bacterial isolates were stored at −80° C in glycerol for further analysis.

Identification of bacteria

The 16S coding region of the rRNA of isolates was amplified using 2 μL of isolate DNA. The primers fD1 (5′-AGA GTT TGA TCC TGG CTC AG-3′ and rD1 (5′-AAG GAG GTG ATC CAG CC-3′) (Weisburg et al. 1991) were used for amplification. Taq polymerase (0.125 μl) as well as the dNTPs (10 mmol/L of each dNTP) and standard Taq buffer (1X) were added to the solution. A negative control without DNA was also included. The amplification was carried out using a PCR System Thermocycler according to the following program: initial denaturation for 5 min at 94° C, followed by 35 cycles of denaturation (30 s at 94 °C), hybridization of the primers (30 s at 55° C) and elongation (1 min at 72 °C), and a final elongation step (7 min at 72° C). After the PCR, the amplified material was added to 3 μL of loading dye (bromophenol blue 0.25% (v/v), glycerol 30% (v/v), EDTA 10 mm, pH 8.0). The mixture was deposited in wells of a horizontal agarose gel at 1% (w/v) containing ethidium bromide (2.5 mL per 50 mL). Gel electrophoresis was carried out in a tank containing Tris-Acetate-EDTA buffer (TAE)

Fig. 2. Study sites in Senegal, redrawn from an illustration from ANACIM (Agence Nationale de l'Aviation Civile et de la Météorologie) map. The colors indicate the differences in rainfall; the colored stars represent the collection sites.



1X, at a voltage of 120 V and an intensity of 35 mA for 20 min. The 1-kb molecular size marker (Pharmacia Biotech) made it possible both to verify the size of the amplified fragment and to determine its concentration. The bands were excised and the DNA was purified using a PureLink™ Quick Gel Extraction Kit (Invitrogen) according to the manufacturer's instructions. The 16S gene was Sanger sequenced by Laragen (Culver City, CA) using the fD1 primer. Closely related sequences in GenBank were retrieved with the Blast algorithm. Sequences obtained have been deposited in the GenBank database under the accession numbers PP125056 to PP125162.

Assays for plant growth promotion

Siderophore and phosphate solubilization activity

The bacterial isolates were qualitatively screened for siderophore production and phosphate solubilization. For the CAS plate assays, bacteria were inoculated onto the surface of Chrome Azurol S agar medium. To evaluate the ability of bacteria to solubilize phosphate in plates, Pikovskaya (PVK) medium contained per liter: glucose, 10 g; $CaHPO_4$, 5 g; $(NH_4)2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4 \cdot H_2O$, 0.002 g; and $FeSO_4 \cdot 7H_2O$, 0.002 g. A total of 20 μL of the bacterial suspension were spotted onto previously prepared plates and incubated at 28° C for 7 days. The developing orange and clear halos around the bacterial colonies after incubation were considered as a positive sign for siderophore production and phosphate solubilization, respectively. The details of the media used for cultivation of the bacterial strains for the experiments above have been previously described (Kaplan et al. 2013).

NaCl sensitivity assay

The ability of the bacteria to grow in NaCl was evaluated on LB agar plates containing 6% sodium chloride. A volume of 20 μL of bacterial culture was inoculated on plates and incubated at 28 °C for 7 days. Bacteria that grew in these conditions were evaluated as being resistant to high salinity.

Carbohydrate polymer degradation (cellulase and amylase production)

The ability of the bacteria to produce cellulase and amylase was determined in LB agar plates containing 0.5% CMC or 0.5% starch, respectively. A volume of 20 μL of bacterial culture was inoculated on the plates and incubated at 28 °C for 3 days. The CMC plates were then flooded with Congo red (1 mg/mL) for 15 min and de-stained with 1 mol/L NaCl for 15 min. Starch plates were flooded with Gram's iodine

(S013) (iodine 1 g, potassium iodide 2 g, in 300 mL of water) that stained the medium yellow to dark brown. The iodine solution was removed after 5 min. Clear halos that developed around the bacterial colonies were considered as a positive sign for cellulase or amylase production.

Seed sterilization

Sterilization procedure

Seeds of pearl millet (*Pennisetum glaucum*) and cowpea (*Vigna unguiculata*) for experiments were immersed in 95% alcohol for 1 min. After decanting the alcohol, the pearl millet and cowpea seeds were sterilized in 10% bleach for 16 and 10 min respectively, and then washed in tap water. After several rinses with sterile demineralized water, the sterilized seeds were placed in Petri dishes containing 0.9% (*w*/*v*) water agar. The plates were sealed with Parafilm and incubated at 30 °C for 2–3 days.

Plant inoculation and growth

Single inoculation on pearl millet and co-inoculation on cowpea

Pearl millet seeds were transplanted into 1 L pots containing a 2:1 mixture of sterilized vermiculite and perlite and singly inoculated with overnight cultures of bacterial isolates. One mL of culture was pipetted onto the root zone of each seedling after transplantation. Plants were grown in a greenhouse in the UCLA Plant Growth Center and irrigated with $\frac{1}{4}$-strength Hoagland's medium without nitrogen (-N). Uninoculated control pots were irrigated with Hoagland's medium with (positive control) or without (negative control) N. Soil control pots contained 10 g of Diohine soil, whereas the bacteria control pots were inoculated with known nitrogen-fixing bacterium *Azospirillum lipoferum* B-4469. All inoculated pots were irrigated with Hoagland's medium without N. Four replicate pots were prepared for each treatment and pearl millet and cowpea plants were inoculated using selected rhizobacteria: RC16 (PP125141), RD23 (PP125135), E2LB5 (PP125059), MKAG7 (PP125078), C1R2A7 (PP125121), NLR2A3 (PP125063), MKAG3 (PP125064), TYAG10 (PP125113), C1R2A3 (PP125133), MKR2A4 (PP125072). The selection was based on the plant growth-promotion assays and plants were grown in greenhouse conditions for 12 weeks.

Cowpeas were grown as described above for pearl millet, except all bacterial-inoculated plants were either singly inoculated with only *Bradyrhizobium elkanii* 20TpCR5 or co-inoculated with *B. elkanii* and selected rhizobacteria.

Pearl millet + Dietzia cinnamea 55

For the treatments, the seeds were bacterized for 3 h by imbibing them in a bacterial suspension, which had been grown for 48 h to contain approximately 10^9 CFU/mL. Post-imbibition, the seeds had an approximate 10^6 CFU/mL adherent bacterial population. Seeds imbibed in LB medium served as the negative control, and the seed treatment was done following Khan et al. (2020). Two experiments were prepared: pots grown under greenhouse conditions and the other in an outside microplot directly in soil.

The greenhouse treatments were grown in sterile Sungro Potting Mix containing mainly Canadian sphagnum peat moss along with a small fraction of coarse perlite and dolomitic limestone. Eight replicates of each treatment, with 10–12 plants in each pot, were prepared. The soil moisture was maintained to approximately 20% by adding water. The plants were 4.5 months old when harvested.

We tested the effectiveness of strain 55 on pearl millet by sowing bacterized and untreated seeds directly in the soil of the experimental microplot (2 m × 2 m) in an outside garden (Hoop house) adjacent to the Plant Growth Center, UCLA, Los Angeles. The seeds were planted in four rows, two for each treatment. Each row contained 60 plants, with an intra- and inter-row spacing of about 20 and 60 cm. Harvesting was carried out after 120 days of sowing, followed by the recording of the plant data.

Plant dry weight measurements were made after drying the plants in a 60 °C oven. The data are presented as mean ± standard deviation (SD).

Single and double inoculation on pearl millet

Using four selected rhizobacteria (RD23, RC16, E2LB5, MKAG7) from the batch above and *Dietzia cinnamea* 55, we performed a greenhouse single and double inoculations in pots, lasting 24 weeks, on pearl millet using the growth conditions described above.

Statistical analysis

Data obtained from plant growth were statistically analysed using one way Analysis of variance (ANOVA) with R software version (64 4.2.0). The Student–Newman–Keuls range test ($P < 0.05$) was performed to indicate the level of differences between the means. The statistical analysis of millet + *Dietzia cinnamea* 55 test was performed using GraphPad Prism software version 5.01 (GraphPad Software, San Diego, CA, United States).

Results

Analysis of soils from three different sites in Senegal

Temperature and rainfall

The temperatures were not very different among the three different sites of soil collection. The average temperature was measured at 26 °C in Kebemer, 27 °C in Diohine, and 28 °C in Paoskoto. In Senegal, rainfall increases from the North to the South (Table S1; Fig. 2). Paoskoto rainfall ranged from 576 to 609 mm, which was higher than that in Diohine where it was between 435 and 462 mm. The lowest amount of rainfall was measured in Kebemer and ranged between 137 and 243 mm. In both the Paoskoto and Diohine areas, there was an average-

Table 1. Factors and nutrients affecting plant growth and potential productivity of crops from different fields in Senegal.

	Diohine	Kebemer	Paoskoto
Salinity (ECe)	0.9 dS/m	0.1 dS/m	0.3 dS/m
Sodium absorption ratio (SAR)*	0.19	0.6	0.31
Boron (B)	0.08 ppm	0.04 ppm	0.11 ppm
Sodium (Na)	0.4 meq/L	0.3 meq/L	0.4 meq/L
pH, slightly acidic	6.3 s.u.	6.5 s.u.	6.2 s.u.
Total exchangeable cations (TEC)	19 meq/kg	6 meq/kg	17 meq/kg
Extractable nutrients/sufficiency factor			
Organic matter	0.79%	0.24%	1.28%
Available N	52 ppm/2.2	13 ppm/0.5	20 ppm/0.6
NO_3-N	33 ppm	1 ppm	6 ppm
NH_4-N	19 ppm	12 ppm	14 ppm
Phosphorus (P); Olsen	2 ppm/0.1	2 ppm/0.1	4 ppm/0.3
Potassium (K)	45 ppm/0.9	20 ppm/0.5	56 ppm/1.0
Calcium (Ca)	275 ppm/0.8	42 ppm/0.3	196 ppm/0.6
Magnesium	63 ppm/1.3	29 ppm/1.4	77 ppm/1.7
Copper	0.1 ppm/0.4	0.0 ppm	0.1 ppm
Zinc	1 ppm/0.7	0.0 ppm/0.4	1 ppm
Manganese	6 ppm/3.8	3 ppm/4.8	26 ppm/13.6
Iron	8 ppm/0.9	3 ppm/1.8	6 ppm/0.7
Boron	0.08 ppm/0.3	0.04 ppm	0.11 ppm

to-above average rainfall, except in the west (FEWS NET 31 August 2018, 13 November 2019).

Physical and chemical properties

None of the collected soils were particularly saline, ECs measured in the three soils were less than 1 dS/m. Both boron and sodium were negligible in the three soils (Table 1). The carbon in soils is low as shown by the level of organic matter varying from 0.24% to 1.28%. For the extractable nutrients, nitrogen levels varied in the soils. Diohine was measured as having optimal available N levels (52 ppm), whereas Paoskoto soils had only medium levels (20 ppm) of N. Kebemer soils were even lower in N (13 ppm). Generally, all three soils had very reduced levels of available phosphorus (2 ppm; Kebemer and Diohine; 4 ppm for Paoskoto). The three soils were also very low in calcium levels, but iron levels were high as is typical of a Dior soil. The pH measurements showed the soil to be slightly acidic, and none of the soils were limed. The capacity of exchangeable cations is low in the three soils demonstrating their limited capacity to retain nutrients in the soil. Details are shown in Table 1.

According the FAO classification (Deckers et al. 1998), in Kebemer soil (semi-arid peanut basin) the hydric and eolienne erosion are low and high, respectively. The salinization is null and chemical and physico-biological degradations are high and very high, respectively. Then, soils are unstructured, chemically exhausted, and vulnerable to wind deflation. In Diohine and Paoskoto soils (center and south peanut basin), hydric and erosion, and salinization are medium. However, chemical and physico-biological degradations are high. These soils are characterized by marginal acidification, compaction and loss of structure, gullying and tan formation.

Microbiological analysis

The different soils exhibited a similar eDNA profile of bacteria; Proteobacteria, Firmicutes, and Actinobacteria being the most prevalent. The two Diohine samples exhibited an almost identical pattern of bacterial sequence diversity, demonstrating that the soil collected for microbiome analysis was consistent when collected at the same site. The dominant microbes were Firmicutes, followed by Proteobacteria and Actinobacteria (Fig. 3). Less abundant phyla included Chloroflexi (*Chloroflexota*) and Planctomycetes (*Planctomycota*). Also, Planctomycetes, Acidobacteria, and Verrucomicrobia were more prominent in the Kebemer and Paoskoto soils based on eDNA analysis.

One major difference between the Pasokoto and Kebemer soils collected in 2019 was a change in the microbial profile in comparison to the 2018-collected Diohine eDNA array where Firmicute eDNA was not as prevalent. Other differences include the increased dominance of eDNA sequences from *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, *Verrucomicrobia*, and *Thaumarchaeota*.

Isolation of bacteria and their use as potential inoculants

Because only those bacteria that are culturable can be used as inoculants, we isolated bacteria from the three Senegalese soils and tested their ability to grow on plates. The most common isolates were Gram-positive *Bacillus* and

Fig. 3. Relative abundance of environmental DNA (eDNA) metagenomic sequences from soils collected in three different sites in Senegal. The eDNA analysis from the Diohine soils were repeated to verify the close correspondence of the results in two distinct runs at different times during the same year.

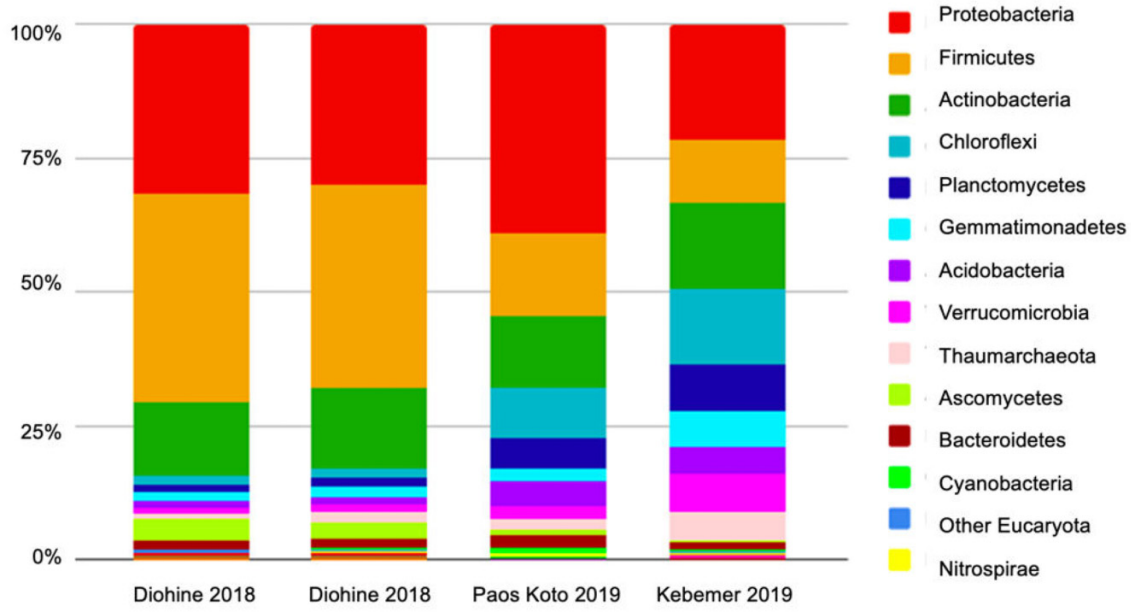


Table 2. Number of bacteria per gram of soil according to the medium used for growth.

	No. of bacteria (colony formed units per g of soil)		
Site	LB	TY	AG
Diohine bush field	2.44×10^3	1.92×10^3	3.56×10^5
Diohine house field	2.54×10^3	2.53×10^3	4.69×10^5
Paoskoto bush field	2.61×10^3	1.82×10^3	1.02×10^5
Paoskoto house field	1.56×10^5	1.17×10^5	1.15×10^5

Note: LB, Luria broth; TY, tryptone yeast extract; AG, arabinose-glucose.

Paenibacillus as well as Actinobacteria (species of *Streptomyces*, *Microbacterium*, *Arthrobacteria*, and *Cellulosimicrobium*). Many of the isolates were Gram-positive: i.e., the Firmicutes, namely *Bacillus* (41 different isolates total), as well as *Lysinibacillus* (2), *Paenibacillus* (1), and *Brevibacillus* (1). Various species of *Staphylococcus* (2) and *Actinobacteria* (20), especially isolates of *Streptomyces*, *Microbacterium*, *Arthrobacteria*, and *Cellulosimicrobium*, were identified from Senegalese soils in addition to two Cytophaga, of the family *Hymenobacteraceae* and genus *Rufibacter*. Of the *Proteobacteria*, 3 *Alphaproteobacteria* and 3 *Gammaproteobacteria* were isolated, representing diverse families that included *Acetobacteraceae, Methylobacteriaceae* and *Sphingomonadaceae,* and *Oxalobacteraceae, Enterobacteriaceae* and *Rhodanobacteraceae,* respectively. A complete list of the soil isolates from all three collected soils, Diohine (orange), Kebemer (yellow), and Paoskoto (blue), is presented in Fig. S1 and Table S2.

Our results showed that bacteria grow better in AG medium (Table 2). In the four soils tested, the number of bacteria per g of soil reached 10^5 in comparison to other culture media where the number was generally 10^3 except for the bacteria grown in LB and TY from the Paoskoto near-to-houses field. Regarding the types, the near-to-houses fields seem to contain a higher number of bacteria. In Diohine, the number of bacteria was also higher in the near-to-houses field even if the difference was not statistically robust. However, in Paoskoto, a notable difference was observed between the near-to-houses and far-from-houses field samples when the bacteria were cultured in LB and TY media.

Physiological potential of Senegal soil isolates for plant growth promotion

A subset of the soil isolates was tested for their potential plant growth-promoting abilities such as the ability to produce siderophores (CAS), solubilize phosphate (PVK) (Fig. 4), degrade cellulose (CMC), tolerate salt (up to 6% NaCl), and digest starch. Of 17 isolates identified and tested, the vast majority were able to solubilize rock phosphate and a large number grew on culture medium containing 6% salt. On the other hand, very few of the isolates degraded starch or hydrolysed carboxymethyl cellulose (CMC) or produced siderophores (Table 3).

Plant inoculation tests

Single inoculation on pearl millet and co-inoculation on cowpea

Pearl millet and cowpea plants were inoculated and compared with uninoculated (negative) and soil-inoculated plants, as well as two positive control strains, *A. lipoferum* B-4469 for pearl millet and *B. elkanii* 20TpCR5 for cowpea, and a + N positive control (Table 4). For pearl millet, rhizobac-

Fig. 4. Plate assays. *Left*. siderophore activity in two of four different isolates from soils in Diohine, Senegal. *Middle*, PVK assay using bromocresol purple results in a color change as well as clearing around the colonies, which indicates phosphate-solubilizing activity that is likely due to organic acid production. *Right*, PVK assay. A clear region around the colonies indicates removal of a phosphate ion from the cloudy medium containing $CaHPO_4$.

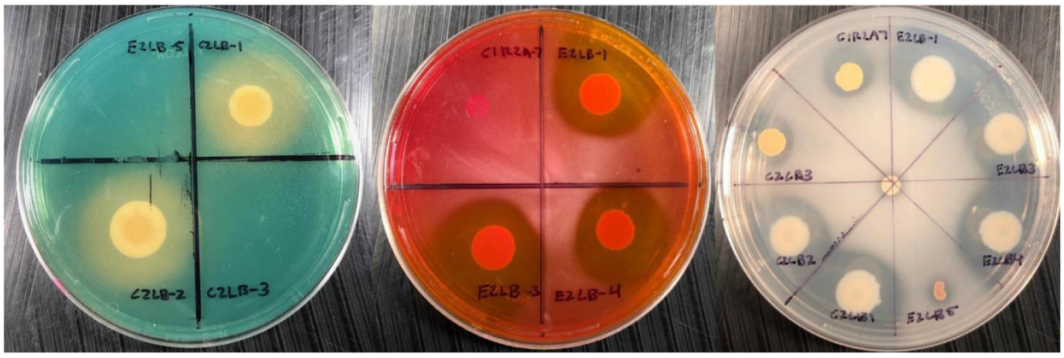


Table 3. Plant growth promoting properties of bacterial isolates from Senegalese soils.

Soil isolate	Strain No./accession number	CAS	PVK	CMC	Growth on 6% NaCl	Starch degradation
3 isolates of *Neobacillus cucumis*	MKAG4 (PP125129)	–	+	–	–	–
	MKR2A6 (PP125130)	–	+	–	–	–
	TYK7 (PP125131)	–	+	N.D.	+	–
Cytobacillus depressus	NLLB3 (PP125122)	+	–	+	–	–
Neobacillus drentensis	MKLB2 (PP125136)	N.D.	+	+	–	–
2 isolates of *Priestia aryabhattai*	MKAGI (PP125144)	–	+	–	–	–
	MKR2A5 (PP125145)	–	+	–	+	–
Neobacillus niacini	AVR2A2 (PP125140)	–	+	N.D.	–	–
Bacillus pumilus	C2R2A1 (PP125098)	–	+	+	+	N.D.
1 isolates of *Bacillus subtilis*	TYLB9 (PP125106)	+	+	N.D.	+	+
2 isolates of *Bacillus tequilensis*	TYLB11 (PP125115)	+	+	ND	+	+
	NLAG2 (PP125111)	–	+	+	+	+
Paenibacillus pueri	MKAG2 (PP125152)	–	+	–	–	+
Pseudarthrobacter phenanthrenivorans	MKAG7 (PP125078)	–	+	–	+	–
Cellulosmicrobium cellulans	MKLB1 (PP125079)	N.D.	+	+	+	–
Microbacterium zeae	MK R2A4 (PP125072)	–	+	–	–	–
Streptomyces fodineus	NL R2A1 (PP125058)	–	+	+	–	N.D.

Note: N.D., not determined.

terium *Peribacillus asahii* RC16 significantly increased plant shoot length by 17%, 16%, and 14% over the negative control, soil-inoculated plants, and plants inoculated with *A. lipoferum* B4469, respectively. No significant difference in dry weight was found between the rhizobacterial treatments and the negative, soil, and *A. lipoferum* sets.

For cowpea co-inoculation, rhizobacterium *Pseudarthrobacter phenanthrenivorans* MKAG7 co-inoculated with *B. elkanii* 20TpCR5 significantly increased plant shoot length by 88%, 51%, and 30% compared to the negative control, the treatments that were inoculated with soil and with *B. elkanii* 20TpCR5 alone, respectively. All the rhizobacteria co-inoculated with *B. elkanii* 20TpCR5 tested significantly improved cowpea dry weight compared to the controls, except for strain *Streptomyces nogalater* MKAG3. The same result was observed for pod weight. However, strains MKAG7 and RC16 separately co-inoculated with *B. elkanii* 20TpCR5 did not significantly increase pod weight compared to *B. elkanii* 20TpCR5 alone.

Pearl millet + *Dietzia cinnamea* 55 single inoculation

Inoculation results are displayed in Table 5 and Fig. 5. The *Dietzia cinnamea* strain 55 increased pearl millet biomass in both the greenhouse as well as the experimental microplot conditions. In the greenhouse experiments, pearl millet inoculated with *Dietzia cinnamea* 55 showed a 13.52% increased dry shoot biomass over that of the control. For the soil microplot experiment, *Dietzia cinnamea* 55 improved both shoot and seed head biomass by 16.4% and 12.9% over the control plants, respectively.

Table 4. Effect of inoculation with different rhizobacterial strains on shoot length and dry weight of pearl millet and shoot length, dry weight, and pod weight of cowpea plants grown for 12 weeks in greenhouse conditions.

	Pearl millet		Cowpea		
Treatments	Shoot_length (cm)	Dry_weight (g)	Shoot_length (cm)	Dry_weight (g)	Pod_weight (g)
Negative_control	27.2 ± 2.7c	0.75 ± 0.18b	8.9 ± 1.7c	0.6 ± 0.23d	0.0 ± 0.0c
Positive_control	60.8 ± 12.8a	3.87 ± 1.70a	51.4 ± 31.1abc	1.5 ± 0.33c	0.15 ± 0.18c
Soil	25.4 ± 2.1c	0.82 ± 0.28b	15.5 ± 11.2bc	1.8 ± 1.12bc	0.01 ± 0.02c
*A. lipoferum*_B4469/*B. elkanii*20TpCR5	28.6 ± 6.4c	0.99 ± 0.21b	26.4 ± 21.3bc	1.8 ± 0.53bc	0.41 ± 0.71bc
RC16 (PP125141)	39.9 ± 4.8b	1.55 ± 0.63b	53.9 ± 38.9ab	3.1 ± 1.18a	0.73 ± 0.53ab
RD23 (PP125135)	35.4 ± 2.7bc	1.33 ± 0.08b	24.8 ± 24.4bc	3.1 ± 0.92a	1.46 ± 0.41a
E2LB5 (PP125059)	32.6 ± 2.9bc	1.16 ± 0.31b	46.5 ± 29.7abc	3.4 ± 1.39a	1.36 ± 0.31a
MKAG7 (PP125078)	29.1 ± 5.2c	1.38 ± 0.38b	78.7 ± 20.16a	3.1 ± 0.84a	0.91 ± 0.45ab
C1R2A7 (PP125121)	33.1 ± 3.7bc	1.06 ± 0.46b	55.7 ± 42.1ab	3.2 ± 0.55a	1.11 ± 0.58a
NLR2A3 (PP125063)	31.8 ± 7.0bc	1.18 ± 0.59b	59.9 ± 32.8ab	3.1 ± 0.84a	1.37 ± 0.75a
MKAG3 (PP125064)	29.5 ± 4.5c	1.02 ± 0.46b	36.1 ± 31.5bc	2.6 ± 0.6ab	1.21 ± 0.28a
TYAG10 (PP125113)	29.4 ± 2.4c	0.91 ± 0.31b	41.6 ± 23.6abc	3.5 ± 1.04a	1.11 ± 0.79a
C1R2A3 (PP125133)	28.9 ± 1.8c	1.10 ± 1.30b	23.1 ± 17.7bc	3.3 ± 0.45a	1.22 ± 0.24a
MKR2A4 (PP125072)	27.1 ± 5.2c	0.88 ± 0.33b	23.6 ± 32.9bc	3.2 ± 0.66a	1.26 ± 0.75a

Note: For each parameter measured in plant species tested, means of values followed by the same letter on each column are not significantly different according to Newman–Keuls test at 5%. Negative and positive control plants were uninoculated. Pearl millet was singly inoculated with bacterial strains, while cowpea was single inoculated with *B. elkanii* or co-inoculated with *B. elkanii* and selected rhizobacteria.

Table 5. Pearl millet plants shoot and seed head biomass after inoculation with *Dietzia cinnamea* 55 in greenhouse and hoophouse soil microplot conditions.

Treatments	Pots millet dry shoot biomass (g)*	Hoophouse millet dry shoot biomass (g)†	Hoophouse millet Seed head dry weight (g)‡
Control	31.4 ± 8.3	52.9 ± 9.1	10.8 ± 4.1
D. cinnamea 55	35.6 ± 7.1	61.6 ± 8.2	12.2 ± 5.4

*Values are means ± SD of 50 plants each at the time of harvesting. Data recorded after 4.5 months of experimental setup. We do not have any harvest data because plants did not develop grains. Plant biomass data was statistically evaluated using unpaired t-test, $P < 0.05$.
†Values are means ± SD of 50 plants each at the time of harvesting.
‡Values are means ± SD of 100 seed heads each at the time of harvesting.
Note: Plant biomass data was statistically evaluated using unpaired t-test, $P < 0.05$. Data recorded after 4 months of experimental setup.

Single and double inoculation on pearl millet

A double inoculation of *Dietzia cinnamea* 55 + MKAG7 significantly increased shoot length, dry weight, and seed head weight over that of uninoculated control (Table 6).

Discussion

In response to climate change, Senegal has been proactive in enhancing soil health by adopting agroecological practices to safeguard its agricultural lands and food production. The detrimental impact of soil degradation due to the persistent use of synthetic pesticides and fertilizers prompted the Senegalese government to adopt a more holistic approach. However, to our knowledge there still appears to be a lack of research examining the soil's current condition and whether its nutrient content or structure can be rehabilitated through the introduction of specific microorganisms, including bacteria and fungi. So far, we do not know for sure what is the best strategy to employ, but our goal was to obtain a window into the identities of culturable microbes that could serve as plant growth promoting-bacteria. To begin to find a solution, we did metagenomic analysis of soil from the groundnut basin in Senegal. Our findings revealed the presence of 14 bacterial phyla, indicating a lower diversity compared to Janssen's 2006 study, which identified at least 32 phylum-level groups from 16S rRNA genes in soil bacteria. Our study primarily identified Proteobacteria, Firmicutes, and Actinobacteria as the most common phyla across all samples. These, along with Acidobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, and Gemmatimonadetes, accounted for an average of 92% of the published soil libraries. Notably, Proteobacteria and Acidobacteria were found to be the most prevalent soil bacteria, as determined by the frequency of 16S rRNA and their genes, echoing Janssen's findings (2006).

Our results showed differences between bacterial phyla in the three sites sampled. Diohine soils display more abundance of Firmicutes, whereas Kebemer and Paoskoto soils had more Protobacteria. This may be explained in part by differing agricultural practices at each site. While intercropping and crop rotation represent the most common practices in the region, variation in crop management in each zone may be responsible for the differences noted in microbial composition. Similar to our results, anthropic insults have been shown to influence the microbial community taxonomic composition in other soils (Galązka et al. 2018;

Fig. 5. Pearl millet plants after inoculation with *Dietzia cinnamea 55* in greenhouse (A) and hoophouse soil microplot conditions (B) with seed head (C).

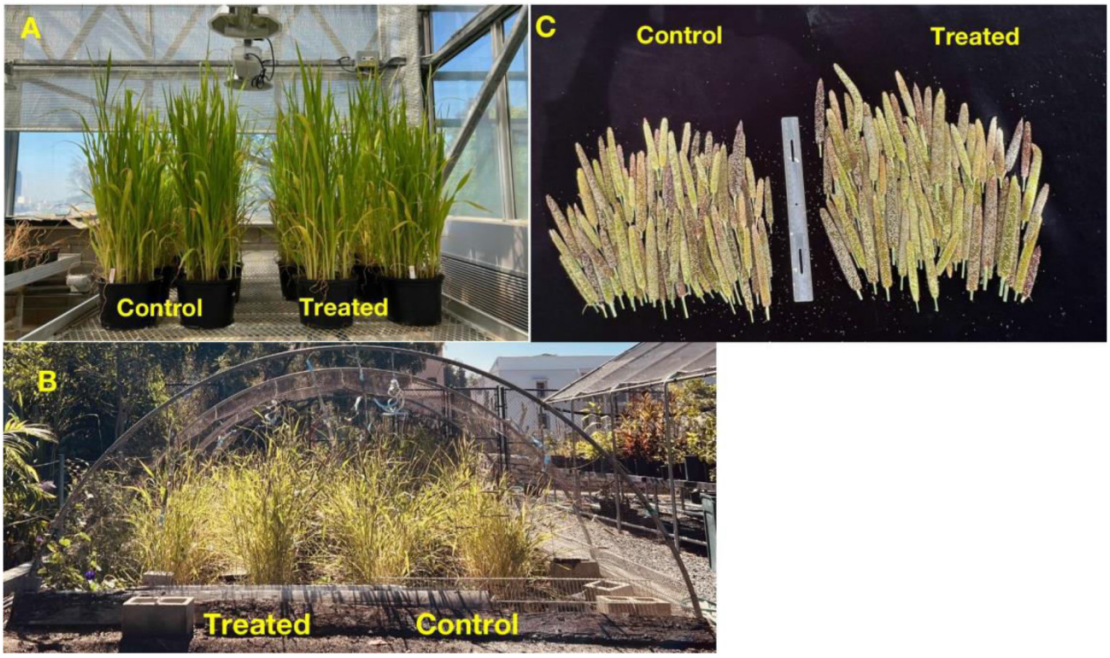


Table 6. Single and double inoculation using different rhizobacterial strains on dry weight, shoot length, and seed head weight of pearl millet plants grown for 24 weeks in greenhouse conditions.

Treatments	Shoot_length (cm)	Dry_weight (g)	Seedhead_weight (g)
Uninoculated	114.2 ± 41.3b	4.7 ± 2.8b	1.0 ± 0.6b
D. cinnamea 55	109.3 ± 36.4b	5.6 ± 2.8b	1.2 ± 0.77b
RD23 (PP125135)	117.4 ± 42.7b	7.1 ± 3.1b	1.6 ± 0.76b
RC16 (PP125141)	113.9 ± 34.5b	5.1 ± 2.6b	0.9 ± 0.59b
E2LB5 (PP125059)	127.2 ± 26.3b	6.7 ± 1.9b	1.2 ± 0.56b
MKAG7 (PP125078)	113.5 ± 33.6b	5.7 ± 2.8b	1.3 ± 0.85b
Dietzia_cinnamea 55 + RD23	115.4 ± 32.6b	5.8 ± 2.6b	1.1 ± 0.58b
Dietzia_cinnamea 55 + RC16	115.8 ± 28.9b	6.1 ± 2.7b	1.1 ± 0.51b
Dietzia_cinnamea 55 + E2LB5	127.1 ± 24.9b	5.9 ± 2.2b	1.3 ± 0.66b
Dietzia_cinnamea 55 + MKAG7	150.4 ± 33.1a	9.6 ± 3.6a	2.0 ± 1.05a

Note: For each parameter tested, means of values followed by the same letter on each column are not significantly different according to Newman–Keuls test at 5%.

Bonomo et al. 2022). Furthermore, it was demonstrated that land-use modes could toughly alter soil microbial assembly processes and co-occurrence network patterns (Yu et al. 2022). Thus, land use is correlated with the nutrient availability that is a major driving force of microbiome composition and diversity in soils as described by Garrido-Sanz et al. (2023).

However, our results display a different response in terms of bacterial community diversity to soil physicochemical characteristics than in some studies. Thomson et al. (2015) demonstrated the impact of soil characteristics in shifting the soil microbial communities. Soil pH and nutrient contents, especially in terms of C, N, and P availability, are crucial factors (Ramirez et al. 2010; Tan et al. 2013) and significantly affect microbial abundance (Lauber et al. 2008; Zhong et al. 2010). In this study, land use is roughly the same except there is a difference regarding types of crops. In the north of the groundnut basin (Kebemer) and the center (Diohine), farmers grow pearl millet, peanut, and cowpea. In the south (Paoskoto), farmers are mostly cropping maize and pearl millet. Similar to our results, other studies have shown microbial community composition is dependent on land use (Merino-Martín et al. 2021), such that organisms with advantageous life-history strategies will become dominant (Fierer et al. 2007). Thus, land use change can generate gradual and cumulative soil disturbance, resulting in soil environments being associated with different land use patterns. This finding corroborates several results in soil microbial community taxonomic composition that display differences because of anthropic insults (Ahmed et al. 2018; Galązka et al. 2018; Bonomo et al. 2022).

Our results also demonstrate that mostly gram-positive bacteria were isolated from Senegal soils. The most common isolates were Gram-positive *Fermicutes* (*Bacillus* and *Paenibacillus*) as well as *Actinobacteria* (species of *Streptomyces*, *Microbacterium*, *Arthrobacteria*, and *Cellulosimicrobium*). The *Firmicutes* associated with plants primarily belong to the classes *Bacilli*, *Clostridia*, *Erysipelotrichi*, *Thermolithobacteria*, and *Mollicutes* (Francis et al. 2010). Common *Actinobacteria* soil inhabitants include *Micromonospora* and *Streptomyces* species and plant commensals (e.g., *Frankia* spp.) (Barka et al. 2016).

Most gram-positive isolates were able to solubilize rock phosphate. Gram-positive bacteria have been described as possessing phosphate solubilization and mineralization abilities (Hanif et al. 2015; Wang et al. 2017). Our findings align with the research conducted by Wang et al. (2022), who demonstrated that certain bacteria, including two Firmicutes-*Bacillus safensis* and *Falsibacillus pallidus*-along with a Proteobacteria, *Pseudomonas moraviensis*, have the capability to solubilize stable and moderately labile phosphorus (P) fractions. This process is crucial for making phosphorus available to plants, which is often locked in insoluble forms in the soil (Francis et al. 2010). Bacterial strains like Bacilli that produce phytase and organic acids are reported to be effective phosphate solubilizers (Vazquez et al. 2000; Ma et al. 2015; Kosty et al. 2020). However, others Gram-positive strains displaying phytase production have been reported (*Brevibacterium*, *Sarcina*, *Paenibacillus*, *Corynebacterium*, and *Micrococcus* strains) (Jorquera et al. 2008).

Four *Bacilli* (1 *C. depressus,* 1 *P. aryabhattai and* 2 *B. subtilis* isolates) showed siderophores production that is crucial for scavenging iron from environmental stocks and delivering it to plants. Similarly, Ma et al. (2015) showed that *Bacillus* is the most abundant genus in soil that is known for siderophore production. Interestingly, in addition to being described as iron transporters, siderophores are decisive mediators of interactions between members of microbial communities and their eukaryotic hosts (Kramer et al. 2020).

The capacity of microorganisms to produce hydrolytic enzymes makes them promising candidates for hydrolysis of complex polymeric substrates (Bhagat and Kokitkar 2021). Cellulose represents one of the most abundant wastes originating from agriculture and industries (Prasad et al. 2014). Our results showed that 4 *Bacilli* (*C. depressus*, *N. drentensis*, *B. pumilus*, *B. subtilis*), 1 *Cellulosmicrobium*, and 1 *Streptomyces* had cellulase activities. Previous studies described production of cellulase in *Bacillus* isolates (Acharya and Chaudhary 2012; Gautam et al. 2012; Bhagat and Kokitkar 2021; Yao et al. 2022). We also analysed the ability of isolates to degrade starch using the starch iodine method. Results showed that 3 isolates of *Bacillus subtilis* and 1 of *Paenibacillus pueri* had amylase activities. Similar results were described for *Paenibacillus amyloliquefaciens* producing amylase to decompose starch (Gong et al. 2023) and in *Bacillus* species as well (Fasiku et al. 2020).

In our study, we found that the rhizobacteria strains RC16 and MKAG7 notably boosted the growth of pearl millet and cowpea shoots, outperforming other controls such as *A. lipoferum* B4469 and *B. elkanii* 20TpCR5. Similarly, *Dietzia cinnamea* 55 was previously shown to improve corn growth and yield in UCLA experiments (Khan et al. 2020). This bacterium also increased the biomass of pearl millet's dry shoots and seed heads under greenhouse and microplot conditions. *Bacillus* sp. MN54 was observed to enhance pearl millet's grain count, weight, and overall yield (Majeed et al. 2022), while *Enterobacter* sp. MN17 and *Stenotrophomonas maltophilia* FA-9 improved the grain quality in field trials (Dawood et al. 2023). Notably, dual inoculation with *Dietzia cinnamea* 55 and MKAG7 significantly elevated shoot length, dry weight, and seed head weight compared to controls and other inoculation combinations. Additionally, a mix of *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Acetobacter* sp., and *Bacillus megaterium* resulted in the highest number of tillers, longest roots, and ear heads when applied at the flag leaf and flowering stages, surpassing the effects of single treatments as reported by Latake et al. (2009).

Conclusion

Relative abundance of environmental DNA (eDNA) metagenomic sequences from soils collected in three different sites in Senegal revealed that Proteobacteria, Firmicutes, and Actinobacteria were the most prevalent. However, the most common bacteria isolated from these soils were Gram-positive *Bacillus* and *Paenibacillus* as well as Actinobacteria. Selected rhizobacterial isolates showed remarkable PGP traits. Interestingly, single inoculations on pearl millet and double inoculations on pearl millet and cowpea revealed that some rhizobacteria isolated from Senegalese soils have the potential to sustainably increase pearl millet and cowpea growth and agricultural productivity in greenhouse and microplot conditions. However, these effects need to be further tested and confirmed in field conditions.

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Supplementary material

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