

Acknowledgements

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Dedication

أكتب هذه الكلمات اليوم لكي أقرأها كلما شعرت بأن الضوء في آخر النفق لن يظهر، ما كان بالأمس حلما أصبح اليوم حقيقة، وها أنا ذا أكتب اهداء أطروحتي للدكتوراه، تلك الرحلة التي ظننت أنها لن تنتهي، سأقطف ثمارها اليوم بإذن الله، قد تبدو نهاية الحكاية ولكنها بداية أجمل فصولها.

سأهدي هذا التخرج لنفسه قبل كل شيء، شكرا لأنك كنت قوية، شكرا على الجهد والمثابرة إلى رفيقة دربي

بشرى....

إلى الذي زين اسمي بأجمل الألقاب، من دعمني بلا حدود وأعطاني بلا مقابل... من علمني أن الدنيا كفاح وسلاحه العلم والمعرفة داعمي الأول في مسيرتي وسندي

وقوتي وملأني بعد الله

إلى فخري واعتزازي

أبي محمد.....

إلى من جعل الله الجنة تحت أقدامها واحتضني قلبها قبل يدها

وسهلت لي الشدائد بدعائها إلى القلب الحنون والشمعة التي

كانت لي في الليالي المظلمات

سر قوتي ونجاحي

أمي فاطمة.....

إلى ضلعي الثابت وأمان أيامي

إلى من شددت عضدي بهم فكانوا لي ينابيع أرتوي منها

إلى خيرة أيامي وصفوتها إلى قرة عيني

إخوتي.. وأخواتي الغاليات: وردة، أمينة، مصطفى، هاجر، مريم، شافية، عصماء..

جدتي الحبيبة.... الزهرة

زوجة أخي... صفية. أزواج أخواتي... إبراهيم (رحمة الله عليه)، جمال، عبد الكريم، عبد القادر، عبد الكريم

صديقتي الغالية... زهراء

أولاد وبنات أخوتي وأخواتي: محمد عبد الغني، سهام، مروة، محمد، أحمد، سجود، ملاك،

فاطمة الزهراء، محمد اسلام، نفيسة، مارية، مصعب، مرام، اياد

وكل من عائلة لعور وبن ساحة

لكل من كان عوناً وسنداً في هذا الطريق... للأصدقاء الأوفياء

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إلى من أفاضني بمشاعره ونصائحه المخلصة

...أهديكم هذا الإنجاز وثمره نجاحي الذي طالما تمنيته...

ها أنا اليوم أكملت واتيتمت أجمل فصوله بفضل سبائه وتعالى

فالحمد لله على ما وهبني.

2022/2026

List of Abbreviations and Acronyms

Rhizosphere soil	RS
Bulk soil	BS
Electrical conductivity	EC
Organic matter	OM
Available phosphorus	AP
Mineral nitrogen	NO ₃
Total nitrogen	TN
Soluble sodium	SNa
Total sodium	TNa
Soluble potassium	SK
Total potassium	TK
Plant growth promoting rhizobacteria	PGPR
Total limestone (calcium carbonate)	CaCO ₃
Magnesium Oxide	MgO
Ammonium Acetate	NH ₄ OAc
Lithium Chloride	LiCl
Volatile Organic Compounds	VOCs
Plant Growth Promoting	PGP
Arbuscular Mycorrhizal Fungi	AMF
Sulfuric acid	H ₂ SO ₄
Sodium hydroxide	NaOH
Boric acid	H ₃ BO ₃
Ammonia	NH ₄
Hexadecyltrimethylammonium	Fe-CAS-HDTMA
Hydrogen Peroxide	H ₂ O ₂
Principal Component Analysis	PCA
Coefficient Of Variation	CV

Milliliter	ml
Microsiemens per centimeter	$\mu\text{S}\cdot\text{cm}^{-1}$.
Parts per million	Ppm
Honestly Significant Difference	HSD
Percent	(%)
Analysis of Variance	ANOVA
Analytical Profile Index	API
Perchloric acid	HClO_4
Nitric acid	HNO_3
Nutrient Agar	GN
Ashby's Mannitol Agar	AM
Yeast Mannitol Agar	YMA
Tricalcium Phosphate	$\text{Ca}_3(\text{PO}_4)_2$
Chrome Azurol S	CAS
Lysogeny Broth	LB
Sodium Bicarbonate	NaHCO_3
Plant Growth Promoting	PGP
Passenger Endophytes	PE
Gram	G
Adenosine Triphosphate	ATP
Tetramethyl-p-phenylenediamine	TMPD
Molar	M
Ribosomal Ribonucleic Acid	rRNA

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INTRODUCTION

Introduction

Agricultural production is increasingly challenged by rapid population growth, limited arable land, and the intensification of both biotic and abiotic stresses such as drought, salinity, nutrient deficiency, and extreme climatic conditions (Bharti & Barnawal, 2019). Feeding the growing population and enhancing agricultural productivity under these constraints represent significant challenges for researchers and farmers worldwide. Conventional agricultural practices, particularly the excessive use of chemical fertilizers and pesticides, have improved crop yields but have also contributed to soil degradation, environmental pollution, and biodiversity loss (Wang *et al.*, 2019; Xing *et al.*, 2025).

In addition, modern agriculture has disrupted natural soil processes, leading to reduced soil fertility, microbial imbalance, and long-term ecosystem instability. Continuous monocropping systems, intensive tillage, and improper irrigation practices have accelerated soil erosion, salinization, and nutrient depletion, especially in fragile ecosystems such as arid and semi-arid regions. These issues highlight the urgent need for sustainable agricultural approaches that maintain productivity while minimizing environmental impact (Hou *et al.*, 2020; Shokri *et al.*, 2025).

Soil is a fundamental, nonrenewable resource that supports plant growth and ecosystem functioning. It acts as a reservoir for nutrients and water, providing essential elements required for plant development. The formation and fertility of soil are governed by complex interactions among physical, chemical, and biological processes influenced by climate, parent material, topography, and living organisms (Schulz *et al.*, 2013; Semenov & Đukić, 2020).

Soil performs multiple ecosystem services, including nutrient cycling, carbon sequestration, water regulation, and habitat provision for microorganisms. These functions are essential for maintaining agricultural productivity and environmental sustainability. The biological component of soil, particularly microbial communities, plays a central role in driving these processes by mediating biochemical transformations and supporting plant growth (Baquerizo *et al.*, 2025; Philippot *et al.*, 2024).

However, soil systems are highly heterogeneous, and their properties vary across spatial and temporal scales due to natural factors and agricultural practices. This heterogeneity significantly affects plant nutrient acquisition, microbial distribution, and overall ecosystem functioning (Kathumno, 2007; Zhou *et al.*, 2012).

Spatial variability refers to the uneven distribution of soil physical, chemical, and biological properties across space. It is a fundamental characteristic of terrestrial ecosystems and a critical determinant of agricultural productivity. Variations in soil influence water availability, nutrient dynamics, root development, and ultimately crop performance (Delgado & Gómez, 2024).

The geographical variability of soil properties is a significant aspect of modern agricultural research and environmental management. Understanding how soil characteristics vary across landscapes is essential for implementing precision agriculture techniques, maximizing resource use, and maintaining agricultural systems. By identifying areas that require specific treatments, like targeted fertilization or soil amendments, mapping spatial variability may improve sustainable agroecosystem management (Acir, 2025; Córdoba *et al.*, 2025).

In arid and semi-arid regions, spatial variability is particularly pronounced due to irregular rainfall patterns, limited vegetation cover, and uneven distribution of organic matter. Small variations in topography can result in significant differences in water accumulation and nutrient availability, leading to patchy plant growth and irregular microbial activity. The effectiveness of plant nutrient acquisition decreases as nutrient availability becomes limited. Therefore, understanding spatial variability is essential for optimizing resource use and improving agricultural productivity (Maestre & Cortina, 2002; Miransari, 2013; Salama *et al.*, 2025).

Soil is considered a crucial area of scientific inquiry, with the rhizosphere being its most active part. Its active microbial community and its interactions with plant roots make it a target for biotechnological applications aimed at improving crop yields and soil health (Roy *et al.*, 2020; Zodge *et al.*, 2024). Understanding these processes better is essential for

preserving the plant cover in arid and semi-arid regions. The rhizosphere, defined as the narrow zone of soil surrounding plant roots, represents one of the most dynamic and biologically active environments in the soil ecosystem (Cantó *et al.*, 2020). It serves as a hotspot for microbial activity and a central interface for interactions between plant roots, soil, and microorganisms (Mendes *et al.*, 2013).

Root exudates, including sugars, amino acids, organic acids, and secondary metabolites, enrich the rhizosphere and create a nutrient-rich environment that supports diverse microbial communities. These microorganisms, collectively referred to as the root microbiome, inhabit the rhizoplane and root endosphere and play a crucial role in plant growth and soil processes (Salwan *et al.*, 2023).

The rhizosphere is characterized by high microbial density and activity compared to bulk soil. Microbial populations in this region can be several times higher due to the continuous supply of nutrients from plant roots. This makes the rhizosphere a key zone for studying plant-microbe interactions and nutrient cycling processes (Maryani & Rogomulyo, 2021).

Plant-rhizosphere interactions are fundamental to plant growth and ecosystem functioning. Plants release root exudates that attract and selectively recruit beneficial microorganisms, while microorganisms influence plant growth through nutrient mobilization, hormone production, and protection against pathogens (Jamil *et al.*, 2022; Hnini *et al.*, 2025).

Environmentally sustainable methods have encouraged the utilization of beneficial soil microorganisms, which improve nutrient absorption, plant health, and resilience to stress (Kumar & Chandra, 2020). Biofertilizers, which utilize microbial activity to convert organic matter into plant-available nutrients, are increasingly recognized as eco-friendly alternatives to chemical fertilizers (Santhosh *et al.*, 2018). Sustainable agriculture, therefore, aims to meet present needs while ensuring long-term ecological balance (Santoyo *et al.*, 2017).

The use of legumes is one of the techniques commonly employed in agroecology to maintain soil fertility, where a positive agronomic effect has been observed in crop rotations that include a legume (Duc, 2010). The fabaceae or Leguminosae, commonly known as the legume family; including peas, beans, lentils, cowpeas, peanuts, etc. Comprising approximately 19,000 species, form the largest plant family in the world and play an important ecological role. Leguminous plants are highly diverse in terms of their morphology, habitat, and ecology (Rivas, 2009). It includes trees, shrubs, and perennial or annual herbaceous plants, easily recognizable by their fruits (legumes) and their compound, stipule-bearing leaves (Judd, 2002). The fabaceae are classified in the subkingdom Plantae, division Magnoliophyta, class Magnoliopsida, and order Fabales (Petit-Aldana, 2014). It is a large and economically important family of flowering plants (Judd, 2002). Their importance as food crops is significant worldwide (Rivas, 2009), but it is not limited to their high protein content. Many scientific studies report the health benefits of consuming a plant-based diet and increasing dietary intake of legumes (Walrand & Rémond, 2017).

As global temperatures rise and rainfall patterns become more unpredictable, the demand for crops that can maintain high photosynthetic rates under stress is likely to increase. Legumes, with their traits for biological nitrogen fixation, may better sustain their photosynthetic activity under adverse conditions, offering a potential buffer against the negative impacts of climate change on crop yields in Africa (Dave *et al.*, 2024).

The legume-rhizobia symbiosis is the most significant biological nitrogen fixation (BNF) process in agriculture, as it can supply up to 80% of the nitrogen required by leguminous crops, thereby reducing reliance on synthetic nitrogen fertilizers (Kebede, 2021).

BNF is a key microbial process that converts atmospheric nitrogen into ammonia, making it available for plant uptake (Dakora *et al.*, 2008). Nitrogen is one of the most important mineral nutrients for plants as it is an integral part of most of the plant physiological processes including photosynthesis and protein synthesis (Alori *et al.*, 2017).

The members of Phyto microbiome, have the capability to substantially reduce the need for soil nitrogen supplements either by fixing atmospheric nitrogen directly through legume-rhizobium interaction or indirectly, by assisting the nitrogen fixers through their

secretions (Naamala & Smith, 2020).

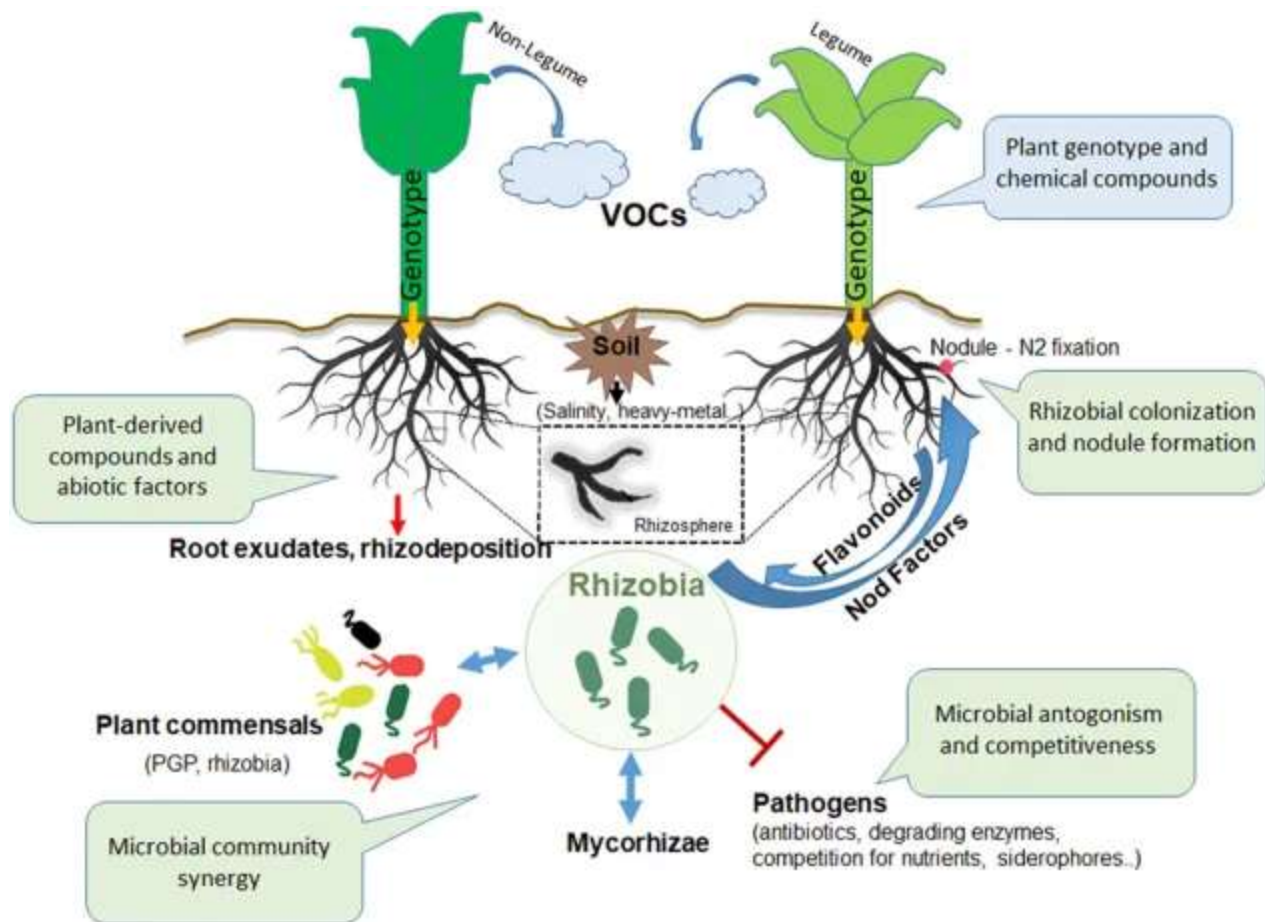
Nitrogen-fixing bacteria (diazotrophs) are generally classified according to their mode of association with plants into three main groups: symbiotic, associative/endophytic, and free-living diazotrophs. Symbiotic diazotrophs establish specialized interactions with their host plants, typically forming nitrogen-fixing root nodules in legumes. This group includes genera such as *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Frankia*, *Burkholderia*, *Azoarcus*, and some *Achromobacter* strains (Babalola, 2010; Pérez-Montaña et al., 2014; Turan et al., 2016). Associative or endophytic diazotrophs colonize the rhizosphere or internal plant tissues without forming specialized nodules and contribute to plant nutrition through biological nitrogen fixation. Representative genera include *Azoarcus*, *Herbaspirillum*, *Gluconacetobacter*, *Azospirillum*, and *Enterobacter*. Free-living diazotrophs inhabit the soil independently of plant tissues and include genera such as *Azotobacter*, *Clostridium* and *Beijerinckia* (Vessey, 2003).

Leguminous plants have a unique ability to form symbiotic relationships with rhizobia, resulting in the formation of root nodules where nitrogen fixation occurs. This process allows legumes to thrive in nitrogen-deficient soils and reduces the need for synthetic fertilizers (Yang *et al.*, 2024).

Rhizobia, as a diverse group of microorganisms, are potential PGPRs (Plant growth promoting rhizobacteria) that are widely distributed throughout the agroecosystem and play an important role in improving the performance of several agricultural crops (Dinesh, 2013).

PGPR are critical components of the rhizosphere microbiome and contribute significantly to plant growth and agricultural productivity (Etesami & Maheshwari, 2018). These bacteria promote plant growth through multiple mechanisms, including nitrogen fixation, phosphorus solubilization, phytohormone production, and stress alleviation. They produce compounds such as auxins, gibberellins, cytokinins, siderophores, and ACC deaminase, which enhance nutrient uptake, stimulate root development, and protect plants from stress and pathogens (Bashan & De-Bashan, 2010; Olanrewaju *et al.*, 2017).

PGPR also play an important role in bioremediation by detoxifying heavy metals and degrading pollutants in contaminated soils. Their ability to adapt to harsh environmental conditions makes them particularly valuable in arid and degraded



ecosystems (Macías-Almazán *et al.*, 2020; Phulpoto *et al.*, 2021).

Figure 1. Actors and interactions in the rhizosphere. Inter-kingdom and intra-kingdom communication involving plants and microbes in the rhizosphere: the consistent role of rhizobia. VOCs, volatile organic compounds; PGP, plant growth promoting; AMF, arbuscular mycorrhizal fungi. (Checcucci & Marchetti, 2020)

Legume-rhizobium symbiotic nitrogen fixation, takes place in an environment where many rhizobium strains are competing for occupying legume root nodules to gain

access to photosynthates, and where the legume attempts to select the best symbiotic partner to maximize nitrogen fixation (Checcucci *et al.*, 2017). Several native legume species play an important ecological role in the Algerian Sahara by contributing to ecosystem stability and soil fertility. Among these species, *Genista saharae* is an indigenous shrub legume naturally distributed in the northeastern Algerian Sahara. It is recognized for its adaptation to arid environments, its contribution to dune stabilization, and its role in combating desertification through biological nitrogen fixation. Previous studies have also demonstrated the diversity of rhizobial populations associated with this species, highlighting the importance of native legumes as reservoirs of beneficial microorganisms in desert ecosystems (Chaïch *et al.*, 2017).

Arid regions are characterized by low rainfall, high temperatures, poor soil fertility, and limited organic matter. Under these harsh conditions, plant survival depends heavily on efficient rhizosphere interactions and microbial associations. Legumes are particularly important in arid ecosystems. Species such as *Retama raetam* and *Arachis hypogaea* are well adapted to arid environments and play a significant role in soil stabilization and ecosystem functioning.

Local observations in the Ghardaïa region also suggests that soils in areas where *Retama raetam* naturally grows exhibit greater fertility, often supporting improved crop growth and productivity.

Retama raetam, also known as white broom or white weeping broom, is a desert shrub native to several countries of North Africa, including Morocco, Algeria, Tunisia, Libya, and Egypt, temperate Asia and in certain Middle Eastern countries (Mechergui *et al.*, 2017). It plays a significant role in soil protection and stabilization against wind or water erosion and provides an important dietary source for livestock species such as camels, goats, and sheep (Laudadio *et al.*, 2009). Additionally, this species represents viable fuel source for humans (Cheriti *et al.*, 2009). It also has medicinal and potential industrial values since its roots are used to treat diarrhea, the leaves are used to help aching joints back pain and eye troubles (Said *et al.*, 2002).

The *Retama* genus comprises four species that are mainly distributed in the

Mediterranean Basin. These plants have been used traditionally for the treatment of different diseases in many parts of the Mediterranean Basin, of which *Retama raetam* is the most applied (León-González *et al.*, 2018).

Retama raetam stabilizes sandy soils, protects against wind and water erosion, and shields the ground from overheating and intense radiation, making it valuable for desert rehabilitation and combating desertification and as a nitrogen-fixing legume, it improves soil fertility (Barakat *et al.*, 2013; Youssef *et al.*, 2024). It also acts as a nurse plant, improving soil moisture, reducing salinity, and facilitating establishment and germination of other native species, thereby shaping plant community structure in arid landscapes (Nouioui *et al.*, 2025).

In addition to native legume species, cultivated legumes represent an important component of arid agroecosystems. *Arachis hypogaea* is recognized as one of the major legume species of strategic importance globally, both for its role in direct human nutrition and for its contributions to the oil industry. In addition to its high oil and protein content, its ability to perform symbiotic nitrogen fixation and its capacity to adapt to semi-arid climatic conditions have made this plant a valuable agricultural product, particularly in supporting rural development in developing countries (Sangh *et al.*, 2025).

Arachis hypogaea is an annual, herbaceous plant belonging to the legume family and the papilionaceous subfamily (Gillier & Silvestre, 1996). Its seeds are cultivated in Asia, Africa, and the Americas (Kochert *et al.*, 1996). Peanuts are the 13th food crop globally, 4th source of edible oil, and 3rd source of vegetable protein, with >95% area in Asia and Africa (Variath & Janila, 2017; Hammouda *et al.*, 2019; Solanki & Nagar, 2020).

In Algeria, peanuts are mainly grown in the northeast (El-Taref and Skikda), in the southeast in the oases (Ghardaïa, Laghouat and El-Oued), and in the southwest (Adrar), covering an area of 2,718 ha and producing approximately 29,950 kg of pods with a yield of about 11 kg/ha. Ghardaïa is the most important region for summer peanut cultivation. It plays an important role in the rural economy (Bissati *et al.*, 2019).

Although extensive research has been conducted on plant-rhizosphere interactions, information regarding the spatial variability of soil properties and the diversity of plant-associated beneficial bacteria in arid ecosystems remains limited, particularly for native and cultivated legumes in southern Algeria. A better understanding of these interactions is essential for elucidating the mechanisms underlying plant adaptation and for identifying microbial resources with potential agricultural value. In this context, the present research initiative seeks to quantify the complexity of the mutualistic relationships established between Fabaceae plants and their rhizosphere in arid environments and their spatial variability, with a particular focus on two legume species: the spontaneous *Retama raetam* and the cultivated *Arachis hypogaea*. A comprehensive understanding of these plant-soil-microbe dynamics is fundamental for promoting sustainable agricultural practices and advancing ecological conservation. In this framework, the present work pursues the following specific objectives:

- To characterize and compare the physicochemical properties of the rhizosphere and bulk soil of *Retama raetam* and *Arachis hypogaea* in arid regions, and to identify the key parameters that differentiate the rhizosphere from the surrounding bulk soil in each species.
- To assess the spatial variability of rhizosphere soil properties using geostatistical approaches, in order to quantify the extent and structure of plant-induced soil heterogeneity at the field scale.
- To isolate and screen rhizosphere-associated and nodule-associated bacteria for plant growth-promoting traits, including biological nitrogen fixation, phosphate solubilization, and siderophore production.
- To highlight species-specific mechanisms of soil modification and microbial diversity.
- To evaluate the potential of the identified PGPR as candidate strains for the development of biofertilizers adapted to arid agricultural systems.
- To contribute to a broader understanding of plant–soil–microbe interactions in North African arid regions, providing a scientific basis for integrating native

Fabaceae species into ecological restoration and sustainable land management strategies.



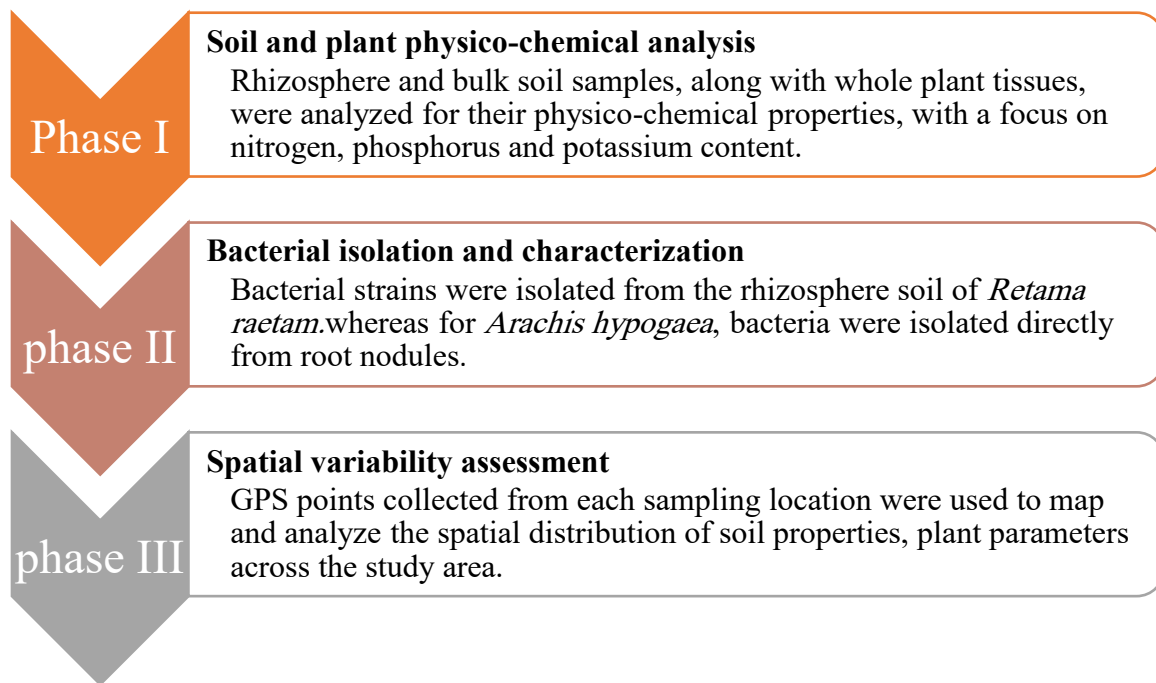
MATERIALS AND METHODS

I. Overview of the study

This study employed a field-based sampling design coupled with laboratory physico-chemical and microbiological analyses to investigate spatial variability and plant rhizosphere interactions in two legume species, from arid regions: the spontaneous shrub *Retama raetam* and the cultivated crop *Arachis hypogaea*. The research was conducted in the Sebseb region of Ghardaïa province, Algeria.

Sampling was carried out according to the phenological cycles of each species. For *Retama raetam*, sampling took place at a single site during spring 2023. For *Arachis hypogaea*, sampling was conducted across three distinct sites in November 2024. At each sampling event, paired rhizosphere and bulk soil samples were collected. Additionally, whole plants were harvested. GPS coordinates were recorded at each sampling point to assess spatial variability.

The study proceeded in three main phases:



II. Study area

The study was carried out in the Sebseb region, located in Ghardaïa Province, in the center of southern Algeria (approximately 32°15'N, 3°26'E) (Figure 2). The region is characterized by an arid Saharan climate with significant temperature fluctuations between

day and night and between seasons. The hot season extends from May to September, with July being the hottest month, reaching 47°C and averaging 36.3°C. In contrast, January is the coldest month, with an average temperature of 9.2°C and an absolute minimum of -1°C. Annual precipitation is scarce and irregular, ranging from 13 to 68 mm, spread over an average of 15 days per year. On average, the region receives around 74.2 mm of rainfall annually with mean maximum and minimum temperatures of 28.5°C and 16.2°C, respectively (ONM, 2023)

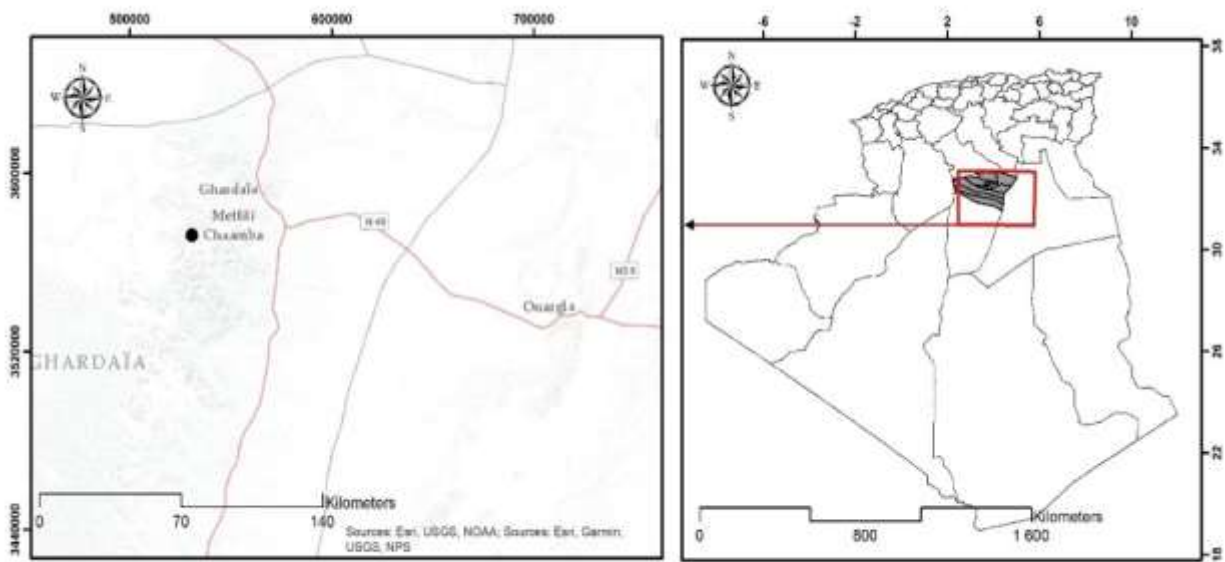


Figure 2. Location of the study area

III. Methodology

III.1. Sampling

III.1.1. Soil samples collection and preparation

Thirty-five (35) soil samples were collected during March 2023, Thirty (30) from the rhizosphere and five from bulk soil of *Retama raetam*. Using a trowel, the rhizosphere soil samples were obtained from areas directly influenced by plant roots. In contrast, bulk soil samples were collected from adjacent areas without direct root influence to provide a

baseline for comparison. Samples were withdrawn at a depth of 20 cm below the surface in Ziplock plastic bags, which were then placed on ice and transported to the laboratory for further analysis. A similar sampling approach was applied for *Arachis hypogaea* L., where thirty-six (36) soil samples were collected from three parcels, Thirty (30) from the rhizosphere and six from bulk soil during November 2024.

Soil samples were air-dried at room temperature and gently crushed to break up aggregates. The dried samples were then sieved through a 2 mm mesh to remove stones, roots, and other debris. The processed soil was homogenized and stored in clean containers for subsequent physico-chemical analysis (Goshu, 2024).



Figure 3. Sampling site of *Retama raetam* (Sebseb, March 2023). (Original)

III.1.2. Plant samples collection and preparation

A total of thirty (30) plants of each species, were collected from the same sites as the corresponding soil samples. Whole plants were carefully uprooted to ensure the collection of all plant parts, Samples were placed in labeled plastic bags and transported to

the laboratory. Plant materials were washed thoroughly with tap water followed by distilled water to remove adhering soil and impurities, then air-dried at room temperature until constant weight was achieved. After drying, roots were separated from the aerial parts, and each fraction was ground separately into a fine powder using a grinder. The ground samples were stored in airtight containers for subsequent analysis.



Figure 4. Sampling site of *Arachis hypogaea* (Sebseb, November 2024). (Original)

III.2. Soil and plant analyses

III.2.1. Soil parameters

III.2.1.1. particle size distribution

particle size distribution was carried out using the Bouyoucos hydrometric method (Mathieu & Pielain, 2003). 40 g sample of air-dried soil was weighed into a 600 ml beaker, and 60 ml of dispersing solution were added. The beaker was covered and left to stand for

24 hours. The contents were then transferred into a mixing container, filled up to three-quarters with water, and continuously shaken overnight in a mechanical shaker. Sample was then transferred into a 1 L graduated cylinder. Sand particles (≥ 0.02 mm) settled after 40 seconds, while particles larger than clay (≥ 0.002 mm) settled after 4 hours. The particles remaining in suspension were measured using a Bouyoucos hydrometer at specified time intervals.

III.2.1.2. pH and Electrical Conductivity

pH and EC focus on measuring the concentration of H^+ ions and soluble salts in a 1:5 aqueous extract of soil. For this purpose, 10 g of air-dried soil are weighed into a 100 ml glass beaker containing 50 ml of distilled water. The sample is then mechanically shaken for one hour and left to settle for 30 minutes. Once settled, the pH electrode is placed into the supernatant at a depth of about 3 cm, while the conductivity cell is immersed without disturbing the sediment. Measurements were taken after the readings have stabilized (Mathieu & Pieltain, 2003).

III.2.1.3. Organic Matter

OM is estimated by loss on ignition, expressed as a percentage of the dry sample mass. Crucibles are first heated at 550 °C for 16 hours, then cooled and weighed. Subsequently, 5 g of soil are placed in each crucible and dried in an oven at 220 °C for 16 hours, followed by cooling and weighing. The crucibles containing the sample are then heated at 450 °C for 4 hours, cooled, and weighed again (Mathieu & Pieltain, 2003).

OM content is calculated using the formula:

$$OM (\%) = ((M1 - M2) / (M1 - M0)) \times 100.$$

- M0: Weight of the empty crucible
- M1: Weight of the crucible with the dried sample
- M2: Weight of the crucible with the sample after calcination.

III.2.1.4. Calcium carbonate content

$CaCO_3$ content is determined by the Bernard method (Mathieu & Pieltain, 2003). using a calcimeter calibrated with 300 mg of $CaCO_3$. 5g of finely ground, air-dried soil is weighed and placed into the calcimeter flask. Then, 7 ml of hydrochloric acid is added, and

the flask is immediately closed and shaken. The volume of CO₂ released is measured by the amount of water displaced in the graduated burette after equilibrium. The percentage of CaCO₃ is calculated using the following equation:

$$\text{CaCO}_3 (\%) = (30 \times V_4) / (V_3 \times P).$$

- V₃: Volume of CO₂ (in ml) released by the sample.
- V₄: Volume of CO₂ (in ml) released by CaCO₃ used for calibration.
- P: Sample mass in grams

III.2.1.5. Total Nitrogen

TN in soil was determined using the Kjeldahl method (FAO, 2021) mixture, sulfuric acid (H₂SO₄), Sodium hydroxide (NaOH), and Boric acid (H₃BO₃). Organic Nitrogen is mineralized by acid digestion, converted to ammonia (NH₄) during alkaline distillation, and quantified by titration with H₂SO₄. TN content is calculated using the following equation:

$$N (\%) = ((V - B) \times N \times V_1 \times 14.01) / (W_t \times V_2 \times 1000) \times 1000.$$

- V: Volume of H₂SO₄ used for the sample (ml).
- B: Blank volume (ml).
- N: Normality of H₂SO₄.
- V₁ : Total digest volume (ml).
- V₂: Volume of digest used for distillation (ml).
- W_t: Weight of soil sample (g).

III.2.1.6. Nitrate

NO₃⁻ is determined by measuring nitrate ions in a 1:5 aqueous soil using a distillation unit followed by titration (Bremner & Keeney, 1965). The method uses magnesium oxide (MgO), Devarda's alloy, and a saturated H₃BO₃ solution. An aliquot of the extract (20 mL) is placed in a distillation flask, and MgO and Devarda's alloy are added before immediate distillation. The process is continued until at least 35 mL of distillate are collected, after which the distillate is titrated with standardized H₂SO₄. Each series includes standards and blanks to ensure accuracy, with a recovery of at least 96%. The concentration of NO₃⁻-N in ppm is calculated using the formula:

$$\text{NO}_3^- \text{-N (ppm)} = ((V - B) \times N \times V_1 \times 14.01 \times 1000) / (W_t \times V_2)$$

- V: Volume of H₂SO₄ used for the sample (ml)
- B: Blank volume (ml).
- N: Normality of the acid.
- V1: Total extract volume (ml).
- V2: Volume used for distillation (ml).
- 14.01: Atomic weight of nitrogen.
- Wt: Weight of the air-dried soil sample (g).

III.2.1.7. Available Phosphorus

AP is determined using the Olsen method (Olsen *et al.*, 1954), a chemical method suitable for alkaline soils. The method uses sodium bicarbonate (NaHCO₃) (0.5 M), H₂SO₄ (5 N), reagents A and B, and a standard solution. Briefly, 5 g of air-dried soil are extracted with 50 mL of NaHCO₃ solution and shaken for 30 minutes at 200 rpm, followed by filtration. An aliquot of the filtrate (5 mL) is then reacted with reagents A and B, diluted to 50 mL, and after 15 minutes, the absorbance is measured at 880 nm. The extractable phosphorus content is calculated from the calibration curve using the equation:

$$\text{Extractable P (ppm)} = \text{ppm P (from calibration curve)} \times (V / Wt) \times (V2 / V1)$$

- V: Total extract volume (ml)
- Wt: Soil weight (g)
- V1: Volume of extract used for measurement (ml)
- V2: Final volume used for measurement (ml).

III.2.1.8. Total Potassium and Sodium

TK and TNa were determined according to the method described by (Mathieu & Pieltain, 2003). The method uses Ammonium Acetate (NH₄OAc), Lithium Chloride (LiCl), and a standard solution. Briefly, 10 g of air-dried soil are extracted with 50 mL of 1 N NH₄OAc solution and shaken for 30 minutes at 200-300 rpm, followed by filtration. The filtrate is adjusted to volume, and potassium and sodium concentrations are determined using flame photometry based on a calibration curve prepared from standard solutions, as shown in the following equations.:

$$\begin{aligned} \text{Extractable K (ppm)} &= \text{ppm K (from calibration curve)} \times V / Wt \text{ and Extractable Na} \\ &(\text{ppm}) = \text{ppm Na (from calibration curve)} \times V / Wt \end{aligned}$$

were,

- V: Total volume of the soil extract (ml)
- Wt: Weight of the air-dried soil (g).

III.2.1.9. Soluble Potassium and Sodium

SK and SNa represent the fraction of K or Na that can be extracted from the soil with water, typically obtained from an aqueous extract of a saturated paste (Mathieu & Pieltain, 2003). soil/water solution is filtered through Whatman No.1 filter paper to remove all soil particles. A series of K and Na standards is prepared to construct a calibration curve. SK and SNa concentrations are then measured using flame photometry.

III.3. Bacterial Isolation and Characterization

For microbiological analysis, samples were collected to isolate bacteria associated with each plant species. Rhizosphere soil samples were collected from *Retama raetam* by carefully removing soil closely adhering to the roots. The soil samples were placed in sterile Ziplock plastic bags and stored at 4 °C until further analysis. In contrast, root nodules were collected from *Arachis hypogaea* plants. The nodules were detached manually from the roots and placed in sterile containers. All samples were transported to the laboratory under appropriate conditions for subsequent bacterial isolation and analysis.

III.3.1. Bacterial Isolation

III.3.1.1. Soil Microorganisms

Culturable soil bacteria were isolated using serial dilution. Aliquots of 0.1 ml from selected dilutions (10^{-1} , 10^{-4} , and 10^{-8}) were aseptically pipetted and inoculated into Petri dishes containing Nutrient Agar (GN) and Ashby's Mannitol Agar (AM). The inoculated plates were incubated at 28 °C for 48–72 hours after which distinct colonies were selected for further purification and analysis.

III.3.1.2. isolation of root-associated bacteria

Nodules from each tube were surface-sterilized by immersion in 60% ethanol for 5–10 seconds, followed by 2 minutes in bleach, and then rinsed thoroughly with sterile distilled water (Vincent, 1970; Beck *et al.*, 1993). A volume of 2 to 3 drops of sterile

distilled water was dispensed into a sterile Petri dish, into which the surface-sterilized nodules were placed. The nodules were crushed using sterile forceps, and the resulting suspension was streaked into Yeast Mannitol Agar (YMA) agar via the streak plate method to obtain single colonies. The inoculated plates were incubated at 28-30 °C for a period of 5-8 days.



Figure 5. Isolation of rhizosphere bacteria from *Retama raetam* rhizosphere soil on GN medium. (Original)

III.3.2. Purification of isolates

Isolates were purified by repeated subculturing on Yeast Extract Mannitol Agar (YMA) and Glucose Nutrient (GN) media using a sterile platinum loop under aseptic conditions until well isolated pure colonies were obtained. The cultures were incubated at 28°C after each subculture.

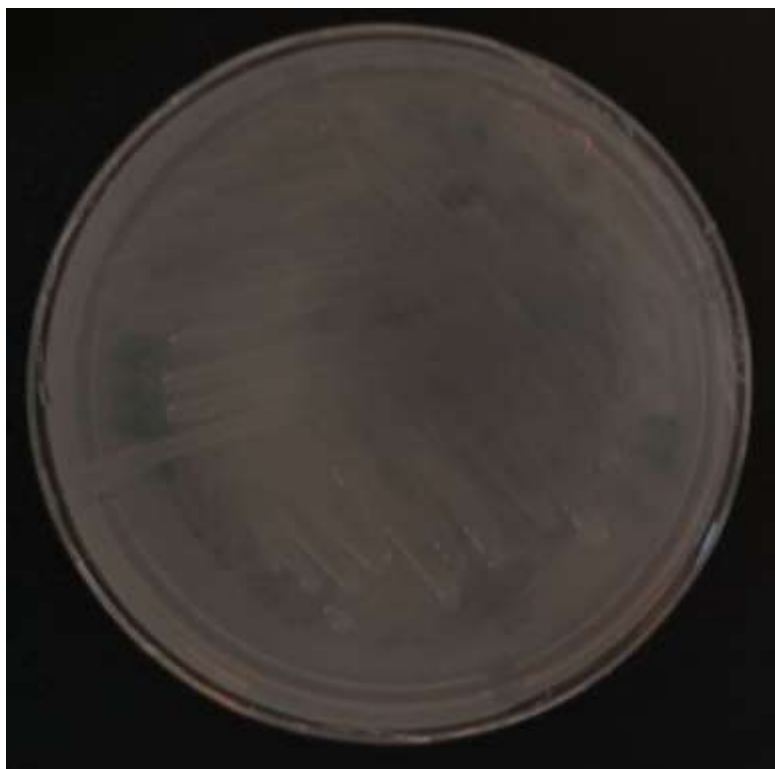


Figure 6. Purification of bacterial Isolates through repeated streaking on YMA medium. (Original)

III.3.3. PGPR Activity

III.3.3.1. Plant Growth Promotion screening

III.3.3.1.1. Phosphate Solubilization

The aim of this test was to evaluate the ability of the isolates to solubilize tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$). The bacterial isolates were inoculated using the spot-inoculation technique on solid PVK medium (Pikovskaya, 1948). After incubation at 28 °C for 10 days, phosphate solubilization was indicated by the formation of a clear halo around the colony.

III.3.3.1.2. Siderophore production

Siderophore production by the isolates was detected using solid medium containing chrome azurol S (CAS) (Schwyn & Neilands, 1987). The principle is based on the medium's initial blue color due to the Hexadecyltrimethylammonium (Fe-CAS-HDTMA) complex, which changes to red-orange when iron is chelated by bacterial siderophores. In

this study, solid LB (Lysogeny Broth) medium was used. After 3 days of incubation, the formation of a red-orange halo around the colony was visually observed, indicating the ability of the isolates to produce siderophores.

III.3.3.1.3. Nitrogen fixation

The nitrogen-fixing ability of the bacterial isolates was evaluated qualitatively using Ashby's mannitol (AM) nitrogen-free medium. Fresh bacterial cultures were streaked onto AM plates under aseptic conditions and incubated at 28 °C for 3-7 days. The ability of the isolates to grow on the nitrogen-free medium, where mannitol served as the carbon source, was considered indicative of their potential nitrogen-fixing capacity. Bacterial growth was recorded as a positive result, whereas the absence of growth was considered negative.

III.3.4. Biochemical identification

Following the screening of plant growth-promoting (PGP) activities, bacterial strains showing positive results for siderophore production and phosphate solubilization were selected for further characterization. Prior to biochemical profiling using API strips, a preliminary identification was performed, including Gram staining as well as catalase and oxidase tests. These preliminary analyses were carried out to guide the selection of the most appropriate API system for each bacterial strain.

III.3.4.1. Preliminary Identification of Bacterial Strains

III.3.4.1.1. Gram Staining

A well-isolated colony was picked, spread on a sterile slide, and heat-fixed. All procedures were performed aseptically under a laminar flow hood. Gram staining was carried out following the method of (Coico, 2005). The slide was first stained with crystal violet for 45 seconds to 1 minute, followed by rinsing and mordanting with Lugol's iodine for the same duration. After rinsing with deionized water, a rapid decolorization with ethanol was performed for 5 to 10 seconds until the smear appeared clear. The slide was then rinsed thoroughly with deionized water to stop the decolorization, followed by counterstaining with fuchsin for 30 seconds to 1 minute. After a gentle rinse with deionized water, the slide was air-dried over a Bunsen burner and observed under an oil

immersion lens at 1000× magnification.

III.3.4.1.2. Catalase test

This test consists of bringing a bacterial colony into contact with a drop of hydrogen peroxide (H₂O₂). The appearance of effervescence (due to the release of oxygen) indicates the presence of catalase (Joffin & Leyval, 2006).

III.3.4.1.3. Oxidase test

This is an identification technique used in bacteriology. It involves detecting cytochrome oxidase, the final enzyme in the respiratory chain, which ensures the transfer of electrons to oxygen or another mineral oxidant. A piece of Whatman No. 3 filter paper is soaked with the substrate (TMPD), then a bacterial colony is applied and crushed using a Pasteur pipette.

- If the paper develops a blue/purple color: the substrate has been oxidized, and the bacterium is oxidase-positive.
- If the paper remains colorless: no reaction has occurred, and the bacterium does not possess the enzyme (Jurtshuk & McQuitty, 1976).

III.3.4.2. Biochemical profiling using API strips

Bacterial isolates were identified using the API gallery system (BioMérieux, France) according to the manufacturer's instructions. Prior to API testing, isolates were characterized by Gram staining and tested for catalase and oxidase activities to select the appropriate API panel. For Gram-negative isolates, API 20E or API 20NE strips were used, while Gram-positive isolates were tested using API 50CH or API Staph strips as appropriate. Pure colonies were suspended in sterile saline to the required turbidity and inoculated into the wells of the API strips. The strips were incubated at 28–30 °C for 18–24 hours, and reactions were interpreted based on color changes. Results were recorded and compared to the API database for preliminary identification of bacterial species.

IV. Statistical analysis

Statistical analysis was performed using the R programming language. Differences between RS and BS were assessed using independent two-sample t-tests, with significance set to $p < 0.05$ (confidence level of 95%). Pearson correlation coefficients were computed

to examine relationships among soil properties. Principal Component Analysis (PCA) was applied to reduce dimensionality, identify key variables influencing soil quality and fertility, and visualize the separation of RS and BS samples. Graphical outputs, including boxplots, correlation matrices, and PCA biplots, were used to illustrate the results. Two-way ANOVA was performed to assess the significance of site, soil type, and their interaction effects on the measured soil variables, as well as the effects of plant species, organ type, and their interaction on the concentrations of nitrogen, phosphorus, potassium, and sodium in plant tissues. Post-hoc pairwise comparisons were conducted using Tukey's HSD test to evaluate differences among sites. PCA was carried out to identify and visualize relationships among soil physicochemical parameters in the rhizosphere, while correlation analysis was performed to detect significant associations among soil properties, with p-values adjusted using the Holm correction method ($n = 30$). Finally, the biochemical profiles of bacterial isolates were analyzed using a hierarchically clustered heatmap, which allowed the identification of distinct metabolic groupings among the recovered strains.

V. Geostatistical Analysis

Geostatistical methods were used to assess the spatial variability of soil parameters. Experimental semi variograms were calculated for each variable to describe spatial dependence, and models (Gaussian, spherical, or exponential) were fitted based on the best agreement with the empirical data. Model selection was guided by minimizing residual sum of squares and maximizing the coefficient of determination (r^2). The nugget-to-sill ratio ($SDR = C_0 / (C_0 + C) \times 100$) was calculated to classify the degree of spatial dependence. The range parameter was used to determine the distance of spatial influence for each soil property. Ordinary kriging was applied to generate spatial distribution maps, allowing visualization of nutrient and property patterns across the study area. These maps were used to identify nutrient hotspots, gradients, and areas of spatial continuity, providing insight into localized rhizosphere processes and broader pedogenic controls. Soil textural classification was determined according to the USDA texture triangle.





RESULTS AND DISCUSSION

I. *Retama reatam* soil

I.1. Comparative Analysis of Rhizosphere and Bulk Soil

Statistical t-tests were used to identify significant differences in attributes between RS and BS. The related boxplots (Figure 07,08) provide a visual representation of the results. Significant changes were found in a number of important soil attributes between RS and BS. The content of EC ($p < 0.0001$) and CaCO_3 ($p < 0.0001$) was substantially greater in BS. In a similar vein, BS had a considerably greater TN content ($p = 0.0069$). RS, on the other hand, has a much lower pH ($p < 0.0001$). Additionally, there was a significant difference in the amounts of TK ($p = 0.01$) and NO_3 ($p = 0.01$), with RS having greater quantities than BS. On the other hand, p-values larger than 0.05 for SK, SNa, AP, and OM indicated no significant changes between the two soils.

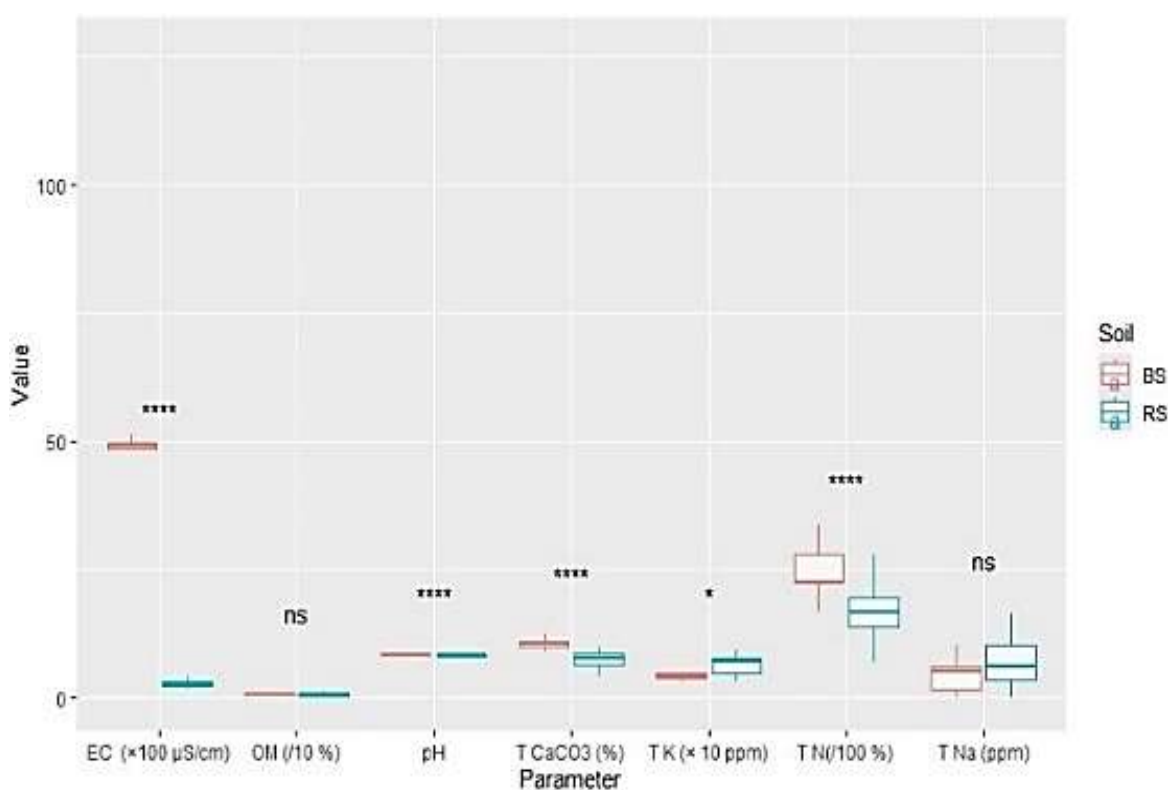


Figure 7. Boxplot showing the significant differences between RS and BS: EC, pH, CaCO_3 , TK, TN, TNa,

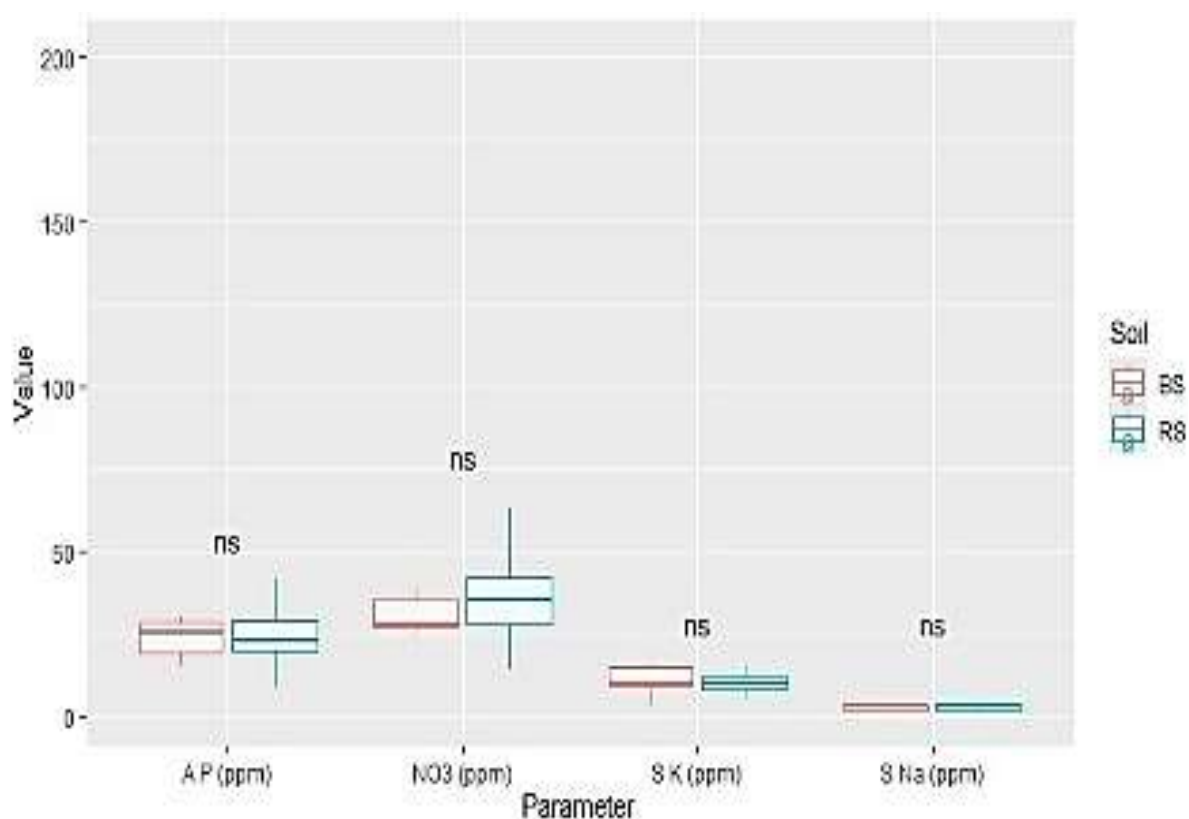


Figure 8. Boxplot showing the significant differences between RS and BS: AP, NO₃, SK and SNa.

I.2. Principal Component and Correlation Analysis

To find correlations between soil physicochemical parameters in the rhizosphere, principal component analysis (PCA) was used. 62.4% of the variance was explained by the first two axis, with Dim1 accounting for 40.2% and Dim2 for 22.2% (Figure 09). EC, TNa, pH, and AP were among the variables that were classified on the positive side of Dim1. CaCO₃, on the other hand, was located on Dim1's negative side. OM and SNa created a close cluster along Dim2, and SK and TK formed another near cluster. NO₃ was found in the negative quadrant of both axes, and TN and NO₃ were isolated from the main clusters.

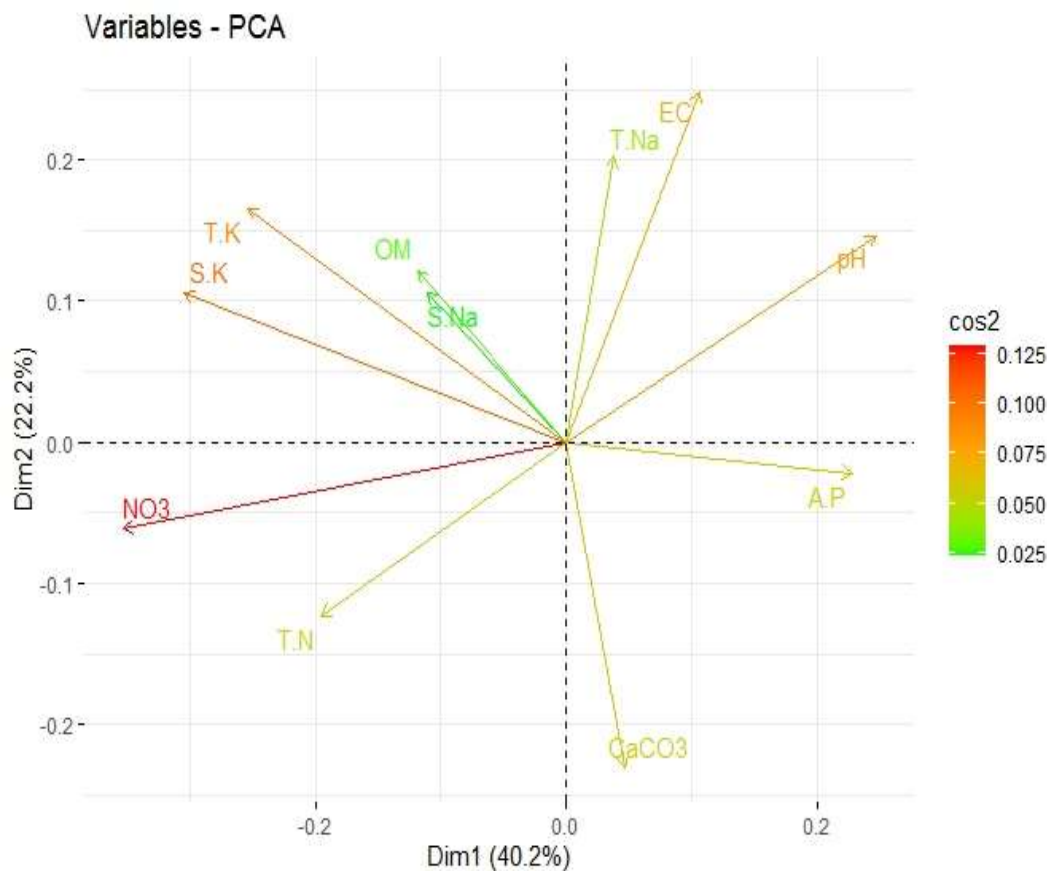


Figure 9. Principal Components Analysis (PCA) of soil parameters

Numerous important connections between rhizosphere soil characteristics were shown using the correlation matrix (figure 10). MO ($r = 0.3624$, $p = 0.0491$), TNa ($r = 0.3670$, $p = 0.0461$), TK ($r = 0.4296$, $p = 0.0178$), and TN ($r = 0.4471$, $p = 0.0132$) were all significantly positively correlated with SNa. There was a strong positive association between SK and NO₃ ($r = 0.4282$, $p = 0.0182$). TK was significantly positively correlated with TNa ($r = 0.4171$, $p = 0.0218$), NO₃ ($r = 0.4655$, $p = 0.0095$), SK ($r = 0.8569$, $p < 0.0001$), and EC ($r = 0.4223$, $p = 0.0201$). TNa had a strong positive connection with both EC ($r = 0.3957$, $p = 0.0304$) and SK ($r = 0.3796$, $p = 0.0385$). (Appendix 02)

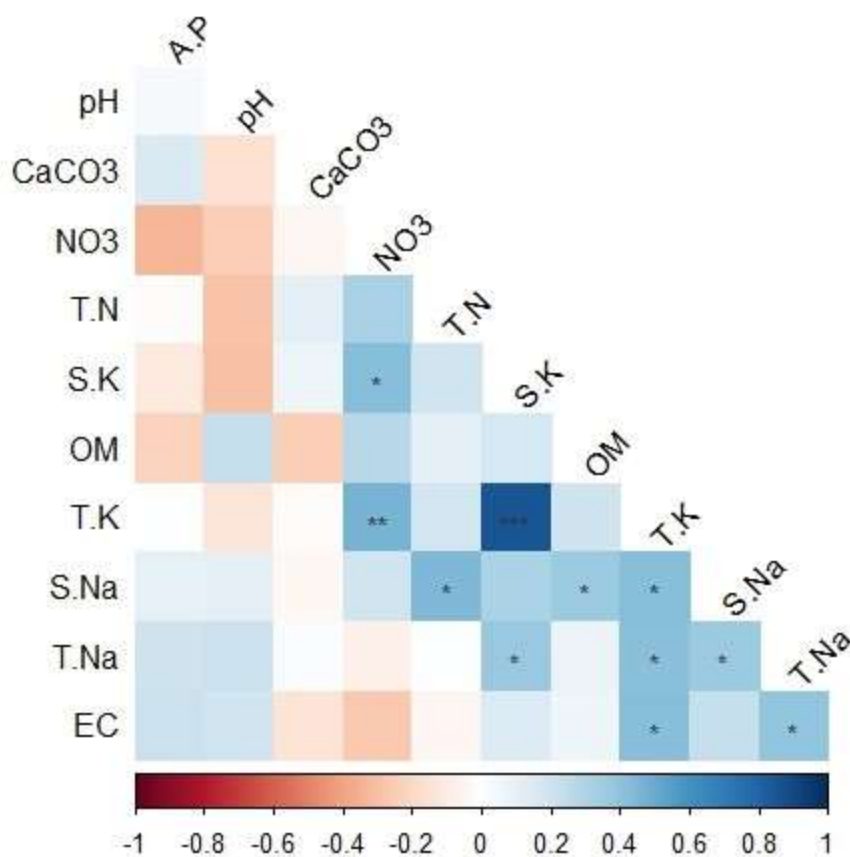


Figure 10. Correlation matrix of rhizosphere soil properties

I.3. Geostatistical analysis of rhizosphere soil parameters

As shown in Table 02, variogram modeling and the coefficient of variation (CV%) were used to assess the regional variability of soil characteristics. While EC, pH, SNa, and TNa were better fitted by spherical models, suggesting differences in spatial continuity, all variables followed a Gaussian model. For every variable, the nugget-to-sill ratios were less than 25%. There was significant variation in the range values. While AP (2119 m), NO₃ (1643 m), and CaCO₃ (20 m) had longer spatial ranges

AP (0.87) and TN (0.87) have high CV values. OM (0.47) and TNa (0.66) showed moderate variability, but pH (0.036) and EC (0.22) had lower CVs.

Table 1. Fitted variogram models and variation properties

Parameter	Model	Nugget	Sill	Range [m]	Nugget/Sill [%]	Coefficient of variation [%]
AP	Gaussian	81	3515946	2119	~0.002%	0.8698258
CaCO ₃	Gaussian	0.83	11	20	7.5%	0.29608311
NO ₃	Gaussian	48	251562	1643	~0.019%	0.3144201
TN	Gaussian	0	0.03	9.1	0%	0.8695164
EC	Spherical	0	3485	10	0%	0.2176384
OM	Gaussian	0	0.06	2.5	0%	0.4712403
pH	Spherical	0.02	0.11	27	18.2%	0.03638240
SK	Gaussian	0	5.3	3.6	0%	0.2611691
SNa	Spherical	0	0.96	4.9	0%	0.3503173
TK	Gaussian	7.7	375	7.2	2.1%	0.2905714
TNa	Spherical	0	17	4	0%	0.6619610

Different patterns of soil property variability throughout the research region were shown by the spatial distribution maps produced by ordinary kriging. The CaCO₃ (Figure 13) content varied from 4.46% to 17.40%, with the south-central region showing the highest concentrations. EC (Figure 12) showed localized salinity hotspots in the northwest, northeast, and southwest, ranging from 197.51 to 500.57 $\mu\text{S}\cdot\text{cm}^{-1}$. AP (Figure 15) levels varied from 9.33 to 127.94 ppm, with the northwest having significantly higher values and the middle and eastern regions having lower concentrations. The south-central region has higher levels of NO₃ (Figure 14), with concentrations ranging from 19.44 to 61.94 ppm. The pH values of the soil (Figure 11) varied somewhat, from 7.55 to 8.49, with the northeastern and central parts having more alkaline zones.

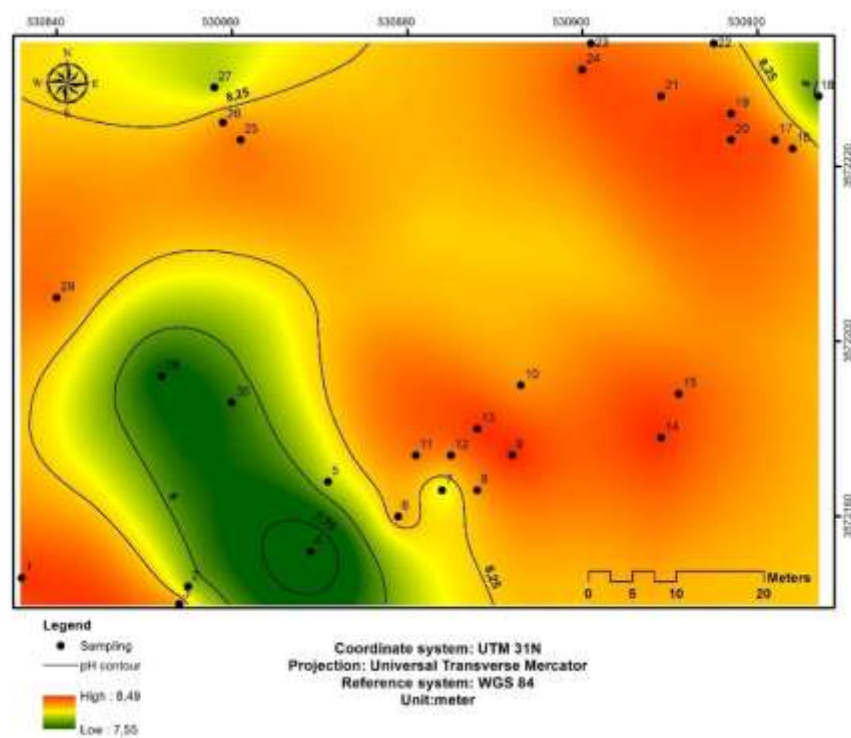


Figure 11. Spatial distribution maps generated by ordinary kriging for pH rhizosphere soil

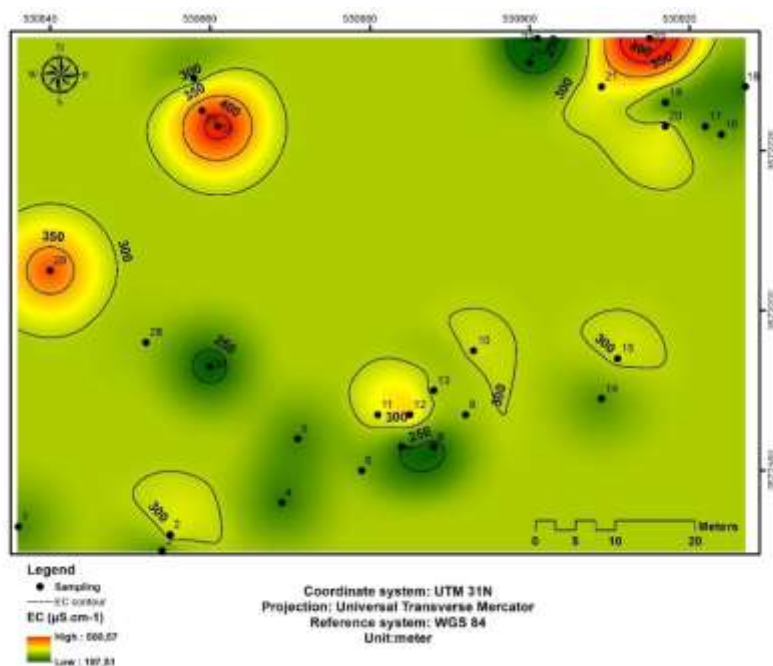


Figure 12. Spatial distribution maps generated by ordinary kriging for EC

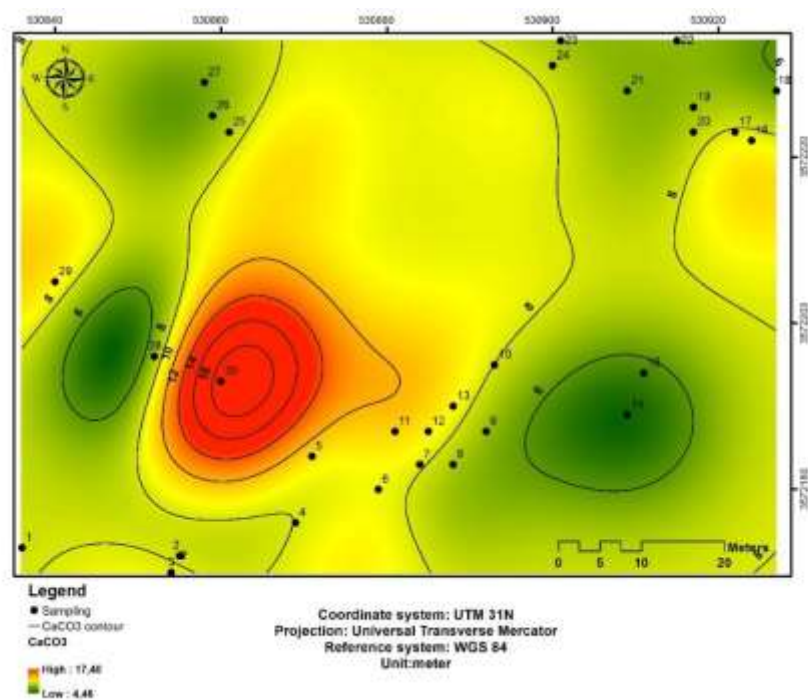


Figure 13. Spatial distribution maps generated by ordinary kriging for CaCO_3

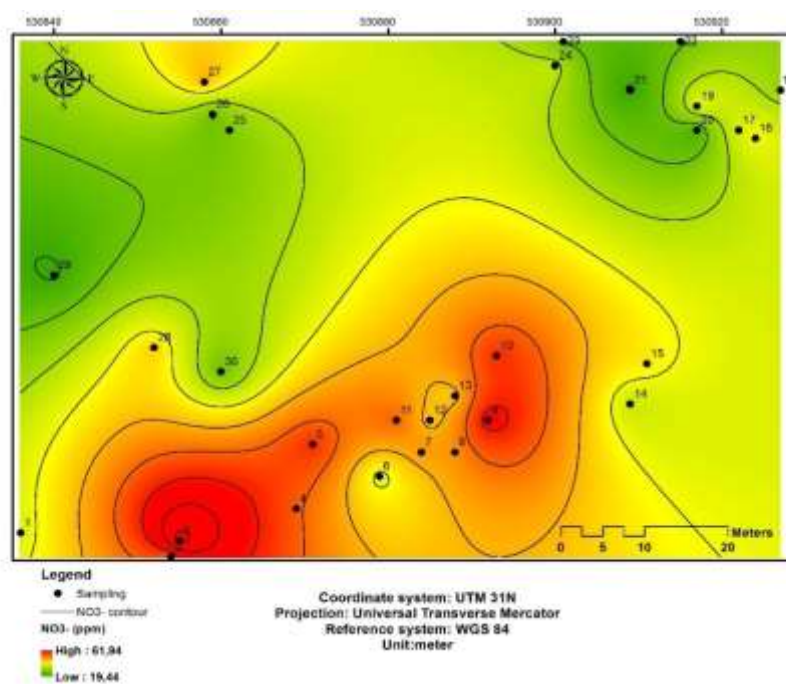


Figure 14. Spatial distribution maps generated by ordinary kriging for NO_3

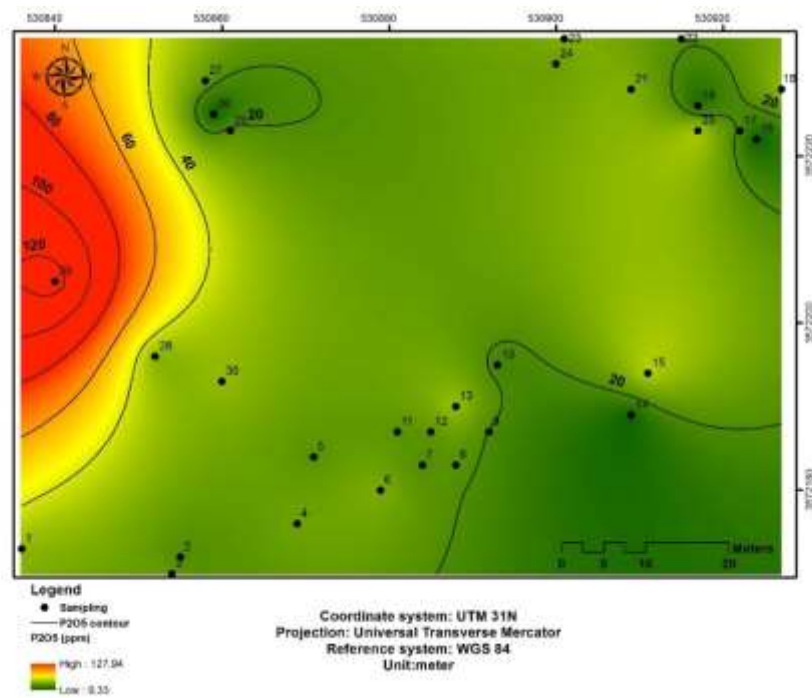


Figure 15. Spatial distribution maps generated by ordinary kriging for P_2O_5

II. Discussion

V.1. *Retama raetam* soil

V.1.1. Rhizosphere and Bulk Soil properties

The microbial community in the rhizosphere is generally more active and diverse than in BS (Maryani & Rogomulyo, 2021), and the chemical environment, particularly EC, is significantly impacted by this activity. Microbial actions alter ion concentrations and nutrient availability, frequently reducing EC in the rhizosphere relative to the surrounding soil. (Nwankwo *et al.*, 2024). Accordingly, our findings revealed substantially greater EC in BS ($p < 0.0001$), a pattern previously documented by earlier research. (Jiang *et al.*, 2024; Li *et al.*, 2024; Nwankwo *et al.*, 2024). Microbial transformations and increased ion uptake are probably the causes of these decreases in rhizosphere EC. Furthermore, techniques like adding legumes as green manure enhance soil organic matter, enhance nutrient cycling, and support sustainable agriculture by stabilizing EC. (Irin *et al.*, 2022). Additionally, there were substantial differences in EC and pH between soil compartments, with RS exhibiting lower values than BS ($p < 0.0001$). Evidence from a variety of agricultural systems, including paddy soils, strongly supports this pattern. (Du *et al.*, 2016), neutral or alkaline soils (Fu *et al.*, 2023), as well as more recent research showing notable acidification of the rhizosphere (Gao *et al.*, 2024; Wei *et al.*, 2023). Microbial activity and root proton release are the main causes of the rhizosphere pH decline, although in species like *Retama raetam*, OM inputs from leaf litter and root residues may produce organic acids during decomposition, intensifying acidification. On the other hand, because of the intricate interactions between rhizosphere processes, the release of some organic anions during decomposition may raise pH by decarboxylation and base release (Michael, 2021; Ritchie & Dolling, 1985). These factors also influence nitrogen dynamics. Our findings showed that RS had significantly higher nitrate concentrations ($p = 0.01$), which is in line with research showing that legume rhizospheres have higher microbial activity, nitrogen fixation by Rhizobiaceae, and increased N-cycling enzyme activity, which results in higher nitrogen availability when compared to BS. (Liu *et al.*, 2022; Wang *et al.*, 2024; Yang *et al.*, 2024). On the other hand, BS had a significantly higher TN content ($p = 0.0069$). This conclusion is consistent with findings from alpine grasslands, where total nitrogen in the

rhizosphere was lower than in BS (Du *et al.*, 2011), indicating that the rhizosphere influence depends heavily on the environment and species. In a similar manner, our study discovered that the CaCO_3 concentration was considerably higher in BS ($p < 0.0001$), which is in line with publications that indicate carbonate content tends to decline in the rhizosphere as a result of root-driven CO_2 enrichment and acidification, with the highest levels retained in BS (Raid *et al.*, 2020). Together, these results demonstrate that distinct chemical fingerprints that distinguish RS from BS in terms of EC, pH, N dynamics, and CaCO_3 distribution are produced by rhizosphere processes driven by root activity, microbial interactions, and organic matter turnover.

V.1.2. Multivariate Analysis

Several interesting relationships between soil characteristics were found in our investigation, which is in line with results from earlier studies. Both OM ($r = 0.3624$, $p = 0.0491$) and TNa ($r = 0.3670$, $p = 0.0461$) were positively correlated with SNa. Numerous studies have shown that soluble and total salt tend to rise in a variety of soil types and situations, including saline and agricultural settings. Similar tendencies have been extensively reported (Levy & Feigenbaum, 1977; Xie *et al.*, 2020). Because sodium exchange activities displace sorbed organic anions and encourage solubilization, sodium salts are also known to increase the release of organic materials (Reemtsma *et al.*, 1999). Furthermore, the importance of soluble sodium to soil structural processes is shown by positive correlations between organic matter and soil aggregate stability, where sodium plays a role alongside organic inputs. (López & Escolar, 1969). Regarding nutrients, there was a significant correlation between SK and NO_3 ($r = 0.4282$, $p = 0.0182$). This correlation was also observed in agricultural soils, where crop uptake and fertilization techniques coordinate the dynamics of K^+ and NO_3^- (Filho *et al.*, 2019; Vidaurre *et al.*, 2023; Mascianica, 1983). This coordination reflects the physiological role of potassium as a counter-ion facilitating nitrate transport in plants (Raddatz *et al.*, 2020). Field evidence further shows synchronized increases of NO_3 and K under fertilization (Filho *et al.*, 2019), as well as positive associations between extractable soil K, NO_3 , and plant performance in vineyards (Vidaurre *et al.*, 2023), and in restored soils where nutrient mineralization links Na and N forms (Wu *et al.*, 2024). TK exhibited strong correlations with SK ($r = 0.8569$,

$p < 0.0001$), NO_3 ($r = 0.4655$, $p = 0.0095$), and EC ($r = 0.4223$, $p = 0.0201$), reinforcing the idea of a dynamic equilibrium among potassium fractions. Similar positive associations among different forms of potassium have been reported in the Lesser Himalayas (Shah *et al.*, 2022), Haryana and North Bihar (Alam *et al.*, 2021; Prabha *et al.*, 2020), and paddy soils of Nagpur (Badole *et al.*, 2020), as well as in acid Alfisols (Sinha *et al.*, 2024). Additionally, TK's link with EC reflects the well-established role of potassium in salinity contexts, with strong correlations ($r \geq 0.83$) documented in arid and saline-sodic soils (Oustan *et al.*, 2007). Similarly, TNa correlated positively with EC ($r = 0.3957$, $p = 0.0304$) and SK ($r = 0.3796$, $p = 0.0385$), in agreement with studies reporting extremely strong associations ($r > 0.95$) between Na, K, and EC in saline soils of Libya and Oman (Al-Busaidi *et al.*, 2022; Chi & Wang, 2010; Idris *et al.*, 2025). Together, our findings highlight the strong links between Na and K dynamics and N and salinity indicators. Our linkages are mediated by soil chemical processes, fertilization techniques, and plant uptake patterns in various conditions.

V.1.3. Rhizosphere soil variability

Ordinary kriging and semi-variogram modeling are frequently used in geostatistical analysis, which has long been acknowledged as a potent method for characterizing soil spatial variability. Spherical and exponential models are frequently found to fit soil qualities the best, according to earlier research. (Chen *et al.*, 2022; Rasool *et al.*, 2020). On the other hand, our findings demonstrated that a spherical model only adequately reflected EC, pH, SNa, and TNa, while a Gaussian model better captured the majority of soil characteristics. These variations show that spatial dependence differs among soil characteristics, highlighting the significance of carefully choosing models to describe gradual vs concentrated patterns of variability. In soil research, both spherical and Gaussian models are well-established because they have been effectively used to characterize spatial patterns of pH, EC, and nutrient distribution in many settings. (Addis & Klik, 2015; Bogunović *et al.*, 2017; Neissian *et al.*, 2022).

The nugget to sill ratio also known as spatial dependence ratio, was calculated for all parameters following (Rosemary *et al.*, 2017). According to the classification of (Cambardella *et al.*, 1994), values below 25% indicate strong spatial dependence, between

25% and 75% moderate and above 75% weak dependence. In our study, all soil properties displayed spatial dependence ratio values lower than 25%, confirming strong spatial dependence. This implies that the variability observed is mainly structured and controlled by soil-forming processes and biological activity, rather than random error or micro-scale heterogeneity.

The spatial ranges of different parameters further illustrate their controlling mechanisms. For instance, OM (2.5 m), SK (3.6 m), and TNa (4 m) exhibited short ranges, suggesting localized variability likely linked to root activity, microbial hotspots, or other small-scale biological processes. Nonetheless, AP (2119 m), NO₃ (1643 m), and CaCO₃ (20 m) had longer ranges, reflecting the influence of broader pedogenic or environmental drivers that extend across the landscape.

The coefficient of variation (CV) provided an additional measure of variability. Following (Nielsen & Bouma, 1985) classification, parameters like pH (0.036) and EC (0.22) were relatively uniform throughout the study area, while AP (0.87) and TN (0.87) showed high variability, probably due to uneven nutrient cycling or plant uptake. Interestingly, even parameters with low CVs, like pH, showed structured patterns in their variograms, demonstrating that uniformity in variance does not necessarily imply spatial randomness (Wilding, 1985).



CONCLUSION AND PERSPECTIVES

Conclusion and Perspectives

Further in-depth studies are envisaged to better elucidate the functional roles of the identified microbial strains, refine our understanding of species-specific rhizosphere processes, and translate these findings into practical solutions for arid land management and sustainable agriculture.

These investigations can be summarized as follows:

- Molecular characterization of the identified bacterial isolates through 16S rRNA gene sequencing and whole-genome analysis to assess functional potential.
- Greenhouse and field trials to evaluate the effectiveness of the PGP strains as biofertilizers under real arid agricultural conditions.
- Metagenomic approaches to overcome the limitations of culture-dependent methods and capture the full microbial diversity associated with both species.
- Continuous monitoring of rhizosphere chemical and biological properties across plant growth stages to understand the dynamics of plant–soil–microbe interactions.
- Integration of remote sensing with geostatistical analysis to upscale rhizosphere variability patterns to the landscape level and map soil fertility hotspots.
- Ecological restoration trials using *Retama raetam* as a nurse plant in degraded arid land soils, quantifying its long-term impact on soil fertility and microbial diversity.
- Climate change simulation studies to assess the resilience of rhizosphere dynamics under increasing drought, temperature, and soil degradation scenarios projected for arid regions.
- Exploration of other native Fabaceae species in Algerian drylands to build a broader regional knowledge base on plant-soil interactions and expand the pool of potential bioinoculant strains.



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APPENDICES

APPENDICES

Appendix 01. Culture media

Table 2. Culture media (For a 1 L volume)

Culture media / Product	YMA	AM	PVK	CAS	LB
Mannitol	10g	20g	/	/	/
K ₂ HPO ₄	0.5g	0.2g	/	/	/
MgSO ₄ 7H ₂ O	0.2g	0.2g	0.41g	/	/
NaCl	0.1g	0.2g	0.2g	/	10 g
K ₂ SO ₄		0.1g			
CaCO ₃		5g			
Yeast extract	0.5g	/	3g	/	5 g
Agar	20g	/	20g	/	/
Ca ₃ HPO ₄	/	/	0.7g	/	/
Glucose	/	/	10g	/	/
KCl	/	/	0.295g	/	/
FeCl ₃	/	/	0,003g	2,7 mg	/
NH ₄ NO ₃	/	/	0.373g		/
HDTMA	/	/	/	0.073 g	/
HCl	/	/	/	10 ml	/
Chrome Azurol S	/	/	/	0.06 g	/
Peptone	/	/	/	/	10 g
PH	6.8	6.8	6.8	6.8	6.8

CAS Medium Preparation

Preparation of the CAS indicator solution

Dissolve 2.7 mg of FeCl₃·6H₂O in 10 ml of 10 mM HCl, then dissolve 0.06 g of Chrome Azurol S (CAS) separately in 50 ml of distilled water. The FeCl₃ solution is then added very slowly, under continuous stirring, to 0.073 g of HDTMA (Hexadecyltrimethylammonium bromide) previously dissolved in 40 ml of distilled water.

The resulting dark blue mixture is adjusted to pH 7. The dark blue solution obtained is then autoclaved at 120°C for 20 minutes.

Preparation of the final CAS medium

In a sterile Erlenmeyer flask, add 100 ml of the CAS-HDTMA solution to 900 ml of LB broth and homogenize thoroughly. Pour the medium into sterile Petri dishes, then inoculate the bacterial isolates.

Appendix 02. tables

Table 3. Correlation matrix of rhizosphere soil properties of *R.raetam*

Var	Ph	EC	CaCO ₃	OM	S Na	S K	T Na	T K	A P	T N	NO ₃
pH	1	0.196	-0.151	0.233	0.111	-0.299	0.219	-0.133	0.032	-0.281	-0.244
EC	0.196	1	-0.142	0.067	0.239	0.148	0.395	0.422	0.217	-0.048	-0.265
CaCO₃	-0.151	-0.142	1	-0.240	-0.036	0.071	0.027	-0.018	0.152	0.110	-0.048
OM	0.233	0.067	-0.240	1	0.362	0.174	0.085	0.209	-0.225	0.119	0.272
S Na	0.111	0.239	-0.036	0.362	1	0.317	0.367	0.429	0.100	0.447	0.196
S K	-0.299	0.148	0.071	0.174	0.317	1	0.379	0.856	-0.118	0.197	0.428
T Na	0.219	0.395	0.027	0.085	0.367	0.379	1	0.417	0.202	0.007	-0.079
T K	-0.133	0.422	-0.018	0.209	0.429	0.856	0.417	1	0.005	0.186	0.465
A P	0.032	0.217	0.152	-0.225	0.100	-0.118	0.202	0.005	1	-0.014	-0.338
T N	-0.281	-0.048	0.110	0.119	0.447	0.197	0.007	0.186	-0.014	1	0.324
NO₃	-0.244	-0.265	-0.048	0.272	0.196	0.428	-0.079	0.465	-0.338	0.324	1

Bold values are significantly different from 0 ($\alpha = 0.05$)

A GIS-based Approach to Assessing the Spatial Variability and Rhizosphere Soil Properties of *Retama raetam* Forssk., Growing in Southern Algeria

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ABSTRACT

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Fabaceae shrubs like *Retama raetam* L. can reshape soil chemistry through rhizosphere-microbe interactions, yet their spatial effects in arid lands remain underexplored. The main objective of the study is to assess and quantify the interactions of rhizosphere soil associated with *Retama raetam* and its spatial variability in the Sebseb region, Algeria. Rhizosphere (RS) and bulk soil (BS) were sampled and analyzed for pH, electrical conductivity (EC), organic matter (OM), CaCO₃, available phosphorus (AP), mineral nitrogen (NO₃), total nitrogen (TN), and soluble/total sodium and potassium (SNa/TNa, SK/TK). RS-BS contrasts, correlations, and spatial structure were assessed using variograms and ordinary kriging. RS/BS comparison revealed significant differences in several characteristics. EC ($p < 0.0001$), CaCO₃ ($p < 0.0001$), and TN ($p = 0.0069$) were higher in BS. RS exhibited a significantly lower pH ($p < 0.0001$), while NO₃ ($p = 0.01$) and TK ($p = 0.01$) levels were higher. All variables fit Gaussian variograms, except EC, pH, SNa, and TNa, which were better fitted in spherical models; nugget: sill $< 25\%$ indicated strong spatial dependence. Kriging maps revealed nutrient hotspots (EC, NO₃, AP) and broader pedogenic patterns (CaCO₃, pH) across Sebseb soils. Rhizosphere soil had lower pH and higher NO₃, while bulk soil harbored more CaCO₃, EC, and TN, highlighting distinct biological and pedogenic processes. Geostatistical analysis indicated strong spatial dependence, with short-range variability linked to rhizosphere effects and longer ranges driven by environmental factors. Overall, *Retama raetam* enhances nutrient availability and soil fertility, supporting its role in sustainable land management.

Keywords: Rhizosphere soil, GIS, kriging, spatial variability, arid ecosystems.

Introduction

Soil is considered a crucial area of scientific inquiry, with the rhizosphere being its most active part. Its active microbial community and its interactions with plant roots make it a target for biotechnological applications aimed at improving crop yields and soil health [1, 2]. Understanding these processes better is essential for preserving the plant cover in arid and semi-arid regions. The rhizosphere, commonly referred to as the "microbial reservoir," is home to diverse microorganisms and

other organisms that engage in complex interactions at the root interface [3, 4], which in turn exert a significant influence on the soil's biological and chemical characteristics [5].

Soil fertility and its management are central to sustainable agriculture, particularly in arid and semi-arid ecosystems where water scarcity and poor soil quality limit crop productivity [6, 7]. Leguminous plants play a key role in enhancing soil fertility through organic matter input and symbiotic nitrogen fixation, thereby supporting agricultural sustainability in fragile environments [8]. They improve soil structure and reduce erosion. This is particularly beneficial in marginal lands where soil fertility is low [9]. Among these, *Retama raetam*, commonly known as Rtem, is a leguminous shrub native to North Africa, Eastern Mediterranean, and Middle East countries, it is widely used in folk medicine, as a powder, an infusion, or a decoction, to treat different diseases such as diabetes [10]. It is also recognized to exhibit antimicrobial activity [11, 12]. This plant has garnered attention for its specialized metabolites, which have potential applications in biopesticides and sustainable agriculture [13].

The *Retama raetam* plant, due to its remarkable germinative ability, its capacity to endure water stress, and its unique root branching pattern, is recommended for use in vegetation restoration in fragile environments. Thanks to their strong symbiotic capacity, *Retama* species, particularly through their associations with nitrogen-fixing rhizobacteria, play a crucial role in biofertilizing saline and nutrient-poor soils, significantly contributing to the nitrogen cycle. These interactions enhance soil fertility and promote sustainable agricultural practices by converting atmospheric nitrogen into mineral nitrogen [14, 8].

Spatial variability of soil parameters represents one of the most fundamental aspects of modern agricultural science and environmental management. Understanding how soil properties vary across landscapes is essential for implementing effective precision agriculture strategies, optimizing resource use, and maintaining sustainable agricultural systems [15]. Spatial variability mapping can inform sustainable agroecosystem management by identifying areas requiring specific interventions, such as targeted fertilization or soil amendments [16]. Local observations in the Sebseb region also suggest that soils in areas where *Retama raetam* naturally grows exhibit greater fertility, often supporting improved crop growth and productivity. Karaoud and Noumi [17] found that *Retama raetam* enhances soil water availability, improves soil nutrient content (organic matter, total carbon, total nitrogen, and available phosphorus), and promotes understory vegetation in arid environments. Despite its importance, the interactions between *Retama raetam* and soil parameters, as well as the spatial variability of these parameters, remain insufficiently understood.

This study is an integral part of a broader research effort to unravel the complex interactions between Fabaceae species and their rhizospheric environment, with a particular focus on Plant Growth-Promoting Rhizobacteria (PGPR). A comprehensive understanding of these plant-soil-microbe dynamics is fundamental for promoting sustainable agricultural practices and advancing ecological conservation. The research initiative seeks to unravel the complexity of how Fabaceae plants and the rhizosphere engage in mutualistic relationships by studying the interactions among rhizosphere and bulk soil parameters and their spatial variability of *Retama raetam* collected in the

region of Sebseb (Algeria), in order to better understand its role in soil fertility and potential contributions to sustainable land management.

Materials and Methods

Study area

The study was carried out in the Sebseb region, located in Ghardaïa Province, in the center of southern Algeria (approximately 32°15'N, 3°26'E) (Figure 1). The region is characterized by an arid Saharan climate with significant temperature fluctuations between day and night and between seasons. The hot season extends from May to September, with July the hottest month, averaging 36.3°C and reaching 47°C. In contrast, January is the coldest month, with an average temperature of 9.2°C and an absolute minimum of -1°C. Annual precipitation is scarce and irregular, ranging from 13 to 68 mm, spread over an average of 15 days per year. On average, the region receives around 74.2 mm of rainfall annually, with mean maximum and minimum temperatures of 28.5°C and 16.2°C, respectively [18].

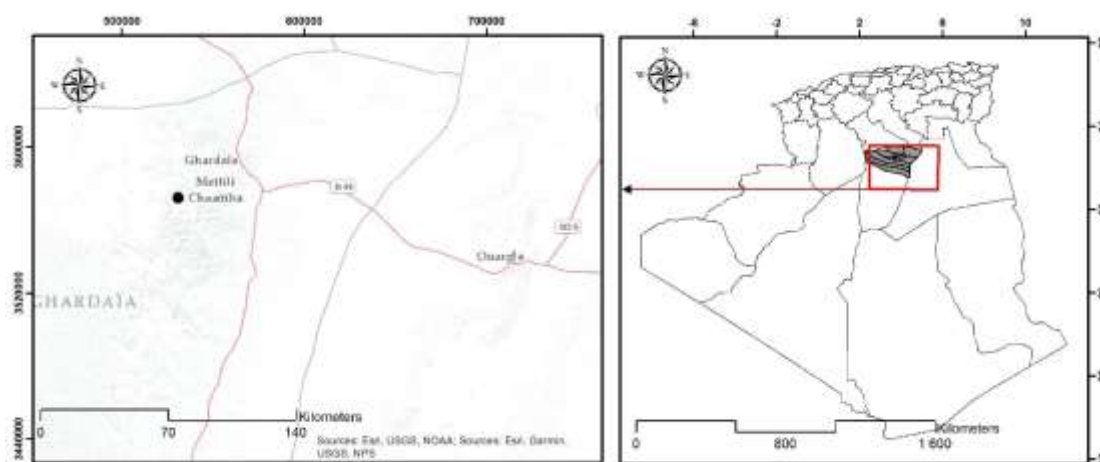


Figure 1. Geographical localization of the study area

Soil samples collection

Thirty-five (35) soil samples were collected during Spring 2023, from the rhizosphere and bulk soil. Using a trowel, the rhizosphere soil samples were obtained from areas directly influenced by plant roots. In contrast, bulk soil samples were collected from adjacent areas without direct root influence to provide a baseline for comparison. Samples were withdrawn at a depth of 20 cm below the surface in Ziplock plastic bags, which were then placed on ice and transported to the laboratory for further analysis.

Soil analysis

Soil samples were sieved through a 2-mm-mesh sieve. pH and EC at 25°C were determined in aqueous extract 1:5 (w/v). The CaCO_3 rate was tested using the Bernard calcimetry method described by [19]. The Loss on Ignition (LOI) method was used to determine OM content in soil. Approximately 5 g of sieved soil, weighed to the nearest 0.0001 g, was placed into pre-weighed porcelain crucibles and then dried in an oven at 220°C overnight. The crucibles containing the oven-dried soil were subjected to a second ignition step at 450°C for 4 hours, cooled, and weighed [19]. TN was measured using the Kjeldahl method from the standard operating procedure for soil nitrogen [20]. 3g of sieved soil was used as the sample size. $\text{NO}_3\text{-N}$ was measured by the steam distillation method using Devarda's Alloy as described by [21]. AP was determined by UV spectrometry at 882-nm using the sodium bicarbonate (NaHCO_3) procedure of Olsen et al [22] as described in ICARDA manual [23]. 0.5M of sodium

bicarbonate solution was used as extractant in 1:10 (w/v) ratio. Calibration concentrations ranged from 0.5 to 10 ppm of dried KH_2PO_4 . TNa and TK were determined using a flame photometer according to the procedure described in the ICARDA manual [23]. 1N Ammonium Acetate (NH_4OAc) was used as the extraction solution with at 1:5 (w/v) ratio. SNa and SK were measured by the same method using an aqueous extract from a saturated paste. NaCl and KCl with concentrations ranging from 10 to 100 ppm were used to calibrate the flame photometer.

Statistical analysis

Statistical analyses were performed using the R programming language (R 4.5.1). Differences between RS and BS were assessed using independent two-sample t-tests, with $p < 0.05$ as the criterion for significance. Pearson correlation coefficients were computed to examine relationships among soil properties. Principal Component Analysis (PCA) was applied to reduce dimensionality, identify key variables influencing soil quality and fertility, and visualize the separation of RS and BS samples. Graphical outputs, including boxplots, correlation matrices, and PCA biplot, were used to illustrate the results.

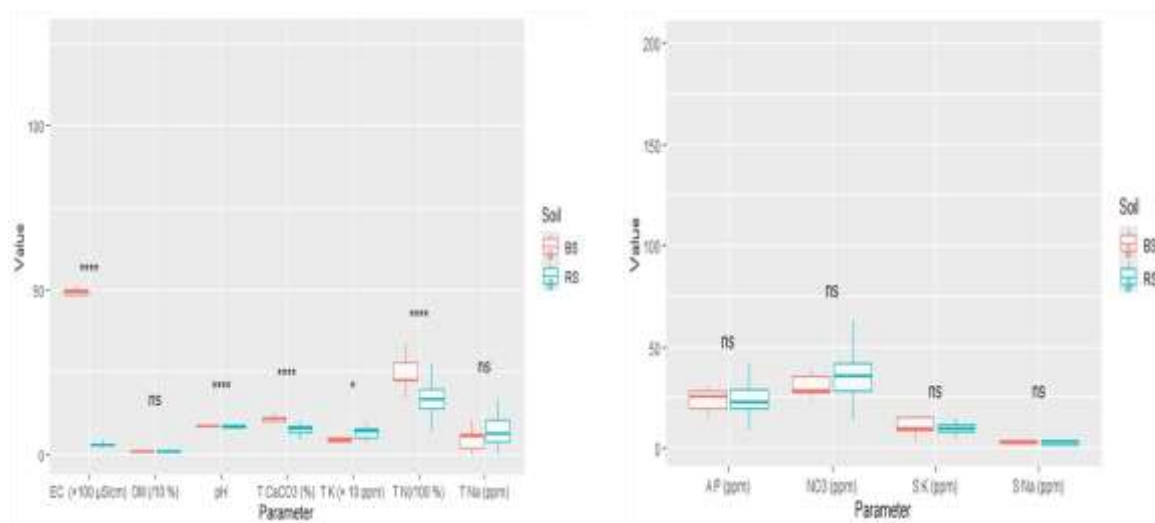
Geostatistical Analysis

Geostatistical methods were used to assess the spatial variability of soil parameters. Experimental semi-variograms were calculated for each variable to describe spatial dependence, and models (Gaussian, spherical, or exponential) were fitted based on the best agreement with the empirical data. Model selection was guided by minimizing residual sum of squares and maximizing the coefficient of determination (R^2). The nugget-to-sill ratio ($SDR = C_0 / (C_0 + C) \times 100$) was calculated to classify the degree of spatial dependence [24]. The range parameter was used to determine the spatial influence distance for each soil property. Ordinary kriging was applied to generate spatial distribution maps, allowing visualization of nutrient and property patterns across the study area. These maps were used to identify nutrient hotspots, gradients, and areas of spatial continuity, providing insight into localized rhizosphere processes and broader pedogenic control.

Results

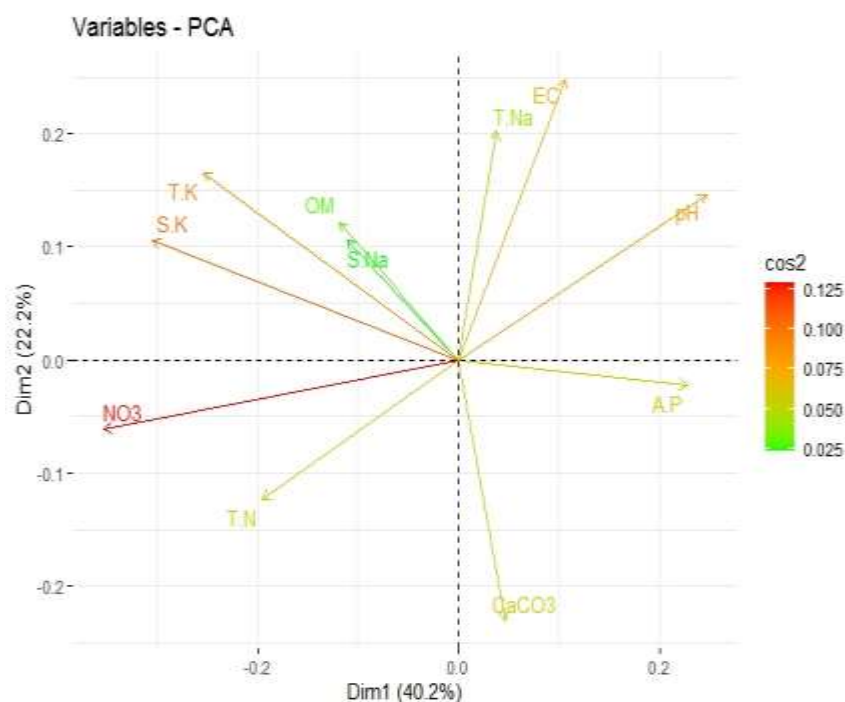
Comparative analysis of rhizosphere and bulk soil

To determine significant differences in properties between RS and BS, statistical t-tests were conducted. The results are visually represented in the corresponding boxplots (Figure 2). The comparison of soil properties between RS and BS revealed significant differences in several key characteristics. EC ($p < 0.0001$) and CaCO_3 ($p < 0.0001$) content were significantly higher in BS. Similarly, TN content was significantly higher in BS ($p = 0.0069$). Whilst RS exhibited a significantly lower pH ($p < 0.0001$). NO_3 ($p = 0.01$) and TK ($p = 0.01$) levels were also significantly different, with RS having higher concentrations than BS. In contrast, SK, SNa, AP, and OM showed no significant differences between the two soils ($p > 0.05$).

(a) EC, OM, pH, CaCO₃, TK, TN and TNa(b) AP, NO₃, SK and SNa**Figure 2.** boxplot showing the significant differences between RS and BS

Principal component analysis and correlation matrix

Principal component analysis (PCA) was performed to identify relationships among soil physicochemical parameters in the rhizosphere. The first two axes explained 62.4% of the total variance, with Dim1 accounting for 40.2% and Dim2 for 22.2% (Figure 3). Variables such as EC, TNa, pH, and AP were grouped on the positive side of Dim1. In contrast, CaCO₃ was positioned on the negative side of Dim1. Along Dim2, OM and SNa clustered closely together, while SK and TK formed another tight group. NO₃ and TN were separated from the main clusters, with NO₃ located in the negative quadrant of both axes.

**Figure 3.** Principal Components Analysis (PCA) of soil parameters

The correlation matrix revealed several significant relationships between rhizosphere soil properties. SNa showed a significant positive correlation with MO ($r = 0.3624$, $p = 0.0491$), TNa ($r = 0.3670$, $p = 0.0461$), TK ($r = 0.4296$, $p = 0.0178$), TN ($r = 0.4471$, $p = 0.0132$). SK showed a significant positive correlation with NO₃ ($r = 0.4282$, $p = 0.0182$). TK showed a significant positive correlation with TNa ($r = 0.4171$, $p = 0.0218$), SK ($r = 0.8569$, $p < 0.0001$), NO₃ ($r = 0.4655$, $p = 0.0095$) and EC ($r = 0.4223$, $p = 0.0201$). TNa showed a significant positive correlation with EC ($r = 0.3957$, $p = 0.0304$), SK ($r = 0.3796$, $p = 0.0385$) (Figure 4).

Geostatistical analysis of rhizosphere soil parameters

The spatial variability of soil parameters was evaluated through variogram modeling and the coefficient of variation (CV%), as summarized in Table 1. All variables followed a Gaussian distribution, whereas EC, pH, SNa, and TNa were better fit by spherical distributions, indicating spatial heterogeneity. The nugget-to-sill ratios for all variables were below 25%. The range values varied substantially. Parameters such as OM (2.5 m), SK (3.6 m), and TNa (4 m) showed limited spatial influence; in contrast, AP (2119 m), NO₃ (1643 m), and CaCO₃ (20 m) had longer spatial ranges. High CV values were observed for AP (0.87) and TN (0.87). Moderate variability was observed for OM (0.47) and TNa (0.66), while pH (0.036) and EC (0.22) had lower CVs.

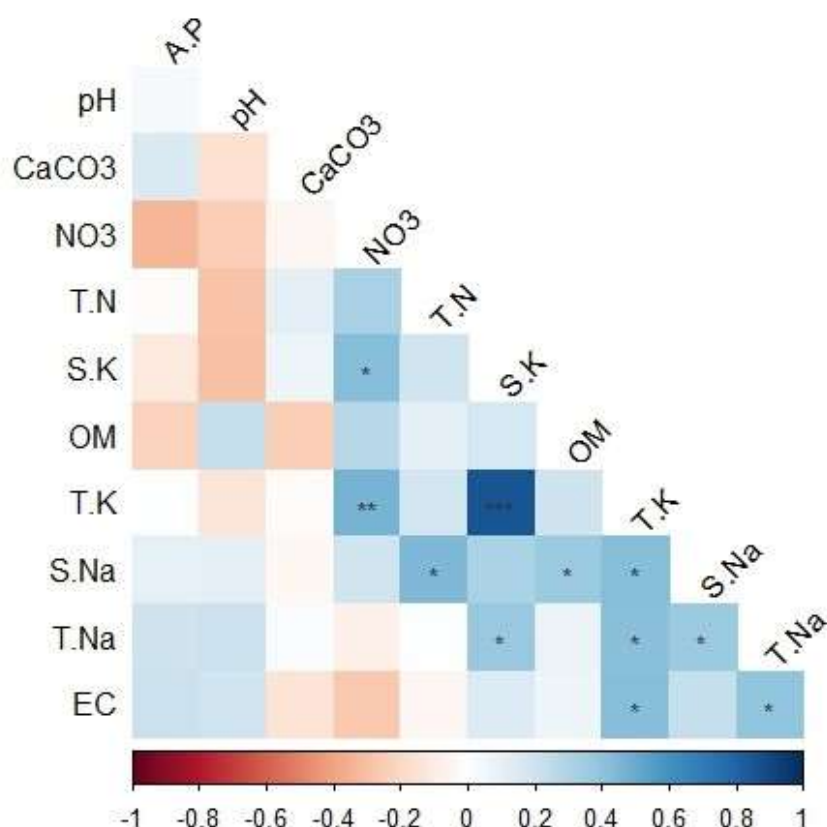
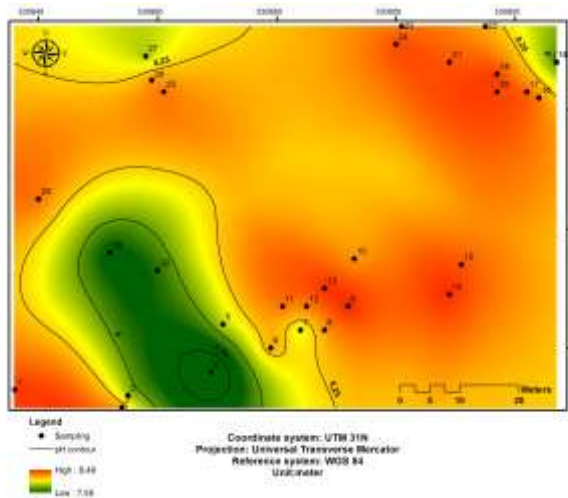


Figure 4. Correlation matrix with significant relationship of rhizosphere soil properties

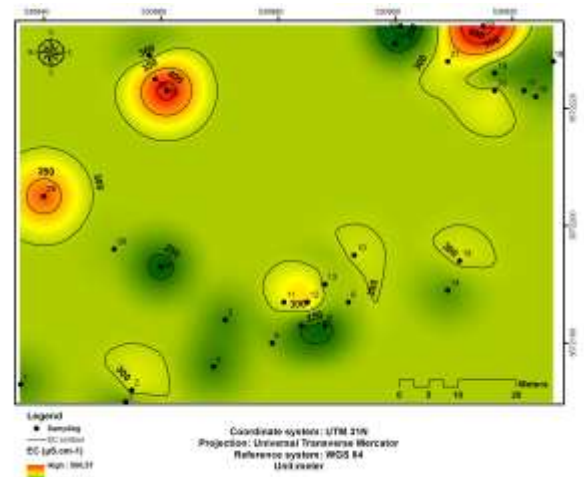
Table 1. Fitted variogram models and variation properties

Parameter	Model	Nugget	Sill	Range (m)	Nugget/Sill (%)	Coefficient of variation (%)
AP	Gaussian	81	3515946	2119	~0.002	86.98
CaCO ₃	Gaussian	0.83	11	20	7.5	29.61
NO ₃	Gaussian	48	251562	1643	~0.019	31.44
TN	Gaussian	0	0.03	9.1	0	86.95
EC	Spherical	0	3485	10	0	21.76
OM	Gaussian	0	0.06	2.5	0	47.12
pH	Spherical	0.02	0.11	27	18.2	3.64
SK	Gaussian	0	5.3	3.6	0	26.12
SNa	Spherical	0	0.96	4.9	0	35.03
TK	Gaussian	7.7	375	7.2	2.1	29.06
TNa	Spherical	0	17	4	0	66.20

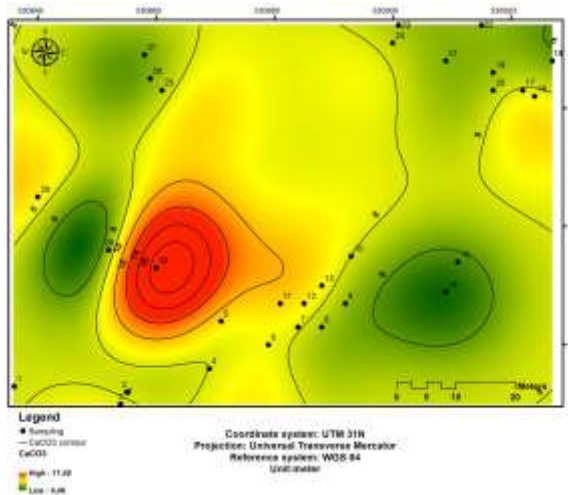
The spatial distribution maps generated by ordinary kriging revealed distinct patterns of soil property variability across the study area. CaCO₃ content ranged from 4.46% to 17.40%, with the highest concentrations observed in the south-central region. EC ranged from 197.51 to 500.57 $\mu\text{S}\cdot\text{cm}^{-1}$, indicating localized salinity hotspots in the northwest, northeast, and southwest. AP levels ranged from 9.33 to 127.94 ppm, with markedly higher values in the northwest, while lower concentrations predominated in the central and eastern regions. NO₃ concentrations ranged from 19.44 to 61.94 ppm, with elevated levels occurring in the south-central area. Soil pH values showed slight variation, ranging from 7.55 to 8.49, with more alkaline zones located in the northeastern and central regions (Figure 5).



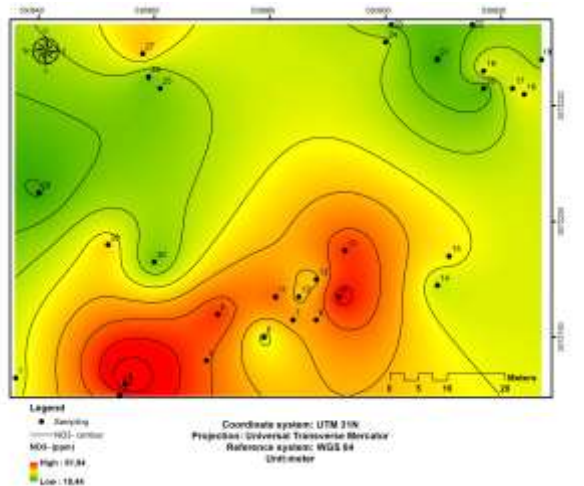
(a) pH



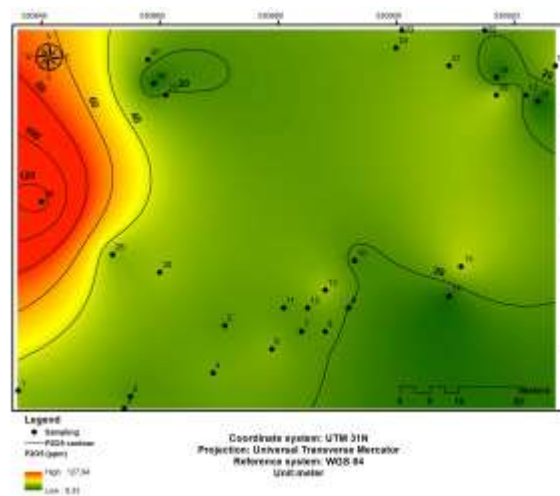
(b) EC



(c) CaCO₃



(d) NO₃⁻



(e) P₂O₅

Figure 5. Spatial distribution maps generated by ordinary kriging for rhizosphere soil parameters

Discussion

Rhizosphere and Bulk Soil properties

The microbial community in the rhizosphere is generally more active and diverse than in bulk soil [25], and this activity strongly influences the chemical environment, including electrical conductivity (EC). Microbial processes modify nutrient availability and ion concentrations, often lowering EC in the rhizosphere compared to the surrounding soil [26]. In line with this, our results showed significantly higher EC in bulk soil ($p < 0.0001$), a trend also reported in previous studies [27, 28]. Such reductions in rhizosphere EC are likely due to enhanced ion uptake and microbial transformations. Additionally, practices such as incorporating legumes as green manure further enrich soil organic matter, improve nutrient cycling, and help stabilize EC while promoting sustainable agriculture [29]. Furthermore, EC and pH also differed significantly between soil compartments, with rhizosphere soil showing lower values than bulk soil ($p < 0.0001$). This pattern is widely supported by evidence across diverse cropping systems, including paddy soils [30], neutral or alkaline soils [31], and more recent studies documenting significant rhizosphere acidification [32, 33]. The decrease in rhizosphere pH is primarily linked to root proton release and microbial activity, while in species such as *Retama raetam*, organic matter inputs from leaf litter and root residues may release organic acids during decomposition, further enhancing acidification. Conversely, the release of certain organic anions during decomposition may increase pH through decarboxylation and base release, reflecting the complex interplay of rhizosphere processes [34, 35]. These conditions also shape nitrogen dynamics: our results indicated that nitrate concentrations were significantly higher in rhizosphere soil ($p = 0.01$), consistent with studies reporting enhanced microbial activity, nitrogen fixation by Rhizobiaceae, and increased N-cycling enzyme activity in legume rhizospheres, leading to greater nitrogen availability compared to bulk soil [36-38]. In contrast, total nitrogen was significantly higher in bulk soil ($p = 0.0069$). This finding agrees with results from alpine grasslands where rhizosphere total nitrogen was lower than in bulk soil [39], suggesting that the rhizosphere effect is highly species- and environment-dependent. Similarly, our study found that CaCO_3 content was significantly higher in bulk soil ($p < 0.0001$), a result consistent with reports showing that carbonate content tends to decrease in the rhizosphere due to root-driven CO_2 enrichment and acidification, with the highest levels retained in bulk soil [40]. Collectively, these findings highlight that rhizosphere processes driven by root activity, microbial interactions, and organic matter turnover create distinct chemical signatures that differentiate rhizosphere from bulk soil in terms of EC, pH, nitrogen dynamics, and CaCO_3 distribution.

Multivariate Analysis

In this study, several significant correlations among soil properties were observed, consistent with previous research. Soluble sodium showed a positive correlation with both organic matter ($r = 0.3624$, $p = 0.0491$) and total sodium ($r = 0.3670$, $p = 0.0461$). Similar patterns have been widely documented, with numerous studies demonstrating that soluble and total sodium tend to increase under varying soil types and conditions, including saline and agricultural environments [41, 42]. Sodium salts are also known to enhance organic matter release, as sodium exchange processes displace sorbed organic anions, thereby promoting solubilization [43]. Moreover, positive associations between organic matter and soil aggregate stability, where sodium plays a role alongside organic inputs, highlight the contribution of soluble sodium to structural processes in soils [44]. In terms of nutrients, soluble potassium correlated positively with nitrate ($r = 0.4282$, $p = 0.0182$), a relationship also reported in agricultural soils, where fertilization practices and crop uptake coordinate K^+ and NO_3^- dynamics [45-47]. This coordination reflects the physiological role of potassium as a counter-ion facilitating nitrate transport in plants [48]. Field evidence further shows synchronized increases of nitrate and potassium under fertilization [45], as well as positive associations between extractable soil potassium, nitrate, and plant performance in vineyards [46], and in restored soils where nutrient mineralization links sodium and nitrogen forms [49]. Total potassium exhibited strong correlations

with soluble potassium ($r = 0.8569$, $p < 0.0001$), NO_3^- ($r = 0.4655$, $p = 0.0095$), and EC ($r = 0.4223$, $p = 0.0201$), reinforcing the idea of a dynamic equilibrium among potassium fractions. Similar positive associations among different forms of potassium have been reported in the Lesser Himalayas [50], Haryana and North Bihar [51, 52], paddy soils of Nagpur [53], and acid Alfisols [54]. Additionally, the link between total potassium and EC reflects the well-established role of potassium in salinity contexts, with strong correlations ($r \geq 0.83$) documented in arid and saline-sodic soils [55]. Similarly, total sodium correlated positively with EC ($r = 0.3957$, $p = 0.0304$) and soluble potassium ($r = 0.3796$, $p = 0.0385$), in agreement with studies reporting extremely strong associations ($r > 0.95$) between sodium, potassium, and EC in saline soils of Libya and Oman [56–58]. Collectively, these results emphasize that sodium and potassium dynamics are closely linked to each other and to nitrogen and salinity indicators, and that these interrelationships are mediated by soil chemical processes, fertilization practices, and plant uptake patterns across environments.

Rhizosphere soil variability

Geostatistical analysis has long been recognized as a powerful approach for characterizing soil spatial variability, with ordinary kriging and semivariogram modeling widely applied in this context. Previous studies often reported that spherical and exponential models provide the best fit for soil properties [59, 60]. In contrast, our results showed that most soil parameters were better represented by a Gaussian model, whereas only EC, pH, soluble sodium, and total sodium were better described by a spherical model. These differences indicate that spatial dependence varies across soil attributes, reinforcing the importance of careful model selection to capture gradual versus localized patterns of variability. Both Gaussian and spherical models are well established in soil science, having been successfully applied to describe the spatial patterns of pH, EC, and nutrient distribution across different environments [61–63].

The nugget-to-sill ratio, also known as the spatial dependence ratio (SDR), was calculated for all parameters as described in [24]. According to the classification of [64], values below 25% indicate strong spatial dependence, between 25% and 75% moderate, and above 75% weak dependence. In our study, all soil properties showed SDR values below 25%, indicating strong spatial dependence. This implies that the observed variability is mainly structured and controlled by soil-forming processes and biological activity, rather than by random error or micro-scale heterogeneity.

The spatial ranges of different parameters further illustrate their controlling mechanisms. For instance, OM (2.5 m), SK (3.6 m), and TNa (4 m) exhibited short ranges, suggesting localized variability likely linked to root activity, microbial hotspots, or other small-scale biological processes. Nonetheless, AP (2119 m), NO_3^- (1643 m), and CaCO_3 (20 m) had longer ranges, reflecting the influence of broader pedogenic or environmental drivers that extend across the landscape.

The coefficient of variation (CV) provided an additional measure of variability. Following [65]'s classification, parameters such as AP (0.87) and TN (0.87) showed high variability, likely due to uneven nutrient cycling or plant uptake. OM (0.47) and TNa (0.66) exhibited moderate variability, while pH (0.036) and EC (0.22) were relatively uniform across the study area. Interestingly, even parameters with low CVs, such as pH, still showed structured patterns in their variograms, indicating that uniform variance does not necessarily imply spatial randomness [66].

CONCLUSION

This study highlights the influence of *Retama raetam* on soil properties and their spatial variability in the arid region of Sebseb, Algeria. Rhizosphere soil was characterized by lower pH and higher nitrate, while bulk soil contained higher EC, CaCO_3 , and total nitrogen, reflecting contrasting biological and pedogenic processes. Strong correlations among sodium, potassium, organic matter, and nitrate confirmed the interconnectedness of nutrient pools.

Geostatistical analysis revealed structured variability, with all parameters fitting Gaussian or spherical models and nugget-to-sill ratios less than 25%, indicating strong spatial dependence. Short-range variability in organic matter, soluble potassium, and total sodium reflected localized rhizosphere processes, whereas longer-range variability in available phosphorus, nitrate, and CaCO₃ was influenced by broader environmental factors.

Overall, *Retama raetam* plays a key ecological role in acidifying soils and enhancing nutrient availability in arid shrublands. The combination of GIS and geostatistical approaches provided valuable insights into multi-scale soil heterogeneity and supports the role of spontaneous Fabaceae shrubs as natural contributors to soil fertility and sustainable land management.

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Département des Sciences Agronomiques



Laboratoire de Valorisation et Conservation des Écosystèmes Arides "LVCEA"
Séminaire national sur l'Agro Système Oasien
(Préservation et Perspectives de Développement)
25 & 26 Février 2025 (SNASO_2025)



Attestation de Participation

Le président du Séminaire national sur l'Agro Système Oasien
(Préservation et Perspectives de Développement) (SNASO_2025) tenue à l'université de Ghardaïa le
25 & 26 Février 2025, atteste que **Mme. LAOUAR Bouchra** a participé à cette manifestation
scientifique par une communication affichée intitulée :

**Contribution des rhizobia associés à l'arachide à la gestion durable des sols agricoles dans la région de
Ghardaïa**

Co-auteurs: BENDIB Fatima Zohra, KRAIMAT Mohamed et BENHAMMOUDA Hicham.

Directeur LVCEA

Président

Doyen de la Faculté SNV-ST

مدير مختبر البحث التلثمين
والحفظ على الأنظمة البيئية الجافة
❖ عليود يوسف ❖





REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTRE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
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FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET SCIENCES DE LA TERRE
SEMINAIRE NATIONAL SUR LES PLANTES SPONTANÉES
LE 29 NOVEMBRE 2023



ATTESTATION DE PARTICIPATION

Le président du Séminaire National sur les Plantes Spontanées (SNPS 2023) atteste que **LAQUAR Bouchra** a présenté une communication affichée intitulée:

Diversité phénotypique des isolats rhizobiens associés à l'arachide (*Arachis hypogaea* L.), cultivée dans la région de Ghardaia

Co-auteur(s): **BENDIB Fatima Zohra, KRAIMAT Mohamed, BENHAMMOUDA Hicham**

Directeur de Laboratoire

مدير مختبر البحوث التثمين
والمحافظة على الأنظمة البيئية الجافة
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CERTIFICATE OF ATTENDANCE

This Certificate is delivered to:

Laouar Bouchra

For his/her participation to 1st international conference on: "Energy Systems, Artificial Intelligence, Plasma, Materials and Environment" from 16-17 October 2024 at university of Ghardaia, Presenting a poster paper entitled:

Phenotypic diversity of rhizobial isolates associated with peanut (*Arachis hypogaea* L.), grown in the region of Ghardaia

Co-authors:

Bendib Fatima Zohra, Kraimat Mohamed, Benhammouda Hicham

October 17, 2024

Chairman of ESAIPME'2024

Pr. AR. BELLADOUAR





People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University of Ghardaïa

Valorization and Conservation of Arid Ecosystems Laboratory "VCAEL"



CERTIFICATE OF ATTENDENCE

is hereby awarded to:

Bouchra LAOUAR

for the participation in the International Congress on the Valorization and Conservation of Arid Ecosystems (ICVCAE) held from 25-27th November 2025 in Ghardaia, Algeria with Displayed communication entitled:

"Unveiling phenotypic diversity in rhizobia associated with peanut (*Arachis hypogaea* L.) cultivated in Ghardaia"

Co-authors: Fatima Zohra BENDIB, Mohamed KRAIMAT, Hicham BENHAMMOUDA

Chairman of Congress



Amel Zerrouini

Director of the LVCEA laboratory



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