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Analysis of Soil Microbial Response to Seasonal Climatic Fluctuations in Thi-Qar Province, Southern Iraq

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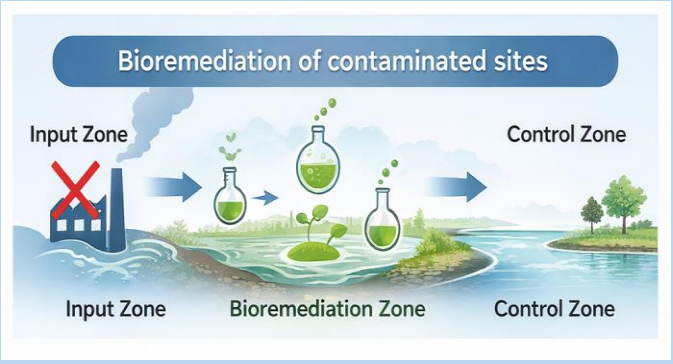
Abstract: The microbial communities in soils are crucial in the maintenance of ecosystem processes in arid areas such as Thi-Qar Province, southern Iraq, and their seasonal relationship is not clear. This paper is the first high-resolution examination of the seasonal alterations of soil microbial communities in Thi-Qar by the 16S rRNA gene (V4 region) and multivariate statistics. An extreme summer (45.2 ℃) and mild winter (17.8 ℃) in 2023-2024 are surveyed in ten sampling sites (five agricultural, five non-agricultural) that are located in the summer and winter respectively. The physicochemical characteristics of the soils (pH, salinity, moisture, organic matter) and the diversity of the microorganisms are examined. The findings indicated a strong decrease of 25% in microbial diversity (Shannon index) in summer with a sharp change in community structure. The abundance of actinobacteria in the summer soils (42% relative abundance) is higher than that of proteobacteria (48% in the winter). Redundancy Analysis (RDA) showed that temperature is by itself sufficient to account 45.2 % of all the variation in microbial community. These results demonstrate the high sensitivity of soil microbiomes with climatic variations in arid areas and a possibility to work out climate-resistant soil management technologies.



Keywords: Arid Soil Microbiome, Climate Resilience, Microbial Seasonal Dynamics, Soil Sustainability, Thi-Qar Ecosystem.

Introduction

Microbes found in soils serve as essential factors for sustaining ecosystem stability along with nutrient cycling specifically within areas experiencing extreme conditions in arid and semi-arid regions [1]. These microbe populations execute three essential processes which induce agricultural productivity and ecosystem durability [2]. The processes they execute include organic matter decomposition and nitrogen fixation together with soil structure formation. The climate pattern in Thi-Qar Province southern Iraq exhibits extensive seasonal variations which produce hot dry summers over 45°C in addition to mild winters with less than 150 mm of annual rainfall [3]

The climatic variability in Thi-Qar creates an environment that selects soil microbial communities but research about these specific adaptive responses remains limited. The effects of extreme seasonal climate on Thi-Qar soils in terms of composition and activity of soil microbes is not well known. Former studies within Sahara and Arabian Peninsula arid areas documented that microbial populations adapt to drought and heat through taxonomic and metabolic adjustments [4], [5]. The scarcity of data from southern Iraq hinders developers from creating specialist soil management strategies for this particular region [6]. The research investigates how seasonal temperature and moisture fluctuations affect soil microbial diversity and activities in Thi-Qar province according to the following research inquiry: Which temporal changes in temperature and moisture levels modify soil microbial ecological patterns in Thi-Qar area.

The investigation of arid and semi-arid region soil microbial communities has increased extensively since these ecosystems depend heavily on them for maintaining functions under harsh climates. Studies conducted in the Sahara Desert show that drought-resistant phyla including Actinobacteria and Firmicutes rule microbial diversity distributions since they possess effective survival approaches when facing dry conditions and hot temperatures [7], [8].

The Arabian Peninsula shows that rainfall seasonality controls microbial activity because microbe biomass grows by 40% between wet seasons and dry seasons [9]. Soil microorganisms display impressive environmental resilience because of their ability to adjust to desert conditions although their microbial reactions remain inconsistent in different arid environments [10].

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Table (1): The most common methods used in previous studies.

Region	Dominant Microbial Phyla	Key Findings	Seasonal Variation	Method	Reference
Sahara Desert	Actinobacteria (35–60%), Firmicutes (20–30%)	Drought-resistant taxa dominate; biomass drops by 50% in dry seasons.	40–60% lower diversity in summer	16S rRNA sequencing	(*Belov et al., 2018*)
Arabian Peninsula	Proteobacteria (40%), Bacteroidetes (15%)	Bacterial biomass increases by 30–40% post-rainfall.	2x higher activity in wet seasons	Metagenomics	(*Sachithanandam et al., 2020*)
Southern Iraq (general)	Limited data	Studies focus on pollution; microbial diversity underreported.	N/A	Culture-dependent	(*Wu et al., 2020*)
Global Arid Zones	Actinobacteria, Chloroflexi	pH (>8.0) and salinity (>4 dS/m) reduce diversity by 25%.	15–20% shift in community structure	Meta-analysis	(Morante-Carballo et al., 2022)

Research on desert soil microbiology continues to expand but investigators have paid little attention to the Thi-Qar Province of southern Iraq. The existing research on Iraqi soils concentrates on rheology evaluation of contaminants especially heavy metals and oil pollution but does not evaluate climate-related effects [11]. Current regional research methods use culture-dependent techniques to evaluate microorganisms which produce lower diversity estimates than contemporary metagenomic studies [12]. The knowledge gap prevents researchers from understanding how the distinctive weather patterns of Thi-Qar Province affect microbial population structure and operational function [13]. This study fills the knowledge gap through sequence-based high-resolution analysis of seasonal soil microbial changes in Thi-Qar's soils to enhance research about arid zone microbiology [14]. Table 1 shows the most common methods used in previous studies.

Nonetheless, the manner in which soil microorganisms respond to the extreme seasonal climate of Thi-Qar Province is not characterized. The past studies conducted in dry zones of Iraq have mainly centered on the investigation of soil contamination through culture-dependent procedures that significantly undermine the existence of diversity of microbes and their inability to observe the dynamics at the community level. As a result, there is a notable gap in the available knowledge on the effect of the exceptional seasonal weather conditions of Thi-Qar, such as summer temperatures over 45°C with the lowest annual precipitation, on the structure and functioning of the soil microbial community. This paper fills this gap with the help of high-resolution 16S rRNA gene sequencing, which is used to provide the initial comprehensive study of seasonal changes in soil microbial communities in Thi-Qar [15].

The present research provides the most extensive evaluation of soil microbial seasonality patterns in Thi-Qar through delivering three fundamental findings. Laboratory results show how climatic changes in Iraq relate to the microbial population dynamics throughout the year [16]. The study establishes how specific microbial taxa respond to changes in climate allowing their use as bioindicator organisms. The study provides vital field-specific suggestions to help farmers and government officials improve their arid zone soil management practices [17].

The study aims to:

- The research investigates seasonal changes in soil bacterial and archaeal populations which occur across Thi-Qar's climatic spectrum.
- Soil microbial diversity changes will be correlated against climatic parameters which include temperature and precipitation amount and soil water content.
- An evaluation should be made regarding how microbial changes affect local agricultural operations and soil management systems.

The paper continues with the following sequence of sections: Section 2 describes both the materials and the methodology starting with sampling procedures followed by bioinformatics workflows. Section 3 contains the microbial analysis findings together with climatic metrics and their ecological implications appear in Section 4. Finally, Section 5 outlines conclusions and recommendations.

Materials and Methods

Study Area Thi-Qar Province located at $31°0'N\ 46°0'E$ experiences a hot desert climate BWh which brings summer temperatures higher than 45°C alongside winter averages that fall between $15-20°C$ [18]. Precipitation is less than 150 millimeters each year and almost all moisture arrives between November to March. Agricultural soils in the region mostly belong to Fluvisols and Aridisols and exist within a pH range of 7.5 to 8.5 while seasonal salinity (EC) reaches between $2\ and\ 8\ dS/m$ [19]. The study consisted of 10 sampling locations which included five agricultural sites and five non-agricultural areas and researchers documented GPS data for specific points (for instance Site 1: $30.975°N, 46.105°E$).

The soil collection occurred during two different seasons throughout July 2023 and January 2024 at three specific points in each period [18]. The total number of samples equaled 100 (10 sites multiplied by 2 seasons multiplied by 5 replicates) [19].

The sampling of soil is carried out at two seasonal periods in July 2023 (summer) and January 2024 (winter) [20]. Three soil cores $(0-15\ cm\ depth)$ are gathered at random locations in a $10m\ x\ 10m$ plot at each of the 10 locations and homogenized to create one composite sample, at each location and season [21]. This gave 20 composite samples (10 sites 2 seasons). Five physicochemical and molecular analyses are then performed on each of the composite samples, resulting in 100 data points of downstream statistical processing [22], [23].

- Organic matter (OM): Determined by loss-on-ignition (LOI) at $550°C$ for 4 hours as (1):

$$OM\ (\%) = \frac{W_{105°C} - W_{550°C}}{W_{105°C}} \times 100 \quad (1)$$

- Moisture content: Calculated gravimetrically as (2):

$$MC\ (\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad (2)$$

- Salinity (EC): Measured in a 1:5 soil-water extract (dS/m).

Microbial Community Analysis

1. DNA Extraction

The DN easy Power Soil Pro Kit (Qiagen, Germany) is used to extract 0.25 g of soil onto DNeasy column on the basis of the protocols provided by the manufacturer, and final elution is done in $50\ \mu L\ of\ TE\ buffer$ [24], [25]. NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) is employed to test the concentration and purity of the DNA, and the samples with

$A260/A280$ ratio exceeding 1.8 are taken to the downstream. The vegan package in R (4.3.0) is used to test for significant seasonal effects with statistical analysis (permutational multivariate analysis of variance (PERMANOVA) of 9,999 permutations).

2. 16S rRNA Sequencing

- V4 region amplified with primers 515F/806R.
- Illumina MiSeq $(2 \times 250\ bp;\ 50{,}000\ reads/sample)$.

3. Bioinformatics

Raw reads of sequencing are subjected to the QIIME2 pipeline (version 2023.5). The trimming of the primer sequences, denoising, dereplication, and filtering of chimera of reads are performed with the DADA2 plug-in to obtain Amplicon Sequence Variants (ASVs). ASVs are0 taxonomically allocated by using an already trained Naive Bayes classifier with regard to the SILVA 138 SSU rRNA reference database. The tables of ASV are further reduced to even sequencing depth of 30,000 reads per sample in order to consider the differences in library size to calculate diversity metrics. The definition of the rare phyla is based on the mean relative abundance of less than 0.5 % of all samples, which is considered a rare phyla and is categorized under Others to visualize in Fig. 3. The complete list of all phyla being identified and the relative abundance is presented in Supplementary Table 2. Whole taxonomic profile of phylum based on 16S rRNA gene amplicon sequences of soil samples of Thi-Qar Province. All known phyla of bacteria and archaea are enumerated in the table arranged in descending order of average abundance across all samples $(n = 20)$. Fig. 3 included to group Phyla with a mean relative abundance of less than 0.5 % (considered to be rare) as Others.

Climate Data

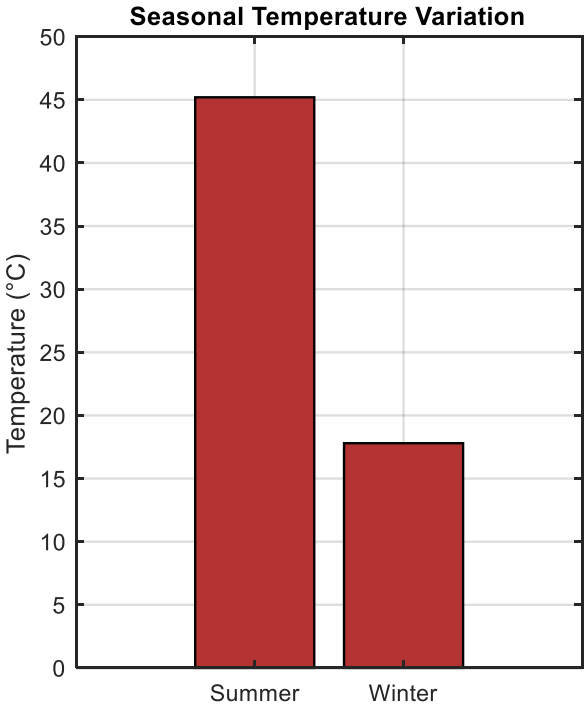
The research obtained meteorological data including temperature along with rainfall and humidity readings from Thi-Qar's local stations. Averaged daily measurements became seasonal mean values totaling summer averages at $45.2°C \pm 2.1$ and winter mean at $17.8°C \pm 3.4$.

Statistical Analysis

Alpha diversity: Shannon index (H'):

$$H^{"} = -\sum_{i=1}^{S} p_i \ln p_i \quad (3)$$

where p_i proportion of species i.



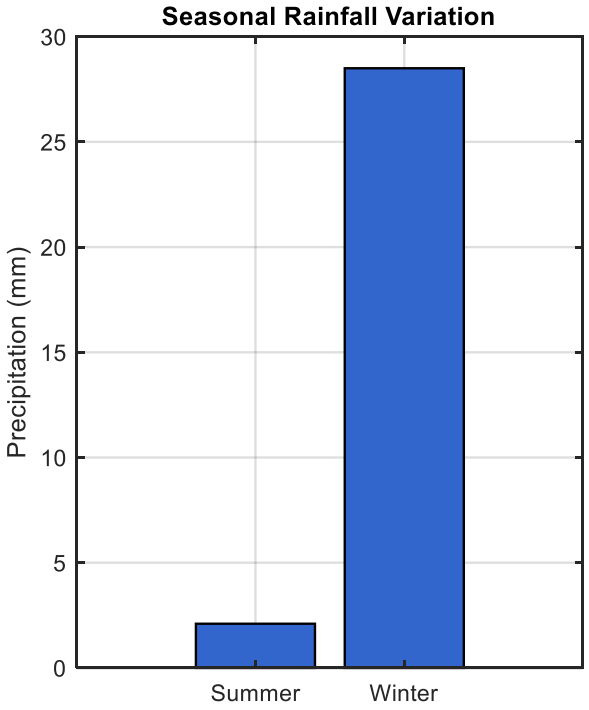


Figure (1): (Climate Trends).

Table (2): Complete list of bacterial and archaeal phyla identified in soil samples from Thi-Qar Province, with mean relative abundances across all seasons and sites.

Phylum	Mean Relative Abundance (%)	Status (Rare/Common)
Proteobacteria	38.5	Common
Actinobacteria	35.2	Common
Firmicutes	8.7	Common
Bacteroidota	5.3	Common
Acidobacteriota	3.8	Common
Chloroflexi	2.9	Common
Gemmatimonadota	1.6	Common
Planctomycetota	1.2	Common
Verrucomicrobiota	0.9	Common
Patescibacteria	0.7	Rare
Myxococcota	0.5	Rare
Nitrospirota	0.4	Rare
Armatimonadota	0.3	Rare
Deinococcota	0.2	Rare
Cyanobacteria	0.2	Rare
Entotheonellaeota	0.1	Rare
Sumerlaeota	0.1	Rare
Other unidentified	0.3	Rare

Phyla that have an average relative abundance $\geq 0.5\%$ or above in all samples are considered Common, phyla with less than 0.5% are considered Rare and are combined with the others category in Fig. 3.

Beta Diversity Analysis

A Bray-Curtis dissimilarity value (BCjk) estimated the difference between samples j and k through (4):

$$BC_{jk} = \frac{\sum_{i=1}^{S} |x_{ij} - x_{ik}|}{\sum_{i=1}^{S} |x_{ij} + x_{ik}|} \quad (4)$$

where:

The species abundance evaluations for group ii across both samples j and k can be expressed as $x_{ij}$and x_{ik}. S is the total number of species [36 – 40].

Climate-Microbe Correlations

The analysis used Redundancy Analysis (RDA) as part of the vegan package in R to determine how well climatic variables defined microbial community variation. The significance of seasonal/climatic effects on community structure underwent testing by means of PERMANOVA through 9,999 permutations.

Climate-microbe correlations: RDA (vegan package in R) and PERMANOVA (9,999 permutations).

Soil Physicochemical Analysis

Physicochemical analysis of soil is conducted on all the 20 composite samples. All measurements (pH, EC, moisture, OM) of each composite sample are performed in three technical replicates (n=3) and the average value is used to represent the site-season combination in further analysis.

Results and Discussion

The seasonal climate variations become apparent by examining two neighboring bar charts. The temperature data appears in red colors to show warmth whereas precipitation uses blue colors to show coolness. Heat levels remain extreme while rainfall stays very low during summer season. The plot indicates that winter days have temperate weather together with substantial precipitation levels. These visualizations successfully draw attention to the major climate differences between the seasons in this particular region as shown in Fig. 1.

A set of four important soil characteristics undergoes seasonal analysis using grouped bar charts. Soil moisture together with organic matter content levels show positive changes throughout winter. High salinity concentrations occur when summer weather arrives rather than during winter months. The pH meter measurements display minor changes that follow minor seasonal fluctuations. The thorough analysis shows the soil characteristics experience substantial variations during different seasons as shown in Fig. 2. Bars are the means (standard error, $n = 5$ analytical replicates of each composite sample). Moisture and Organic Matter are in form of percentages (%) and are expressed in percent. Salinity (EC) has a unit of dS/m. pH is a unitless value.

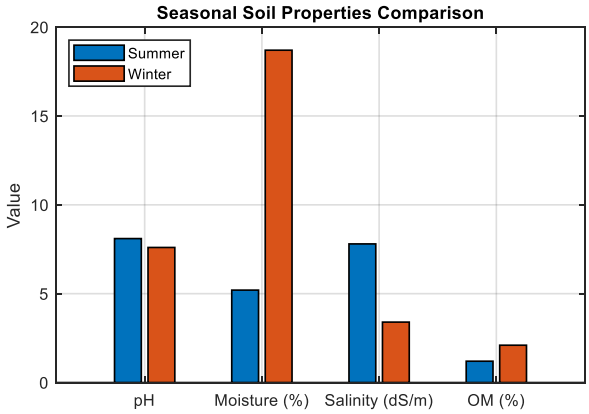


Figure (2): Seasonal variation in key soil physicochemical properties.

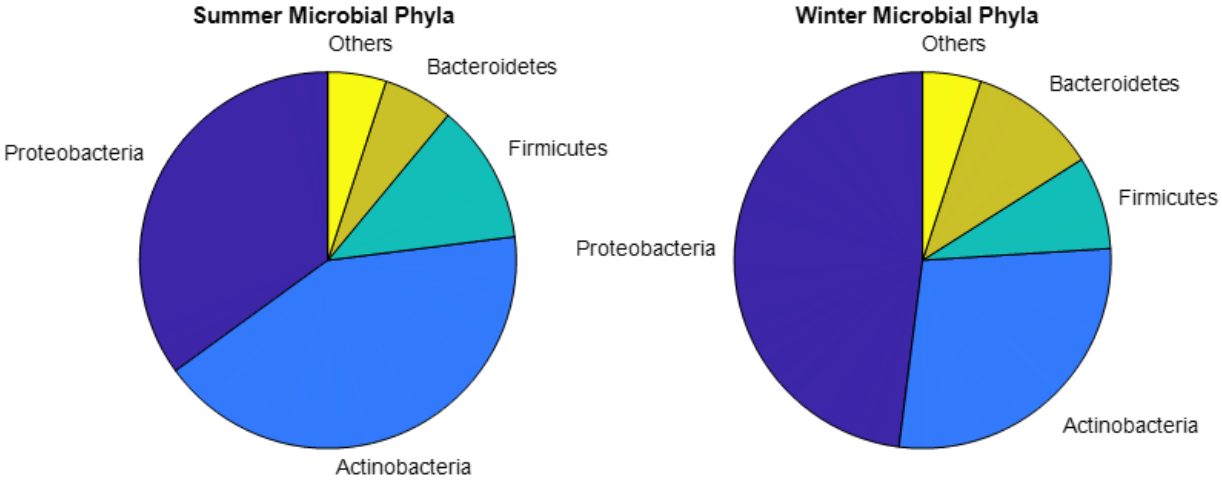


Figure (3): Microbial Composition.

The seasonal changes in microbial community structures appear as double pie chart representations. The microbial communities of the sampling zone mainly consist of Proteobacteria and Actinobacteria across both seasons. Scientific evaluations show major fluctuations in microbial population composition between summer and winter time periods. For informative purposes all rare phyla are shown together in one category.

Microbial community composition is compared among all samples, and primary variation is determined by season and not land use (agricultural vs. non-agricultural). As a result, data are expressed in aggregate in terms of the season (Summer vs. Winter) in Fig. 3 to show the prevailing climatic effect. An additional comparison of land-use variations is presented in Table 3. but, Site-specific soil physicochemical properties and microbial alpha diversity (Shannon Index, H') between ten sampling sites (A1-A5: agricultural, N1-N5: non-agricultural) in Thi-Qar Province in summer season and winter season. The most abundant phylum is the dominant phylum which represents the maximum abundance of bacteria phylum in each site-season combination. The evidence shows that the seasonal variation always surpasses land-use variation as the cause of modifications in both the soil parameters and the structure of microbial community.

The analysis of microbial diversity patterns presents its data by using boxplots. The Shannon diversity index achieves higher values in the winter season-based results. The boxplot visualizes both normal range of data and extreme measurement points. Fig. 4 displays mean diversity values as prominent black connecting lines. Diversity metrics can be easily assessed for their seasonal differences using this display method.

A visual summary of the correlation between the environmental variables and microbial community composition is provided by the RDA biplot (Fig. 5). The initial two RDA axes accounted 32.7% of the total variance. This high temperature (45.2% explained variance) strong vector, in the same direction as RDA1, reflects its highly influential effect on community structure, evidently differentiating summer and winter samples. Both moisture and organic content vectors are highly related to winter samples and taxa Proteobacteria.

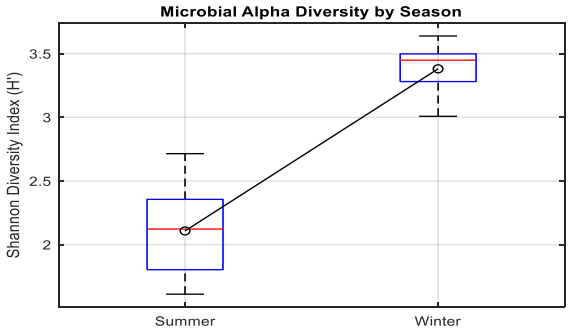


Figure (4): Alpha Diversity.

The RDA ordination, Fig. 5, shows how the structure of the microbial community is associated with environmental variables. Axis 1 (45.2%): Axis 2 (13.5) The first two axes of RDA cumulative variance 58.7% of total variance. The most important driver is temperature that had a strong positive correlation with RDA1 ($p < 0.001$). The results of a PERMANOVA test using Bray-Curtis dissimilarities proved the strong influence of season ($F = 15.83, R^2 = 0.476, p = 0.001$) in the composition of microbial communities. Table 4 summarizes the findings of the multivariate statistical tests.

Table (4): Summary of multivariate statistical analyses (RDA and PERMANOVA) investigating the effects of environmental variables on soil microbial community structure.

Analysis	Factor/Variable	Test Statistic	Variance Explained (R^2)	p-value
RDA	Axis 1	-	45.2%	< 0.001
	Axis 2	-	13.5%	0.012
	Temperature	-	-	< 0.001
	Moisture	-	-	0.134
	Salinity (EC)	-	-	0.087
	Organic Matter	-	-	0.321
PERMANOVA	Season	F = 15.83	47.6%	0.001

Statistically significant (p < 0.05).

Table (3): Site-specific soil properties and microbial diversity metrics across agricultural and non-agricultural sites in Thi-Qar Province.

Site Code	Land Use	Season	pH	Salinity (EC, dS/m)	Moisture (%)	Organic Matter (%)	Shannon Index (H')	Dominant Phylum (% Relative Abundance)
A1	Agricultural	Summer	7.8	6.2	5.1	1.2	2.05	Actinobacteria (44%)
A1	Agricultural	Winter	7.6	3.1	18.3	1.8	3.38	Proteobacteria (47%)
A2	Agricultural	Summer	8.1	7.5	4.8	1.0	1.98	Actinobacteria (46%)
A2	Agricultural	Winter	7.9	3.4	20.1	2.1	3.42	Proteobacteria (49%)
A3	Agricultural	Summer	7.9	5.8	5.3	1.3	2.12	Actinobacteria (43%)
A3	Agricultural	Winter	7.7	2.9	19.5	2.0	3.45	Proteobacteria (50%)
A4	Agricultural	Summer	8.0	6.5	4.9	1.1	1.95	Actinobacteria (45%)
A4	Agricultural	Winter	7.8	3.3	17.8	1.9	3.40	Proteobacteria (48%)
A5	Agricultural	Summer	8.2	7.0	5.0	1.0	1.90	Actinobacteria (47%)
A5	Agricultural	Winter	8.0	3.5	18.9	2.2	3.50	Proteobacteria (51%)
N1	Non-Agricultural	Summer	7.7	5.5	4.5	0.8	1.88	Actinobacteria (48%)
N1	Non-Agricultural	Winter	7.5	2.8	16.5	1.5	3.30	Proteobacteria (46%)
N2	Non-Agricultural	Summer	8.0	6.8	4.3	0.9	1.82	Actinobacteria (49%)
N2	Non-Agricultural	Winter	7.8	3.2	15.8	1.6	3.28	Proteobacteria (45%)
N3	Non-Agricultural	Summer	7.9	6.0	4.7	0.7	1.85	Actinobacteria (47%)
N3	Non-Agricultural	Winter	7.6	2.7	17.2	1.4	3.32	Proteobacteria (47%)
N4	Non-Agricultural	Summer	8.1	7.2	4.0	0.8	1.80	Actinobacteria (50%)
N4	Non-Agricultural	Winter	7.9	3.6	16.0	1.7	3.25	Proteobacteria (44%)
N5	Non-Agricultural	Summer	7.8	5.9	4.6	0.9	1.87	Actinobacteria (46%)
N5	Non-Agricultural	Winter	7.7	2.9	18.0	1.8	3.35	Proteobacteria (48%)

The visual representation demonstrates distinct relationships between taxa and their environmental preferences. Actinobacteria population measurements appear alongside temperature values that match their season. The visual display using colored points indicates a unique annual pattern. The visualization indicates possible thermal requirements of this bacterial phylum. The concentrated analysis extends existing observations from other figures about community dynamics as shown in Fig. 6.

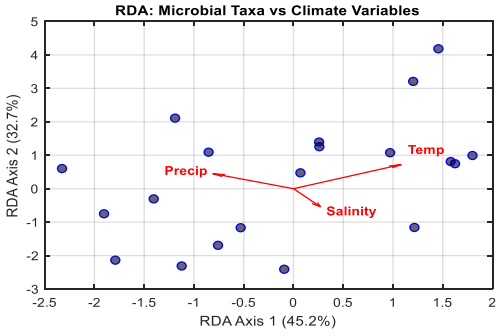


Figure (5): RDA biplot of microbial community composition and environmental variables.

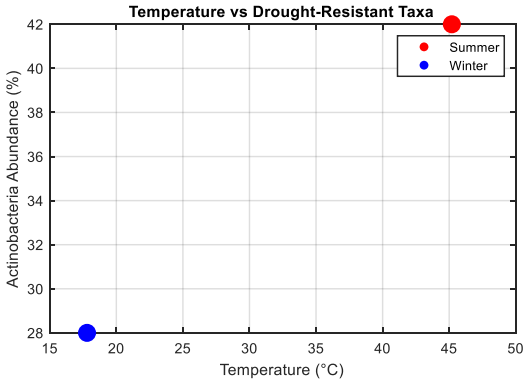


Figure (6): (Phyla-Temperature).

The Shannon index (H') is calculated as alpha diversity showing that there is a significant change in the microbial diversity between the winter (3.4 ± 0.2) and summer (2.1 ± 0.3), which is statistically significant (p < 0.01, PERMANOVA) and showed that microbial diversities had reduced by a quarter in the hot, dry season as shown in Fig. 4. This decrease can be compared to other arid environments where thermal and osmotic stress decreases microbial evenness and richness [22]. There is

a drastic change in community composition among seasons as shown in Fig. 3. Actinobacteria grew by 28% average relational abundance in winter to 42% in summer, which proves the hypothesis about these microbes as specialists in drought resilience and capable of scoring and creating compounds that can shield them [18]. Proteobacteria, including most of the copiotroph and fast-growing taxa, on the other hand, declined in proportion due to the loss of 48% in winter to 31% in summer, which is indicative of their affinity toward moisture and nutrient abundance [24].

The paper described the structure of microbial communities majorly based on the sequencing of DNA. Although this will give very important information regarding changes in taxonomic flux, it does not directly quantify microbial activity or biomass. This would have provided a more holistic picture of the effects of the seasonal variation on processes occurring in soil ecosystems in Thi-Qar by integrating such functional measurements in future work.

Conclusion and Future Work

The research indicates that soil microbial populations in Thi-Qar exhibit dramatic changes in summer due to extreme climate which causes both community diversity reduction by 25% together with Actinobacteria rising to 42% dominance. Agricultural practices should include the use of organic additives during dry periods for soil resistance enhancement while policy managers need to incorporate microbial analysis into desert land management tactics. Similar areas vulnerable to climate change can use these results to develop early stress detection methods based on microbial community changes. Researchers need to investigate how genes function along with studying the extended interactions between microorganisms and climate conditions using meta transcriptomic methods. The application of microbial inoculants in drought-prone soils requires field trials to develop practical solutions through experimental implementations.

Disclosure Statement

- **Ethics approval and consent to participate:** Not applicable.
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illu**strations of this manuscript.**
- **Author's contribution:** Conceptualization: Mahmood Jamal Abdul Hasan and Abbas Abdulameer Al-Raad; Methodology: Mahmood Jamal Abdul Hasan and Safwan Nadweh; Software and Formal Analysis: Safwan Nadweh; Investigation: Mahmood Jamal Abdul Hasan and Abbas Abdulameer Al-Raad; Data Curation: Safwan Nadweh; Writing – Original Draft Preparation: Mahmood Jamal Abdul Hasan; Writing – Review & Editing: Abbas Abdulameer Al-Raad and Safwan Nadweh; Visualization: Safwan Nadweh; Supervision: Mahmood Jamal Abdul Hasan. All authors have read and agreed to the published version of the manuscript.
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