

Biological assessment and soil health sustainability using the shannon microbial diversity index in Al-Dulab soils, Babylon, Iraq

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Abstract

This study evaluates soil health in the Dulab region, Babylon, Iraq, using the Shannon microbial diversity index as a quantitative biological indicator. A total of 48 agricultural soil samples were collected and analyzed to determine microbial density (CFU/g). The results revealed values ranging from 1.08×10^7 to 2.92×10^7 CFU/g, with a mean of 1.99×10^7 CFU/g. The Shannon index (H) was calculated based on relative microbial abundance. The relative proportions (pj) ranged from 0.0141 to 0.0380, while individual contributions (Hj) ranged from 0.0430 to 0.1264. The overall Shannon index reached $H=3.63$, approaching the theoretical maximum $H_{\max}=3.871$. The normalized index value was $B=0.94$, indicating high microbial homogeneity and excellent soil health. The findings confirm that the studied soils exhibit high microbial activity and a well-balanced microbial community, reflecting strong ecological stability and sustainability.

Keywords: Shannon Index, Soil Health, Microbial Diversity, Soil Sustainability, Agricultural Soils

Introduction

Soil represents a highly complex and dynamic ecosystem in which microbial communities play a fundamental role in maintaining fertility, ecological balance, and long-term sustainability. These microorganisms are directly involved in essential biogeochemical processes, including organic matter decomposition and nutrient cycling, particularly nitrogen and phosphorus, which are critical for plant growth and agricultural productivity (Hartmann, et al. 2023).

Recent studies have emphasized that soil should be viewed as an integrated living system, governed by interactions between its physical structure and microbial activity. Microbial diversity, in particular, has emerged as a key indicator of soil health, reflecting the ecosystem's resilience, stability, and capacity to withstand environmental stress (Cui, et al. 2023).

The Shannon Index is one of the most widely used

measures for assessing biodiversity, as it combines species richness with their relative distribution within a biological community. It has been extensively used in soil science to assess microbial community dynamics under varying environmental and management conditions (He, et al. 2023).

Moreover, contemporary studies highlight that microbial diversity is not only indicative of biological status but also closely linked to functional efficiency and ecosystem services. Variations in agricultural practices, crop systems, and environmental gradients significantly influence microbial community structure, thereby affecting soil fertility and sustainability (Zong, et al. 2024).

Microbial diversity may increase in some agricultural systems despite the deterioration of some physical properties, indicating the complexity of the relationship between biodiversity and soil health (Xiang, et al. 2024).

Materials and Methods

Study Area

The study was conducted in the Dulab region, located in Babylon, central Iraq. The area is characterized by a semi-arid climate, with high summer temperatures and low annual rainfall. Geographically, the study area extends between:

Longitude: 44°27'24.740"– 44°29'10.576" E.
Latitude: 32°25'53.171"– 32°27'49.381" N.

The soils are predominantly alluvial and relatively undeveloped, with low salinity, high calcium carbonate content, and good organic matter levels due to continuous agricultural practices.

These conditions provide a favorable environment for the growth of microorganisms, as agricultural soils with good organic content support higher microbial diversity compared to degraded soils (Zhang, et al. 2024).

Sample Collection

A total of 48 soil samples were collected from different sites at a depth of 0–30 cm. Samples were placed in sterile containers and transported to the laboratory under controlled conditions for further analysis.

Microbial Analysis

Microbial density was determined using the standard Plate Count Method. Colony-forming units were calculated according to the following equation:

CFU = Number of colonies × Dilution factor (1)

Where:

CFU/g = Colony-forming units per gram of soil.
Number of colonies = colonies observed after incubation.
Dilution factor = applied dilution prior to plating.

Shannon Index Calculation

The Shannon diversity index (Xie, et al. 2023) was calculated using

$H = - \sum_{j=1}^S p_j \cdot \ln(p_j)$ (2)

Where:

H = Shannon diversity index.
S = Total number of unique species (richness).
p_j: The relative abundance for each species.

The normalized Shannon index was calculated as:

$B = \frac{H - H_{min}}{H_{max} - H_{min}}$ (3)

Where:

B = Normalized Shannon index
H_{min} = Minimum value (typically 0)

H_{max} = Maximum value (when the abundance of all species is equal ln(S)).

Statistical Analysis

Statistical analysis included Mean, Standard deviation (SD), Normal distribution test, Coefficient of variation (CV), CV was calculated using:

$CV = \frac{SD}{Mean} \times 100$ (4)

Results and Discussion

Microbial Density

Table 1 shows the numerical distribution of bacterial colonies in the studied samples, where values ranged between 1.08×10⁷ and 2.92×10⁷ CFU/g, indicating a relatively high biological activity within the soil.

This activity is a direct indicator of the efficiency of biological processes such as the decomposition of organic matter and mineralization of nutrients, which are essential for maintaining soil fertility and sustainability, as increased microbial density is associated with higher rates of biochemical activity (Zhang, et al. 2024).

Spatial variation in the distribution of values is observed, reflecting the heterogeneity of soil properties at the microscopic level. Environmental factors such as moisture, aeration, and organic matter content differ between various locations.

This aligns with what the scientific literature (Lu,

Table 1: Microbial density in soil samples from the study area

Site No.	Microbial Density CFU/g 1×10 ⁷	Site No.	Microbial Density CFU/g 1×10 ⁷
1	1.60	25	1.36
2	1.32	26	2.04
3	2.04	27	2.52
4	2.16	28	2.36
5	2.56	29	1.96
6	1.28	30	2.12
7	2.80	31	1.20
8	1.76	32	1.40
9	1.48	33	2.00
10	1.44	34	2.60
11	1.80	35	2.48
12	2.72	36	2.88
13	2.40	37	1.72
14	2.20	38	1.08
15	1.20	39	1.16
16	1.68	40	1.88
17	2.08	41	2.68
18	1.56	42	2.92
19	2.52	43	2.44
20	2.32	44	2.00
21	1.76	45	2.28
22	1.96	46	1.88
23	2.04	47	2.64
24	1.68	48	2.0

et al. 2024) has indicated: that this spatial variation is a natural characteristic of ecosystems and contributes to enhancing biodiversity and increasing ecosystem resilience.

Despite this variation, the absence of anomalous values suggests relative stability in the microbial community, reflecting a balanced ecosystem capable of maintaining its vital functions. This has been confirmed by recent studies in soil environment (Chen, et al. 2021).

Statistical Analysis

Table 2 shows that the mean microbial density was 2.00×10^7 CFU/g, a level reflecting high biological activity in the soil. This activity is an important indicator of soil fertility, as microorganisms play a fundamental role in converting nutrients into forms available to plants, thus enhancing agricultural productivity and being a key

Table 2 - Statistical analysis of microbial density (CFU/g)

Value	Indicator
48	Number of Samples (S)
76.6 $\approx$	Total CFU
2.00×10^7	Mean
2.00×10^7	Medium
4.97×10^7	Standard Deviation
24.85 %	CV %
1.08×10^7	Minimum Value
2.92×10^7	Maximum Value

factor in soil health and sustainability (Yang, et al. 2022).

The median value was 2.00 CFU/g, very close to the mean, indicating a relatively homogeneous distribution of the samples, with no significant variations or extreme measurements affecting the mean.

The closeness of the mean to the median suggests a high degree of homogeneity in the data distribution. This was confirmed by (Lal, 2020), who stated that a balanced distribution of biological characteristics is an indicator of soil ecosystem stability.

The standard deviation was 4.97×10^7 CFU/g, reflecting the natural variation between samples. Soils can have patches of high microbial activity and others of lower activity due to locational factors such as organic content, water, and sun exposure (Chen et al., 2024).

The minimum values were 1.08×10^7 CFU/g and the maximum values were 2.92×10^7 CFU/g. This confirms the presence of good microbial diversity with natural variation, reflecting the different environmental conditions within the field. The coefficient of variation (CV) was 24.85%, reflecting moderate variability. This indicates a relative stability of the soil microbial environment with natural variations and moderate variability within

environmentally acceptable limits.

Values below 30% reflect relative stability in environmental characteristics, indicating that the studied soil has a balance between homogeneity and heterogeneity, and that this variability is considered normal in field studies (Philippot et al., 2024). Microbial density is influenced by factors such as humidity, the type of previous crop, organic content, improved aeration, and the absence of high salinity (Wu et al., 2023).

This aligns with the findings of (Jiao et al., 2022), who indicated that soil microbial communities are among the most sensitive indicators of environmental changes, and that higher microbial density is directly linked to improved biogeochemical processes, particularly carbon and nitrogen cycles.

Increased microbial activity in agricultural soils is associated with higher efficiency in organic matter decomposition and nutrient release, which enhances soil fertility and long-term sustainability (Zhao et al., 2024).

Shannon Index and Normalization

The results in **Tables 3** and **4** indicate that the Shannon index reached 3.63, a value indicating a high level of soil biodiversity and reflecting a rich and balanced microbial community. This index is one of the most important tools used in assessing biodiversity, as it combines the number of species with their relative distribution, a point emphasized by (Shannon 1948) in the theoretical basis of the index.

Table 3: Shannon index and normalized values for the soil samples

Values	Indices
48	Number of Samples (S)
76.6 $\approx$	Total CFU
3.63	Shannon (H)
3.871	$H_{\max} = \ln(48)$
0.94	$(B = H/H_{\max})$

A high Shannon index value indicates that the soil not only contains a large number of microorganisms but also that their distribution is relatively balanced, thus enhancing the efficiency of biological processes within the soil, such as nitrogen fixation and the decomposition of organic matter (Huang et al., 2023).

This is also attributed to the fact that lower salinity contributes to reducing the osmotic pressure on microorganisms, allowing for increased density and improved homogeneity. Furthermore, the high organic matter content provides a continuous source of carbon and energy, which supports the stability of the microbial community. Continuous agricultural activity and the associated root growth of plants lead to the formation of

Table 4: Shannon index, normalized values, and percentages for the soil samples

Site No.	pj	H _j	B normalized	Site No.	pj	H _j	B normalized
1	0.0209	0.0650	0.0168	25	0.0179	0.0582	0.0151
2	0.0173	0.0566	0.0146	26	0.0267	0.0880	0.0228
3	0.0267	0.0880	0.0228	27	0.0330	0.1144	0.0296
4	0.0283	0.0920	0.0238	28	0.0309	0.1073	0.0277
5	0.0335	0.1158	0.0299	29	0.0257	0.0809	0.0209
6	0.0168	0.0546	0.0141	30	0.0278	0.0925	0.0239
7	0.0367	0.1231	0.0318	31	0.0158	0.0488	0.0126
8	0.0231	0.0732	0.0189	32	0.0184	0.0559	0.0145
9	0.0194	0.0614	0.0159	33	0.0261	0.0856	0.0221
10	0.0189	0.0599	0.0155	34	0.0339	0.1179	0.0305
11	0.0236	0.0744	0.0192	35	0.0323	0.1109	0.0287
12	0.0357	0.1194	0.0309	36	0.0375	0.1246	0.0322
13	0.0315	0.1086	0.0281	37	0.0224	0.0710	0.0184
14	0.0289	0.0977	0.0253	38	0.0141	0.0430	0.0111
15	0.0158	0.0488	0.0126	39	0.0151	0.0459	0.0119
16	0.0221	0.0699	0.0181	40	0.0245	0.0769	0.0199
17	0.0274	0.0901	0.0233	41	0.0349	0.1202	0.0311
18	0.0205	0.0646	0.0167	42	0.0380	0.1264	0.0327
19	0.0330	0.1144	0.0296	43	0.0318	0.1097	0.0284
20	0.0303	0.1051	0.0272	44	0.0261	0.0856	0.0221
21	0.0231	0.0732	0.0189	45	0.0297	0.0999	0.0258
22	0.0257	0.0809	0.0209	46	0.0245	0.0769	0.0199
23	0.0267	0.0880	0.0228	47	0.0344	0.1187	0.0307
24	0.0221	0.0699	0.0181	48	0.0261	0.0856	0.0209

an active rhizosphere rich in root exudates, which are an important food source for microorganisms. The rhizosphere is one of the most microbially diverse environments, due to its richness in soluble organic compounds (Philippot et al., 2013).

A high Shannon index does not necessarily indicate the complete absence of pollution. (Fierer et al., 2020) explained that some pollutants may not only reduce the total number of microorganisms but may also alter the composition of the microbial community in terms of dominant species.

Therefore, the use of this index should be within an integrated soil assessment system that includes other chemical and physical indicators such as EC, pH, and organic matter.

Thus, the soil in the Al-Dulab area exhibits excellent biological health, and the Shannon index has proven its effectiveness as a quantitative tool for assessing soil health in Iraqi agricultural environments.

Soil Health Classification

The normalized value of Shannon index $B = 0.94$,

as shown in Table 3, indicates that the studied soil falls within the very high health category, according to Table 4. This reflects a balanced and biodiverse microbial system. This high level of diversity indicates the efficiency of biological processes within the soil, such as organic matter decomposition and nutrient cycling.

Furthermore, the value's proximity to 1 suggests a relative homogeneity in the distribution of microorganisms, which enhances the stability of the ecosystem and its ability to withstand environmental stresses. This result is a strong indicator that the soil is not degraded and enjoys a high degree of biological sustainability (Álvarez et al., 2024).

This was confirmed by (Bünemann et al., 2018), who stated that bioindicators, especially those based on microbial diversity, are more sensitive than traditional chemical indicators in the early detection of soil degradation.

The relationship between biodiversity and soil health is close, as soils with high biodiversity are better able to withstand environmental stresses and achieve long-term stability (Singh et al., 2023).

Table 5 - Soil Health Classification

Environmental Interpretation	Normalized value (B)	Soil Health Rating
High biodiversity and a stable ecosystem	> 0.75	Very high
A reasonable balance with some variation	0.75 – 0.50	Medium to good
Low biological activity	< 0.50	Low

Integrated Analysis

The combined results of Tables 1, 2, 3, and 4 indicate a positive relationship between microbial density and biodiversity. High values of CFU/g microbial density were associated with an increased Shannon index (H) and high homogeneity in printed values (B).

This suggests that the soil is biologically healthy and capable of sustainably supporting agricultural activity. This correlation reflects the fact that soils with sufficient nutrients support the growth of diverse microbial species, leading to increased biodiversity (Delgado-Baquerizo et al., 2018).

Moderate spatial variation contributes to this diversity by providing different environments that support diverse microbial communities, thus enhancing the stability of the soil ecosystem (Hu et al., 2021).

Conclusions

It is concluded that the soil in the study area possesses excellent biological health, and that microbial diversity constitutes a strong and reliable indicator for assessing soil health and sustainability.

The Shannon Index of Microbial Diversity can be an effective tool for assessing soil health and guiding agricultural practices, particularly when combined with chemical and physical indicators for the early detection of any potential degradation.

Maintaining high levels of organic matter and sustainably managing organic waste promotes soil health and preserves its biodiversity, thus contributing to the long-term sustainability of agricultural production.

Acknowledgments

We thank Al-Qasim Green University for technical support. Huda Hameedi thanks Babylon University for financial support.

Conflicts of Interest

The authors declare no conflict of interest.

References

Álvarez, V.E., El Mujtar, V.A., Salles, J.F., Jia, X., Castán, E., Cardozo, A.G., Tiftonell, P.A. 2024. Micro-environmental variation in soil microbial biodiversity in forest frontier ecosystems: implications for sustainability assessments. *Sustainability* 16: 1236.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Brussaard, L. 2018. Soil quality – A critical review. *Soil Biology & Biochemistry* 120: 105-125.

Chen, L.F., He, Z.B., Wu, X.R., Du, J., Zhu, X., Lin, P.F. 2021. Linkages between soil respiration and microbial communities following afforestation of alpine grasslands in the northeastern Tibetan plateau. *Applied Soil Ecology*

161: 103882.

Chen, Y., Wang, J., Liu, S., Zhang, R., Li, X. 2024. Soil microbial diversity and ecosystem stability under environmental gradients. *Soil Biology & Biochemistry* 185: 109145.

Cui, S., Xiao, Y., Zhou, Y., Wu, P., Cui, L., Zheng, G. 2023. Variations in diversity, composition, and species interactions of soil microbial community in response to increased N deposition and precipitation intensity in a temperate grassland. *Ecological Processes* 12: 35.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N. 2018. A global atlas of the dominant bacteria found in soil. *Science* 359: 320-325.

Fierer, N., Wood, S.A., de Mesquita, C.P.B., Bradford, M.A. 2020. How microbes can, and cannot, be used to assess soil health. *Soil Biology & Biochemistry* 148: 107879.

Hartmann, M., Six, J. 2023. Soil structure and microbiome functions: A framework for understanding soil as a living system. *Nature Reviews Earth & Environment* 4: 389-403.

He, J.Z., Shen, J.P., Zhang, L.M., Zhu, Y.G., Zheng, Y.M., Xu, M.G., Di, H.J. 2023. Microbial diversity and soil health: Implications for sustainable agriculture. *Soil & Tillage Research* 221: 105484.

Hu, W., Ran, J., Dong, L., Du, Q., Ji, M., Yao, S. 2021. Aridity-driven shift in biodiversity-soil multifunctionality relationships. *Nature Communications* 12: 5350.

Huang, B., Fang, W.S., Gu, Q., Tilocca, B. 2023. Editorial: soil microbiome community and functional succession mechanism driven by different factors in agricultural ecology. *Frontiers in Microbiology* 14: 1276119.

Jiao, S., Lu, Y., Wei, G. 2022. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. *Global Change Biology* 28: 140-153.

Lal, R. 2020. Soil health and sustainability. *Agronomy Journal* 112: 4565-4573.

Lu, Y., He, J., Liu, M., Zhou, X. 2024. Soil microbial diversity as an indicator of soil health in agricultural systems. *Agriculture, Ecosystems & Environment* 345: 108413.

Philippot, L., Chenu, C., Kappler, A., Rillig, M.C., Fierer, N. 2023. The interplay between microbial communities and soil properties. *Nature Reviews Microbiology* 22: 226-239.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H. 2013. Going back to the roots: The microbial ecology of the rhizosphere. *Nature Reviews Microbiology* 11: 789-799.

Shannon, C.E. 1948. A mathematical theory of communication. *Bell System Technical Journal* 27: 379-423.

Singh, B.K., Quince, C., Macdonald, C.A., Khachane, A.N. 2023. Soil microbial community structure and function in relation to soil health. *Nature Communications* 14: 4532.

Wu, H., Guo, Z., Peng, C., Li, Y., Zhang, W. 2023. Spatial variability of soil microbes. *Ecological Indicators* 154: 110567.

Xiang, X., Xue, K., Liu, Y., Zhou, J. 2024. Agricultural practices influence soil microbial diversity in semi-arid regions. *Frontiers in Microbiology* 15: 1187302.

Xie, Y., Zhang, X., Li, Q., Huang, M., Zhou, J. 2023. Soil health assessment. *Geoderma* 430: 116287.

Yang, F., Huang, M.B., Li, C.H., Wu, X.F., Fang, L.C. 2022. Vegetation restoration increases the diversity of bacterial communities in deep soils. *Applied Soil Ecology* 180: 104631.

Zhang, H., Li, P., Wang, J. 2024. Soil microbial diversity as a bioindicator of soil quality in arid and semi-arid agroecosystems. *Applied Soil Ecology* 190: 104918.

Zhang, T., Chen, H., Zhao, Y., Sun, Q., Wang, L. 2024. Soil microbial biomass and nutrient cycling. *Applied Soil Ecology* 195: 105232.

Zhao, L., Wang, R., Sun, K., Li, M., Zhou, P. 2024. Soil resilience and microbial diversity. *Science of the Total Environment* 912: 168746.

Zong, Y., Feng, Y., Chen, H. 2024. Linking microbial diversity and soil functions in agricultural landscapes. *Science of the Total Environment* 892: 164062.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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