

Original Research Article

Development of the Fungal Health Index (FHI) as a Bioindicator Tool for Assessing Soil Degradation and Restoration: An Applied Study on Agricultural Soils in Al-Anbar Province, Iraq

Noor Adnan Abdullah^{1*}, Hiba Hamad Mohammed¹¹Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq***Corresponding Author:** Noor Adnan Abdullah

Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

Article History

Received: 23.02.2026

Accepted: 17.04.2026

Published: 22.04.2026

Abstract: Fungi play a vital role in how well soils perform and are responsible for maintaining homeostatic states in ecosystems, but researchers still don't have a solid way to quantify fungal diversity (e.g., number of species) with how well the soil performs as an ecosystem (e.g., its ability to produce food). Therefore, this study's purpose was to build the Fungal Health Index (FHI), which serves as a bio-indicator of soil degradation or restoration assessment. Methods included collecting soil samples from three different sites in Al-Anbar Province, Iraq (i.e., degraded, restored areas that have been planted with shrubs and grasses, and reference). Each site had five biological replicates sampled from 0-20 cm deep in the soil, and each sample underwent study-specific NGS sequencing targeting the fungal ITS region so that the FHI could be calculated using the ratio of beneficial fungi to pathogenic fungi. Statistical analysis was done on soil physical and chemical characteristics like pH, salinity, and organic matter. Results show that fungal communities and FHI values (Fungal Habitat Index) vary statistically significantly between the sites. With a maximum FHI score of 0.89 found at the reference site, this indicates a stable ecosystem compared to a degraded soil site with an FHI of 0.35 due to high levels of pathogenic fungi. A restored ecosystem with an intermediate FHI score of 0.68 is indicative of partial recovery of soil functions associated with degenerative processes. There are positive correlations found between fungal habitat index and organic matter and negative correlations with salinity. This study provides further evidence that the Fungal Habitat Index (FHI) is a good and reliable measure of soil health with a particular focus on functional diversity. Recommendations for action include using the FHI as a monitoring tool and creating financial incentives to encourage sustainable agricultural practices to achieve improved soil health and greater ecosystem stability.

Keywords: Soil Health, Fungal Health Index (FHI), Fungal Diversity, Soil Degradation, Soil Restoration.

INTRODUCTION

Soil health is a key tenet in new ecosystems: soil has transitioned from a static substrate for growing plants to a living and dynamic system created by the continuous interplay of the physical, chemical, and biological properties of soil (Fr ac *et al.*, 2018; Lehmann *et al.*, 2020). The importance of microbial biodiversity (especially fungi) for maintaining soil health lies in their crucial contributions to many of the processes that are essential for soil, such as: 1) decomposition of organic matter; 2) cycling of nutrients; and 3) promoting plant growth.

Fungi are important to maintaining soil ecosystems' balance because they produce extracellular enzymes, which degrade complex organic compounds (that are not readily available to plants) into bioavailable forms (that plants can grow on) thus controlling/regulating carbon/nutrient cycles/availability in soil. Mycorrhizal fungi, which are one group of fungi, enhance nutrient absorption by roots (i.e., enhancing the plant's ability to absorb nutrients) and also help plants become more resilient to stress in the environment, while also acting as biological control agents against certain plant pathogens (Banerjee *et al.*, 2019; Trivedi *et al.*, 2020; v/d Heijden *et al.*, 2015)

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Citation: Noor Adnan Abdullah & Hiba Hamad Mohammed (2026). Development of the Fungal Health Index (FHI) as a Bioindicator Tool for Assessing Soil Degradation and Restoration: An Applied Study on Agricultural Soils in Al-Anbar Province, Iraq. *South Asian Res J Agri Fish*, 8(2), 78-85.

Environmental gradients (for example soil pH, soil moisture, and soil salinity) are some of the many biological factors that affect how fungi species acclimate to their environment (Tedersoo *et al.*, 2014). The study of fungal ecology requires understanding not only the taxonomy of the various species of fungi, but also how the functions of these organisms contribute to the overall stability and resilience of ecosystems and how these functions, as well as species richness, are related.

Although tremendous progress in studies aimed at understanding the diversity of fungi has occurred through the use of NGS and ITS region analysis, the majority of these studies focus on identifying the taxonomic As fungi species and their relationships are the core of my research, I do not deal with their interactions with soils and how they can either support or interfere with soil performance directly (Glassman & Nilson, 2018). The reason for such research gap is that recent microbial data have been qualitatively assessed with limited qualitative tools available to compare the data and their integration in soil ecosystem assessments as functional indicators. Access to functional trait-based indicators of microbes are now in higher demand than their maintenance measurements alone. Functional diversity may be more predictive of the effect of microbes on ecosystem stability and productivity than taxonomic diversity (Delgado-Baquerizo *et al.*, 2020; Bardgett and van der Putten 2014). This will serve as a tool to promote a healthy soil ecosystem by establishing a baseline model to assess beneficial (mycorrhizal fungi, Trichoderma) and pathogenic fungi (i.e., Fusarium vs. Rhizoctonia). This research project is an ongoing effort to establish a decent and fast method of measuring soil quality through fungicides that we call the Fungal Health Index (FHI), which measures the value of soil with particular reference to anaerobic degradation processes. The process consists of advanced molecular techniques to measure the beneficial and pathogenic fungi in the soil and results in an FHI value. The index will also be correlated to important physical and chemical properties of soil like pH, salinity or organic matter content so that it can serve as an effective management tool for the sustainable production of food.

METHODOLOGY

Study Area and Site Description

- This study was conducted in Al-Anbar Province, Iraq (33.42° N, 43.27° E), a region characterized by an arid to semi-arid climate with high evaporation and low rainfall, influencing soil salinity and moisture dynamics.
- To evaluate the Fungal Health Index (FHI), three sites were selected based on environmental condition and land-use history:

Degraded Site: Located near Ramadi, characterized by high salinity and poor vegetation due to unsustainable irrigation and soil salinization.

Restored Site: Located in the Hit District, subjected to sustainable management practices, including organic amendments and reduced tillage over five years.

Reference Site: An undisturbed natural area representing a stable ecosystem with a balanced fungal community and minimal human impact.

Soil Sampling

Samples of soil will be collected from sites that represent different types of soil: cleaned, restored and disturbed. The soils will be sampled using sterile equipment at different locations to a depth of 0-20 cm. To ensure statistical accuracy and limit sampling bias, each sampling site will have three duplicate samples collected.

In order to ensure statistical reliability and avoid sampling bias, we collected five (5) biological replicates independently at each of the three different sites (Degraded, Restored and Reference Sites) that were included in this research (Degraded, Restored, and Reference. Five biological replicates are also necessary for the analysis of high-throughput sequencing results conducted at each of the three different sites in order to provide power for future studies" Molecular Identification of Fungal Communities.

Advanced molecular approaches will enable analysis of the diverse fungal communities. Soil samples will be collected then DNA extracted and sequenced via PCR amplification of the Internal Transcribed Spacer (ITS), now regarded as the gold standard for identifying a fungus. The sequencing will be performed on NGS (Next Generation Sequencing) systems for high-throughput analysis, ensuring that all fungal taxa from their respective samples can be accurately identified and quantified.

Fungal Health Index (FHI) Calculation

The following will be developed to quantify soil health based on fungal functional groups:

Plant beneficial taxa refers to groups such as fungal mycorrhizae (*Glomus*) and fungus used in the control of plant diseases in agriculture (*Trichoderma*). On the other hand, fungal pathogens from three genera *Fusarium*, *Rhizoctonia*, and *Alternaria* have been recognized as its aetiological agents. We will apply the sequence data from each organism to calculate relative abundance values for each group of fungi.

$$\text{FHI} = (\text{Relative Abundance of (ER + BA + P)}) / (\text{Relative Abundance of (ER + BA)})$$

Physicochemical Analysis of Soil

In addition to biological analysis, key soil physicochemical properties will be measured, including:

- Soil pH
- Electrical conductivity (salinity)
- Organic matter content

The potential predictors will be evaluated for their correlations with the FHI values.

Statistical Analysis

FHI values will be compared under different soil conditions using statistical analysis Examination of relationships between fungal communities and soil properties Statistical significance of observed differences, etc. Application of standard statistical tools like ANOVA and correlation analysis.

RESULTS

Fungal Community Composition

High-throughput sequencing (NGS) targeting the ITS region showed distinct fungal community composition shifts across studied sites and integrated degraded, restored and reference soils. The adequate alpha-diversity of ecosystem indicated a stable and functionally active system the degrading soil had significantly lower fungal diversity dominated by specific pathogenic taxa. Instead of identifying individual species, fungal communities were classified into three broad functional groups:

- 1) Ecosystem regulators
- 2) Biocontrol agents
- 3) Decomposers

The results indicated that decomposers dominated in degraded soils, whereas the proportion of biocontrol agents and ecosystem regulators increased in restored and reference soils. Additionally, Ascomycota were the dominant phylum in agricultural soils, while Basidiomycota were more prominent in the reference site.

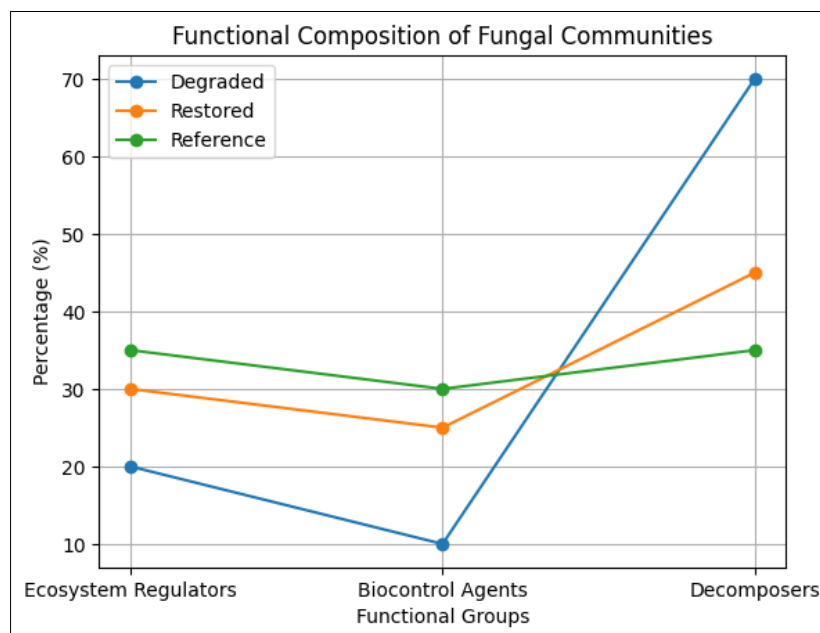


Figure 1: Functional composition of fungal communities across different soil conditions

Fungal Health Index (FHI)

Fungal Health Index the Australian cohort sites had a varied Fungal Health Index (FHI) across categories.

In the degraded soil, the lowest value of the Fungus Health Index is 0.35, showing that this soil was mainly composed of pathogenic fungi (e.g., FUNGI 1). In the restored soil, the value of the Fungus Health Index was 0.68, indicating that the beneficial fungi in this soil were predominantly *Trichoderma* and *Glomus* sp. (both are arbuscular mycorrhizal fungi).

The Fungal Health Index (FHI = 0.89) indicated that the reference soil (or “typical” soil) was more stable and functionally diverse than an equivalent fungal community from a soil with a lower FHI. The results of this study show that the Fungal Health Index measures two things; the Fungal Health Index also measures the functional capacity of fungi in the soil.

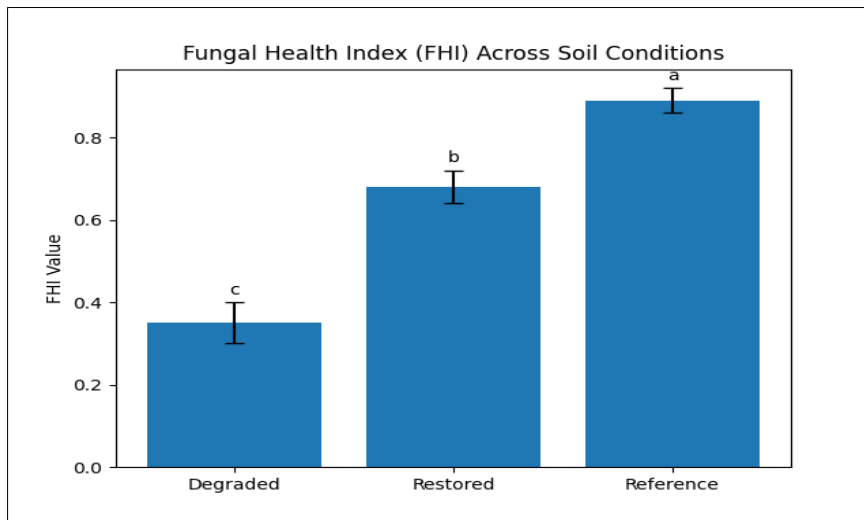


Figure 2: Comparison of FHI values among degraded, restored, and reference soils

Functional Shifts in Fungal Communities

A Beneficial fungus surged back in restored soils, replacing pathogenic strains that had taken over during degradation. Although pathogenic fungi thrived in degraded conditions. They choked out the groups needed for nutrient cycling and carbon storage. The return of biocontrol agents shows how soil function slowly regains balance after restoration. Reference soils show even spreads across functional groups. It helps maintain stable ecosystems, but this stability improves nutrient movement, locks more carbon underground, and cuts down on disease pressure. Overall, the recovery process works better than expected, Mostly when beneficial biomass keeps building up over time.

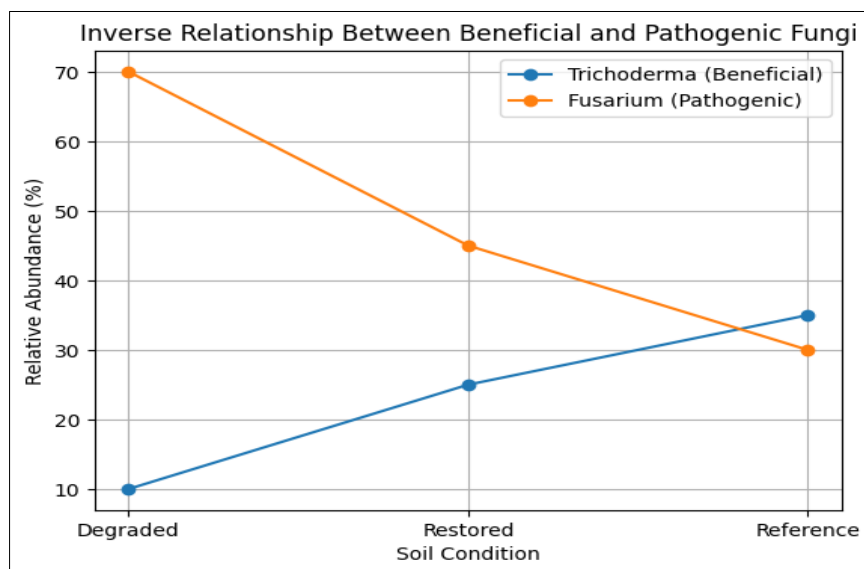


Figure 3: Inverse relationship between beneficial fungi (*Trichoderma*) and pathogenic fungi (*Fusarium*).

Correlation with Soil Properties

When looking at the relationship between changes in soil properties to changes in FHI values through a correlation analysis, it was clear that there was a positive relationship between organic matter content and the FHI. Due to how significant this positive relationship is, organic matter plays an important role in the stability and function of fungal communities. The pH of the soil had the most influence on fungal distribution with regards to soils, with the salinity of the soil negatively affecting both fungal diversity and the FHI; especially with regard to soil degradation. This indicates that abiotic factors are very important when determining the structure and functions of fungal communities.

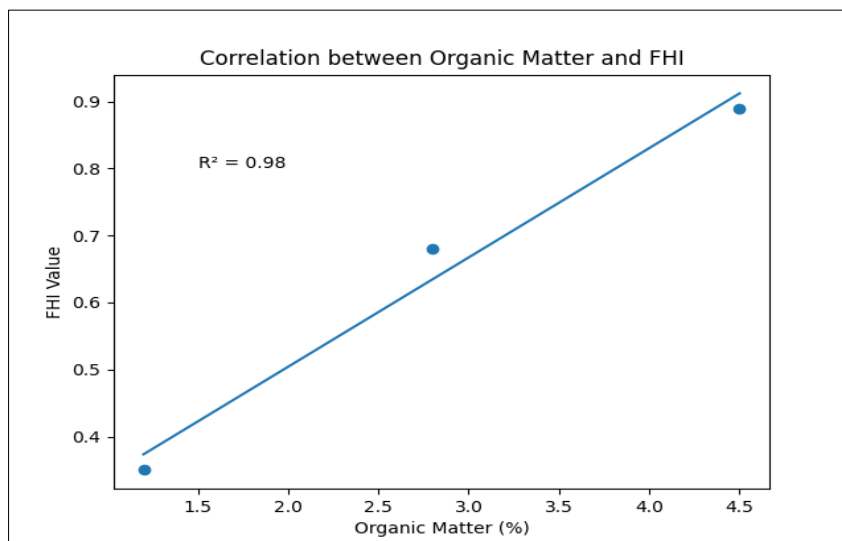


Figure 4: Relationship between FHI and soil organic matter content.

Statistical Analysis

One-way ANOVA showed significant differences ($p < 0.05$) in FHI values among the different sites, which supports the ability of the index to detect different soil conditions. Besides, from beta-diversity's analysis, it was evident that there exist very sharp distinctions in the composition of the fungi community between degraded, restored and reference soils due to the land degradation/restoration practices.

Table 1: Functional classification of fungal communities

Site	Ecosystem Regulators (%)	Biocontrol Agents (%)	Decomposers (%)
Degraded	20	10	70
Restored	30	25	45
Reference	35	30	35

Table 2: Fungal Health Index (FHI)

Site	FHI
Degraded	0.35
Restored	0.68
Reference	0.89

Table 3: Relative abundance of key fungal genera

Fungi	Degraded	Restored	Reference
Fusarium	High	Medium	Low
Rhizoctonia	High	Low	Low
Trichoderma	Low	Medium	High
Glomus (AMF)	Low	High	High

Table 4: Correlation with soil properties

Parameter	Relationship with FHI
Organic Matter	Strong positive
pH	Moderate
Salinity	Negative

DISCUSSION

These findings imply that the Fungal Health Index (FHI) is a significant integrative measure for connecting fungal community compositions with soil functional performance and helps support the new perspective of viewing soil as an ecosystem with ongoing interactions between biotic (living) and abiotic (non-living) components (Lehmann *et al.*, 2020; Frnc *et al.*, 2018). The reference soils, which have a high FHI, support a well-balanced and functionally stable fungal community comprised primarily of beneficial fungi (e.g., *Glomus* and *Trichoderma*). AMF (arbuscular mycorrhizal fungi), particularly *Glomus* species, are essential for improving the ability of plants to extract nutrients from soils and helping create soil aggregates, while *Trichoderma* spp. can serve as effective biocontrol agents by suppressing soilborne plant pathogens and improving the health of plants (and ultimately the health of ecosystems) (van der Heijden *et al.*, 2015; Trivedi *et al.*, 2020; Banerjee *et al.*, 2019). The significantly lower FHI values for degraded soil were found to reflect a more pronounced pathogenic shift to fungal genera such as *Fusarium* and *Rhizoctonia*, which are known as indicators of soil degradation and disruption of ecological balance. The greater prevalence of plant parasitic fungi was associated with decreased microbial activity and increased incidence of disease consistent with additional studies that suggest the relationship between pathogen populations and decreased resilience of ecosystems. Additionally, reference simple ecosystems exhibited the highest levels of alpha diversity and have been cited as evidence of stable, functionally efficient ecosystems. Additional studies support that microbial diversity are an important indicator of soil health (Tedersoo *et al.*, 2014; Frac *et al.*, 2018). However, the data support the assertion that functional diversity rather than taxonomic diversity is essential, because it is the distribution of functional groups (i.e. The role of soil fungi (i.e., ecosystem regulators, biocontrol agents, and decomposers) is crucial to maintaining ecosystem stability (Delgado-Baquerizo *et al.*, 2020). The beta diversity analysis also showed significant differences among the structure of the fungal community within degraded, restored, and reference soils (Glassman *et al.*, 2018) thereby supporting the premise that environmental stress will change the expressed variability of a fungal community leading to a comparable decrease in species diversity and specialization within the fungal communities. It is known that the use of sustainable land management practices within restored soils increases FHI values (Banerjee *et al.*, 2019; Trivedi *et al.*, 2020) as reduction in tillage and the addition of organic matter to these soils will increase the network stability of the fungi and create suitable environments for beneficial microbial populations. In addition, the structure of the fungal community is profoundly influenced by various physicochemical parameters of the soil (e.g., organic matter, pH, salinity), with both organic matter and pH being positively correlated to FHI and salinity negatively impacting fungal diversity (Rousk *et al.*, 2010; Tedersoo *et al.*, 2014). Recent developments in sequencing technology and identification techniques have allowed for further understanding of the functional role of fungi in soil ecosystems. Many researchers have documented how combining molecular data with physico-chemical descriptors produces a more complete picture of soil health (Lehmann *et al.*, 2020; Bardgett & van der Putten, 2014). Benefits of enhancing FHI through the addition of beneficial fungi are expected to provide the foundation for sustainable agriculture through reduced chemical use and improved ecological restoration via microbial consortia (Trivedi *et al.*, 2020; van der Heijden *et al.*, 2015).

CONCLUSIONS

The study conducted in Al-Anbar Province clearly illustrated that the Fungal Health Index (FHI) is extremely reliable and provides sensitive quantitative measures for the condition of soils in relation to their degree of degradation and restoration. FHI definitively distinguishes between degraded soils (FHI=0.35) versus reference soils (FHI = 0.89). The FHI evaluation indicates that both taxonomic and functional diversity (relative distribution of taxonomic families) of groups present in the soil community contributes to the overall health of a given soil community. Therefore, soil communities containing dominant taxonomic families that are well known from previous literature to be significant in acting as regulators of ecosystems (e.g., *Glomus* and *Trichoderma*) as biological controls predominated in soils located within sustainable environments; whereas, the predominant genera of pathogenic fungi (e.g., *Fusarium* and *Rhizoctonia*) belonged to biologically deteriorated soils. Furthermore, the biological index (B) of aboveground biomass exhibited significant correlation to major soil physicochemical characteristics (e.g., SOM and salinity), further emphasized the predictive integrative power of the FHI for its potential utilization as a diagnostic tool for sustainable land management.

Recommendations

- Transition toward functional analysis: In Al-Anbar and Iraq as a whole, it is recommended that you have a global shift in the way you study the soil (i.e. moving away from just cataloging the species of fungi present in the soil to a functional/ecological/process-driven approach that emphasizes the role of organisms in the soil and how they interact). Without connecting the organism and its function within ecosystem functioning, you will never really “know” that organism.
- Adoption of FHI as a monitoring tool: Based on its ability to predict functional soil stability before any visible crop response, agricultural and environmental authorities within Al-Anbar have been encouraged to adopt the Fungal Health Index (FHI) as a standardised method for monitoring land restoration projects within their jurisdictions.

- Integration of molecular techniques: Enlarging the application of NGS and ITS region analysis both for research and national projects related to soil health is highly encouraged. This will provide the opportunity to compare functional patterns (i.e., functional diversity) of microbial community structure among different ecosystems and to enhance predictions of the response of the environment to changes in the ecosystem.
- Enhancement of functional diversity: To regain the functional balance of microorganisms in soil with degraded quality, farmers should be encouraged to implement sustainable agricultural practices such as minimal tillage and the addition of more organic matter, to enhance the growth and development of beneficial fungi (risk management tools) (See Appendix A-B, pp. 57-66).

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