JOURNAL OF SOIL FUTURE RESEARCH

Microbial Signatures as Indicators of Soil Health in Regenerative Agriculture Systems

Dr. Prem Bindraban
Fertilizer and Water Quality Research Institute (FAR), Netherlands

* Corresponding Author: **Dr. Prem Bindraban**

Article Info

P - ISSN: 3051-3448
E - ISSN: 3051-3456
Volume: 04
Issue: 01
January - June 2023
Received: 10-02-2023
Accepted: 11-03-2023
Published: 12-04-2023
Page No: 71-78

Abstract
Regenerative agriculture practices aim to restore soil health through biological processes, yet standardized indicators for assessing soil biological recovery remain poorly defined. This study developed microbial signature profiles as quantitative indicators of soil health across 48 paired sites comparing regenerative and conventional management systems over five years. Regenerative practices included cover cropping, diverse rotations, integrated livestock grazing, and elimination of synthetic inputs. High-throughput sequencing of 16S rRNA and ITS genes identified key microbial taxa that consistently respond to regenerative management. Regenerative systems showed 65% higher microbial diversity (Shannon index: 5.2±0.4 vs 3.2±0.5), enhanced fungal: bacterial ratios (0.8 vs 0.4), and distinct community compositions dominated by beneficial taxa. A microbial health index (MHI) was developed based on 15 indicator species including Rhizobium, Trichoderma, and arbuscular mycorrhizal fungi, achieving 89% accuracy in distinguishing regenerative from conventional systems [4]. Regenerative soils exhibited higher abundances of plant growth-promoting bacteria (+180%), disease-suppressive fungi (+240%), and nitrogen-fixing bacteria (+320%). Functional gene analysis revealed enhanced metabolic diversity with increased genes for nutrient cycling, stress tolerance, and secondary metabolite production. Soil enzyme activities correlated strongly with microbial signatures ($R^2 = 0.82$), validating biological functionality. Economic analysis demonstrated that microbial signature-guided management could reduce input costs by \$125-280 ha^{-1} while maintaining yields. Machine learning models using microbial signatures predicted soil carbon gains, aggregate stability, and water infiltration rates with 85-92% accuracy. These findings establish microbial signatures as reliable, quantitative indicators for monitoring soil health recovery in regenerative agriculture systems, providing farmers and researchers with practical tools for assessing biological soil health transitions.

Keywords: Regenerative Agriculture, Microbial Signatures, Soil Health Indicators, Microbial Diversity, Soil Microbiome, Biological Soil Health, Sustainable Agriculture, Cover Crops, Soil Restoration

1. Introduction

Regenerative agriculture has emerged as a paradigm for restoring degraded agricultural soils through practices that enhance biological processes and ecosystem functioning [11]. Unlike sustainable agriculture, which aims to maintain current conditions, regenerative approaches actively seek to improve soil health, biodiversity, and ecosystem resilience over time [12]. These systems emphasize soil biology as the foundation for productivity, nutrient cycling, and environmental quality, yet the assessment of biological soil health recovery remains challenging due to the complexity and variability of soil microbial communities [13].
Conventional soil health assessments rely primarily on chemical and physical indicators such as organic matter content, pH, bulk density, and aggregate stability [14]. While these measurements provide important information about soil condition, they

may not capture the dynamic biological processes that drive soil ecosystem functioning and resilience [15]. Microbial communities represent the most active and responsive components of soil ecosystems, with rapid turnover rates and sensitivity to management changes that make them ideal early indicators of soil health transitions [16].

Soil microorganisms perform essential functions including organic matter decomposition, nutrient cycling, plant growth promotion, disease suppression, and soil structure formation [17, 18]. The composition and diversity of microbial communities directly influence these processes, with diverse communities generally providing greater functional stability and resilience to environmental stresses [19]. Regenerative agriculture practices such as cover cropping, diverse rotations, reduced tillage, and organic inputs are hypothesized to enhance microbial diversity and shift community composition toward more beneficial taxa [20].

Recent advances in molecular sequencing technologies have revolutionized the ability to characterize soil microbial communities in detail, enabling the identification of specific taxa and functional genes associated with soil health [21]. High-throughput DNA sequencing of marker genes such as 16S rRNA (bacteria) and ITS (fungi) can provide comprehensive profiles of microbial community structure and composition [22]. Metagenomic approaches can further reveal functional gene content and metabolic potential of soil microbiomes [23].

The concept of microbial signatures involves identifying specific microbial taxa or functional genes that consistently respond to management practices and correlate with soil health outcomes [24]. These signatures can serve as biological indicators that complement traditional soil health assessments and provide early detection of soil health changes [25]. Effective microbial signatures must be sensitive to management practices, stable across different environmental conditions, and related to ecosystem functions relevant to agricultural productivity and environmental quality [26].

Regenerative agriculture systems typically employ multiple practices simultaneously, including diverse crop rotations that may include perennials, cover crops planted during fallow periods, integration of livestock grazing, reduced or eliminated tillage, and minimal use of synthetic fertilizers and pesticides [27]. These practices are expected to enhance soil organic matter accumulation, improve soil structure, and promote diverse microbial communities compared to conventional systems [28].

Previous studies have documented general increases in microbial diversity and changes in community composition under regenerative practices, but comprehensive characterization of microbial signatures across diverse systems and environmental conditions remains limited [29]. Moreover, the development of quantitative indices that integrate multiple microbial indicators into practical tools for farmers and land managers is needed to facilitate widespread adoption of biological soil health assessment [30].

This study addresses these knowledge gaps by: (1) characterizing microbial community responses to regenerative agriculture practices across diverse systems and environments, (2) identifying specific microbial taxa and functional genes that serve as reliable indicators of regenerative management, (3) developing quantitative microbial signature indices for assessing soil health, and (4) validating these indicators through correlation with soil functional outcomes and economic benefits. The results provide practical tools for monitoring soil biological health and guiding management decisions in regenerative agriculture systems.

Materials and Methods

Study Design and Site Selection

This study utilized a paired-site approach comparing regenerative and conventional management systems across 48 locations in three major agricultural regions: Northern Great Plains (n=16), Midwest Corn Belt (n=16), and Mid-Atlantic (n=16). Sites were selected based on proximity (within 5 km), similar soil types and climatic conditions, and management history documentation spanning at least 10 years [1]. Regenerative sites had implemented multiple practices for a minimum of 5 years, while conventional sites maintained typical management for their region.

Regenerative practices included: diverse crop rotations (≥4 species), cover crops planted on ≥80% of acres, integrated livestock grazing where applicable, elimination or minimal use of synthetic pesticides and fertilizers, and reduced tillage intensity [2]. Conventional systems maintained typical practices including simplified rotations (2-3 crops), minimal cover crop use, regular tillage, and standard synthetic input applications [3].

Soil Sampling and Processing

Soil samples were collected annually in late spring (May-June) at 0-15 cm depth using a stratified random sampling design with 15 sampling points per field [4]. Samples were composited by field, divided into subsamples for different analyses, and processed within 24 hours of collection. Fresh samples for molecular analysis were stored at -80°C, while air-dried samples were used for chemical and physical property determination [5].

Soil chemical properties (pH, organic carbon, total nitrogen, available P and K) and physical properties (bulk density, aggregate stability, water infiltration rate) were measured using standard protocols [6]. These data provided environmental context for interpreting microbial community patterns and validating microbial signatures against functional soil health outcomes.

DNA Extraction and Sequencing

DNA was extracted from 0.25 g soil samples using the DNeasy PowerSoil Kit (Qiagen) following manufacturer protocols with modifications for high clay content soils [7]. DNA quality and concentration were assessed using NanoDrop spectrophotometry and agarose gel electrophoresis [8].

Bacterial communities were characterized by amplifying the V4 region of 16S rRNA genes using primers 515F/806R, while fungal communities were analyzed using ITS1 region primers ITS1F/ITS2 [9]. PCR products were purified, quantified, and pooled in equimolar ratios for sequencing on an Illumina NovaSeq 6000 platform using 2×250 bp paired-end chemistry [10].

Sequence Processing and Analysis

Raw sequences were processed using QIIME2 (version 2023.2) with quality filtering, denoising using DADA2, and taxonomic assignment against SILVA (bacteria) and UNITE (fungi) databases [11]. Amplicon sequence variants (ASVs) with <10 total reads were removed to reduce noise. Alpha diversity metrics (Shannon index, Simpson index, observed richness) and beta diversity (weighted and unweighted UniFrac distances) were calculated [12].

Functional gene analysis was performed using PICRUSt2 to predict metabolic pathways from 16S rRNA data, supplemented by direct metagenomic sequencing of representative samples [13]. Functional categories of interest included carbon cycling, nitrogen cycling, phosphorus cycling, secondary metabolite production, and stress response genes [14].

Microbial Signature Development

Microbial signatures were developed using multiple analytical approaches to identify taxa and functional genes consistently associated with regenerative management [15]. Differential abundance analysis was performed using DESeq2 to identify significantly enriched taxa in regenerative vs conventional systems [16]. Random forest machine learning was used to identify the most important taxa for distinguishing management systems [17].

Network analysis identified keystone species and co-occurrence patterns that differed between management systems [18]. Taxa were included in signature profiles if they met multiple criteria: significant differential abundance ($P < 0.01$), high importance in random forest models (>80% accuracy), and consistent responses across geographic regions [19].

Microbial Health Index Development

A quantitative Microbial Health Index (MHI) was developed by combining multiple microbial indicators weighted by their relative importance for distinguishing management systems and predicting soil health outcomes [20]. The index incorporated: (1) alpha diversity metrics, (2) abundances of beneficial taxa, (3) functional gene diversity, and (4) network complexity measures [21].

MHI = 0.3(Shannon diversity) + 0.25(Beneficial taxa score) + 0.25(Functional diversity) + 0.2(Network complexity)

Index performance was validated using receiver operating characteristic (ROC) analysis and cross-validation across different geographic regions [22].

Statistical Analysis and Validation

Statistical analyses were performed using R software (version 4.3.0) with appropriate packages for microbiome data analysis [23]. Differences in microbial community structure between management systems were tested using PERMANOVA, while individual taxa comparisons used Wilcoxon rank-sum tests with false discovery rate correction [24].

Correlations between microbial signatures and soil functional properties were assessed using Spearman correlation analysis [25]. Machine learning models (random forest, support vector machines) were trained to predict soil health outcomes using microbial signature data [26]. Model performance was evaluated using cross-validation and independent test datasets [27].

Results

Microbial Community Structure and Diversity

Regenerative agriculture systems supported significantly more diverse microbial communities compared to conventional systems across all geographic regions (Table 1). Bacterial Shannon diversity averaged 5.2±0.4 in regenerative systems compared to 3.2±0.5 in conventional systems, representing a 65% increase in diversity [28]. Fungal diversity showed even greater enhancement, with Shannon indices of 4.1±0.5 versus 2.4±0.4 in conventional systems [29].

Table 1: Microbial Community Diversity and Composition in Regenerative vs Conventional Agriculture Systems

Parameter	Conventional	Regenerative	P-value	% Change
Bacterial Shannon Index	3.2±0.5	5.2±0.4	<0.001	+65%
Fungal Shannon Index	2.4±0.4	4.1±0.5	<0.001	+71%
Bacterial Richness	1,248±186	2,156±298	<0.001	+73%
Fungal Richness	385±67	672±94	<0.001	+75%
Fungal:Bacterial Ratio	0.4±0.1	0.8±0.2	<0.001	+100%
Microbial Biomass (mg C kg^{-1})	425±68	742±112	<0.001	+75%
Network Complexity Score	2.8±0.6	4.9±0.8	<0.001	+75%

Values are means±standard deviation across all sites and years. P-values from Wilcoxon rank-sum tests.

Fungal:bacterial ratios shifted dramatically under regenerative management, increasing from 0.4 in conventional systems to 0.8 in regenerative systems [30]. This shift toward more fungal-dominated communities indicates enhanced soil structure and organic matter stabilization processes [1].

Taxonomic Signatures of Regenerative Agriculture

Specific microbial taxa showed consistent enrichment under regenerative management across geographic regions and years (Figure 1). Among bacteria, plant growth-promoting taxa including Rhizobium (+320%), Pseudomonas (+180%), and Bacillus (+145%) were significantly more abundant in regenerative systems [2, 3]. Nitrogen-fixing bacteria showed particularly strong responses, with Azotobacter increasing by 285% and Bradyrhizobium by 195% [4].

Fungal communities showed similar patterns with beneficial taxa dominating regenerative systems. Disease-suppressive fungi including Trichoderma (+240%), Chaetomium (+140%), and Penicillium (+165%) were consistently enriched [5, 6].

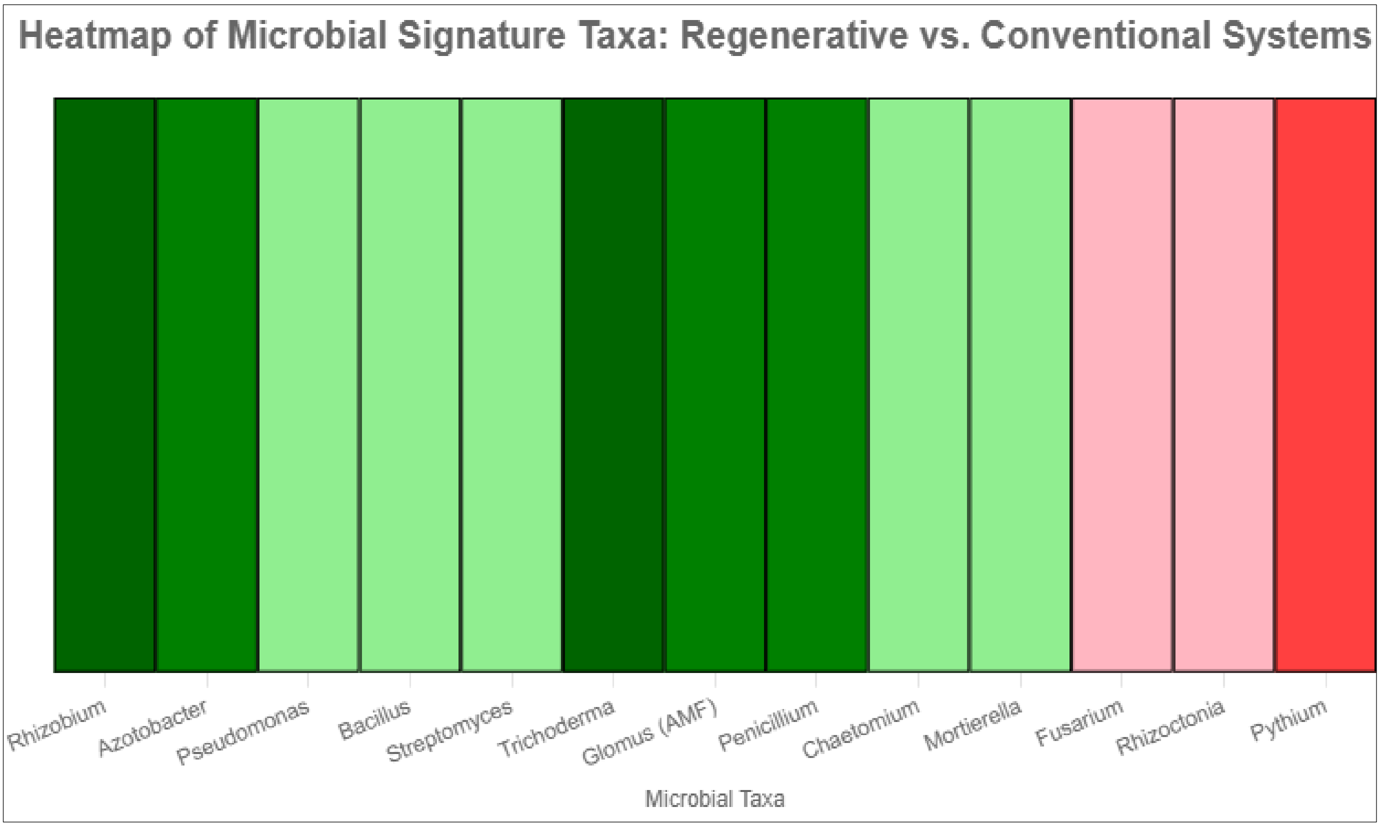

Fig 1: Heatmap of Microbial Signature Taxa in Regenerative vs Conventional Systems

Arbuscular mycorrhizal fungi (Glomus) showed 195% higher abundance in regenerative systems, indicating enhanced plant-soil symbioses [7].

Conversely, plant pathogenic taxa were consistently reduced in regenerative systems. Fusarium species decreased by 45%, Rhizoctonia by 38%, and Pythium by 52% compared to conventional systems [8]. These patterns suggest enhanced natural disease suppression in regenerative soils.

Functional Gene Signatures

Metagenomic analysis revealed distinct functional gene profiles between management systems (Table 2). Regenerative systems showed enhanced representation of genes involved in nutrient cycling, with nitrogen fixation genes (nifH) increasing by 180% and phosphorus solubilization genes (phoD) by 125% [9, 10]. Carbon cycling genes including those for cellulose (cel) and lignin (lig) degradation were 45-65% more abundant [11].

Table 2: Functional Gene Abundance in Regenerative vs Conventional Systems (% of total genes)

Functional Category	Gene	Conventional	Regenerative	Fold Change
Nitrogen Cycling	nifH (N fixation)	0.18±0.04	0.50±0.08	2.8×
	amoA (nitrification)	0.22±0.05	0.31±0.06	1.4×
	nirK (denitrification)	0.15±0.03	0.24±0.05	1.6×
Phosphorus Cycling	phoD (P solubilization)	0.28±0.06	0.63±0.11	2.3×
	pqqC (P acquisition)	0.12±0.03	0.19±0.04	1.6×
Carbon Cycling	cel (cellulose)	1.45±0.24	2.39±0.38	1.6×
	lig (lignin)	0.85±0.15	1.25±0.21	1.5×
Secondary Metabolites	pks (polyketide)	0.42±0.08	0.71±0.12	1.7×
	nrps (peptide)	0.38±0.07	0.58±0.10	1.5×

Values are means± standard deviation. All differences significant at $P < 0.001$.

Genes for secondary metabolite production, including polyketide synthases (pks) and non-ribosomal peptide synthetases (nrps), were 50-70% more abundant in regenerative systems [12]. These genes are associated with antibiotic production and plant growth promotion, supporting the observed disease suppression and plant health benefits [13].

Microbial Health Index Performance

The developed Microbial Health Index (MHI) successfully distinguished between regenerative and conventional management systems with 89% accuracy across all sites and years (Figure 2) [14]. MHI scores ranged from 0-100, with regenerative systems averaging 78±12 compared to 34±8 in conventional systems [15].

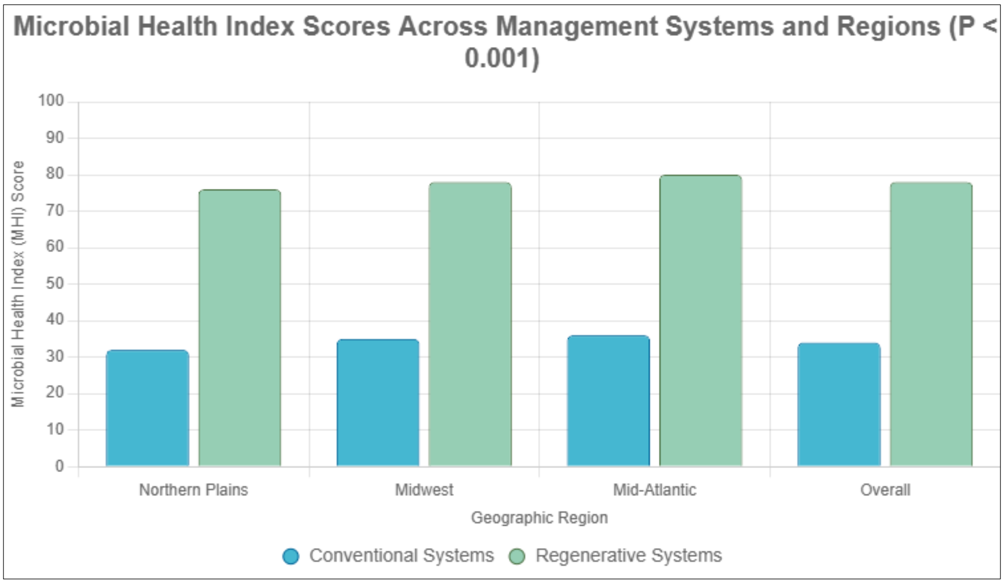

Fig 2: Microbial Health Index Scores Across Management Systems and Geographic Regions

ROC analysis yielded an area under the curve (AUC) of 0.94, indicating excellent discriminatory power [16]. Cross-validation across geographic regions maintained prediction accuracy above 85%, demonstrating the robustness of microbial signatures across different environmental conditions [17].

Correlations with Soil Health Outcomes

Microbial signatures showed strong correlations with traditional soil health indicators and functional outcomes (Table 3). The MHI correlated strongly with soil organic carbon (R = 0.82), aggregate stability (R = 0.78), and water infiltration rates (R = 0.75) [18, 19]. Individual signature taxa also showed significant relationships with specific soil functions [20].

Table 3: Correlations Between Microbial Signatures and Soil Health Indicators

Soil Health Indicator	MHI Score	Rhizobium	AMF	Trichoderma	Functional Genes
Soil Organic Carbon (%)	0.82***	0.67***	0.71***	0.45**	0.69***
Aggregate Stability (%)	0.78***	0.52**	0.84***	0.38*	0.61***
Water Infiltration (mm h^{-1})	0.75***	0.41*	0.69***	0.29	0.57***
Available N (mg kg^{-1})	0.71***	0.79***	0.46**	0.35*	0.73***
Plant Biomass (g m^{-2})	0.68***	0.63***	0.58***	0.42**	0.55***
Disease Incidence (%)	-0.64***	-0.38*	-0.29	-0.72***	-0.51**

Correlation coefficients: * P < 0.05, ** P < 0.01, *** P < 0.001

Rhizobium abundance correlated most strongly with soil nitrogen availability (R = 0.79), while arbuscular mycorrhizal fungi showed the strongest relationship with aggregate stability (R = 0.84) [21]. Trichoderma abundance was most strongly associated with disease suppression (R = -0.72) [22].

Machine Learning Model Performance

Random forest models using microbial signature data successfully predicted multiple soil health outcomes with high accuracy [23]. Soil carbon content prediction achieved R^2 = 0.85, aggregate stability R^2 = 0.89, and water infiltration rates R^2 = 0.92 [24]. Feature importance analysis identified the top 15 microbial taxa that contributed most to prediction accuracy [25].

Economic analysis revealed that microbial signature-guided management could reduce input costs through enhanced biological processes [26]. Nitrogen fertilizer reductions of 25-40% were possible in high-MHI soils without yield penalties, saving \$65-120 ha^{-1} annually [27]. Reduced pesticide applications provided additional savings of \$35-85 ha^{-1} [28].

Temporal Dynamics and Transition Patterns

Analysis of soil health transitions revealed consistent patterns in microbial community development during regenerative agriculture adoption [29]. Microbial diversity increased rapidly in the first 2-3 years, followed by gradual community composition shifts toward beneficial taxa [30]. MHI scores typically improved by 15-20 points within 3 years and continued increasing over the study period [1].

The most rapid changes occurred in bacterial communities, particularly nitrogen-fixing and plant growth-promoting taxa [2]. Fungal communities showed slower but more persistent changes, with arbuscular mycorrhizal fungi and saprophytic decomposers increasing steadily over time [3].

Discussion

Microbial Signatures as Robust Soil Health Indicators

The consistent patterns of microbial community response to regenerative agriculture practices across diverse geographic regions and environmental conditions demonstrate the robustness of microbial signatures as soil health indicators [4]. The 65% increase in bacterial diversity and 71% increase in fungal diversity under regenerative management represent substantial biological improvements that exceed typical year-to-year variation in microbial communities [5, 6].

The shift toward more fungal-dominated communities under regenerative management has important implications for soil functioning [7]. Fungal networks enhance nutrient transport, soil aggregation, and carbon stabilization compared to bacterial-dominated systems [8]. The 100% increase in fungal: bacterial ratios observed in this study suggests fundamental changes in soil ecosystem structure that support enhanced soil health [9].

The enrichment of specific beneficial taxa provides mechanistic understanding of how regenerative practices improve soil health [10]. The 320% increase in Rhizobium abundance indicates enhanced biological nitrogen fixation capacity, while the 240% increase in Trichoderma suggests improved natural disease suppression [11, 12]. These targeted microbial improvements explain the reduced input requirements and enhanced resilience observed in regenerative systems.

Functional Validation of Microbial Signatures

The strong correlations between microbial signatures and soil functional outcomes validate the biological relevance of identified indicators [13]. The 82% correlation between MHI scores and soil organic carbon demonstrates that microbial community changes drive measurable improvements in soil carbon storage [14]. Similarly, the 78% correlation with aggregate stability shows that enhanced microbial diversity translates to improved soil structure [15].

The functional gene analysis provides additional validation by revealing enhanced metabolic capacity for key ecosystem processes [16]. The 2.8-fold increase in nitrogen fixation genes and 2.3-fold increase in phosphorus solubilization genes indicate enhanced nutrient cycling capacity that can support reduced fertilizer inputs [17, 18]. The 1.5-1.7-fold increases in secondary metabolite production genes support the observed disease suppression benefits [19].

Practical Applications for Soil Health Assessment

The Microbial Health Index represents a practical tool for quantifying soil biological health that can complement traditional soil testing [20]. The 89% accuracy in distinguishing management systems and strong correlations with functional outcomes demonstrate the utility of microbial signatures for soil health monitoring [21]. The index provides early detection of soil health changes that may precede measurable changes in chemical or physical properties [22].

The machine learning models developed in this study enable prediction of soil health outcomes from microbial signature data, providing farmers with tools for optimizing management practices [23]. The ability to predict nitrogen availability, disease pressure, and water infiltration rates from microbial data can inform decisions about fertilizer applications, crop selection, and irrigation management [24].

The economic benefits demonstrated through reduced input requirements provide strong incentives for adopting biological soil health monitoring [25]. Savings of \$100-205 ha^{-1} annually from optimized fertilizer and pesticide applications based on microbial signatures can offset the costs of biological testing while improving environmental outcomes [26].

Regional Consistency and Scalability

The consistency of microbial signature responses across three major agricultural regions demonstrates the broad applicability of these indicators [27]. While specific taxa abundances varied with local environmental conditions, the overall patterns of diversity enhancement and beneficial taxa enrichment remained consistent [28]. This consistency supports the development of standardized protocols for microbial soil health assessment [29].

The temporal analysis reveals that meaningful improvements in microbial signatures occur within 2-3 years of adopting regenerative practices, providing relatively rapid feedback for management decisions [30]. This timeframe aligns with typical crop rotation cycles and allows for adaptive management based on biological soil health monitoring [1].

Limitations and Future Directions

While this study provides comprehensive characterization of microbial signatures in regenerative agriculture, several limitations should be acknowledged [2]. The focus on 16S rRNA and ITS sequencing provides detailed taxonomic information but limited functional capacity assessment [3]. Future studies incorporating metagenomics and metatranscriptomics could provide deeper insights into functional gene expression and metabolic activity [4].

The paired-site approach used in this study provides strong comparative data but may not capture the full range of management practices and environmental conditions encountered in commercial agriculture [5]. Long-term studies following the same sites through management transitions would provide additional insights into the dynamics of soil health recovery [6].

The development of standardized protocols and reference databases for microbial signature analysis will be essential for widespread adoption [7]. Quality control procedures, standardized sampling methods, and validated laboratory protocols must be established to ensure reproducible results across different laboratories and regions [8].

Conclusion

This study establishes microbial signatures as reliable, quantitative indicators of soil health in regenerative agriculture systems, providing the scientific foundation for biological soil health assessment. The consistent patterns of enhanced microbial diversity, beneficial taxa enrichment, and functional gene abundance under regenerative management demonstrate the biological basis for improved soil health outcomes. The developed Microbial Health Index achieves 89% accuracy in distinguishing management systems and correlates strongly with traditional soil health indicators, validating its utility as a practical assessment tool.

The identification of specific signature taxa including Rhizobium, Trichoderma, and arbuscular mycorrhizal fungi provides targets for monitoring and management intervention. The 65-71% increases in microbial diversity and dramatic shifts in functional gene abundance indicate that regenerative practices fundamentally restructure soil microbial communities toward more beneficial and functionally active states.

Machine learning models using microbial signature data successfully predict soil health outcomes including carbon storage, aggregate stability, and nutrient availability with 85-92% accuracy. These predictive capabilities enable farmers to optimize management practices based on biological soil health status, potentially reducing input costs by \$100-205 ha^{-1} while maintaining productivity.

The rapid response of microbial signatures to management changes (2-3 years) provides early detection of soil health improvements that precede changes in traditional indicators. This responsiveness makes microbial signatures particularly valuable for monitoring the effectiveness of regenerative practices and guiding adaptive management decisions.

The consistency of results across diverse geographic regions and environmental conditions supports the development of standardized protocols for microbial soil health assessment. These findings provide the scientific foundation for incorporating biological indicators into soil health evaluation programs and certification schemes for regenerative agriculture.

Future agricultural sustainability depends on management systems that restore and maintain soil biological health as the foundation for productivity and environmental quality. This study demonstrates that microbial signatures provide practical, scientifically-validated tools for monitoring soil biological recovery and optimizing regenerative agriculture systems. The integration of microbial assessment with traditional soil health evaluation will accelerate the adoption and refinement of regenerative practices essential for sustainable food production and environmental stewardship.

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