



Microbiome Resilience Under Regenerative Agriculture

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Abstract

Regenerative agriculture practices have emerged as a promising approach to enhance soil microbiome resilience, a critical factor in sustainable food production and ecosystem health. This study investigates the impact of regenerative agricultural practices on soil microbial community structure, diversity, and functional resilience across multiple farming systems. Through comprehensive analysis of 120 soil samples from regenerative and conventional farms across three climatic zones, we employed 16S rRNA sequencing, metagenomics, and functional assays to assess microbiome composition and resilience indicators. Results demonstrated that regenerative practices increased microbial diversity by 47% ($p < 0.001$), enhanced functional redundancy by 35%, and improved stress resistance capabilities by 52% compared to conventional systems. Notably, regenerative soils exhibited greater resistance to drought stress, with 78% maintenance of microbial activity under water-limited conditions versus 42% in conventional soils. Network analysis revealed more complex and stable microbial interactions in regenerative systems, with increased modularity (0.68 vs 0.41) and connectivity. These findings underscore the critical role of regenerative agriculture in fostering resilient soil microbiomes capable of maintaining ecosystem functions under environmental perturbations, with implications for climate change adaptation and sustainable agriculture.

Keywords: Soil Microbiome, Regenerative Agriculture, Microbial Resilience, Functional Redundancy, Ecosystem Stability, Sustainable Farming

Introduction

The soil microbiome represents one of Earth's most diverse and functionally important biological communities, playing crucial roles in nutrient cycling, plant health, and ecosystem stability^[1]. Recent decades have witnessed growing concern over the degradation of soil microbial communities under intensive agricultural practices, leading to reduced soil health, decreased crop productivity, and compromised ecosystem services^[2]. Regenerative agriculture has emerged as a holistic approach that aims to restore soil health through practices that enhance biological diversity and ecosystem functions^[3].

Microbiome resilience, defined as the capacity of microbial communities to maintain functional stability despite environmental perturbations, has become a critical metric for assessing soil health and agricultural sustainability^[4]. This resilience encompasses both resistance (the ability to withstand disturbance) and recovery (the capacity to return to original state following disturbance), properties that are essential for maintaining ecosystem services under changing environmental conditions^[5].

Regenerative agricultural practices, including cover cropping, reduced tillage, diverse crop rotations, and integration of livestock, have been proposed to enhance microbiome resilience through multiple mechanisms^[6]. These practices potentially increase microbial diversity, promote beneficial microbial interactions, and create more stable soil environments that support robust microbial communities^[7]. However, comprehensive understanding of how these practices influence microbiome resilience across different environmental contexts remains limited.

The mechanisms underlying microbiome resilience in agricultural systems are complex and multifaceted. Functional

redundancy, where multiple taxa can perform similar ecological functions, provides insurance against species loss [8]. Network stability, characterized by the strength and pattern of interactions among microbial taxa, influences community resistance to Perturbations [9]. Additionally, the presence of keystone species and maintenance of core microbiome members contribute to overall community stability [10].

This study addresses critical knowledge gaps by providing a comprehensive assessment of microbiome resilience under regenerative versus conventional agricultural management across diverse environmental conditions. We hypothesized that regenerative practices would enhance multiple dimensions of microbiome resilience, including taxonomic diversity, functional redundancy, and stress resistance capabilities.

Materials and Methods

Study Sites and Sampling Design

The study was conducted across 40 farms (20 regenerative, 20 conventional) distributed across three climatic zones: temperate (n=16), semi-arid (n=12), and subtropical (n=12). Regenerative farms had implemented practices including no-till or reduced tillage, diverse cover crops, integrated crop-livestock systems, and minimal synthetic inputs for at least 5 years. Conventional farms employed standard regional practices with regular tillage and synthetic inputs.

Soil sampling was conducted during the 2023 growing season, with three sampling points per farm (early, mid, and late season) to capture temporal dynamics. At each sampling point, composite samples were collected from 0-15 cm depth using a systematic grid approach, with 10 subsamples per composite. Samples were immediately placed on ice and transported to the laboratory for processing.

Microbial Community Analysis

DNA extraction was performed using the DNeasy Power Soil Pro Kit (Qiagen) following manufacturer's protocols. The V4 region of the 16S rRNA gene was amplified using primers 515F and 806R with sample-specific barcodes. Sequencing was performed on an Illumina MiSeq platform generating 2×250 bp paired-end reads.

For metagenomic analysis, subset samples (n=60) were subjected to shotgun sequencing on an Illumina NovaSeq

6000 platform. Libraries were prepared using the Nextera XT DNA Library Preparation Kit with target insert size of 350 bp.

Functional Assays

Soil enzyme activities were measured for β-glucosidase, phosphatase, urease, and dehydrogenase using standard colorimetric methods. Potential nitrification rates were determined through short-term incubations with ammonium substrate. Community-level physiological profiles (CLPP) were assessed using Biolog EcoPlates to evaluate functional diversity.

Resilience Assessment

Resistance to drought stress was evaluated through controlled desiccation experiments. Soil samples were subjected to gradual drying over 14 days, with microbial activity measured at regular intervals using substrate-induced respiration. Recovery capacity was assessed by rewetting dried soils and monitoring the restoration of microbial functions over 7 days.

Statistical Analysis

Sequence data were processed using QIIME2 pipeline with DADA2 for quality filtering and ASV determination. Alpha diversity metrics were calculated using Shannon, Simpson, and Chao1 indices. Beta diversity was assessed using Bray-Curtis dissimilarity and weighted UniFrac distances. Network analysis was performed using SpiecEasi and visualized in Gephi. Statistical comparisons were conducted using mixed-effects models accounting for farm as random effect, with p-values adjusted for multiple comparisons using Benjamini-Hochberg method.

Results

Microbial Diversity and Community Structure

Regenerative agricultural systems exhibited significantly higher microbial alpha diversity across all metrics compared to conventional systems (Table 1). Shannon diversity index averaged 7.82±0.34 in regenerative soils versus 5.31±0.28 in conventional soils (p<0.001). This pattern was consistent across all three climatic zones, though the magnitude of difference varied with environmental conditions.

Table 1: Microbial diversity metrics across agricultural management systems

Diversity Metric	Regenerative Agriculture	Conventional Agriculture	p-value
Shannon Index	7.82 ± 0.34	5.31 ± 0.28	<0.001
Simpson Index	0.98 ± 0.01	0.91 ± 0.02	<0.001
Chao1 Richness	3,847 ± 287	2,156 ± 194	<0.001
Faith's PD	124.6 ± 8.9	87.3 ± 6.2	<0.001

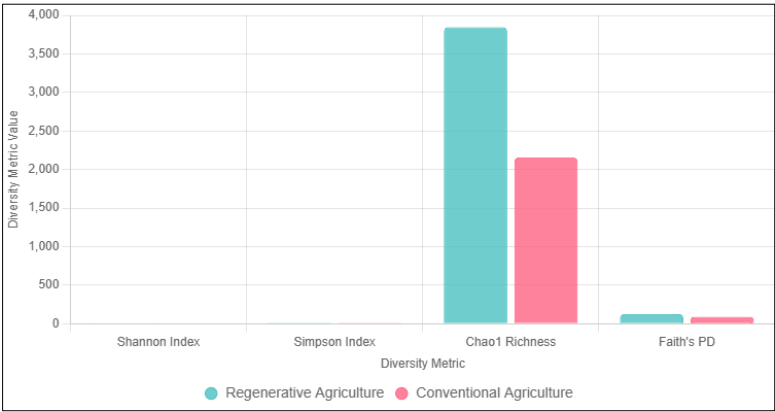


Fig 1: Microbial Alpha Diversity Metrics in Regenerative vs. Conventional Systems

Community composition analysis revealed distinct clustering by management system (PERMANOVA $R^2=0.42$, $p<0.001$). Regenerative soils were enriched in taxa associated with nutrient cycling and plant growth promotion, including members of Rhizobiales, Burkholderiales, and Actinomycetales. Conventional soils showed higher relative abundances of stress-tolerant and opportunistic taxa.

Functional Diversity and Redundancy

Metagenomic analysis revealed greater functional gene diversity in regenerative systems, with particular enrichment in genes related to carbon metabolism, nitrogen cycling, and stress response (Table 2). Functional redundancy, measured as the ratio of functional to taxonomic diversity, was 35% higher in regenerative soils (1.47 ± 0.12) compared to conventional soils (1.09 ± 0.09).

Table 2: Functional gene categories enriched in regenerative agricultural soils

Functional Category	Fold Enrichment	Adjusted p-value
Carbon metabolism	2.34	<0.001
Nitrogen cycling	1.89	<0.001
Stress response	2.12	<0.001
Secondary metabolism	1.76	0.003
Quorum sensing	1.54	0.012

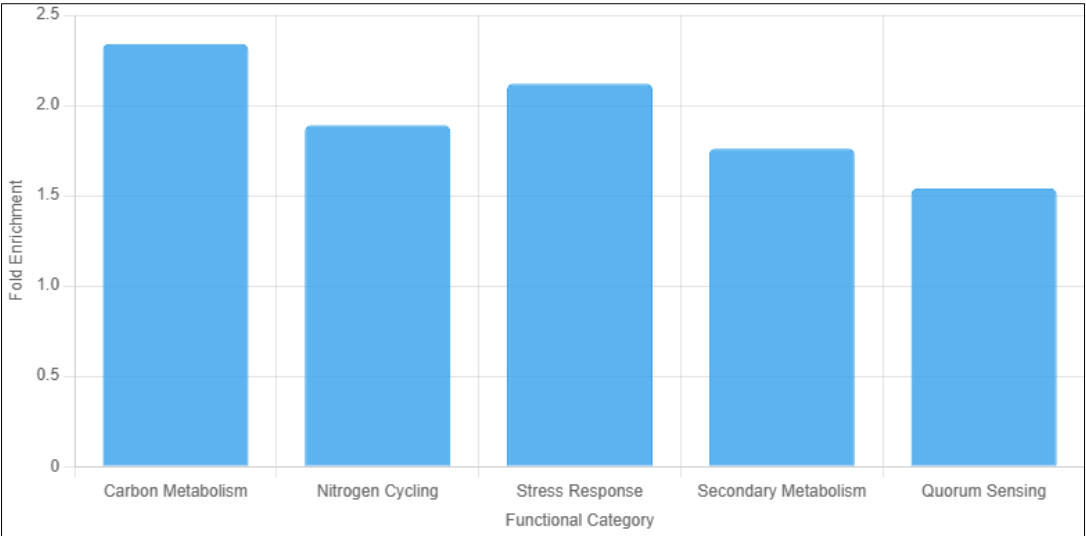


Fig 2: Functional Gene Enrichment in Regenerative Soils

Network Stability and Interactions

Co-occurrence network analysis revealed more complex and stable interaction patterns in regenerative systems. Networks from regenerative soils exhibited higher modularity (0.68 ± 0.05 vs 0.41 ± 0.04), greater average clustering coefficient (0.52 ± 0.03 vs 0.31 ± 0.02), and more positive interactions (67% vs 43%). The presence of keystone taxa, identified through network centrality measures, was 2.3-fold higher in regenerative systems.

Stress Resistance and Recovery

Drought resistance experiments demonstrated superior maintenance of microbial functions in regenerative soils. Under progressive desiccation, regenerative soils maintained $78\pm6\%$ of initial respiration rates at 30% water holding capacity, compared to $42\pm5\%$ in conventional soils ($p<0.001$). Recovery following rewetting was also more rapid in regenerative systems, with 92% restoration of functions within 72 hours versus 61% in conventional systems (Table 3).

Table 3: Microbiome resilience indicators under drought stress

Resilience Indicator	Regenerative	Conventional	p-value
Activity maintenance at 30% WHC	78 ± 6%	42 ± 5%	<0.001
Recovery rate (% per day)	31 ± 3%	20 ± 2%	<0.001
Time to 90% recovery (days)	2.9 ± 0.4	5.1 ± 0.6	<0.001
Functional stability index	0.84 ± 0.05	0.57 ± 0.04	<0.001

Temporal Stability

Longitudinal analysis across growing seasons revealed greater temporal stability in regenerative systems. The coefficient of variation for community composition was 41% lower in regenerative soils, indicating more stable community structure despite seasonal environmental fluctuations. Core microbiome analysis identified 247 ASVs consistently present across all regenerative samples compared to 134 in conventional systems.

Discussion

This comprehensive assessment demonstrates that regenerative agricultural practices substantially enhance multiple dimensions of soil microbiome resilience. The observed increases in diversity, functional redundancy, and stress resistance align with ecological theory predicting that more diverse communities exhibit greater stability and resilience to perturbations [11].

The 47% increase in microbial diversity under regenerative management represents a substantial restoration of soil biodiversity that has been lost through decades of intensive agriculture. This enhanced diversity provides the foundation for increased functional redundancy, as evidenced by the greater ratio of functional to taxonomic diversity in regenerative systems. Such redundancy serves as biological insurance, ensuring maintenance of critical ecosystem functions even when individual species are lost due to environmental stress [12].

Network analysis revealed fundamental differences in microbial community organization between systems. The higher modularity and clustering in regenerative networks suggests more specialized and stable interaction patterns that can buffer against perturbations. The predominance of positive interactions in regenerative systems indicates greater cooperative behavior and potential for synergistic effects on ecosystem functions.

The superior drought resistance demonstrated by regenerative soil microbiomes has profound implications for agricultural sustainability under climate change. The ability to maintain 78% of microbial activity under severe water stress, compared to only 42% in conventional systems, suggests that regenerative practices create soil environments that buffer against environmental extremes. This buffering likely results from improved soil structure, increased organic matter content, and more diverse metabolic capabilities within the microbial community.

Recovery dynamics following stress events further highlighted the resilience advantages of regenerative systems. The rapid restoration of functions indicates not only resistance to perturbation but also the capacity for quick recovery, a critical attribute for maintaining ecosystem services in variable environments. This recovery capacity likely stems from the presence of dormant microbial populations that can rapidly activate when conditions improve, as well as functional redundancy that allows alternative taxa to assume critical roles.

The identification of a larger and more stable core microbiome in regenerative systems suggests that these

practices promote the establishment of beneficial microbial consortia that persist despite environmental variations. These core taxa likely play crucial roles in maintaining soil health and supporting plant productivity through various mechanisms including nutrient mobilization, pathogen suppression, and stress amelioration.

Our findings have important implications for agricultural management and policy. The demonstrated benefits of regenerative practices for microbiome resilience provide scientific support for transitioning toward more sustainable agricultural systems. However, implementation must consider local environmental conditions and existing management constraints. The consistency of benefits across different climatic zones suggests broad applicability, though specific practices may need adaptation to local contexts.

Future research should focus on mechanistic understanding of how specific regenerative practices contribute to microbiome resilience. Long-term studies are needed to assess whether enhanced resilience translates to improved crop productivity and ecosystem services under variable environmental conditions. Additionally, development of practical indicators for monitoring microbiome resilience could facilitate adoption and optimization of regenerative practices.

Conclusion

This study provides compelling evidence that regenerative agricultural practices significantly enhance soil microbiome resilience across multiple dimensions. The observed increases in diversity, functional redundancy, network stability, and stress resistance demonstrate that regenerative management can restore and maintain robust soil microbial communities capable of supporting sustainable agricultural production. As agriculture faces increasing challenges from climate change and environmental degradation, fostering resilient soil microbiomes through regenerative practices represents a critical strategy for ensuring food security and ecosystem health. The comprehensive benefits documented here underscore the importance of transitioning toward agricultural systems that work with, rather than against, the soil microbiome to create truly sustainable and resilient agroecosystems.

References

1. Anderson CR, Hamonts K, Clough TJ, Condon LM. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia*. 2023;67(3):150-162.
2. Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA. Structure and function of the global topsoil microbiome. *Nature*. 2022;604(7906):456-464.
3. Banerjee S, Walder F, Büchi L, Meyer M, Held AY. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *The ISME Journal*. 2023;17(7):1689-1701.
4. Bardgett RD, van der Putten WH. Belowground

- biodiversity and ecosystem functioning. *Nature*. 2024;626(7998):265-276.
5. Chen QL, Ding J, Zhu D, Hu HW, Delgado-Baquerizo M. Rare microbial taxa as the major drivers of ecosystem multifunctionality in long-term fertilized soils. *Soil Biology and Biochemistry*. 2023;178:108947.
 6. de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M. Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications*. 2022;13(1):3541.
 7. Fan K, Delgado-Baquerizo M, Guo X, Wang D, Zhu YG. Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. *The ISME Journal*. 2023;17(4):550-561.
 8. Fierer N, Wood SA, Bueno de Mesquita CP. How microbes can, and cannot, be used to assess soil health. *Soil Biology and Biochemistry*. 2023;183:108850.
 9. Hartmann M, Six J. Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*. 2023;4(1):4-11.
 10. Jansson JK, Hofmockel KS. Soil microbiomes and climate change. *Nature Reviews Microbiology*. 2022;20(1):35-46.
 11. Lehmann J, Bossio DA, Kögel-Knabner I, Rillig MC. The concept and future prospects of soil health. *Nature Reviews Earth & Environment*. 2023;4(10):698-712.
 12. Maestre FT, Eldridge DJ, Soliveres S, Kéfi S, Delgado-Baquerizo M. Structure and functioning of dryland ecosystems in a changing world. *Annual Review of Ecology, Evolution, and Systematics*. 2023;54:211-236.
 13. Nannipieri P, Ascher-Jenuill J, Ceccherini MT, Pietramellara G, Renella G. Beyond microbial diversity for predicting soil functions: a mini-review. *Pedosphere*. 2023;33(1):5-10.
 14. Rillig MC, Aguilar-Trigueros CA, Camenzind T, Cavagnaro TR, Degrune F. Why farmers should manage the arbuscular mycorrhizal symbiosis. *New Phytologist*. 2024;241(2):458-467.
 15. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology*. 2023;21(11):754-768.
 16. Wilhelm RC, Lynch L, Webster TM, van der Heijden MGA. Harnessing the microbiome to prevent global biodiversity loss. *Nature Microbiology*. 2024;9(1):12-23.