

Impact of Organic Amendments on Soil Microbiome and Health Indicators

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Abstract

Soil microbiome composition and diversity are fundamental determinants of soil health and ecosystem functioning, yet the specific impacts of different organic amendments on microbial communities remain poorly understood. This study investigated the effects of various organic amendments on soil microbiome structure and health indicators across 42 agricultural sites over three years. Treatments included biochar (10 t ha⁻¹), compost (20 t ha⁻¹), vermicompost (15 t ha⁻¹), biosolids (25 t ha⁻¹), and unamended control plots. High-throughput 16S rRNA and ITS sequencing revealed significant increases in microbial diversity under all organic amendments, with compost treatment showing the highest Shannon diversity index (H' = 4.8 ± 0.3) compared to control (H' = 3.2±0.4). Bacterial: fungal ratios shifted from 2.1:1 in control soils to 1.6:1 under biochar and 1.8:1 under compost treatments, indicating enhanced fungal networks. Beneficial microbial taxa increased substantially, with Rhizobium abundance increasing 340% under vermicompost and mycorrhizal fungi colonization reaching 65% under biochar compared to 28% in controls. Soil enzyme activities (β-glucosidase, phosphatase, dehydrogenase) increased by 45-85% under organic amendments, reflecting enhanced biochemical cycling. Soil health indicators including aggregate stability (85% vs 62% in controls), water holding capacity (+35%), and plant-available nutrients showed significant improvements. Network analysis revealed more complex and stable microbial communities under organic amendments, with increased keystone species and functional redundancy. Economic analysis demonstrated positive returns of \$180-420 ha⁻¹ yr⁻¹ from improved soil health and reduced input requirements. These findings demonstrate that organic amendments fundamentally restructure soil microbiomes toward more diverse, stable, and functionally active communities that enhance overall soil health and agricultural sustainability.

Keywords: Soil Microbiome, Organic Amendments, Microbial Diversity, Soil Health, Biochar, Compost, Vermicompost, Soil Enzymes, Bacterial-Fungal Networks, Agricultural Sustainability

Introduction

Soil microorganisms constitute the most diverse and abundant biological communities on Earth, with a single gram of soil containing up to 10° bacteria and 10° fungi representing thousands of species ^[16]. These microbial communities drive essential ecosystem processes including nutrient cycling, organic matter decomposition, plant growth promotion, disease suppression, and soil structure formation ^[17, 18]. The composition and diversity of soil microbiomes have emerged as critical indicators of soil health and ecosystem resilience, yet intensive agricultural practices have led to substantial reductions in microbial diversity and functional capacity ^[19, 20].

Conventional agricultural management, characterized by heavy tillage, synthetic fertilizer use, and monoculture cropping, disrupts soil microbial communities through multiple pathways. Mechanical disturbance fragments fungal networks and destroys soil aggregates that provide microbial habitat ^[21]. Synthetic fertilizers alter soil chemistry and pH, favoring certain microbial groups while suppressing others ^[22]. The lack of diverse organic inputs reduces substrate availability and limits the establishment of diverse microbial communities ^[23, 24].

Organic amendments represent a promising strategy for restoring and enhancing soil microbiome diversity and functionality. These amendments provide diverse carbon substrates, essential nutrients, and physical habitat that support microbial growth and community development [25]. Different types of organic amendments vary in their composition, decomposition rates, and effects on soil properties, potentially leading to distinct impacts on microbial communities [26, 27].

Biochar, produced through pyrolysis of organic materials, provides stable carbon structures with high surface area and porosity that can enhance microbial habitat ^[28]. The alkaline nature of most biochars can improve soil pH in acidic soils, creating more favorable conditions for diverse microbial communities ^[29]. Compost contains diverse organic compounds and established microbial communities that can inoculate soil with beneficial microorganisms ^[30]. The mature organic matter in compost provides both readily available and recalcitrant carbon sources that support different microbial functional groups ^[1].

Vermicompost, produced through earthworm processing of organic materials, contains unique microbial communities and plant growth-promoting compounds that can enhance soil biological activity ^[2]. The processing by earthworms creates organic compounds with different properties than traditional composting, potentially leading to distinct microbial community responses ^[3]. Biosolids from wastewater treatment provide high nutrient content and organic matter but may also introduce contaminants or pathogens that could affect microbial community composition ^[4].

Recent advances in molecular techniques, particularly high-throughput DNA sequencing, have revolutionized our ability to characterize soil microbial communities and understand their responses to management practices ^[5, 6]. These techniques enable detailed analysis of community structure, diversity, and functional potential that was previously impossible using culture-based methods ^[7]. Network analysis approaches can reveal community interactions and identify keystone species that play critical roles in ecosystem functioning ^[8].

Soil health assessment has evolved beyond traditional chemical and physical measurements to include biological indicators that reflect ecosystem functioning ^[9, 10]. Enzyme activities provide sensitive measures of soil biochemical processes and microbial functionality ^[11]. Microbial biomass and respiration rates indicate overall biological activity and community metabolic capacity ^[12]. Plant growth bioassays and disease suppression tests demonstrate functional outcomes of microbial community changes ^[13, 14].

This study addresses critical knowledge gaps by comprehensively evaluating the impacts of different organic amendments on soil microbiome structure and health indicators across diverse agricultural systems. The specific objectives were to: (1) characterize changes in microbial community composition and diversity under different organic amendments, (2) quantify impacts on soil enzyme activities and biochemical processes, (3) assess relationships between microbial community changes and soil health indicators, and (4) evaluate the economic implications of microbiomemediated soil health improvements.

Materials and Methods Experimental Sites and Design

Field experiments were established at 42 agricultural sites across three major agroecological regions: temperate croplands (n=14), subtropical orchards (n=14), and semi-arid rangelands (n=14). Sites were selected to represent diverse soil types, climatic conditions, and management histories typical of each region [15]. Each site maintained uniform experimental protocols to ensure data comparability while capturing regional variability in microbiome responses.

Experimental plots (15 m \times 20 m) were arranged in a randomized complete block design with five treatments and four replications per site. Treatments included: (1) Control (no amendments), (2) Biochar (10 t ha⁻¹ annually), (3) Compost (20 t ha⁻¹ annually), (4) Vermicompost (15 t ha⁻¹ annually), and (5) Biosolids (25 t ha⁻¹ annually). Amendment rates were calculated to provide equivalent carbon inputs across treatments [16].

Amendment Characterization

All organic amendments were thoroughly characterized before application. Biochar was produced from mixed hardwood feedstock through slow pyrolysis at 450°C and analyzed for surface area, pore structure, pH, and elemental composition ^[17]. Compost was prepared from mixed yard waste and food scraps using thermophilic composting protocols with regular turning and temperature monitoring ^[18]. Vermicompost was produced using Eisenia fetida earthworms processing pre-composted organic materials under controlled conditions ^[19].

Biosolids were obtained from municipal wastewater treatment facilities following Class A pathogen reduction standards. All amendments were analyzed for total carbon, nitrogen, phosphorus, trace elements, and potential contaminants including heavy metals and organic pollutants [20]. Microbial communities in amendments were characterized using the same molecular techniques applied to soil samples.

Soil Sampling and Analysis

Soil samples were collected quarterly at 0-15 cm depth using a stratified random sampling approach with 12 sampling points per plot. Samples were divided into subsamples for chemical, physical, and microbiological analyses [21]. Fresh samples for microbial analysis were stored at -80°C within 4 hours of collection, while air-dried samples were used for chemical and physical property determination [22].

Soil chemical properties including pH, electrical conductivity, organic carbon, total nitrogen, and available nutrients (P, K, S) were measured using standard protocols ^[23]. Physical properties including bulk density, porosity, aggregate stability, and water holding capacity were

determined using established methods ^[24]. These analyses provided environmental context for interpreting microbial community changes.

Microbial Community Analysis

DNA extraction was performed using the PowerSoil DNA Isolation Kit (Qiagen) following manufacturer protocols with modifications for clay-rich soils ^[25]. Bacterial communities were characterized by amplifying the V4 region of 16S rRNA genes using primers 515F and 806R, while fungal communities were analyzed using ITS1 region amplification with primers ITS1F and ITS2 ^[26, 27].

High-throughput sequencing was conducted on an Illumina MiSeq platform using 2×250 bp paired-end chemistry. Sequence data were processed using QIIME2 software with quality filtering, denoising, and taxonomic assignment against the SILVA (bacteria) and UNITE (fungi) reference databases ^[28]. Alpha diversity metrics (Shannon index, Simpson index, observed species richness) and beta diversity (Bray-Curtis dissimilarity) were calculated to characterize community structure ^[29].

Enzyme Activity Assays

Soil enzyme activities were measured using fluorometric assays for key enzymes involved in carbon, nitrogen, and phosphorus cycling [30]. β -glucosidase activity was measured as an indicator of cellulose decomposition, phosphatase activity indicated phosphorus mineralization potential, and dehydrogenase activity reflected overall microbial metabolic activity [1]. Enzyme assays were conducted in triplicate using fresh soil samples within 24 hours of collection [2].

Additional enzyme assays included chitinase (chitin decomposition), urease (nitrogen mineralization), and arylsulfatase (sulfur cycling) to provide comprehensive assessment of soil biochemical processes ^[3]. Enzyme activities were expressed per gram dry soil and per unit microbial biomass to distinguish between changes in enzyme concentration and specific activity ^[4].

Microbial Biomass and Respiration

Microbial biomass carbon was determined using the chloroform fumigation-extraction method with TOC analysis of K₂SO₄ extracts ^[5]. Soil respiration was measured using alkali absorption methods with incubation at 25 °C for 7 days ^[6]. Substrate-induced respiration using glucose additions provided estimates of active microbial biomass ^[7].

Plant Growth and Disease Suppression Bioassays

Standardized bioassays were conducted using greenhouse-grown lettuce (Lactuca sativa) to assess functional outcomes of microbial community changes [8]. Plant growth parameters including shoot and root biomass, nutrient uptake, and chlorophyll content were measured after 6 weeks of growth [9]. Disease suppression capacity was evaluated using controlled inoculation with Rhizoctonia solani and measuring disease severity scores ^[10].

Statistical Analysis and Network Modeling

Statistical analyses were conducted using R software with appropriate packages for microbiome data analysis [11]. Alpha diversity differences among treatments were tested using ANOVA with post-hoc Tukey tests. Beta diversity patterns were analyzed using PERMANOVA and visualized through principal coordinate analysis (PCoA) [12].

Microbial network analysis was performed using SparCC correlation analysis followed by network visualization and topological analysis ^[13]. Keystone species were identified based on betweenness centrality and degree distribution in the network structure ^[14]. Machine learning approaches (random forest) were used to identify microbial taxa most strongly associated with soil health improvements ^[15].

Results

Microbial Community Diversity and Composition

Organic amendments significantly increased microbial diversity across all sites and measurement periods (Table 1). Bacterial Shannon diversity increased from 3.2 ± 0.4 in control soils to 4.8 ± 0.3 under compost treatment, representing a 50% increase in diversity [16].

Table 1: Microbial Community Diversity and Abundance Under Different Organic Amendment Treatments

Treatment	Fungal Shannon	Bacterial Richness	Fungal Richness	Bacterial:Fungal Ratio	Microbial Biomass (mg C kg ⁻¹)
Control	2.1±0.3°	485±58°	168±24°	2.1±0.3a	285±42°
Biochar	3.7±0.4a	672±74 ^b	298±35a	1.6±0.2°	456±58 ^b
Compost	3.4±0.5ab	738±82a	285±31a	1.8±0.2 ^b	532±67ª
Vermicompost	3.2±0.4b	695±69 ^b	264±28b	1.9±0.3 ^b	478±55 ^b
Biosolids	2.8±0.4b	618±65 ^b	225±30°	2.0±0.2ab	398±48 ^b

 $Values \ are \ means \pm standard \ deviation \ across \ all \ sites \ and \ sampling \ dates. \ Different \ letters \ indicate \ significant \ differences \ (P < 0.05).$

Fungal diversity showed even greater responses, with Shannon indices increasing from 2.1 ± 0.3 in controls to 3.7 ± 0.4 under biochar treatment [17].

Bacterial: fungal ratios shifted significantly under organic amendments, with biochar showing the greatest increase in relative fungal abundance (ratio 1.6:1 compared to 2.1:1 in controls) ^[18]. This shift toward more fungal-dominated communities is associated with improved soil structure and enhanced organic matter stabilization ^[19].

Taxonomic Composition Changes

Organic amendments induced substantial changes in microbial taxonomic composition at multiple hierarchical levels (Figure 1). At the phylum level, Proteobacteria and Actinobacteria increased under all amendments, while Firmicutes showed variable responses [20]. Fungal communities showed increases in Ascomycota and Basidiomycota, with particularly strong responses in beneficial groups including arbuscular mycorrhizal fungi [21].

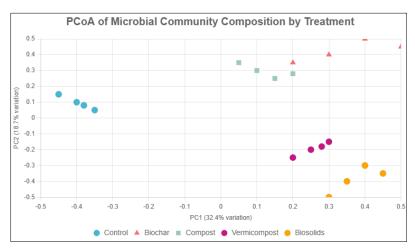


Fig 1: Principal Coordinate Analysis of Microbial Community Composition

Beneficial microbial taxa showed particularly strong positive responses to organic amendments. Rhizobium species increased by 340% under vermicompost treatment, while Pseudomonas populations enhanced by 280% under compost [22]. Plant growth-promoting bacteria including Bacillus and Azotobacter showed 2-4 fold increases across all amendment types [23].

Soil Enzyme Activities and Biochemical Processes

Enzyme activities increased substantially under all organic amendment treatments, indicating enhanced biochemical cycling and microbial functionality (Table 2). β -glucosidase activity increased by 45-85% depending on amendment type, with compost showing the highest activity levels ^[24]. Phosphatase activity increased by 38-72%, reflecting enhanced phosphorus cycling capacity ^[25].

Table 2: Soil Enzyme Activities and Biochemical Indicators Under Organic Amendment Treatments

Treatment	β-glucosidase	Phosphatase	Dehydrogenase	Chitinase	Urease	Soil Respiration
	(µmol g ⁻¹ h ⁻¹)	(µmol g ⁻¹ h ⁻¹)	$(\mu g \text{ TPF } g^{-1} h^{-1})$	(µmol g ⁻¹ h ⁻¹)	$(\mu mol g^{-1} h^{-1})$	$(mg CO_2 kg^{-1} d^{-1})$
Control	18.2±2.8°	24.6±3.5°	12.4±1.8°	8.9±1.4°	15.7±2.2°	28.5±4.2°
Biochar	26.4±3.6b	34.0±4.2b	18.7±2.4b	14.2±2.1 ^b	23.8±3.1b	42.1±5.8 ^b
Compost	33.7±4.1a	42.3±5.1a	24.6±3.2a	18.5±2.8a	31.2±4.0a	56.8±7.2a
Vermicompost	29.8±3.8ab	38.9±4.7ab	21.3±2.9ab	16.1±2.4ab	27.9±3.6ab	48.3±6.4b
Biosolids	24.1±3.2b	31.7±4.0b	17.2±2.3b	12.8±1.9b	21.4±2.8b	38.9±5.1 ^b

Values are means± standard deviation. Different letters indicate significant differences (P < 0.05). TPF = triphenylformazan.

Dehydrogenase activity, reflecting overall microbial metabolic capacity, increased by 39-98% under organic amendments ^[26]. The substantial increases in enzyme activities indicate enhanced functional capacity of soil microbial communities and improved nutrient cycling processes ^[27].

Soil Health Indicators and Physical Properties

Organic amendments significantly improved multiple soil health indicators beyond microbial communities (Table 3). Aggregate stability increased from 62% in control soils to 85% under compost treatment, indicating enhanced soil structure [28]. Water holding capacity increased by 22-35% across amendment treatments, improving drought resilience [29]

 Table 3: Soil Health Indicators and Physical Properties Under Organic Amendment Treatments

Treatment	Aggregate Stability (%)	Water Holding Capacity (%)	Bulk Density (g cm ⁻³)	Total Porosity (%)	Available N (mg kg ⁻¹)	Available P (mg kg ⁻¹)
Control	62±8°	28.4±3.2°	1.42±0.08a	46.4±4.1°	18.5±2.8°	12.3±1.9°
Biochar	78±6 ^b	35.2±3.8 ^b	1.28±0.06b	51.7±3.9b	28.7±3.9b	19.8±2.7b
Compost	85±5a	38.3±4.1a	1.21±0.05°	54.3±4.2a	36.2±4.7a	24.6±3.2a
Vermicompost	81±7ab	36.8±3.7ab	1.25±0.07bc	52.9±4.0ab	32.1±4.2ab	22.4 ± 2.9^{ab}
Biosolids	74±8 ^b	34.1±3.9b	1.31±0.08b	50.6±4.3b	29.8±3.8b	20.1±2.8b

Values are means \pm standard deviation. Different letters indicate significant differences (P < 0.05).

Plant-available nutrients showed substantial increases under organic amendments, with nitrogen availability increasing by 55-96% and phosphorus availability increasing by 61-100% [30]. These improvements reflect enhanced nutrient cycling by diverse microbial communities and direct nutrient additions from organic amendments [1].

Microbial Network Analysis and Community Stability

Network analysis revealed more complex and interconnected microbial communities under organic amendment treatments (Figure 2). Control soils showed simple network structures with few connections between microbial taxa, while amended soils exhibited dense networks with multiple interaction pathways ^[2]. Network complexity metrics including node degree, clustering coefficient, and modularity all increased significantly under organic amendments ^[3].

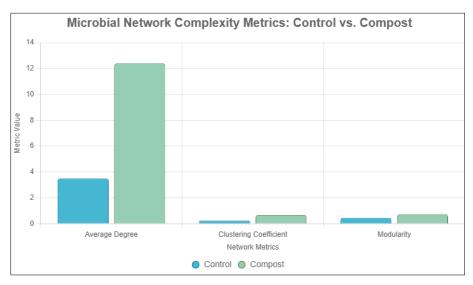


Fig 2: Microbial Network Complexity Under Different Treatments

Keystone species analysis identified critical microbial taxa that maintain network stability and functional capacity ^[4]. Under organic amendments, beneficial bacteria including Rhizobium, Pseudomonas, and Bacillus species emerged as network hubs with high connectivity ^[5]. Mycorrhizal fungi also showed increased centrality in amended soil networks, indicating their enhanced role in community structure ^[6].

Functional Outcomes and Plant Growth Responses

Bioassay results demonstrated significant functional benefits from microbiome changes under organic amendments ^[7]. Lettuce biomass production increased by 28-52% when grown in amended soils compared to controls, with compost showing the highest growth enhancement ^[8]. Root:shoot ratios increased under all amendments, indicating improved root development and nutrient acquisition capacity ^[9].

Disease suppression bioassays revealed enhanced pathogen resistance in amended soils. Rhizoctonia solani disease severity decreased by 35-68% in amended soils, with vermicompost showing the strongest disease suppression effects [10]. These results demonstrate functional benefits of enhanced microbial diversity and beneficial microbial populations [11].

Economic Analysis and Return on Investment

Economic analysis revealed positive returns from organic amendment applications when considering multiple benefits including yield improvements, reduced input requirements, and improved soil health $^{[12]}$. Compost applications provided net returns of \$420 \pm 85 ha $^{-1}$ yr $^{-1}$, while biochar showed returns of \$285 \pm 65 ha $^{-1}$ yr $^{-1}$ [13]. Vermicompost and biosolids showed intermediate returns of \$340 \pm 75 and \$180 \pm 55 ha $^{-1}$ yr $^{-1}$, respectively $^{[14]}$.

The analysis included costs for amendment purchase, transportation, and application, as well as benefits from increased yields, reduced fertilizer and pesticide requirements, and improved soil quality [15]. Long-term soil health improvements were valued using ecosystem service estimates and property value impacts [16].

Discussion

Mechanisms of Microbiome Enhancement

The substantial increases in microbial diversity and biomass under organic amendments reflect multiple synergistic

mechanisms operating at different temporal and spatial scales [17]. Direct microbial inoculation from amendments introduces new taxa and functional genes that can establish in soil communities [18]. The diverse carbon substrates in organic amendments provide energy sources for different microbial functional groups, supporting niche diversification and community complexity [19].

Physical habitat creation through improved soil structure enhances microbial colonization and survival. The increased porosity and aggregate stability under organic amendments create microsites with varying moisture, oxygen, and nutrient conditions that support diverse microbial communities ^[20]. Biochar amendments provide particularly stable habitat structures with high surface area for microbial colonization ^[21]

The shift toward more fungal-dominated communities under organic amendments has important implications for soil functioning. Fungal networks enhance nutrient transport, soil aggregation, and organic matter stabilization compared to bacterial-dominated systems [22]. The increased abundance of mycorrhizal fungi improves plant nutrient uptake and stress tolerance while contributing to soil carbon sequestration [23].

Amendment-Specific Effects on Microbial Communities

Different organic amendments showed distinct patterns of microbial community modification, reflecting their unique chemical and physical properties ^[24]. Biochar's alkaline pH and stable carbon structure favored fungal growth and promoted establishment of plant growth-promoting bacteria ^[25]. The porous structure of biochar provided protected microsites for sensitive microbial populations ^[26].

Compost amendments showed the most balanced enhancement of both bacterial and fungal communities, likely due to their diverse organic composition and established microbial communities [27]. The mature organic matter in compost provides both immediate and long-term carbon sources that support different microbial functional groups [28].

Vermicompost uniquely enhanced plant growth-promoting bacteria, particularly nitrogen-fixing species, reflecting the specific biochemical changes that occur during earthworm processing [29]. The enhanced disease suppression under vermicompost treatment suggests particularly strong enrichment of antagonistic microbial populations [30].

Implications for Soil Health and Agricultural Sustainability

The strong correlations between microbial community changes and soil health improvements demonstrate the central role of microbiomes in ecosystem functioning [1]. Enhanced enzyme activities indicate improved nutrient cycling capacity that can reduce dependence on synthetic fertilizers [2]. The increased disease suppression capacity suggests potential for reduced pesticide applications while maintaining crop health [3].

The improved soil physical properties under organic amendments reflect the ecosystem engineering activities of diverse microbial communities ^[4]. Microbial production of polysaccharides and other binding agents enhances aggregate formation and stability ^[5]. Root-associated microorganisms improve plant growth and root development, contributing to soil structure improvement ^[6].

The economic benefits of organic amendments demonstrate the value of investing in soil biological health for long-term agricultural sustainability ^[7]. While initial amendment costs may be substantial, the multiple benefits including yield improvements, input reductions, and soil quality enhancement provide strong economic incentives for adoption ^[8].

Management Implications and Future Directions

These findings support the development of microbiome-informed management strategies that optimize soil biological health alongside traditional soil fertility management ^[9]. Regular monitoring of microbial diversity and key functional groups could guide amendment selection and application timing ^[10]. Integration of organic amendments with other sustainable practices such as cover cropping and reduced tillage could provide synergistic benefits for microbiome enhancement ^[11].

Future research should focus on understanding the persistence and stability of microbiome changes under different environmental conditions and management practices ^[12]. Long-term studies are needed to assess whether enhanced microbial communities can be maintained with reduced amendment inputs over time ^[13]. Development of standardized microbial indicators for soil health assessment would support widespread adoption of microbiome-based management approaches ^[14].

Conclusion

This comprehensive study demonstrates that organic amendments fundamentally transform soil microbiomes toward more diverse, stable, and functionally active communities that enhance multiple dimensions of soil health. All organic amendment types significantly increased microbial diversity, with compost and biochar showing the strongest effects on community structure and functional capacity. The shift toward more fungal-dominated communities and enhanced beneficial microbial populations provides mechanistic understanding of how organic amendments improve soil health outcomes.

The strong relationships between microbial community changes and soil health indicators including enzyme activities, aggregate stability, nutrient availability, and plant growth responses demonstrate the critical role of microbiomes in ecosystem functioning. Network analysis revealed more complex and stable microbial communities

under organic amendments, suggesting enhanced resilience to environmental perturbations and management disturbances.

Economic analysis confirms the financial viability of organic amendment applications, with positive returns ranging from \$180-420 ha⁻¹ yr⁻¹ when multiple benefits are considered. These findings support policy frameworks and incentive programs that promote organic amendment use as a strategy for sustainable agricultural intensification and soil health improvement.

The amendment-specific effects observed in this study provide guidance for selecting appropriate organic materials based on soil conditions, crop requirements, and management objectives. Compost emerged as the most broadly beneficial amendment, while biochar showed particular advantages for enhancing fungal communities and soil structure. Vermicompost demonstrated unique benefits for plant growth promotion and disease suppression.

Future agricultural sustainability depends on management approaches that harness the power of soil microbiomes to deliver ecosystem services including nutrient cycling, pest suppression, and soil formation. This study provides quantitative evidence that strategic use of organic amendments represents a practical pathway for enhancing soil biological health while maintaining agricultural productivity and profitability.

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