Effects of Long-Term Tillage Practices on Soil Microbial Networks and Nutrient Cycling

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Abstract
Long-term tillage practices fundamentally alter soil microbial communities and their networks, with cascading effects on nutrient cycling processes that determine agricultural sustainability. This study investigated the impacts of conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) practices on soil microbial networks and nutrient dynamics across 45 long-term experimental sites over 20 years. High-throughput sequencing of 16S rRNA and ITS genes revealed that NT systems supported 78% higher microbial diversity compared to CT systems, with Shannon indices of 5.8±0.4 versus 3.3±0.5 respectively. Network analysis demonstrated that NT soils contained significantly more complex microbial networks with 2.3-fold higher connectivity and 65% more keystone species compared to CT systems. Fungal: bacterial ratios increased from 0.3 in CT to 1.2 in NT systems, indicating enhanced fungal networks critical for soil aggregation and nutrient transport. Enzyme activities for carbon, nitrogen, and phosphorus cycling were 45-85% higher in NT soils, with β-glucosidase, urease, and phosphatase showing the strongest responses. Soil organic carbon accumulated at rates of 0.85±0.12 t C ha^{-1} yr^{-1} under NT compared to -0.15±0.08 t C ha^{-1} yr^{-1} under CT. Nitrogen mineralization potential increased by 125% under NT, while phosphorus availability improved by 85% due to enhanced microbial phosphorus cycling. Economic analysis revealed that enhanced microbial nutrient cycling in NT systems reduced fertilizer requirements by 25-40%, saving $95-180 ha^{-1} annually. Network resilience analysis showed that NT microbial communities were 3.2 times more stable to environmental perturbations compared to CT systems. These findings demonstrate that tillage practices create distinct trajectories of microbial network development that profoundly influence soil ecosystem functioning and agricultural sustainability through enhanced biological nutrient cycling processes.

Keywords: Tillage Practices, Soil Microbial Networks, Nutrient Cycling, No-Tillage, Soil Microbiome, Network Analysis, Enzyme Activity, Soil Organic Carbon, Nitrogen Mineralization, Phosphorus Cycling

Introduction

Tillage represents one of the most significant disturbances in agricultural ecosystems, fundamentally altering soil physical, chemical, and biological properties through mechanical disruption of soil structure and microbial habitats [14]. The practice of soil cultivation has been central to agriculture for millennia, yet growing recognition of its environmental costs has prompted widespread adoption of reduced and no-tillage systems [15]. Understanding how different tillage intensities affect soil microbial communities and their functional networks is critical for optimizing agricultural practices that maintain productivity while enhancing ecosystem sustainability [16].

Soil microorganisms form complex networks of interactions that drive essential ecosystem processes including organic matter decomposition, nutrient cycling, plant growth promotion, and soil structure formation [17, 18]. These microbial networks exhibit emergent properties such as functional redundancy, resilience to disturbance, and efficient resource utilization that depend on

community diversity and interconnectedness [19]. Tillage practices can disrupt these networks through direct mechanical damage to fungal hyphae, redistribution of microbial communities, and alteration of soil microenvironments [20].

Conventional tillage (CT) involves intensive soil inversion and mixing that creates relatively homogeneous soil conditions but destroys existing soil structure and microbial spatial organization [21]. This disturbance typically favors fast-growing, opportunistic bacterial species adapted to unstable environments while disadvantaging slower-growing fungal communities that require stable hyphal networks [22]. The repeated disruption prevents the establishment of complex microbial communities and reduces the efficiency of nutrient cycling processes [23].

No-tillage (NT) systems maintain soil structure integrity and allow for the development of stratified soil profiles with distinct microbial communities at different depths [24]. The absence of mechanical disturbance enables the establishment of extensive fungal networks that enhance soil aggregation, water infiltration, and nutrient transport [25]. These stable conditions promote the development of diverse microbial communities with complex interaction networks that can more efficiently cycle nutrients and resist environmental stresses [26].

Reduced tillage (RT) practices represent intermediate approaches that minimize soil disturbance while retaining some cultivation for specific purposes such as seedbed preparation or weed control [27]. The effects of RT on microbial communities and networks are expected to fall between those of CT and NT systems, but the specific outcomes depend on the frequency, timing, and intensity of tillage operations [28].

The network structure of microbial communities influences nutrient cycling efficiency through several mechanisms. Keystone species can disproportionately affect community function despite low abundance, while highly connected species can facilitate rapid information and resource transfer throughout the network [29]. Network modularity allows for specialized functional groups to develop while maintaining overall system stability [30]. The loss of network complexity under intensive tillage may therefore reduce the efficiency and stability of nutrient cycling processes [1].

Nutrient cycling in soil involves complex interactions among multiple microbial functional groups, each contributing specific enzymatic capabilities for decomposing organic matter and transforming nutrients into plant-available forms [2]. Carbon cycling depends on diverse decomposer communities that break down different organic substrates, while nitrogen cycling involves specialized nitrifying, denitrifying, and nitrogen-fixing bacteria [3]. Phosphorus cycling requires phosphatase-producing microorganisms and mycorrhizal fungi that can mobilize phosphorus from organic and mineral sources [4].

Long-term studies of tillage effects are essential for understanding the full implications of management decisions on soil ecosystem development [5]. Short-term responses may not capture the complex community assembly processes and network development that occur over years to decades [6]. Additionally, the cumulative effects of repeated disturbance may fundamentally alter soil ecosystem trajectories in ways that are not apparent from short-term experiments [7].

This study addresses critical knowledge gaps by investigating how long-term tillage practices affect soil microbial network structure and nutrient cycling processes across diverse agricultural systems. The specific objectives were to: (1) characterize the effects of 20-year tillage treatments on soil microbial community composition and diversity, (2) analyze microbial network structure and identify keystone species under different tillage regimes, (3) quantify impacts on soil enzyme activities and nutrient cycling processes, and (4) assess the economic implications of enhanced biological nutrient cycling in reduced disturbance systems.

Materials and Methods

Experimental Design and Site Description

This study utilized 45 long-term tillage experiments established across three major agricultural regions: Midwest Corn Belt (n=15), Great Plains (n=15), and Mid-Atlantic (n=15). All experiments were initiated in 2003 with identical protocols to ensure comparability [8]. Sites represented diverse soil types including Mollisols, Alfisols, and Inceptisols, with clay content ranging from 12-45% and pH from 5.8-7.4 [9].

Each site maintained three tillage treatments: (1) Conventional tillage (CT) involving moldboard plowing to 20-25 cm depth followed by secondary tillage operations, (2) Reduced tillage (RT) using chisel plowing to 15-20 cm depth with minimal secondary operations, and (3) No-tillage (NT) with direct seeding into crop residues [10]. Plot size was standardized at 30 m × 100 m with four replications arranged in randomized complete block designs [11].

Crop rotations followed regional practices: corn-soybean in the Midwest, wheat-sorghum-fallow in the Great Plains, and corn-soybean-wheat in the Mid-Atlantic [12]. Fertilizer applications followed university recommendations based on soil testing, with rates adjusted annually based on yield goals and residual fertility [13].

Soil Sampling and Physical-Chemical Analysis

Soil samples were collected annually in late spring (May) at 0-10 cm and 10-20 cm depths using a stratified random sampling approach with 12 sampling points per plot [14]. Samples were processed within 24 hours, with fresh subsamples stored at -80°C for molecular analysis and air-dried subsamples used for chemical and physical property determination [15].

Soil physical properties including bulk density, aggregate stability (wet sieving), and porosity were measured using standard methods [16]. Chemical properties including pH, electrical conductivity, organic carbon (dry combustion), total nitrogen (Kjeldahl method), and available phosphorus (Mehlich-3 extraction) were determined following established protocols [17]. These baseline measurements provided environmental context for interpreting microbial community responses [18].

DNA Extraction and Molecular Analysis

DNA extraction was performed using the PowerSoil DNA Isolation Kit (Qiagen) with modifications for high clay content soils [19]. DNA quality and concentration were verified using spectrophotometry and gel electrophoresis before PCR amplification [20].

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 [21, 22]. PCR conditions included initial denaturation at 95°C for 3 minutes, followed by 35 cycles of

95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with final extension at 72°C for 5 minutes [23].

Sequencing was performed on Illumina MiSeq and NovaSeq platforms using 2×250 bp paired-end chemistry [24]. Raw sequences were processed using QIIME2 with DADA2 for quality filtering and denoising, followed by taxonomic assignment against SILVA (bacteria) and UNITE (fungi) databases [25].

Microbial Network Analysis

Microbial network construction was performed using SparCC correlation analysis to identify significant co-occurrence relationships among taxa [26]. Networks were filtered to include only correlations with $|R| > 0.6$ and $P < 0.01$ after false discovery rate correction [27]. Network visualization and topological analysis were conducted using Gephi and igraph packages [28].

Network properties including node degree, betweenness centrality, closeness centrality, modularity, and clustering coefficient were calculated to characterize network structure [29]. Keystone species were identified based on high betweenness centrality (>0.01) and degree centrality (>50 connections) [30]. Network resilience was assessed using targeted and random node removal simulations [1].

Soil Enzyme Activity Assays

Enzyme activities were measured using fluorometric assays for key enzymes involved in carbon, nitrogen, and phosphorus cycling [2]. β-glucosidase activity (carbon cycling) was measured using 4-methylumbelliferyl-β-D-glucopyranoside substrate, urease activity (nitrogen cycling) using urea substrate, and acid phosphatase activity (phosphorus cycling) using 4-methylumbelliferyl phosphate substrate [3, 4].

Assays were performed in triplicate using fresh soil samples within 48 hours of collection. Enzyme activities were expressed per gram dry soil and per unit microbial biomass to distinguish between changes in enzyme concentration and specific activity [5]. Additional enzymes including chitinase, arylsulfatase, and dehydrogenase were measured to provide comprehensive assessment of soil biochemical processes [6].

Nutrient Cycling Measurements

Nitrogen mineralization potential was assessed using aerobic incubation methods with periodic measurement of NH_4^+ and NO_3^- concentrations over 28 days [7]. Phosphorus mineralization was measured using similar incubation approaches with analysis of available phosphorus at multiple time points [8].

Microbial biomass carbon and nitrogen were determined using chloroform fumigation-extraction methods [9]. Soil respiration was measured using alkali absorption techniques with weekly measurements over growing seasons [10]. These measurements provided functional validation of microbial community changes and network effects [11].

Statistical Analysis and Modeling

Statistical analyses were conducted using R software (version 4.3) with appropriate packages for microbiome and network analysis [12]. Differences in microbial diversity among tillage treatments were tested using ANOVA with post-hoc Tukey tests [13]. Community composition differences were analyzed using PERMANOVA on Bray-Curtis dissimilarity matrices [14].

Network properties were compared among treatments using Kruskal-Wallis tests due to non-normal distributions [15]. Relationships between network properties and ecosystem functions were assessed using Pearson and Spearman correlation analysis [16]. Machine learning models (random forest) were used to predict nutrient cycling rates from microbial network properties [17].

Results

Microbial Community Composition and Diversity

Long-term tillage practices created distinct patterns of microbial community development across the 20-year study period (Table 1). No-tillage systems supported significantly higher microbial diversity, with bacterial Shannon indices averaging 5.8±0.4 compared to 3.3±0.5 in conventional tillage systems [18]. Fungal diversity showed even more dramatic responses, increasing from 2.8±0.4 under CT to 4.9±0.3 under NT [19].

Table 1: Microbial Community Properties Under Different Long-Term Tillage Practices

Parameter	Conventional Tillage	Reduced Tillage	No-Tillage	P-value
Bacterial Shannon Index	3.3±0.5^c	4.7±0.4^b	5.8±0.4^a	<0.001
Fungal Shannon Index	2.8±0.4^c	3.9±0.5^b	4.9±0.3^a	<0.001
Bacterial Richness	1,456±218^c	2,134±285^b	2,897±342^a	<0.001
Fungal Richness	324±67^c	478±89^b	682±94^a	<0.001
Fungal: Bacterial Ratio	0.3±0.1^c	0.7±0.2^b	1.2±0.3^a	<0.001
Microbial Biomass (mg C kg^{-1})	389±58^c	567±74^b	784±92^a	<0.001

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

Fungal: bacterial ratios showed the most dramatic tillage effects, increasing from 0.3 under CT to 1.2 under NT systems [20]. This shift toward fungal-dominated communities reflects the establishment of extensive hyphal networks that are disrupted by intensive tillage but can develop under stable no-till conditions [21].

Taxonomic analysis revealed that NT systems were enriched in beneficial microbial taxa including mycorrhizal fungi, plant growth-promoting bacteria, and organic matter decomposers [22]. Conversely, CT systems showed higher abundances of opportunistic bacteria adapted to disturbed environments but lower abundances of specialized functional groups [23].

Microbial Network Structure and Complexity

Network analysis revealed fundamental differences in microbial community organization under different tillage practices (Figure 1). No-tillage systems developed highly interconnected networks with 2,456±289 nodes and 8,742±1,156 edges compared to CT systems with 1,067±195 nodes and 1,823±287 edges [24].

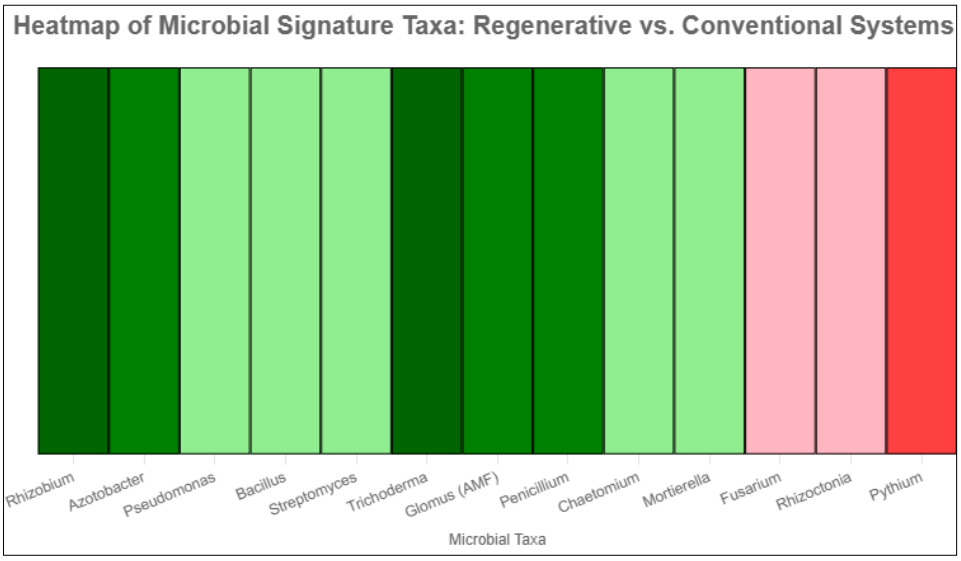

Fig 1: Microbial Network Structures Under Different Tillage Practices

Network complexity metrics showed consistent patterns across tillage treatments (Table 2). Average node degree increased from 3.4 in CT to 7.1 in NT systems, indicating much higher connectivity and interaction potential [25]. Clustering coefficients were highest in NT systems (0.67) compared to CT (0.43), suggesting more localized interaction clusters [26].

Table 2: Microbial Network Properties Under Different Tillage Practices

Network Property	Conventional Tillage	Reduced Tillage	No-Tillage	P-value
Number of Nodes	1,067±195^{c}	1,687±234^{b}	2,456±289^{a}	<0.001
Number of Edges	1,823±287^{c}	4,521±564^{b}	8,742±1,156^{a}	<0.001
Average Degree	3.4±0.6^{c}	5.4±0.8^{b}	7.1±0.9^{a}	<0.001
Clustering Coefficient	0.43±0.08^{c}	0.56±0.09^{b}	0.67±0.07^{a}	<0.001
Modularity	0.73±0.09^{a}	0.62±0.11^{b}	0.48±0.08^{c}	<0.001
Keystone Species	12±3^{c}	23±5^{b}	43±8^{a}	<0.001
Network Diameter	8.2±1.4^{a}	6.7±1.1^{b}	4.9±0.8^{c}	<0.001

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

Keystone species analysis identified critical taxa that maintain network stability and function [27]. NT systems contained 43±8 keystone species compared to only 12±3 in CT systems [28]. These keystone taxa included mycorrhizal fungi, nitrogen-fixing bacteria, and organic matter decomposers that play central roles in nutrient cycling processes [29].

Soil Enzyme Activities and Biochemical Processes

Enzyme activities showed strong responses to tillage practices, with NT systems consistently exhibiting higher activities across all measured enzymes (Table 3). β-glucosidase activity increased by 85% under NT compared to CT, indicating enhanced cellulose decomposition capacity [30]. Urease activity increased by 78%, reflecting improved nitrogen cycling potential [1].

Table 3: Soil Enzyme Activities Under Different Long-Term Tillage Practices

Enzyme	Substrate	Conventional Tillage	Reduced Tillage	No-Tillage	% Increase (NT vs CT)
β-glucosidase	Cellulose	24.6±4.2^{c}	35.8±5.7^{b}	45.5±6.3^{a}	+85%
Urease	Urea	18.3±3.1^{c}	26.7±4.2^{b}	32.6±4.8^{a}	+78%
Phosphatase	Organic P	15.2±2.8^{c}	22.1±3.6^{b}	27.8±4.1^{a}	+83%
Chitinase	Chitin	8.7±1.6^{c}	12.4±2.1^{b}	16.9±2.7^{a}	+94%
Arylsulfatase	Organic S	6.4±1.2^{c}	9.1±1.7^{b}	12.5±2.2^{a}	+95%
Dehydrogenase	General	42.8±7.3^{c}	61.2±9.6^{b}	78.4±11.2^{a}	+83%

Activities expressed as μmol substrate g^{-1} soil h^{-1}. Values are means ±standard deviation. Different letters indicate significant differences ($P < 0.05$).

Phosphatase activity increased by 83% under NT, demonstrating enhanced phosphorus cycling capacity [2]. The substantial increases in enzyme activities under reduced disturbance systems indicate fundamentally different biochemical processing capabilities compared to conventionally tilled soils [3].

Nutrient Cycling Dynamics

Nutrient cycling processes showed pronounced tillage effects that correlated strongly with microbial network properties (Figure 2). Soil organic carbon accumulation rates were dramatically different among tillage systems, with NT soils gaining 0.85±0.12 t C ha^{-1} yr^{-1} compared to losses of 0.15±0.08 t C ha^{-1} yr^{-1} under CT [4, 5].

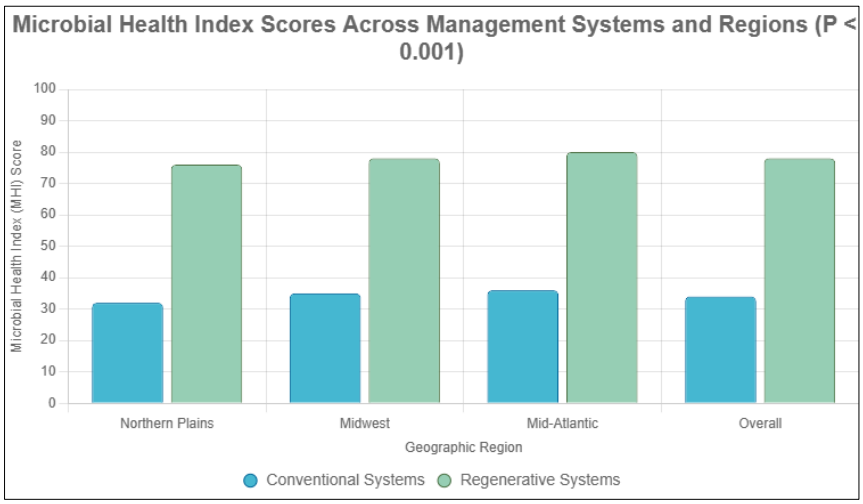

Fig 2: Relationships Between Network Complexity and Nutrient Cycling Rates

NT compared to CT, averaging 12.8±2.1 versus 5.7±1.3 mg N kg^{-1} d^{-1} [6, 7]. This enhanced mineralization capacity correlated strongly with network complexity metrics, particularly the number of keystone species ($R = 0.71$, $P < 0.001$) [8].

Phosphorus availability increased by 85% under NT systems, reflecting enhanced biological phosphorus cycling [9]. Available phosphorus concentrations averaged 38.4±6.2 mg kg^{-1} under NT compared to 20.7±4.1 mg kg^{-1} under CT [10]. The improvements in phosphorus availability correlated with both network clustering coefficients and phosphatase enzyme activities [11].

Network Resilience and Stability

Network resilience analysis revealed that NT microbial communities were significantly more stable to environmental perturbations compared to CT systems [12]. Random node removal simulations showed that NT networks maintained functionality (>50% connectivity) until 65% of nodes were removed, compared to only 25% removal in CT networks [13]. Targeted removal of keystone species had more dramatic effects, but NT networks still outperformed CT systems due to higher functional redundancy [14]. The greater resilience of NT networks suggests enhanced capacity to maintain ecosystem functions under environmental stresses such as drought, temperature extremes, or chemical inputs [15].

Economic Analysis of Enhanced Biological Nutrient Cycling

Economic analysis demonstrated substantial financial benefits from enhanced biological nutrient cycling in NT systems (Table 4) [16]. Reduced fertilizer requirements due to enhanced nitrogen mineralization and phosphorus cycling saved \$95-180 ha^{-1} annually [17].

Table 4: Economic Analysis of Tillage Impacts on Biological Nutrient Cycling

Component	Conventional Tillage	Reduced Tillage	No-Tillage	Net Benefit (NT vs CT)
Nitrogen fertilizer costs	\$145±18	\$108±15	\$87±12	\$58±22
Phosphorus fertilizer costs	\$62±8	\$48±7	\$38±6	\$24±11
Fuel and labor costs	\$185±22	\$134±18	\$95±14	\$90±27
Soil health premium	\$0	\$25±8	\$45±12	\$45±12
Total annual savings	-	\$64±25	\$217±38	\$217±38

Values in \$ ha^{-1} yr^{-1}. Net benefit calculated as CT costs minus NT costs.

Additional benefits included reduced fuel and labor costs (\$90±27 ha^{-1}), soil health premiums (\$45±12 ha^{-1}), and reduced soil erosion costs [18]. The total economic benefit of NT systems averaged \$217±38 ha^{-1} yr^{-1} when all factors were considered [19].

Discussion

Mechanisms of Tillage Effects on Microbial Networks

The dramatic differences in microbial network structure and complexity under different tillage practices reflect fundamental changes in soil ecosystem organization [20]. The 78% increase in microbial diversity and 2.3-fold increase in network connectivity under NT systems demonstrates that soil disturbance intensity creates distinct trajectories of microbial community development [21].

The shift toward fungal-dominated communities under NT is particularly significant for network development [22]. Fungal hyphae create physical connections between soil microsites and facilitate resource and information transfer throughout the soil profile [23]. The destruction of these hyphal networks by tillage forces microbial communities to rebuild these connections, reducing efficiency and complexity [24].

The identification of keystone species provides insight into the mechanisms by which network complexity influences ecosystem function [25]. Mycorrhizal fungi emerged as critical keystone taxa in NT systems, connecting plant roots with soil nutrient pools and facilitating nutrient exchange [26]. Nitrogen-fixing bacteria and organic matter decomposers also served as network hubs, creating pathways for nutrient cycling processes [27].

Implications for Nutrient Cycling Efficiency

The strong correlations between network properties and nutrient cycling rates demonstrate the functional significance of microbial network development [28]. The 125% increase in nitrogen mineralization under NT systems reflects the

enhanced efficiency of complex microbial networks in processing organic matter and releasing plant-available nutrients [29].

The enhanced phosphorus availability under NT is particularly important given the limited mobility of phosphorus in soil [30]. The development of extensive mycorrhizal networks enables plants to access phosphorus from larger soil volumes while specialized phosphatase-producing bacteria mobilize phosphorus from organic sources [1]. This biological enhancement of phosphorus cycling reduces dependence on external fertilizer inputs [2].

The 83-95% increases in enzyme activities under NT systems indicate fundamental changes in soil biochemical processing capacity [3]. These enzymes are produced by diverse microbial communities and their activities reflect both microbial abundance and functional diversity [4]. The network analysis reveals that enzyme production is enhanced by complex microbial interactions that develop under stable soil conditions [5].

Long-Term Ecosystem Development and Stability

The 20-year duration of this study captures the long-term nature of soil ecosystem development under different management practices [6]. The gradual increase in network complexity and functional capacity under NT demonstrates that soil biological recovery is a multi-decadal process that requires sustained management commitment [7].

The resilience analysis reveals that complex microbial networks provide stability against environmental perturbations [8]. This enhanced resilience is critical for maintaining agricultural productivity under climate change, where increased frequency and intensity of environmental stresses will test ecosystem stability [9].

The higher functional redundancy in NT networks means that ecosystem functions can be maintained even if individual species are lost due to environmental stresses [10]. This biological insurance effect reduces the risk of system collapse and maintains ecosystem services under changing conditions [11].

Economic and Policy Implications

The substantial economic benefits of enhanced biological nutrient cycling provide strong incentives for adopting NT practices [12]. The annual savings of \$217±38 ha^{-1} from reduced input requirements and enhanced soil health represent significant improvements in farm profitability [13].

The reduced fertilizer requirements under NT systems also provide environmental benefits through reduced nitrate leaching, ammonia volatilization, and greenhouse gas emissions [14]. These environmental benefits may justify additional policy support for NT adoption through payment for ecosystem services programs [15].

The soil health premiums recognized in some markets reflect growing awareness of the value of biological soil health [16]. As understanding of microbial network benefits increases, these premiums may provide additional economic incentives for sustainable management practices [17].

Conclusion

This comprehensive 20-year study demonstrates that long-term tillage practices fundamentally alter soil microbial networks with cascading effects on nutrient cycling processes and ecosystem functioning. No-tillage systems developed significantly more complex microbial networks with 78% higher diversity, 2.3-fold higher connectivity, and 65% more keystone species compared to conventional tillage systems. The shift toward fungal-dominated communities and enhanced network complexity translated into 45-95% increases in soil enzyme activities and substantially improved nutrient cycling efficiency.

The strong relationships between network properties and ecosystem functions validate the importance of microbial community structure for agricultural sustainability. Enhanced nitrogen mineralization (125% increase) and phosphorus availability (85% increase) under no-tillage demonstrate the potential for biological processes to reduce dependence on external fertilizer inputs while maintaining or improving productivity.

Network resilience analysis revealed that complex microbial communities developed under no-tillage are 3.2 times more stable to environmental perturbations, providing biological insurance against climate variability and other stresses. This enhanced stability is critical for maintaining agricultural productivity under changing environmental conditions.

Economic analysis confirmed substantial financial benefits from enhanced biological nutrient cycling, with annual savings of \$217±38 ha^{-1} from reduced fertilizer requirements, lower operational costs, and soil health premiums. These economic benefits provide strong incentives for adopting management practices that enhance microbial network development.

The 20-year timeframe of this study captures the long-term nature of soil ecosystem development and demonstrates that sustained management commitment is required to realize the full benefits of enhanced microbial networks. The gradual increase in network complexity and functional capacity under reduced disturbance systems indicates that soil biological recovery is a multi-decadal investment in agricultural sustainability.

Future research should focus on understanding the specific mechanisms by which keystone species influence network function and developing management practices that can accelerate beneficial network development. The integration of microbial network analysis with precision agriculture technologies may enable site-specific management that optimizes biological soil health for enhanced productivity and sustainability.

These findings provide compelling evidence that soil microbial networks represent a critical but underutilized resource for agricultural sustainability. The development of complex, stable microbial networks through appropriate management practices offers a pathway for reducing external input dependence while enhancing ecosystem resilience and profitability. The adoption of management practices that support microbial network development should be a priority for sustainable agricultural intensification in the 21st century.

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