Long-Term Assessment of Natural Farming Systems on Nutrient Cycling and Soil Enzyme Activity: Implications for Soil Health, Microbial Dynamics, and Sustainable Crop Productivity

Dr. Lucas Ferreira da Silva [1*], **Dr. Jonathan P Reed** [2]
Department of Agroecology, Federal Rural University of Pernambuco, Brazil
Institute for Sustainable Agriculture, University of Florida, Gainesville, USA

* Corresponding Author: **Dr. Lucas Ferreira da Silva**

Article Info

E-ISSN: 3107-7145
Volume: 02
Issue: 01
Received: 17-11-2025
Accepted: 19-12-2025
Published: 21-01-2026
Page No: 28-37

Abstract

Natural farming systems have gained global recognition as sustainable alternatives to conventional agriculture, yet long-term empirical evidence on their effects on soil biological processes remains limited. This review synthesizes findings from long-term field experiments and observational studies examining the impacts of natural farming practices—including Zero Budget Natural Farming (ZBNF), cow-based formulations (Jeevamrut, Beejamrut), botanical extracts, and indigenous microbial consortia—on nutrient cycling dynamics and soil enzyme activities. The 42-year DOK trial in Switzerland demonstrates that organic management practices enrich functional genes involved in organic phosphorus acquisition, nitrate transformation, and organic matter degradation, fundamentally altering soil metabolic potential. Long-term studies from Vertisols in central India reveal that organic farming practices achieve the highest soil organic carbon concentrations while significantly enhancing enzymatic indices including dehydrogenase, urease, and phosphatase activities compared to conventional systems. Research from the 19-year pearl millet-wheat experiment at Hisar, India, shows that organic amendments produce microbial biomass carbon ranging from 202–491 mg/kg and dehydrogenase activity reaching 63.7 µg TPF/g/24h, with pressmud demonstrating superior impacts on soil microbial properties. The 10-year Danish trials on perennial cropping systems document that deep-rooted perennials enhance nutrient cycling through increased hydrolytic enzyme activity while suppressing oxidative enzymes, thereby stabilizing soil organic carbon. Biodynamic and organic soils consistently exhibit higher microbial counts and enzyme activities compared to conventional soils, with spent mushroom substrate amendments further amplifying these biological indicators. This review establishes that soil enzymes—dehydrogenase, urease, phosphatase, β-glucosidase, and catalase—serve as sensitive early indicators of soil health restoration under natural farming. The geometric mean of enzyme activities and biological activity index emerge as robust integrative metrics for assessing agroecosystem sustainability. Long-term natural farming systems demonstrate enhanced nutrient cycling efficiency, improved soil microbial biomass, and greater soil resilience despite occasionally lower available nutrient concentrations compared to conventional systems. These findings have profound implications for global agroecological transitions, carbon sequestration strategies, and climate-resilient agriculture. Future research priorities include standardizing enzyme assay protocols, establishing baseline values across agroecological zones, and integrating enzyme indicators into soil health monitoring frameworks.

Keywords: Natural farming; nutrient cycling; soil enzyme activity; soil microbial biomass; sustainable agriculture; agroecology; soil health indicators

1. Introduction

Natural farming systems, encompassing Zero Budget Natural Farming (ZBNF), indigenous natural practices, and organic-input-based agriculture, have emerged as transformative approaches for addressing the dual challenges of soil degradation and climate change. These systems fundamentally differ from conventional agriculture in their reliance on on-farm biological inputs, cow-based formulations, botanical extracts, and indigenous microbial consortia rather than synthetic fertilizers and pesticides [6]. The Andhra Pradesh Community Managed Natural Farming program, launched in 2016, exemplifies this transition, with small and marginal farmers adopting practices that substitute chemical inputs with indigenous microbial consortia including Jeevamritha

Ghanajeevamritha, Beejamritha, and Panchagavya, complemented by intercropping and mulching [6].

Soil biological health constitutes the foundation of productive and resilient agricultural systems. Unlike chemical and physical properties that respond slowly to management changes, soil biological indicators provide early and sensitive measures of ecosystem function. Soil microorganisms drive essential processes including organic matter decomposition, nutrient mineralization, nitrogen fixation, and aggregate formation [1]. The metabolic potential of soil microbial communities—their collective capacity to carry out biogeochemical transformations—determines the efficiency of nutrient cycling and the availability of essential elements for plant uptake.

Nutrient cycling in natural farming systems operates through fundamentally different mechanisms than in conventional agriculture. Rather than relying on soluble synthetic fertilizers that supply nutrients directly to plants, natural farming emphasizes the activation of soil biological processes that release nutrients from organic reserves through mineralization. This approach depends on complex interactions among soil organic matter, microbial communities, and plant roots. The 42-year DOK trial comparing organic and conventional management systems revealed that manure fertilization is the main factor altering soil metabolic potential, with organic practices enriching functional genes involved in organic phosphorus acquisition, nitrate transformation, and organic degradation [1]. These findings demonstrate that long-term management shapes the fundamental genetic capacity of soils to cycle nutrients.

Soil enzymes serve as indispensable bio-indicators of soil health because they catalyze rate-limiting steps in nutrient cycling and reflect the metabolic activity of microbial communities. Dehydrogenase activity indicates overall microbial oxidative activity; urease governs nitrogen mineralization from organic urea; phosphatases mediate organic phosphorus mineralization; β-glucosidase participates in carbon cycling by hydrolyzing cellulose; and catalase protects cells from oxidative damage [2][3]. The geometric mean of enzyme activities and biological activity index integrate multiple enzyme responses into single metrics that correlate strongly with soil fertility and crop productivity [2].

Despite growing adoption of natural farming worldwide, critical gaps persist in long-term assessment of its effects on soil biological processes. Most studies focus on short-term responses or compare natural farming with conventional systems without adequate temporal replication. The 19-year pearl millet-wheat experiment at CCS Haryana Agricultural University provides valuable insights into how organic amendments influence microbial biomass carbon, microbial biomass nitrogen, soil microbial quotient, and dehydrogenase activity over nearly two decades [3]. Similarly, the 10-year Danish trial on perennial cropping systems documents shifts in enzyme balance and carbon stability that emerge only after extended periods [4].

The objective of this review is to synthesize evidence from long-term field experiments and observational studies on the effects of natural farming systems on nutrient cycling dynamics and soil enzyme activities. Specific aims include: (1) characterizing indigenous nutrient management practices and their effects on soil biological processes; (2) examining long-term trends in nitrogen, phosphorus, potassium, and carbon cycling under natural farming; (3) evaluating soil enzyme activities as indicators of soil health restoration; (4) comparing long-term outcomes between natural farming and other systems; and (5) identifying implications for sustainability and future research priorities. The scope encompasses peer-reviewed studies from diverse agroecological contexts, with emphasis on field experiments exceeding five years duration and employing rigorous methodological approaches.

2. Natural Farming Systems and Soil Biological Processes

2.1. Indigenous Nutrient Management Practices

Natural farming systems employ a diverse array of indigenous preparations that supply nutrients, enhance microbial activity, and protect crops through biological mechanisms. The Andhra Pradesh community-managed natural farming initiative has systematized the use of several key formulations derived from locally available materials [6].

Jeevamritha is a fermented microbial consortium prepared from cow dung, cow urine, jaggery, pulse flour, and soil from the farm bund. This liquid formulation serves as both a nutrient source and a microbial inoculant, containing diverse populations of heterotrophic bacteria, nitrogen-fixers, and phosphorus-solubilizing microorganisms. Regular application of Jeevamritha to soil activates indigenous microbes, accelerates organic matter decomposition, and enhances nutrient availability throughout the crop growing season [6]. Ghanajeevamritha represents the solid equivalent, prepared by fermenting similar ingredients with dry organic matter and applied as a basal soil amendment.

Beejamrut is specifically designed for seed treatment, protecting germinating seeds from soil-borne pathogens while providing initial microbial inoculum. Prepared from cow dung, cow urine, lime, and soil, this formulation coats seeds with beneficial microorganisms that colonize the developing rhizosphere [6]. Panchagavya, meaning "five products of the cow," combines cow dung, urine, milk, curd, and ghee with fermentation aids including jaggery and tender coconut water. This versatile preparation serves as both a soil amendment and foliar spray, supplying nutrients and growth-promoting substances.

Botanical extracts prepared from neem (Azadirachta indica), pungam (Pongamia pinnata), and other locally available plants complement microbial formulations by providing pest-repellent properties and additional organic inputs. These extracts are typically fermented before application, enhancing their nutrient content and microbial load [6].

On-farm microbial inoculants derived from forest soils or well-maintained farm soils serve as sources of diverse native microorganisms adapted to local conditions. The "forest as mother" concept recognizes that undisturbed ecosystems harbor microbial consortia that can be multiplied and introduced to agricultural soils to enhance biological diversity and function.

2.2. Soil Organic Matter Dynamics Under Natural Farming

Soil organic matter (SOM) dynamics under natural farming differ fundamentally from those in conventional systems due to continuous organic inputs and minimal soil disturbance. The 10-year Danish study on perennial cropping systems documented that replacing annual crops with deep-rooted tall fescue fundamentally reshaped soil biology [4]. In the topsoil

(0-20 cm), perennial grasses boosted the activity of nitrogen- and phosphorus-related hydrolytic enzymes—proteins produced by microbes that act like biological scissors to cut up complex organic matter and free nutrients. With biomass harvested three times annually for biorefineries, substantial nutrients were removed from fields. Faced with this nutrient shortage, microbes adapted by producing more enzymes to unlock nutrients directly from soil organic matter.

Simultaneously, the activity of oxidative enzymes that normally attack tough compounds such as lignin was suppressed under perennial systems. This subtle shift in enzyme balance created a double advantage: nutrients became more available to plants while the most resistant forms of carbon in the soil were left intact [4]. Lignin, a complex substance found in plant cell walls, decomposes slowly and plays a crucial role in soil carbon storage. When activity of lignin-decomposing enzymes is suppressed, more carbon remains in the ground for extended periods.

In deeper layers (20-50 cm), soils under tall fescue held more microbial biomass carbon—a clear sign that extensive root systems were feeding microbial life far belowground. Analyses revealed a tendency toward greater amounts of mineral-associated organic carbon, the type that binds tightly to soil particles and can remain stable for decades or centuries [4]. Using advanced infrared spectroscopy, the research team documented clear shifts in chemical forms of soil organic carbon: carbon became more stable in topsoil, while deep roots appeared to feed and protect long-lasting carbon pools in subsoil.

Rhizosphere interactions in natural farming systems amplify these organic matter dynamics. Plant roots release exudates that recruit specific microbial communities, which in turn enhance nutrient availability and promote aggregate formation. The resulting soil structure physically protects organic matter from decomposition while facilitating water infiltration and root penetration.

3. Long-Term Effects on Nutrient Cycling

3.1. Nitrogen Dynamics

Nitrogen cycling in natural farming systems operates through biological pathways fundamentally distinct from the synthetic nitrogen inputs characteristic of conventional agriculture. The DOK trial's metagenomic analysis revealed that organic management practices enrich functional genes involved in nitrate transformation and organic nitrogen acquisition [1]. These genetic adaptations translate into altered nitrogen cycling processes including biological nitrogen fixation, mineralization-immobilization turnover, and nitrification.

Biological nitrogen fixation by free-living and symbiotic bacteria assumes primary importance in natural farming systems where synthetic nitrogen fertilizers are absent. Indigenous microbial consortia including Jeevamritha contain diverse diazotrophs that contribute fixed nitrogen to the soil-plant system [6]. Leguminous crops integrated into rotations or intercropping systems enhance nitrogen inputs through rhizobial symbioses, with residual nitrogen benefiting subsequent crops.

Mineralization and immobilization dynamics determine the availability of nitrogen from organic sources. Soil microorganisms decompose organic amendments, releasing ammonium through ammonification. This ammonium may be taken up by plants, immobilized into microbial biomass, or converted to nitrate through nitrification. The balance between mineralization and immobilization depends on the carbon-to-nitrogen ratio of organic inputs and the activity of microbial communities. The 42-year DOK trial demonstrated that organic management maintains higher rates of organic nitrogen mineralization compared to conventional systems, sustaining crop nitrogen supply without synthetic inputs [1].

Ammonification and nitrification rates under natural farming reflect the activity of specific microbial groups and enzymes. Urease catalyzes the hydrolysis of urea from organic amendments to ammonia and carbon dioxide. Long-term organic amendments in the Hisar experiment produced urease activity reaching 97.6 μg $NH4^{+}$-N/g/h under pressmud application, attributed to higher nitrogen content in this amendment [3]. Interestingly, dehydrogenase and urease activity decreased where organic manures were applied in conjunction with NP fertilizers compared to solitary organic manure application, suggesting that synthetic nitrogen suppresses biological nitrogen cycling processes [3].

3.2. Phosphorus Cycling

Phosphorus dynamics in natural farming systems emphasize organic phosphorus mineralization through phosphatase enzymes rather than supply of soluble inorganic phosphates. Alkaline and acid phosphatases produced by soil microorganisms and plant roots hydrolyze organic phosphorus compounds, releasing phosphate ions for plant uptake. The DOK trial's metagenomic analysis revealed significant enrichment of genes involved in organic phosphorus acquisition under organic management [1].

Phosphatase activity responses to natural farming inputs are well-documented across long-term experiments. In the 19-year Hisar study, alkaline phosphatase activity among organic manures applied alone followed the order: poultry manure > farmyard manure > pressmud [3]. However, when organic manures were combined with NP fertilizers, alkaline phosphatase activity increased compared to solitary organic applications—a contrasting pattern to that observed for dehydrogenase and urease. This suggests differential regulation of phosphorus-acquiring enzymes compared to those involved in carbon and nitrogen cycling.

The study on Vertisols in central India documented significantly higher phosphatase activities under organic farming and natural preparation treatments compared to conventional systems [2]. These enzymatic responses correlated strongly with available phosphorus concentrations, confirming that phosphatase-mediated mineralization contributes meaningfully to crop phosphorus supply. Importantly, organic management maintained phosphorus availability despite absence of soluble phosphate fertilizers, demonstrating the efficacy of biological phosphorus cycling.

Organic phosphorus mineralization depends on the quality and quantity of organic inputs. Plant residues, manures, and microbial inoculants supply organic phosphorus compounds that serve as substrates for phosphatases. The continuous application of organic amendments builds soil organic phosphorus pools that sustain mineralization over extended periods.

3.3. Potassium and Micronutrient Dynamics

Potassium cycling in natural farming systems operates through different mechanisms than nitrogen and phosphorus, reflecting potassium's occurrence as a cation rather than component of organic compounds. Soil potassium exists in soluble, exchangeable, non-exchangeable, and mineral forms, with plant availability governed by equilibrium among these pools. Organic matter influences potassium availability primarily through effects on cation exchange capacity and microbial mobilization.

The survey of ZBNF and conventional farming systems in Solan District, Himachal Pradesh, revealed that conventional farming recorded higher available potassium (10.27%) compared to ZBNF [7]. This difference reflects the continuous application of potassium-containing synthetic fertilizers in conventional systems versus reliance on organic cycling in natural farming. However, the ZBNF system recorded 22.85% higher organic carbon compared to conventional farming [7], indicating that while immediately available potassium may be lower, the capacity to retain and slowly release potassium through cation exchange is enhanced.

Micronutrient dynamics under natural farming reflect complex interactions among organic matter complexation, microbial mobilization, and plant uptake. The Solan District study found higher leaf micronutrients under conventional farming compared to ZBNF [7], again indicating slower release from organic sources. However, the study on Vertisols in central India showed that integrated crop management with natural pest control maintained adequate micronutrient availability [2]. Long-term organic amendments build soil organic matter that chelates micronutrients, reducing leaching losses while maintaining plant availability.

Microbial mechanisms of micronutrient mobilization include production of organic acids, siderophores, and enzymes that solubilize micronutrients from mineral sources and organic complexes. Indigenous microbial consortia in natural farming systems include diverse organisms capable of these transformations, contributing to micronutrient supply.

3.4. Soil Organic Carbon Turnover

Soil organic carbon (SOC) turnover in natural farming systems represents the balance between carbon inputs from organic amendments and crop residues versus losses through decomposition and erosion. Long-term experiments consistently demonstrate higher SOC concentrations under organic management compared to conventional systems. The Solan District study documented 22.85% higher organic carbon under ZBNF compared to conventional farming [7]. The Vertisol study similarly found that organic farming practice achieved the highest soil organic carbon among all treatments [2].

The 10-year Danish perennial cropping trial provided mechanistic insights into how perennial root systems alter SOC dynamics [4]. Deep roots of tall fescue stimulated microbial life in subsoil while simultaneously protecting carbon through suppression of oxidative enzymes. The shift toward mineral-associated organic carbon in deeper layers indicates formation of stable carbon pools with long mean residence times. Advanced infrared spectroscopy documented clear shifts in chemical forms of soil organic carbon under perennial systems, with topsoil carbon becoming more stable over time.

Carbon sequestration potential of natural farming systems depends on management practices including residue retention, organic amendment application, and reduced tillage. The Kenyan smallholder study modeled SOC trajectories under alternative management scenarios, finding that erosion control, residue retention, and incorporation of high biomass legumes (lablab) resulted in at least 50% more SOC than current trends or residue export scenarios after 50 modeled years [8]. Combining these interventions showed promise for stabilizing SOC trajectories over multi-decadal horizons.

The relationship between SOC and nutrient cycling operates through multiple mechanisms. Soil organic matter serves as both source and sink for nutrients, releasing them through mineralization while retaining them through cation exchange and complexation. Higher SOC under natural farming thus supports more efficient nutrient cycling while contributing to climate change mitigation through carbon sequestration.

4. Soil Enzyme Activity as Indicators of Soil Health

4.1. Dehydrogenase Activity

Dehydrogenase enzymes catalyze oxidation-reduction reactions in soil microorganisms, transferring electrons from organic substrates to electron acceptors. Because dehydrogenases occur only within living cells, their activity serves as a direct measure of overall microbial oxidative activity and metabolic state. Dehydrogenase activity thus integrates the physiological status of the entire microbial community.

Long-term natural farming consistently elevates dehydrogenase activity compared to conventional systems. In the 19-year Hisar experiment, dehydrogenase activity reached 63.7 µg TPF/g/24h under farmyard manure application at 15 t/ha, statistically comparable to farmyard manure with nitrogen fertilizer and pressmud at 7.5 t/ha [3]. These elevated activities indicate vigorous microbial metabolism sustained by continuous organic inputs.

The Vertisol study documented significantly higher dehydrogenase activity under organic farming and natural preparation treatments compared to control and integrated crop management with chemical pesticides [2]. Dehydrogenase responded sensitively to management differences, distinguishing among treatments with similar nutrient availability but different biological inputs. This sensitivity recommends dehydrogenase as an early indicator of soil health changes following transition to natural farming.

Temporal dynamics of dehydrogenase activity reflect seasonal patterns of temperature, moisture, and organic matter availability. The Slovenian study comparing farming systems found that biodynamic soil maintained stable enzyme activities throughout the year, indicating greater biological stability compared to conventional soils that fluctuated seasonally [5]. This stability suggests that diverse microbial communities in natural farming systems buffer against environmental variation.

4.2. Urease Activity

Urease catalyzes the hydrolysis of urea to ammonia and carbon dioxide, the rate-limiting step in nitrogen mineralization from organic urea-containing compounds. This enzyme plays a critical role in nitrogen cycling by converting organic nitrogen to plant-available ammonium. Urease activity reflects both the potential for nitrogen mineralization from recent organic inputs and the historical accumulation of stabilized organic nitrogen.

The 19-year Hisar experiment documented urease activity reaching 97.6 µg $NH4^+$-N/g/h under pressmud application at 7.5 t/ha [3]. This elevated activity corresponded to the higher nitrogen content (3.23%) in pressmud compared to other organic amendments. Importantly, urease activity decreased where organic manures were applied in conjunction with NP fertilizers compared to solitary organic manure application [3], suggesting that synthetic nitrogen suppresses biological nitrogen cycling enzymes.

Research on spent mushroom substrate amendments demonstrated significant increases in urease activity following organic fertilizer application, with responses varying by farming system [5]. Biodynamic and organic soils showed greater urease responses to organic amendments compared to conventional soils, indicating higher biological capacity to utilize added organic substrates. The geometric mean of enzyme activities, including urease, correlated strongly with soil organic carbon and microbial biomass.

Urease activity in natural farming systems supports efficient nitrogen cycling from cow-based formulations including Jeevamrut and Panchagavya, which contain urea from cow urine. The rapid hydrolysis of this urea by soil urease releases ammonium that can be taken up directly by plants or further transformed through nitrification. Synchrony between urea hydrolysis and plant nitrogen demand depends on timing of application and environmental conditions.

4.3. Phosphatase Activity

Phosphatases hydrolyze organic phosphorus compounds, releasing inorganic phosphate for plant uptake. Acid phosphatases predominate in acid soils while alkaline phosphatases dominate in alkaline and calcareous soils. Together, these enzymes mediate the transformation of organic phosphorus—often comprising 30-80% of total soil phosphorus—into plant-available forms.

The DOK trial's metagenomic analysis revealed significant enrichment of genes involved in organic phosphorus acquisition under organic management [1]. This genetic capacity translates into elevated phosphatase activities documented across long-term experiments. The 19-year Hisar study found that alkaline phosphatase activity among organic manures applied alone followed the order: poultry manure > farmyard manure > pressmud [3]. Interestingly, combining organic manures with NP fertilizers increased alkaline phosphatase activity compared to solitary organic applications, suggesting that phosphorus limitation may stimulate phosphatase production.

The Vertisol study documented significantly higher phosphatase activities under organic farming and natural preparation treatments [2]. These enzymatic responses correlated strongly with available phosphorus concentrations, confirming that phosphatase-mediated mineralization contributes meaningfully to crop phosphorus supply. Enzymatic indices including biological activity index and geometric mean of enzyme activities were greater in organic farming and natural preparation compared to control, with phosphatase contributing importantly to these integrative metrics.

Research on continuous cowpea cropping demonstrated that combining organic fertilizer with soil conditioner significantly enhanced phosphatase activity while reducing urease activity [9]. This differential response reflects the specific constraints of continuous cropping systems, where phosphorus availability often limits productivity while excess nitrogen may accumulate. Phosphatase responses to organic amendments thus indicate targeted alleviation of phosphorus limitation.

4.4. β-Glucosidase Activity

β-glucosidase catalyzes the hydrolysis of cellobiose to glucose, the final step in cellulose decomposition. This enzyme plays a critical role in carbon cycling by completing the breakdown of plant cell wall polysaccharides and releasing glucose that fuels microbial metabolism. β-glucosidase activity thus indicates the potential for organic matter decomposition and carbon mineralization.

Research comparing farming systems documented significant effects of management on β-glucosidase activity, with biodynamic soils showing highest activities followed by organic and conventional soils [5]. Spent mushroom substrate amendments significantly increased β-glucosidase activity, especially in biodynamic and organic soils, demonstrating that these systems possess greater capacity to respond to organic inputs. Seasonal variations affected β-glucosidase in all soils, but biodynamic soils maintained more stable activity throughout the year.

The 10-year Danish perennial cropping trial revealed nuanced effects on glycoside hydrolases involved in carbon cycling [4]. While nitrogen- and phosphorus-related hydrolytic enzymes increased under perennial grasses, the balance among different carbon-acquiring enzymes shifted. These changes in enzyme profiles indicate altered carbon cycling pathways that favor nutrient acquisition while protecting stable carbon pools.

β-glucosidase responses to natural farming inputs reflect the quality and quantity of organic materials applied. Cellulose-rich residues including crop stover and straw stimulate β-glucosidase production as microorganisms seek to access carbon substrates. Over long periods, sustained β-glucosidase activity maintains rapid carbon cycling that supports microbial biomass and nutrient mineralization.

4.5. Catalase Activity

Catalase decomposes hydrogen peroxide, a toxic byproduct of aerobic metabolism, to water and oxygen. This enzyme protects microbial cells from oxidative damage and indicates the overall intensity of aerobic biological activity. Catalase activity thus serves as a complementary indicator to dehydrogenase, reflecting both microbial abundance and metabolic status.

The Vertisol study included catalase among the suite of enzymes elevated under organic farming and natural preparation treatments [2]. Catalase responses paralleled those of dehydrogenase, urease, and phosphatase, confirming that natural farming enhances overall biological activity rather than specific functions in isolation. The geometric mean of enzyme activities, incorporating catalase with other enzymes, provided robust discrimination among treatments.

Catalase activity in natural farming systems supports healthy microbial communities capable of sustained metabolic activity. By protecting against oxidative stress, catalase enables microorganisms to maintain function under variable environmental conditions including wet-dry cycles and temperature fluctuations. This protective function contributes to the biological stability observed in biodynamic and organic soils [5].

The relationship between catalase and soil organic matter reflects the physical protection of microbial cells within aggregates and organic matrices. Soils with higher organic carbon typically exhibit greater catalase activity, indicating larger and more active microbial communities. The 22.85% higher organic carbon under ZBNF documented in Solan District [7] would thus predict correspondingly higher catalase activity, though direct measurements were not reported in this study.

5. Comparative Long-Term Outcomes: Natural Farming vs Other Systems

Comparative assessment of natural farming against conventional and integrated systems reveals consistent patterns in biological soil health indicators despite variability in nutrient availability and crop yields. The Vertisol study in central India provides direct comparison among control, natural preparation, organic farming, integrated crop management with natural pest control, and integrated crop management with chemical pesticides [2]. Organic farming achieved the lowest soil pH and electrical conductivity while attaining the highest soil organic carbon. Enzymatic indices, including biological activity index and geometric mean of enzyme activities, were significantly greater under organic farming and natural preparation compared to control and integrated crop management with chemical pesticides.

The DOK trial's metagenomic analysis revealed fundamental differences in metabolic potential between organic and conventional systems [1]. Manure fertilization emerged as the main factor altering soil metabolic potential, but organic management practices—including omission of synthetic pesticides and mineral fertilization—induced additional changes such as enriching functional genes involved in organic phosphorus acquisition, nitrate transformation, organic degradation, and non-hydrolytic carbohydrate cleavage. Conventional systems receiving mineral fertilization and chemical plant protection enriched genes associated with inorganic nutrient acquisition and transcriptional activity. These genetic differences indicate that cropping systems shape the fundamental capacity of soils to cycle nutrients and regulate ecosystem processes.

The Slovenian study comparing conventional-integrated, organic, and biodynamic farming found that biodynamic soil showed the highest microbial counts and enzyme activities, followed by organic and conventional soils [5]. Spent mushroom substrate significantly increased microbial numbers and enzyme activities, especially in biodynamic and organic soils, demonstrating that these systems possess greater capacity to benefit from organic inputs. Seasonal variations affected all microorganisms and most enzymes in all soils, but biodynamic soil maintained stable activity throughout the year, indicating greater biological resilience.

Nutrient use efficiency under natural farming operates through different mechanisms than in conventional systems. While conventional farming in Solan District recorded higher available nitrogen, phosphorus, and potassium (5.21%, 14.69%, and 10.27% higher respectively) compared to ZBNF [7], these differences reflect continuous synthetic fertilizer inputs rather than inherent system efficiency. The ZBNF system achieved 22.85% higher organic carbon and substantially higher microbial counts (45.72×10^5 cfu/g bacteria, 6.73×10^3 cfu/g fungi, and 9.28×10^3 cfu/g actinomycetes) compared to conventional farming [7], indicating greater biological capacity for nutrient cycling from organic sources.

Soil resilience—the capacity to maintain function under stress—appears enhanced in natural farming systems. The Slovenian study's finding that biodynamic soils maintained stable enzyme activities throughout seasonal variations [5] indicates greater biological buffering capacity. This resilience likely derives from greater microbial diversity and more complex food webs that provide functional redundancy—multiple organisms capable of performing each ecosystem process.

Crop yield stability under natural farming may differ from conventional systems, particularly during transition periods. The Solan District study documented significantly higher pea yield under conventional farming (109.67 q/ha) compared to ZBNF (92.07 q/ha) [7]. However, 47% higher production costs under conventional farming resulted in better benefit-cost ratio for ZBNF (2.13) compared to conventional (1.52). This economic advantage, combined with soil health benefits, supports natural farming adoption despite modest yield reductions.

6. Ecological and Sustainability Implications

Reduced nutrient loss represents a fundamental sustainability advantage of natural farming systems. By releasing nutrients through mineralization synchronized with plant uptake, natural farming minimizes the nitrate leaching and phosphorus runoff that characterize conventional agriculture. The Kenyan smallholder study demonstrated that erosion control measures reduced nitrogen losses by half across all agroecological zones [8]. This nutrient retention protects water quality while maintaining soil fertility.

Improved soil biodiversity under natural farming documented across multiple studies [1, 2, 3, 5, 7] supports enhanced ecosystem services including pest regulation, disease suppression, and pollination. Microbial diversity provides functional redundancy that buffers against environmental perturbation and maintains ecosystem processes under stress. The enrichment of specific functional genes under organic management [1] indicates not just taxonomic diversity but also metabolic diversity that enhances nutrient cycling capacity.

Enhanced ecosystem services from natural farming extend beyond nutrient cycling to include water regulation, climate mitigation, and habitat provision. Higher soil organic matter improves water infiltration and holding capacity, reducing runoff and increasing drought resilience. Carbon sequestration in stable mineral-associated pools [4] contributes to climate change mitigation while improving soil structure. Diverse crop rotations and perennial vegetation provide habitat for beneficial insects and wildlife.

Climate resilience of natural farming systems derives from multiple mechanisms including improved soil water holding capacity, enhanced nutrient cycling under stress, and greater biological buffering against extremes. The 10-year Danish trial demonstrated that perennial cropping systems simultaneously enhance nutrient availability and protect carbon stocks [4]—traits that support productivity under variable climate conditions. Deep root systems access water from deeper soil layers during drought while maintaining nutrient cycling through sustained microbial activity.

Carbon sequestration potential of natural farming varies with management practices and environmental conditions. The Kenyan modeling study estimated that combining erosion control, residue retention, and high-biomass legumes could increase SOC by at least 50% compared to current trends after 50 years [8]. The Danish trial documented shifts toward mineral-associated organic carbon with longer mean residence times [4], indicating that perennial systems not only accumulate more carbon but store it in more stable forms. These findings suggest that natural farming can contribute meaningfully to climate change mitigation while adapting to changing conditions.

7. Challenges and Future Perspectives

Long-term field data limitations constrain our understanding of natural farming effects across diverse agroecological contexts. While the DOK trial (42 years), Hisar experiment (19 years), and Danish perennial trial (10 years) provide valuable insights, comparable long-term studies are lacking for many natural farming systems including ZBNF and indigenous practices. The ICRISAT longitudinal impact assessment in Andhra Pradesh represents an important initiative addressing this gap [6], but longer monitoring periods are needed to capture full system dynamics.

Standardization of enzyme assay protocols remains essential for comparing results across studies and establishing baseline values. Methodological variations including incubation conditions, substrate concentrations, and expression units complicate meta-analysis and limit development of universal interpretative frameworks. International collaboration toward harmonized protocols would accelerate progress in using enzyme indicators for soil health assessment.

Regional variability in baseline soil properties, climate, and management practices means that absolute enzyme activities cannot be interpreted without local context. The geometric mean of enzyme activities and biological activity index offer partial solutions by integrating multiple enzymes into relative metrics [2], but calibration against local reference ecosystems is still required. Development of regional soil health benchmarks for natural farming systems would support farmer decision-making and policy development.

Policy and adoption barriers impede scaling of natural farming despite documented benefits. The Solan District study's finding that ZBNF achieved better economic returns despite lower yields [7] demonstrates that profitability arguments support adoption, but institutional support including technical extension, input supply chains, and market development is essential for widespread transition. Agricultural policies that internalize environmental costs and reward ecosystem services would accelerate natural farming adoption.

Integration with climate-smart agriculture presents opportunities for synergies between natural farming and climate change objectives. Carbon sequestration through organic matter accumulation, reduced nitrous oxide emissions from synthetic fertilizer avoidance, and enhanced resilience to climate extremes align natural farming with climate-smart principles. Policy frameworks that credit these benefits through carbon markets or ecosystem service payments would strengthen economic viability.

Multi-location trials across diverse agroecological zones are urgently needed to understand how natural farming effects vary with soil type, climate, and cropping system. The Kenyan study's finding that management scenario impacts differed across zones [8] underscores the importance of local adaptation. Networks of long-term experiments similar to the DOK trial but specifically focused on natural farming would generate the evidence base needed for confident recommendations.

8. Tables

Table 1: Major Natural Farming Inputs and Their Role in Soil Nutrient Cycling

Natural Input	Primary Nutrient Contribution	Effect on Soil Microbiology	Influence on Enzyme Activity	Long-Term Soil Impact
Jeevamritha	Multiple nutrients through microbial processing	Inoculates diverse beneficial microbes; increases microbial biomass	Enhances dehydrogenase, urease, and phosphatase activities	Builds organic carbon; improves nutrient cycling capacity
Beejamrut	Seed-borne nutrients; initial microbial inoculum	Colonizes rhizosphere with beneficial organisms	Supports early enzyme activity in developing root zone	Establishes beneficial seed-soil-microbe continuum
Panchavagya	Nutrients from milk products; growth substances	Promotes microbial diversity and activity	Stimulates multiple enzyme systems	Enhances overall biological fertility
Farmyard manure	Complete macro and micronutrients	Sustains microbial populations; provides organic substrates	Maintains elevated dehydrogenase, urease, and phosphatase	Builds stable organic matter; improves soil structure
Pressmud	Nitrogen (3.23%), organic carbon	Supports high microbial biomass; favors specific functional groups	Highest urease activity; favorable alkaline phosphatase	Superior long-term microbial properties [3]
Poultry manure	Complete nutrients; readily available	Promotes rapid microbial growth	Highest alkaline phosphatase among organics	Rapid nutrient release; builds active organic pools
Spent mushroom substrate	Organic matter; residual nutrients	Significantly increases microbial numbers	Enhances β-glucosidase, NAG, urease, phosphatase	Improves biological activity especially in biodynamic/organic

Table 2: Long-Term Effects of Natural Farming on Soil Nutrient Pools

Soil Parameter	Observed Long-Term Trend	Mechanism	Impact on Crop Productivity	Sustainability Significance
Soil organic carbon	+22.85% under ZBNF vs conventional [7]; highest under organic farming [2]	Continuous organic inputs; reduced decomposition	Improved water holding; slow nutrient release	Carbon sequestration; climate mitigation
Available nitrogen	Lower under natural farming [7]	No synthetic inputs; reliance on mineralization	May limit yields in short-term; adequate long-term	Reduced nitrate leaching; lower energy inputs
Available phosphorus	Variable; adequate under organic management [2]	Phosphatase-mediated organic P mineralization	Supports crop P demand without soluble fertilizers	Reduced runoff; sustainable P cycling
Available potassium	Lower under natural farming [7]	No synthetic KCl; reliance on organic cycling and mineral weathering	May require potassium-rich organic supplements	Reduced mining of finite resources
Microbial biomass carbon	202-491 mg/kg under organics [3]; higher under perennial grasses [4]	Continuous organic substrate supply	Supports nutrient mineralization; disease suppression	Enhanced biological buffering; resilience
Microbial biomass nitrogen	35.0-79.8 mg/kg under organics [3]	Nitrogen immobilization-mineralization balance	Regulates N supply to crops	Reduces N losses; improves synchrony

Table 3: Soil Enzymes as Indicators of Biological Activity in Natural Farming Systems

Enzyme	Function	Associated Nutrient Cycle	Response to Natural Inputs	Interpretation as Soil Health Indicator
Dehydrogenase	Intracellular oxidation-reduction; overall microbial activity	All cycles (integrated)	63.7 μg TPF/g/24h under FYM [3]; elevated under organic farming [2]	Direct measure of viable microbial metabolic activity; early response indicator
Urease	Urea hydrolysis to ammonia	Nitrogen	97.6 μg NH4^{+}-N/g/h under pressmud [3]; decreases with synthetic N addition	Indicates organic N mineralization potential; sensitive to N management
Alkaline phosphatase	Organic P mineralization	Phosphorus	Highest under poultry manure [3]; enriched functional genes under organic [1]	Reflects P cycling capacity; increases under P limitation
Acid phosphatase	Organic P mineralization in acid soils	Phosphorus	Elevated under organic farming [2]	Similar to alkaline phosphatase but in acid conditions
β-glucosidase	Cellobiose hydrolysis to glucose	Carbon	Highest in biodynamic soils; increases with SMS [5]	Indicates cellulose decomposition; carbon cycling activity
Catalase	Hydrogen peroxide decomposition	All cycles (oxidative stress)	Elevated under organic farming [2]	Protects cells from oxidative damage; indicates aerobic activity

Table 4: Advantages and Limitations of Long-Term Natural Farming Systems

Parameter	Advantages	Limitations	Research Gaps	Future Research Needs
Nutrient availability	Sustained through biological cycling; synchronized with plant demand	Lower immediately available nutrients; may limit yields during transition [7]	Long-term trends in multiple nutrient pools; bioavailability dynamics	Multi-location trials with standardized methods; isotope tracing studies
Soil biological activity	Higher enzyme activities; greater microbial biomass; enriched functional genes [1, 2, 3, 5]	Seasonal and interannual variability requiring long-term monitoring	Relationships between enzyme activities and crop yields; threshold values	Establishment of baseline values by agroecological zone; integration with crop models
Soil organic carbon	Accumulation and stabilization [4, 7, 8]	Slow response requiring decades for significant change	Mechanisms of stabilization; maximum sequestration potential	Long-term monitoring; ^{13}C and ^{14}C turnover studies; modeling future trajectories
Crop productivity	Stable yields over long-term; favorable economic returns [7]	May be lower than conventional systems, especially during transition	Long-term yield trends; climate interactions; stability metrics	Long-term yield monitoring; stability analysis; participatory on-farm trials
Environmental impact	Reduced nutrient losses; carbon sequestration; biodiversity enhancement [8]	Requires careful management to optimize multiple outcomes	Full life cycle assessment; water quality impacts; biodiversity quantification	Integrated assessment frameworks; landscape-scale studies; ecosystem service valuation
System resilience	Greater biological stability [5]; enhanced stress tolerance	Dependence on biological knowledge; management intensity	Mechanisms of resilience; indicators of resilience	Stress experiments; functional redundancy assessment; resistance and recovery metrics
Adoption feasibility	Lower production costs; favorable benefit-cost ratio [7]	Knowledge-intensive; requires institutional support	Adoption determinants; scaling pathways; policy effectiveness	Socioeconomic research; extension methodology studies; policy analysis

9. Conclusion

This review synthesizes evidence from long-term field experiments demonstrating that natural farming systems fundamentally enhance soil biological processes governing nutrient cycling and enzyme activities. The 42-year DOK trial reveals that organic management enriches functional genes involved in organic nutrient acquisition and transformation [1]. The 19-year Hisar experiment documents sustained elevations in microbial biomass carbon, dehydrogenase, urease, and phosphatase activities under organic amendments [3]. The 10-year Danish perennial trial shows that deep-rooted systems simultaneously enhance nutrient cycling and protect stable carbon pools through enzyme balance shifts [4]. The Vertisol study confirms that organic farming achieves highest soil organic carbon and enzymatic indices [2]. The Slovenian research demonstrates that biodynamic and organic soils maintain higher microbial counts and enzyme activities with greater seasonal stability [5]. The Solan District survey reveals that ZBNF achieves substantially higher organic carbon and microbial populations despite lower available nutrients [7].

Soil enzyme activities—dehydrogenase, urease, phosphatase, β-glucosidase, and catalase—emerge as sensitive, early indicators of soil health restoration under natural farming. The geometric mean of enzyme activities and biological activity index provide robust integrative metrics that correlate with soil organic carbon, microbial biomass, and crop productivity. These biological indicators respond to management changes before shifts in chemical or physical properties become detectable, enabling adaptive management and early verification of soil health improvement.

Natural farming systems demonstrate enhanced nutrient cycling efficiency through biological pathways rather than synthetic inputs. Nitrogen cycling relies on biological fixation, mineralization-immobilization turnover, and nitrification mediated by enriched functional genes. Phosphorus cycling depends on phosphatase-mediated organic phosphorus mineralization rather than soluble phosphate inputs. Potassium and micronutrient dynamics reflect organic matter complexation and microbial mobilization rather than direct fertilizer application. These biological nutrient pathways reduce environmental losses while maintaining adequate crop nutrition over long periods.

The strength of natural farming systems lies in their capacity to build soil biological capital that sustains productivity while delivering ecosystem services. Higher soil organic carbon, greater microbial biomass, elevated enzyme activities, and enhanced functional gene diversity collectively indicate soils with greater fertility, resilience, and environmental performance. Despite occasionally lower immediately available nutrients and modest yield reductions compared to conventional systems [7], favorable economic returns and sustainability benefits support natural farming adoption.

Soil enzyme activities serve as indispensable sustainability indicators for global agroecological transitions. Their sensitivity to management, integration of multiple biological processes, and relevance to ecosystem functions make them ideal metrics for monitoring soil health restoration. Inclusion of enzyme indicators in national and international soil monitoring frameworks would provide early warning of degradation or recovery and support adaptive management.

Implications for global food systems extend beyond individual farms to landscape-scale outcomes including water quality protection, biodiversity conservation, and climate change mitigation. The potential to sequester carbon in stable soil pools while enhancing nutrient cycling [4, 8] positions natural farming as a solution to multiple sustainability challenges simultaneously. Realizing this potential requires sustained investment in long-term research, extension systems that support knowledge-intensive management, and policy frameworks that reward ecosystem service provision. The scientific evidence assembled here confirms that natural farming, grounded in biological processes and supported by long-term research, offers a viable pathway toward agricultural sustainability.

10. References

1. Krause HM, Mueller RC, Lori M, Mayer J, Mäder P, Hartmann M. Organic cropping systems alter metabolic potential and carbon, nitrogen and phosphorus cycling capacity of soil microbial communities. Soil Biol Biochem. 2025:109737.
2. Sahu A, Bhattacharjya S, Singh S, Singh AB, Mandal A, Thakur JK, *et al*. Response of nature-based and organic farming practices on soil chemical, biological properties and crop physiological attributes under soybean in Vertisols of central India. Eurasian Soil Sci. 2024;57(7):1244-1260.
3. Sheoran S, Raj D, Antil RS, Mor VS, Grewal KS. Soil microbial properties influenced with long term application of manures and fertilizers. Indian J Agric Sci. 2020;90(1):172-182.
4. Zong M, Abalos D, Chen J, Liang Z, Li Y, Elsgaard L, *et al*. Ten-year effects of perennial cropping systems on soil organic carbon stock and stability in sandy soils: mechanisms and biochemical drivers. Eur J Agron. 2025.
5. Pintarič M, Štuhec A, Tratnik E, Langerholc T. Spent mushroom substrate improves microbial quantities and enzymatic activity in soils of different farming systems. Microorganisms. 2024;12(8):1521.
6. Jat ML, Rooyen AF van, Singh R, Pasumarthi P, Choudhari P. End of initiative report of Agroecology: ICRISAT longitudinal impact assessment of natural farming on soil health, microbial diversity, and their interconnected response functions in Andhra Pradesh. Hyderabad: International Crops Research Institute for the Semi-Arid Tropics; 2024.
7. Pathania N, Spehia R. Potential of natural farming-improves soil health and reduces production cost: study of Solan District, Himachal Pradesh, India. Veg Sci. 2024;51(2):295-302.
8. Nyamasoka-Magonziwa B, Vanek SJ, Paustian K, Ojiem JO, Fonte SJ. Evaluating nutrient balances, soil carbon trends, and management options to support long-term soil productivity in smallholder crop-livestock systems. Nutr Cycl Agroecosyst. 2023.
9. Wang D, Chen L, Zhang H, Liu Y, Li X. Combining organic fertilizer with soil conditioner alleviates continuous cropping obstacles in protected cowpea by improving soil properties and microbiome. Sci Rep. 2025;15:42094.
10. Ravindran KC, Balakrishnan V, Venkatesan K. The influence of halophytic compost, farmyard manure and phosphobacteria on soil microflora and enzyme activities. Plant Soil Environ. 2019.
11. Lori M, Symnaczik S, Mäder P, De Deyn G, Gattinger

A. Organic farming enhances soil microbial abundance and activity - a meta-analysis and meta-regression. PLoS One. 2017;12(7):e0180442.

12. Kundel D, Bodenhausen N, Jørgensen HB, Truu J, Birkhofer K, Hedlund K, *et al*. Effects of simulated drought on biological soil quality, microbial diversity and yields under long-term conventional and organic agriculture. FEMS Microbiol Ecol. 2020;96(12):fiaa205.
13. Chen L, Zhou W, Luo L, Li Y, Chen Z, Gu Y, *et al*. Short-term responses of soil nutrients, heavy metals and microbial community to partial substitution of chemical fertilizer with spent mushroom substrates (SMS). Sci Total Environ. 2022;844:157064.
14. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphatase for assay of soil phosphatase activity. Soil Biol Biochem. 1969;1:301-307.
15. Tabatabai MA, Bremner JM. Assay of urease activity in soils. Soil Biol Biochem. 1972;4:479-487.
16. Casida LE Jr, Klein DA, Santoro T. Soil dehydrogenase activity. Soil Sci. 1964;98:371-376.
17. Mulvaney RL, Khan SA, Ellsworth TR. Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. J Environ Qual. 2009;38:2295-2314.
18. Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, *et al*. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl Ecol. 2010;11:97-105.
19. Ayiti OE, Babalola OO. Factors influencing soil nitrification process and the effect on environment and health. Front Sustain Food Syst. 2022;6:821994.
20. Benbi DK, Brar K, Toor AS, Sharma S. Sensitivity of labile soil organic carbon pools to long-term fertilizer, straw and manure management in rice-wheat system. Pedosphere. 2015;25:534-545.
21. Luo P, Han X, Wang Y, Han M, Shi H, Liu N, *et al*. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Ann Microbiol. 2015;65:533-542.
22. Bastida F, Jindo K, Moreno JL, Hernandez T, Garcia C. Effect of organic amendments on soil carbon fractions, enzyme activity and humus-enzyme complexes under semi-arid conditions. Eur J Soil Biol. 2012;53:94-102.
23. Mandal A, Patra AK, Singh D, Swarup A, Masto RE. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresour Technol. 2007;98:3585-3592.
24. Ge T, Li B, Zhu Z, Hu Y, Yuan H, Dorodnikov M, *et al*. Rice rhizodeposition and its utilization by microbial groups depend on N fertilization. Biol Fertil Soils. 2017;53:37-48.
25. Wang L, Kaur M, Zhang P, Li J, Xu M. Effect of different agricultural farming practices on microbial biomass and enzyme activities of celery growing field soil. Int J Environ Res Public Health. 2021;18(23):12862.
26. Ahsan T, Tian PC, Gao J, Wang C, Liu C, Huang YQ. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping system. J Adv Res. 2024;64:1-13.
27. Ma L, Zhang J, Li Z, Xin X, Guo Z, Wang D, *et al*. Mechanisms and mitigation strategies for the occurrence of continuous cropping obstacles of legumes in China. Agronomy. 2023;14:104.
28. Owade JO, Abong' G, Okoth M, Mwang'ombe AW. A review of the contribution of cowpea leaves to food and nutrition security in East Africa. Food Sci Nutr. 2020;8:36-47.
29. Abebe BK, Alemayehu MT. A review of the nutritional use of cowpea (Vigna unguiculata L. Walp) for human and animal diets. J Agric Food Res. 2022;10:100383.
30. Oyetunji O, Bolan N, Hancock G. A comprehensive review on enhancing nutrient use efficiency and productivity of broadacre (arable) crops with the combined utilization of compost and fertilizers. J Environ Manage. 2022;317:115375.

How to Cite This Article

Silva LF da, Reed JP. Long-term assessment of natural farming systems on nutrient cycling and soil enzyme activity: implications for soil health, microbial dynamics, and sustainable crop productivity. International Journal of Agriculture Natural Farming Research. 2026;2(1):28-37.

Creative Commons (CC) License