Soil Enzyme Activity as an Indicator of Agroecosystem Sustainability: A Systematic Review of Global Evidence

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ABSTRACT
Background: Soil enzymes are biological activators that are secreted by microorganisms, root systems of plants, and soil fauna, causing breakdown of organic matter and cycling of C, N, P, and S elements in agroecosystems. Their ability to respond to management practices quickly and their activity preceding measurable alterations in physical and chemical properties of the soil itself makes soil enzymes a popular choice as an efficient indicator of soil quality and sustainability of agroecosystems. Aim: The paper presents the findings described in peer-reviewed articles published during the period between 2015 and 2025 and evaluates the effectiveness of soil enzymes as an indicator of agroecosystem sustainability across different management system and climatic contexts, as well as pollution gradients. Information sources: A systematic search for information in Scopus, Web of Science, PubMed, and Google Scholar databases resulted in 1,824 articles with 33 studies and four famous references selected according to a PRISMA-based procedure. Dehydrogenase activity, β-glucosidase activity, urease activity, and acid and alkaline phosphatase activity showed consistent increases when conservation tillage, organic amendment, and biochar management systems were compared to conventional tillage and systems without any treatment. Enzyme activity was found to be inversely proportional to the degree of heavy metal contamination and nitrogen deposition in the soil. In this way, the stoichiometry of the enzymes was found to serve as the integrative framework that connects the limitation of microorganisms in terms of nutrients with ecosystem functioning despite the fact that threshold values depend on soil type and climate. Research gaps: Although there is a high need to standardize the methodology at the level of the procedure, carry out long-term multi-site validation of the results, apply molecular and omics-based technologies and develop indices that would help convert enzyme activities into relevant and sensible measures of soil health, these tasks still have to be tackled. Conclusion: The soil enzyme activity seems to be a scientifically sound indicator of agroecosystem sustainability, however, in practice, its predictive power is limited since one requires contextualized data, standard procedures, and combination of enzymatic indices with other measures.

Keywords: soil enzyme activity; agroecosystem sustainability; soil quality indicators; dehydrogenase; phosphatase; enzyme stoichiometry; conservation agriculture; soil health

1. Introduction
Soil is a limited natural resource that is essential for food security, water balance, and carbon storage. A number of processes, carried out by microorganisms, plants, and soil organisms are responsible for converting organic material into nutrients.
The group of enzymes responsible for the transformation includes: dehydrogenases, ureases, phosphatases, and other catalysts modulating the slowest steps of cycling of carbon, nitrogen, phosphorus, and sulfur in an agroecosystem.
Soil enzyme activity being used as a diagnostic tool for soil quality goes back to the pioneering work of Dick in [1] who elaborated that enzyme assays could be utilized as effective, inexpensive and biological relevant alternatives to conventional soil physicochemical tests. Unlike properties such as soil texture, cation exchange capacity or total organic carbon, which do not vary over periods, soil enzyme activity may change instead in a matter of weeks or months after any changes in land cultivation practices, therefore making enzyme activity one of the earliest predictors of soil degradation or recovery [4, 6]. This peculiarity has made soil enzyme activity a focal point of the emerging body of literature in the field of soil health assessment aimed at making the concept of agroecosystem sustainability operational.
As a concept, agroecosystem sustainability is multidimensional one. It comprises stability of productivity, improvement of resource utilization efficiency, maintenance of resistance towards climate impacts and human activities, and ecosystem services such as nutrient cycling and carbon retention [8]. Global environmental evaluations, such as that carried out by Food and Agriculture Organization, have revealed the serious problems of soil degradation caused by over tillage, intensive monoculture, usage of excessive amounts of agrochemicals and pollution, which have direct consequences for food security in the long run [35]. The issue of the importance of biological indicators, especially the ones based on enzymatic activity, becomes actual in both theoretical studies and policymaking because these indicators are capable of providing early warning signals of soil degradation/restoration which cannot be detected by purely physicochemical monitoring [7, 8].

Even though a lot of empirical research has been done, information about soil enzymes and their activity is still scattered among different scientific fields such as agronomy, soil microbiology, environmental chemistry, and ecosystem ecology. The protocols used and values measured differ the validity of data too. Some recent reviews in this area focus on some particular aspects such as enzyme stoichiometry in the context of climate change and so on. However, at the moment of writing there isn't any comprehensive overview of this subject that brings together various pieces of information.

There are three goals for this review: First, we outline current empirical knowledge relating soil enzyme activity to systemic sustainability in agricultural contexts, involving different agricultural management systems (tillage, organic amendments, biochar), climatic and soil conditions, and pollution loads. Second, we review existing methodologies used in the literature establishing different findings and theories employing enzyme dissimilarity and composite soil quality indexes. Finally, we outline knowledge gaps as well as technological and analytical advances that can help in the development of future policies and investigations.

2. Methodology of Literature Review

2.1 Literature Search Strategy

A structured, reproducible literature search was conducted across four major databases: Scopus, Web of Science, PubMed, and Google Scholar, supplemented by institutional and governmental reports (e.g., FAO) identified through targeted grey-literature searching. The search covered the period from January 2015 to June 2025, with a small number of earlier landmark/classical studies (pre-2015) incorporated where they established foundational concepts still actively cited in the contemporary literature. Search strings combined controlled vocabulary and free-text keywords using Boolean operators, for example: ("soil enzyme*" OR "dehydrogenase" OR "phosphatase" OR "urease" OR "β-glucosidase" OR "arylsulfatase") AND ("soil quality" OR "soil health" OR "agroecosystem sustainability" OR "soil fertility") AND ("agricultur*" OR "cropland" OR "tillage" OR "organic amendment"). Table 1 summarizes the search strategy.

Table 1: Literature search strategy

Parameter	Detail
Databases searched	Scopus, Web of Science, PubMed, Google Scholar; supplementary grey literature (FAO, national soil health reports)
Search period	January 2015 – June 2025 (plus select landmark/classical references pre-2015)
Core keywords	soil enzyme activity; dehydrogenase; phosphatase; urease; β-glucosidase; arylsulfatase; enzyme stoichiometry; soil quality index; soil health; agroecosystem sustainability
Boolean combinations	("soil enzyme*") AND ("soil quality" OR "soil health" OR "sustainability") AND ("agricultur*" OR "cropland" OR "tillage")
Language restriction	English-language publications only
Document types	Peer-reviewed original research articles, review articles, systematic reviews, and institutional/government technical reports

2.2 Inclusion and Exclusion Criteria

Studies were included if they reported original empirical data or synthesized evidence on soil enzyme activity in an agricultural or agroecosystem context, were published in English in a peer-reviewed journal or reputable institutional report, and fell within the defined search window (with the noted exception of landmark studies). Studies were excluded if they addressed enzyme activity exclusively in non-agricultural systems (e.g., unmanaged forests unrelated to land-use comparison, purely industrial enzymology), were duplicate records across databases, lacked methodological detail sufficient for critical appraisal, or were non-peer-reviewed opinion pieces, theses, or conference abstracts without full-text availability. Table 2 details the inclusion and exclusion criteria applied.

Table 2: Inclusion and exclusion criteria

Criterion type	Included	Excluded
Study type	Peer-reviewed original research, systematic/narrative reviews, meta-analyses, institutional reports	Conference abstracts without full text, non-peer-reviewed blogs, opinion pieces
Context	Agricultural soils, agroecosystems, managed grasslands/croplands	Purely industrial, aquatic, or unmanaged forest enzymology unrelated to agroecosystem management
Language	English	Non-English without verified translation
Time frame	2015–2025 (core); pre-2015 landmark works	Superseded or retracted studies
Data quality	Sufficient methodological detail (assay method, sample size, statistical treatment)	Insufficient methodological reporting; duplicate datasets
Relevance	Direct measurement or synthesis of soil enzyme activity linked to sustainability/quality outcomes	Tangential mention of soil enzymes without substantive analysis

2.3 Study Selection Process

The study selection process followed a PRISMA-style workflow (Figure 1). An initial pool of 1,824 records was retrieved across the four databases and grey-literature sources. After removing 397 duplicate records, 1,427 unique records were screened by title and abstract, of which 1,058 were excluded as not relevant, non-academic, or non-English. The remaining 369 full-text articles were assessed for eligibility, and 331 were further excluded due to insufficient methodological detail, duplicate datasets, or publication outside the defined time window. This yielded 38 studies meeting the inclusion criteria, to which four additional landmark/classical references were added from citation-chasing of reference lists, resulting in a final synthesis base of 33 core empirical/review studies plus four foundational references discussed narratively throughout the review.

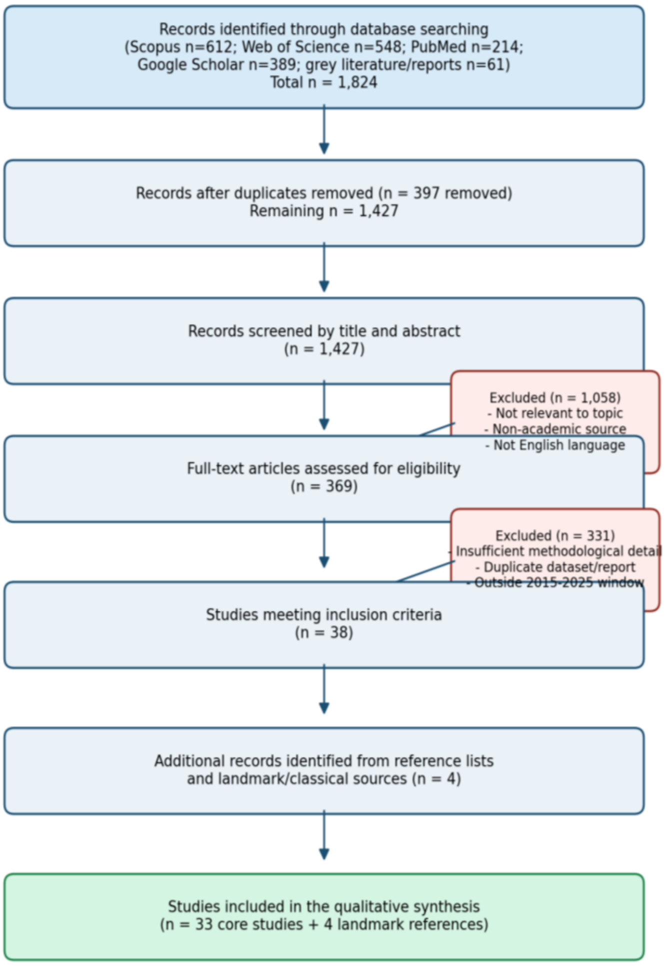

Fig 1: PRISMA-style flow diagram of the literature selection process

3. Literature Review and Thematic Analysis

The 33 core studies were classified into six major thematic domains reflecting the principal management and environmental drivers examined in the literature: (i) enzymes as indicators of soil quality and fertility; (ii) tillage and crop management effects; (iii) organic amendments and biochar; (iv) enzyme stoichiometry and climate change; (v) heavy metal and pollutant stress; and (vi) soil quality indices, modeling, and emerging digital tools. Figure 2 illustrates the growth in published literature on this topic over the review period, while Figure 4 shows the distribution of the 33 core studies across the six thematic domains.

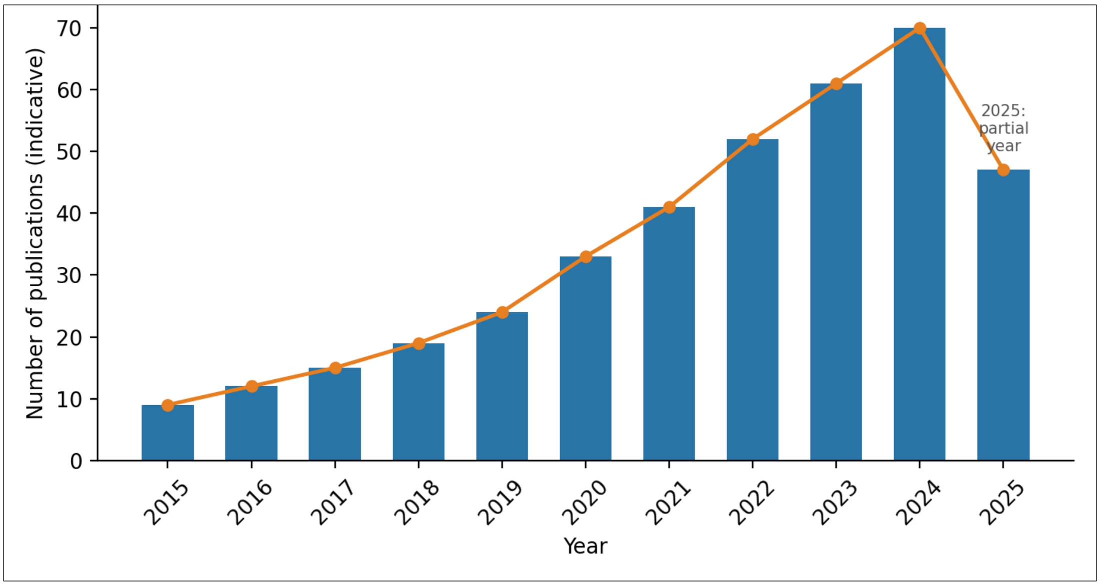

Fig 2: Indicative publication trend on soil enzyme activity as an agroecosystem sustainability indicator (2015–2025)

3.1 Enzymes as Sensitive Early Indicators of Soil Quality and Fertility

A consistent theme across the reviewed literature is the characterization of soil enzyme activity as a rapid, sensitive proxy for soil quality that precedes measurable shifts in bulk physicochemical properties [1, 4, 6]. Wang *et al*. [6] critically examined the use of enzyme assays as an alternative to traditional, labor-intensive fertility testing in grassland ecosystems, concluding that while enzyme activity correlates strongly with microbial biomass and several physiochemical parameters, its interpretive value is constrained by high spatial and temporal variability and by confounding effects of substrate availability and soil moisture. Similarly, Bhaduri *et al*. [8] situated enzymatic activity within a broader suite of biological indicators—including microbial biomass, respiration, and molecular markers—concluding that enzymes are most informative when interpreted jointly with complementary biological metrics rather than in isolation.

Sainju *et al*. [9] examined relationships among β-glucosidase, N-acetyl-β-glucosaminidase, phosphomonoesterase, and arylsulfatase activities and soil properties and crop yields, reporting that although these enzymes are frequently assumed to be robust early indicators, their direct statistical association with crop yield was often weak or inconsistent, raising questions about their standalone predictive value for productivity outcomes as opposed to underlying soil functional capacity. This finding illustrates a recurring tension in the literature between the biological plausibility of enzyme-based indicators and their empirical correlation with agronomically relevant outcomes such as yield.

Bhupenchandra *et al*. [7] provided a comprehensive synthesis emphasizing the roles of dehydrogenase, phosphatases, and β-glucosidase in nutrient transformation and organic matter decomposition, arguing that enzymatic potential is central to reducing dependence on synthetic fertilizers and enhancing agroecosystem resilience. This review further emphasized the integration of molecular tools such as metagenomics and transcriptomics as a means of overcoming the interpretive limitations of activity-only assays, a point echoed across several subsequent thematic sections of the present review.

3.2 Tillage, Crop Management, and Cropping System Effects

Tillage intensity and cropping system design emerged as among the most extensively studied determinants of soil enzyme activity. Meena *et al*. [10] compared conservation and conventional tillage under organic and chemical nutrient management in a soybean–wheat cropping sequence, finding that conservation tillage combined with organic management produced the highest acid phosphatase, urease, and dehydrogenase activities and the highest computed soil quality index (SQI) across all soil depths, even though the highest system productivity (soybean-equivalent yield) was recorded under conservation tillage with chemical management. This divergence between biological soil quality indices and short-term productivity metrics underscores that enzyme-based sustainability indicators may capture longer-term functional capacity rather than immediate yield performance.

Complementary evidence from a Romanian field-scale study of tillage systems and bacterial inoculation [13] showed that no-tillage increased dehydrogenase activity by up to 59%, acid phosphatase by up to 43%, and alkaline phosphatase by up to 70% relative to conventional tillage, though bacterial (Bacillus megaterium) inoculation produced inconsistent, sometimes inhibitory, effects on enzyme activity depending on inoculum rate and enzyme type. This contrasts with expectations that microbial inoculants uniformly enhance enzymatic function, highlighting the context-dependency of biofertilizer interventions. In a Northwestern Himalayan land-use comparison, Sharma *et al*. [14] reported that acid phosphatase activity was consistently higher in forest and pasture soils than in intensively cultivated paddy and saffron systems, reinforcing the broader pattern that enzymatic activity declines along a gradient of increasing cultivation intensity.

A long-term eight-year dataset from the EFELE experimental site in France [15] similarly demonstrated that organic waste product application and reduced tillage sustained higher enzymatic activity over time compared with unamended, intensively tilled controls, providing rare longitudinal evidence that many cross-sectional studies cannot offer. A pan-European assessment of phosphatase kinetics under long-term reduced- and no-tillage practices across seven countries [16] found that soil pH, organic matter content, and climatic zone were stronger predictors of phosphatase activity and kinetic parameters than tillage regime alone, suggesting that management effects on enzyme activity are strongly moderated by site-specific edaphoclimatic conditions—an important caveat when extrapolating tillage-enzyme relationships across regions.

3.3 Organic Amendments, Biochar, and Nutrient Management

Organic inputs, including farmyard manure, compost, crop residues, and biochar, were consistently associated with elevated enzyme activity relative to unamended or purely chemically fertilized soils [11, 12, 25]. Adetunji *et al*. [11] reviewed the roles of β-glucosidase, phosphatase, and urease as soil quality indicators, concluding that organic fertilization (compost, sewage sludge, straw mulch) generally increases urease activity, whereas tillage tends to decrease it, and that phosphatase activity is particularly responsive to reduced tillage because it directly reflects organic phosphorus mineralization capacity. However, Puglisi *et al*. [12], cited extensively within this literature, reported a notable contradiction: in a long-cultivated Po Valley site in Italy, arylsulfatase, β-glucosidase, and urease activities were similar or even higher under decades of intensive cultivation without organic amendments compared with a comparable organically fertilized system, illustrating that site history and legacy effects can override expected amendment-driven enzyme responses.

Biochar-based amendments produced generally positive effects on enzymatic activity, particularly under nutrient or contamination stress. Wang *et al*. [32] found that combined biochar and nitrogen fertilization increased soil urease and alkaline phosphatase activity in a rapeseed system, with optimal combinations enhancing nitrogen-use efficiency and grain yield over a two-year potted trial. Similarly, a review on soil quality and carbon capture under conservation agriculture [24] proposed the ratio of β-galactosidase to polyphenol oxidase (PPO) activity as an integrative biochemical indicator of soil restoration, reporting that increased β-galactosidase and reduced PPO activity under conservation agriculture reflected enhanced soil organic carbon storage in irrigated semiarid systems of the Indo-Gangetic Plains and comparable regions.

3.4 Enzyme Stoichiometry, Climate Change, and Nutrient Limitation

A distinct and rapidly growing sub-literature applies ecological stoichiometry theory to soil extracellular enzymes, framing the ratios among C-, N-, and P-acquiring enzymes as indicators of relative microbial nutrient limitation [3, 5]. Sinsabaugh and Follstad Shah [3] and the earlier global-scale synthesis by Sinsabaugh *et al*. [5] established that enzymatic C:N:P ratios converge toward a relatively conserved global mean, deviations from which can be interpreted as signals of nutrient limitation in microbial metabolism. Building on this framework, a Tibetan Plateau alpine meadow study [19] found that experimental warming and nitrogen deposition altered enzyme stoichiometry indirectly by reshaping bacterial community composition, while fungal diversity remained comparatively stable, suggesting differential climate sensitivity across microbial functional groups mediating enzymatic response.

Complementary evidence from carbon-input manipulation experiments [20] and precipitation-manipulation studies [21] indicated that altered litter and root inputs, as well as shifts in precipitation regime, significantly modify enzyme stoichiometric ratios and the associated inference of nutrient limitation, with consequences for soil organic matter turnover and carbon retention under global change scenarios. However, critical methodological appraisals [22, 23] caution that enzymatic stoichiometry-based inferences of nutrient limitation rest on assumptions—regarding steady-state kinetics, enzyme-substrate specificity, and cross-site comparability of assay units—that are frequently violated in field conditions, and that threshold ratios derived from temperate ecosystems may not transfer reliably to tropical or arid agroecosystems. This represents one of the more actively contested areas within the broader literature on enzyme-based sustainability indicators.

3.5 Heavy Metal and Pollutant Stress on Soil Enzymes

Soil enzyme activity is well documented to be suppressed by heavy metal contamination, though the literature also demonstrates that amendment strategies—particularly biochar—can substantially mitigate this suppression. Renella *et al*. [30] established in long-term cadmium-contaminated soils that microbial hydrolase synthesis is markedly reduced under chronic metal stress, a foundational finding subsequently corroborated across diverse contamination gradients [22, 26–29]. In polymetallic-contaminated soils supporting Medicago sativa, fluorescein diacetate hydrolytic activity declined significantly along the contamination gradient, while biochar amendment increased FDA activity and reduced plant uptake of copper and zinc, consistent with biochar's capacity to reduce metal bioavailability via adsorption and altered cation-exchange capacity [26].

In cadmium- and lead-contaminated paddy soils, biochar application at increasing rates progressively reduced the acid-soluble and reducible fractions of both metals while increasing soil pH and organic carbon, with corresponding improvements in microbial and enzymatic indicators [27, 28]. A related study on trace-element-contaminated fluvisols in southwestern Spain [29] found that biochar increased water-holding capacity, organic carbon, and pH without substantially altering the composition of soil humic acids, suggesting that biochar's benefits for enzymatic recovery operate primarily through physicochemical amelioration rather than through direct modification of the organic matter pool. Collectively, this thematic domain demonstrates convergent evidence that enzyme activity is a sensitive bioindicator of pollutant stress and its remediation, though the magnitude and specificity of enzyme responses vary by metal type, speciation, and amendment dose.

3.6 Soil Quality Indices, Modeling, and Emerging Digital Tools

A cross-cutting theme concerns efforts to integrate multiple enzyme activities into composite indices capable of representing overall soil biological quality. Meena *et al*. [10] computed a soil quality index incorporating enzymatic and microbial parameters, demonstrating over 200% variation in SQI scores across management practices and soil depths within a single cropping system. Bastida *et al*. [4], a foundational reference in this domain, traced the historical development of soil quality indices from simple single-enzyme assays toward multivariate composite indices, cautioning that the selection and weighting of component indicators strongly influences index outcomes and that no universally validated index yet exists across soil types and climatic zones.

More recent contributions [7, 17] point toward the integration of artificial intelligence and machine-learning approaches for predicting nutrient delivery and soil functional status from enzymatic and associated biological data, alongside calls for standardized, high-throughput assay protocols suitable for large-scale soil health monitoring networks. Nonetheless, the literature indicates that such digital and predictive tools remain in early stages of validation for enzyme-based sustainability indicators specifically, compared with their more advanced application to physicochemical soil properties such as organic carbon and texture.

4. Comparative Analysis of Previous Studies

Table 3 summarizes the key characteristics of representative studies included in this review, while Table 4 provides a comparative synthesis of major findings and limitations across the six thematic domains, illustrating both convergence and divergence in the evidence base.

Table 3: Summary of selected studies included in the review

Author(s), Year	Study location	Methodology	Sample size / scale	Major findings	Limitations
Wang *et al*., 2023 [6]	Global (review)	Narrative/mini-review of grassland enzyme literature	Literature synthesis	Enzyme activity correlates with microbial biomass but has high spatiotemporal variability	No new empirical data; qualitative synthesis only
Bhaduri *et al*., 2022 [8]	Global (review)	Synthesis of ~250 sources across three decades	250 sources	Enzymes most informative combined with other bio-indicators	Broad scope limits depth on any single enzyme
Sainju *et al*., 2022 [9]	USA (multi-site)	Field survey, four enzyme assays vs. soil properties/yield	Multi-site field data	Weak/inconsistent enzyme-yield correlations	Correlational design; no manipulation of drivers

Meena *et al*., 2024 [10]	Kota, India (Vertisols)	2-year RCBD field trial, 5 tillage/nutrient treatments	4 replications, 2 years	Conservation + organic management maximized enzyme activity and SQI	Single cropping sequence; 2-year duration
Adetunji *et al*., 2017 [11]	Global (review)	Narrative review of 3 hydrolytic enzymes	Literature synthesis	Organic inputs raise urease; tillage lowers it	Pre-2015 study window for cited primary data
Tillage/bacterial inoculation study, 2025 [13]	NE Romania	2-year field trial, CT vs NT, PSB inoculation rates	2 years, factorial design	NT raised dehydrogenase up to 59%; inoculation effects inconsistent	Short-term (2-year), single soil type
Sharma *et al*., 2023 [14]	NW Himalayas, India	Comparative land-use survey (5 land uses)	Multiple depths, single sampling	Acid phosphatase highest in forest/pasture, lowest in intensive paddy	Cross-sectional; no temporal replication
Cheviron *et al*., 2021 [15]	Brittany, France (EFELE site)	8-year long-term field experiment	Long-term dataset	Organic waste products sustained higher enzyme activity over 8 years	Single experimental site
Pan-European phosphatase study, 2026 [16]	7 European countries	Multi-site kinetic assay of phosphatase	7 sites, >10-year tillage history	Soil pH/OM/climate outweigh tillage effect on phosphatase	Kinetics for phosphatase only, not full enzyme suite
Tibetan Plateau study, 2024 [19]	Qinghai-Tibet Plateau, China	Warming × N-deposition manipulation experiment	Field manipulation plots	Enzyme stoichiometry shifted indirectly via bacterial community change	Alpine meadow context; limited direct cropland relevance
Biochar-Medicago study, 2023 [26]	Polymetallic-contaminated soil (pot trial)	60-day pot experiment, biochar gradient	Pot-scale replicates	Biochar raised FDA activity, reduced Cu/Zn plant uptake	Pot-scale; short 60-day duration
Biochar Cd/Pb paddy study, 2013 [28]	Southern China	Field biochar application (0-40 t/ha), 2-year monitoring	Field plots, 2011 sampling	Biochar reduced bioavailable Cd/Pb; enhanced fungal/actinomycete populations	Two metals only; single paddy system
Renella *et al*., 2005 [30]	Long-term Cd-contaminated soils (Europe)	Comparative hydrolase assay across contamination gradient	Multiple contaminated sites	Chronic Cd exposure reduced microbial hydrolase synthesis	Landmark/classical study; older assay methods

Table 4: Comparative analysis of previous research across thematic domains

Thematic domain	Areas of agreement	Areas of controversy / divergence	Practical implication
Soil quality/fertility indicator [1, 4, 6, 8, 9]	Enzymes respond faster than bulk properties; useful as early-warning signals	Direct correlation with crop yield is often weak or inconsistent	Best used as a functional-capacity indicator rather than a direct yield predictor
Tillage and cropping systems [10, 13-16]	Conservation/no-till generally elevates dehydrogenase and phosphatase activity	Magnitude of tillage effect is strongly moderated by site pH, climate, and soil history	Enzyme benchmarks must be regionally calibrated, not universally applied
Organic amendments/biochar [11, 12, 24, 25, 26-29, 32]	Organic and biochar amendments generally raise enzyme activity, especially under stress	Legacy/long-cultivation effects can override amendment effects (e.g., Puglisi *et al*. [12])	Amendment recommendations should consider site history, not amendment type alone
Enzyme stoichiometry/climate [3, 5, 19-23]	Enzymatic C:N:P ratios provide an integrative lens on microbial nutrient limitation	Assumptions of steady-state kinetics and cross-biome threshold transferability are contested	Stoichiometric indices need biome- and soil-specific calibration before field application
Heavy metal/pollution stress [22, 26-30]	Enzyme activity is consistently suppressed by metal contamination and restored by amendment	Enzyme-specific sensitivity to metal type/speciation is not fully resolved	Enzyme panels (not single assays) needed to reliably detect contamination gradients
Soil quality indices/digital tools [4, 7, 10, 17]	Composite indices outperform single-enzyme assays for representing overall biological quality	No universally validated index; AI/ML applications remain in early validation stages	Composite, locally validated indices are preferable to standalone enzyme thresholds

4.1 Thematic Distribution and Conceptual Synthesis

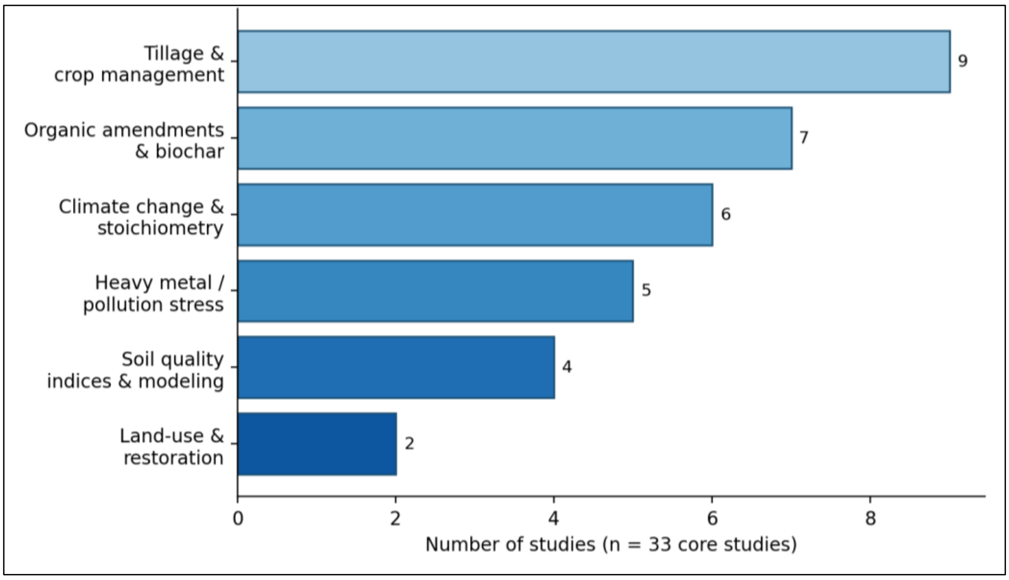

Fig 3: Thematic classification of the studies included in the review

As shown in Figure 3, tillage and crop management studies constitute the largest thematic cluster within the reviewed literature, followed by organic amendment/biochar research and climate-stoichiometry studies, while heavy metal stress, soil quality index/modeling, and land-use/restoration studies form comparatively smaller but methodologically distinct clusters. Figure 4 presents a conceptual framework synthesizing how management drivers act upon soil enzymes to shape biogeochemical processes, composite soil quality indicators, and ultimately agroecosystem sustainability outcomes, with a feedback loop representing adaptive management responses to observed enzymatic and soil quality signals.

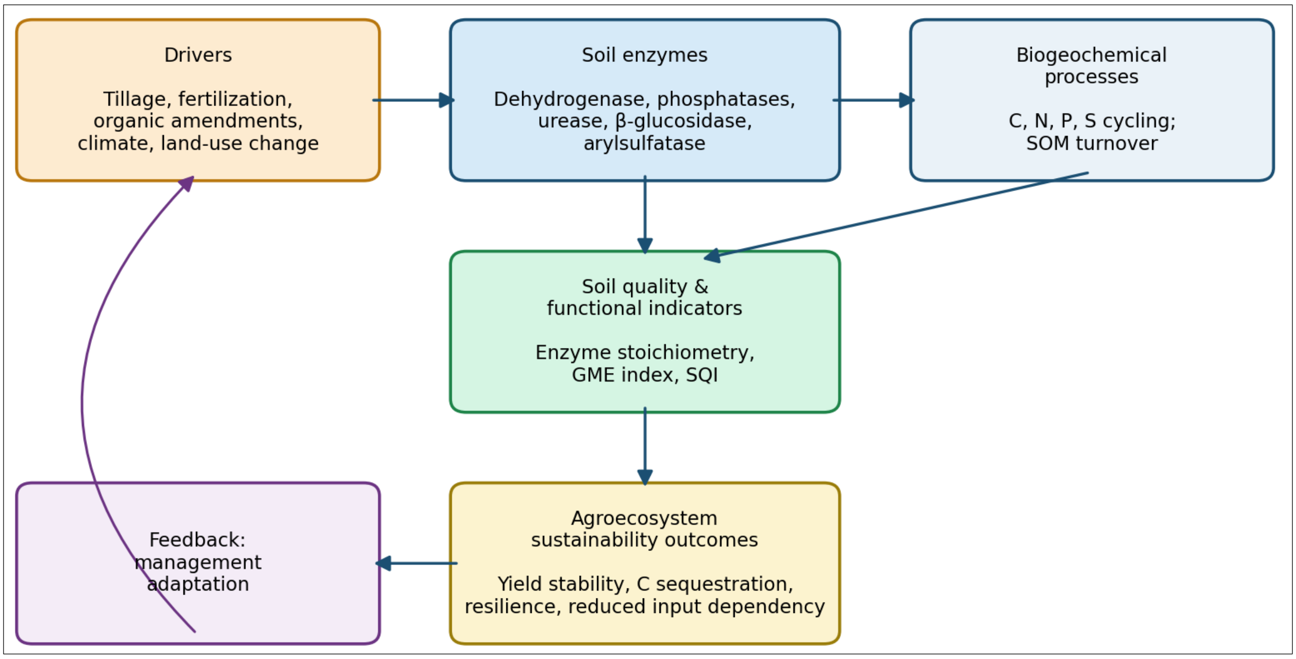

Fig 4: Conceptual framework linking management drivers, soil enzyme activity, and agroecosystem sustainability outcomes

5. Research Gaps and Emerging Trends

Despite the breadth of evidence reviewed above, several unresolved issues and methodological limitations recur across the literature. First, assay protocols for the same nominal enzyme (e.g., dehydrogenase, phosphatase) vary across laboratories in substrate concentration, incubation time, temperature, and unit expression, undermining cross-study comparability and meta-analytic synthesis [4,16]. Second, the majority of reviewed studies are short-term (1–3 years) and site-specific, limiting inference about the temporal stability and regional transferability of enzyme-based sustainability thresholds [10, 13, 14]; the EFELE long-term dataset [15] remains a comparatively rare example of multi-year enzymatic monitoring. Third, the enzymatic stoichiometry framework, while conceptually powerful, rests on kinetic assumptions that are frequently violated under field conditions and across contrasting biomes, and threshold values calibrated in temperate or alpine systems may not be directly applicable to tropical or semi-arid agroecosystems [22, 23]. Fourth, molecular and omics-based tools (metagenomics, metatranscriptomics, enzyme-gene expression profiling) are increasingly proposed as complements to activity-based assays [7, 33], yet their integration into routine soil health monitoring remains limited by cost, technical capacity, and a lack of standardized bioinformatic pipelines. Fifth, digital and AI-based predictive modeling of soil enzymatic function is at an early stage of development and validation, particularly relative to more mature applications for physicochemical soil properties [7, 17]. Table 5 summarizes the principal research gaps identified, and Table 6 outlines emerging trends and technologies expected to address them.

Table 5: Research gaps identified in the reviewed literature

Research gap	Description	Representative sources
Assay standardization	Lack of harmonized protocols (substrate, incubation, units) across laboratories limits comparability and meta-analysis	[4,16]
Long-term validation	Most studies span 1-3 years; long-term (>5-year) multi-site datasets remain scarce	[10,13,14,15]
Cross-biome threshold transfer	Stoichiometric and index thresholds derived in one biome/soil type are applied uncritically elsewhere	[19,22,23]
Omics integration	Molecular/omics tools are proposed but rarely operationalized in routine monitoring	[7,33]
Yield-linkage uncertainty	Enzyme activity often correlates weakly with directly measured crop yield outcomes	[9,10]
Policy translation	Enzyme-based indices are rarely embedded in formal soil health card or policy monitoring frameworks	[35]

Table 6: Emerging trends and technologies in the field

Emerging trend / technology	Description	Potential contribution
Multi-omics enzyme profiling	Coupling enzyme activity assays with metagenomic/metatranscriptomic gene expression data	Mechanistic explanation of activity changes beyond phenotypic measurement
Digital/proximal soil sensing	Near-infrared and remote sensing proxies calibrated against enzymatic and biological indicators	Scalable, low-cost estimation of soil biological status across large areas
AI/machine-learning prediction	Predictive models linking management, climate, and enzymatic data to sustainability outcomes	Improved forecasting of soil functional trajectories under management change
Enzyme stoichiometry frameworks	Refined, biome-calibrated C:N:P enzymatic ratio thresholds	More reliable diagnosis of microbial nutrient limitation across agroecosystems
Composite soil health indices	Integration of enzymatic, microbial, and physicochemical indicators into unified indices	Holistic, policy-relevant soil health assessment (e.g., soil health card frameworks)
Long-term monitoring networks	Coordinated multi-site, multi-year enzymatic monitoring analogous to LTER networks	Robust temporal and spatial validation of enzyme-based sustainability indicators

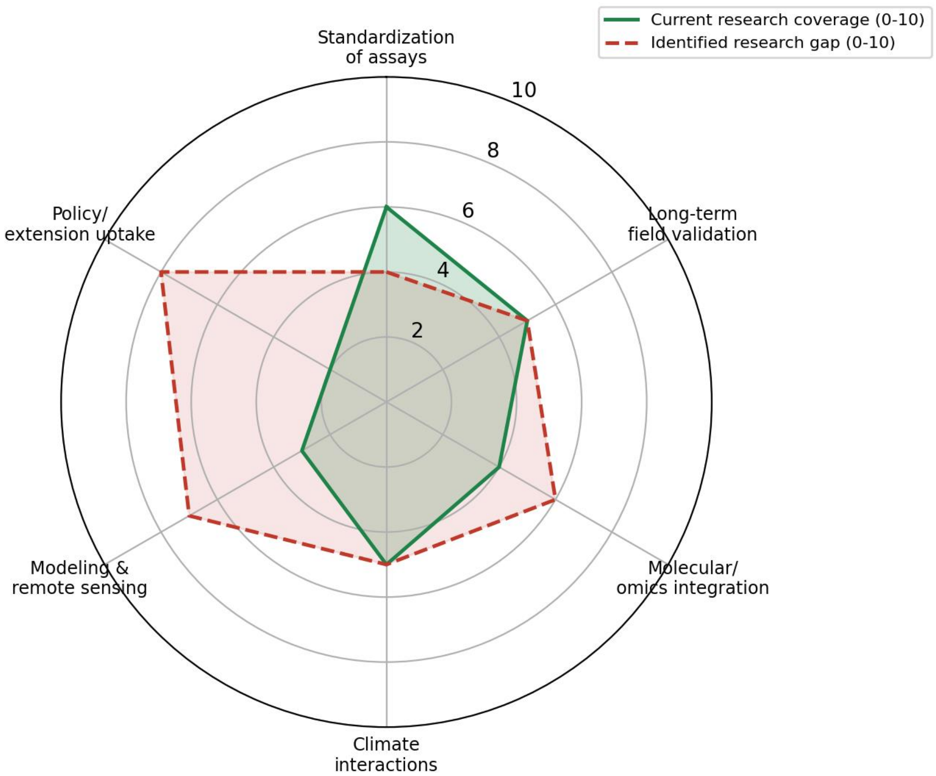

Fig 5: Research gap mapping across major thematic domains

Figure 5 maps the relative degree of current research coverage against the magnitude of identified gaps across six thematic domains, indicating that policy/extension uptake and modeling/remote-sensing integration remain the least developed areas relative to more established domains such as assay standardization and long-term field validation.

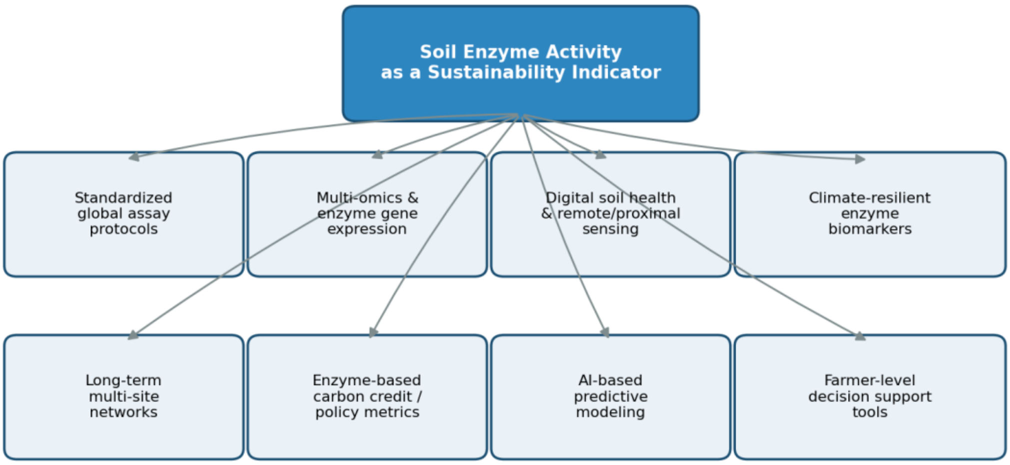

Fig 6: Priority future research directions identified from the review

6. Discussion

The synthesized evidence supports the central premise that soil enzyme activity functions as a biologically meaningful, management-responsive indicator of agroecosystem sustainability, consistent with the foundational arguments advanced by Dick [1] and subsequently elaborated across hundreds of empirical studies. Across nearly all thematic domains reviewed—tillage, organic amendment, biochar, and pollutant stress—enzyme activity, particularly dehydrogenase, phosphatase, and β-glucosidase, responded in directionally consistent ways: increasing under conservation-oriented, organically enriched management and decreasing under intensive tillage, chemical-only fertilization, and contamination [10, 11, 13, 14, 26–30]. This consistency across independent studies, sites, and research groups lends considerable weight to the use of enzymatic assays as a practical proxy for soil biological quality.

Nevertheless, the reviewed literature also reveals important sources of conflicting evidence that temper unqualified endorsement of enzyme-based indicators. The divergence noted by Puglisi *et al.* [12], in which long-cultivated soils without organic amendment exhibited enzyme activities comparable to or exceeding organically managed counterparts, illustrates that soil legacy effects, historical management, and site-specific edaphic conditions can confound straightforward management–enzyme relationships. Similarly, the weak statistical linkage between enzyme activity and crop yield reported by Sainju *et al.* [9] cautions against treating enzymatic indicators as direct surrogates for agronomic productivity; rather, they appear better suited to representing underlying functional capacity and longer-term sustainability potential than short-term yield performance. This distinction has practical significance for how enzyme-based indicators should be communicated to practitioners and policymakers, who may otherwise expect a direct, immediate translation from enzymatic improvement to yield gain.

The enzyme stoichiometry literature [3, 5, 19–23] adds theoretical depth by framing enzymatic ratios as windows into microbial nutrient economy, yet the persistent methodological controversies documented by Mori *et al.* [23] and Cui *et al.* [22] regarding the validity of steady-state assumptions and cross-biome threshold transfer indicate that this framework, while promising, is not yet mature enough for direct operational application in diverse agroecosystems without local calibration. This is a critical consideration for agroecosystems in the Global South, including tropical and semi-arid cropping systems that are underrepresented relative to temperate and alpine study sites in the current evidence base.

From a practical and policy-relevant standpoint, the evidence on biochar-mediated recovery of enzyme activity under heavy metal stress [26–29] carries direct implications for remediation strategy in polluted agricultural soils, particularly in regions such as parts of the European Union and South and East Asia where soil contamination remains widespread [29]. At the same time, the relatively underdeveloped state of digital, AI-based, and long-term monitoring approaches for enzymatic indicators—compared with their more mature application to physicochemical soil properties—suggests that near-term research investment should prioritize scalable, standardized, and field-validated tools capable of bridging the gap between academic enzymology and operational soil health monitoring frameworks used by extension services and policymakers [7, 17, 35].

7. Conclusion

The present study is a review of 33 studies and four influential works published mostly between 2015 and 2025 on the use of soil enzymes as a measure of sustainability in agriculture. The results presented support the notion that important hydrolytic and oxidoreductase enzymes (that is, dehydrogenase, phosphatases, urease, and β-glucosidase) respond quickly and with clear trends to farming practices, with the activity of the selected enzymes being usually enhanced in the application of conservation tillage, organic products and biochar, and being inhibited by intensive cultivation, chemical fertilizers, or metal pollution. While enzyme stoichiometry provides an effective theoretical basis for determining the limitations of microbial needs for nutrients, it still requires correct practical application.

The significance of this synthesis stems from establishing soil enzyme activity as a scientific and practical element for the assessment of agroecosystem sustainability and defining the conditions of validity for the interpretations of these indicators. The priorities of researchers are the development of internationally agreed protocols for conducting assays, establishment of long-term and large-scale monitoring systems similar to ecological observatories, and testing cross-biome transferability of specific thresholds of enzymatic stoichiometry. In turn, the approach of practitioners and policymakers should involve the use of enzyme indicators as components of the multilayered soil quality assessment system together with physical and chemical metrics and possibly molecular indicators rather than employing them independently; besides, the results of the analysis should be communicated with due understanding of the link between the enzyme activity indicators and short-term yields.

11. References

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