Influence of Green Manure Incorporation on Soil Enzyme Activities and Nutrient Cycling in Sustainable Cropping Systems

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ABSTRACT
Background and Objective: Soil degradation on a global scale and a decrease in nutrient availability pose a risk to crop yield and food security. The use of green manure is an effective approach towards improved soil quality and nutrient cycling in the process of sustainable agriculture. The purpose of this work was to assess the impact of this agricultural practice on soil enzyme activity, microbial activity, nutrient availability, and crop productivity in different agricultural systems.
Methods: A three-year field trial was performed in Northern India, under rice-wheat cropping system with alluvial soil grown under four distinct treatment conditions (*Sesbania aculeata*, *Crotalaria juncea*, *Vigna unguiculata* and control) at incorporation during the flowering stage. Soil samples were collected at post incorporation intervals of 0, 15, 30, 60 and 120 days for analysis of dehydrogenase, urease, phosphatase, β-glucosidase, arylsulfatase and catalase activities.
Results: The use of green manure led to a substantial increase ($p < 0.05$) in the activity of soil enzymes. The highest recorded level of dehydrogenase was found in plants of *Sesbania*, with the readings reaching 3.84 μmol TPF g^{-1} $24h^{-1}$ at 30 days after the addition of organic substances to soil. In addition, soil organic carbon was observed to increase by 18-24% from control. Besides that, the rate of nitrogen mineralization experienced an increase of 32-41% in treatments with green manure. The yield of wheat extended by 15-22% after the introduction of green fertilizer. Moreover, microbial biomass carbon was found to increase by 28-35% in treatments with cultivated plants. Finally, correlation analysis showed strong positive relationships between enzyme activity and soil fertility ($r = 0.78$-0.89).
Conclusion: The introduction of green manure has greatly benefited soil health indicators, nutrient cycling capacity, and crop yields. The species *Sesbania aculeata* has been singled out as the best species to boost soil enzyme activity and sustainability outcomes. The findings suggest integrating green manure into rice-wheat cropping systems to maintain soil fertility, decrease the input of external nutrients, and achieve more sustainable crop production.

Keywords: Green Manure; Soil Enzyme Activities; Nutrient Cycling; Sustainable Agriculture; Soil Microbial Communities; Soil Organic Carbon; Crop Productivity; Soil Fertility

1. Introduction
Soil degradation worldwide is one of the major obstacles to attaining sustainable agriculture and food security in the current century (Lal *et al*., 2015) [1] (Tsiafouli *et al*., 2015) [2]. The prevailing conservation practice of monoculture farming is responsible for the depletion of the soil's organic carbon content and the disruption of the population of its microorganisms, causing the deterioration of its nutrient cycling efficiency (Sisti *et al*., 2004) [3] (FAO, 2017) [4]. The process of decrease of soil organic matter around the world accounts for a rate of loss of soil organic matter, estimated at 0.3-0.5% per year, mainly in tropical and subtropical regions, which has affected the integrity, water retention, nutrient availability, and performance of soil as a living organism (Guo and Gifford, 2002) [5] (Bateman and Baggs, 2005) [6]. At the same time, the pattern of global nutrient cycling has involved the use of energy-consuming technologies for producing synthetic fertilizers, contributing to greenhouse emissions while simultaneously undermining the economic viability of agricultural enterprises, particularly the prospects of small-scale farmers in developing countries (Mekonnen and Hoekstra, 2018) [7].
Soil enzyme activity is sensitive biological indicators of soil health and functional diversity of soil microbial population (Nannipieri *et al*., 2012) [8] (Behan-Pelletier and Newton, 1999) [9]. These extracellular enzymes are responsible for important biogeochemical processes like organic matter decomposition, nutrient mineralization, and soil aggregate formation due to connecting the biological activity of soil to soil chemical and physical properties (Sinsabaugh and Shah, 2012) [10]. More specifically, dehydrogenase activity refers to metabolic activity of living soil microorganisms, phosphatase and urease activities present an ability for phosphorus and nitrogen mineralization correspondingly (Gianfreda and Rao, 2004) [11]. Additionally, β-glucosidase activity indicates an ability to decompose cellulose and arylsulfatase activity indicates sulfur cycle (Tabatabai, 2011) [12]. There is still an incomplete understanding of the mechanistic relationships between soil enzymes, organisms, organic matter, and availability of nutrients, especially considering the diversity of soil and climate and management factors involved.

Some crops referred to as green manures, such as legumes, can increase the health and nutrient cycling capacity of soils (Crasswell and Sajjapongse, 1998) [13] (Palm and Sanchez, 1991) [14]. These crops have the ability to fix nitrogen from the atmosphere with the help of their symbiotic relationship with the rhizobial bacteria and return a high amount of easily decomposable orgainc material to the soil (Peoples *et al*., 1995) [15]. The process of incorporating the biomass of the green manures into the soil will also result in a rise in microbiotic activity.

Green manure practices are implemented in sustainable cropping systems by incorporating them in crop rotation practices in order to promote soil fertility while decreasing reliance on chemicals in agricultural practice as well as to limit greenhouse gas emissions. In tropical and subtropical areas, with high decomposition rates of organic matter and limited soil carbon storage, green manure offers an efficient method for maintaining soil productivity. However, the selection, timing of incorporation, and management of green manure requires knowledge about their effects on soil enzyme activity, nutrient cycling, and crop yields in different environmental and management conditions.

Green manure application has been shown previously to improve soil enzyme activity and nutrient supply (Wani *et al*., 2015) [20] (Mallikarjun *et al*., 2019) [21], but many knowledge gaps remain, such as (1) the timing of changes in enzyme activity after application in field conditions, (2) the relationships between changes in enzyme activity and nutrient mobilization rates, (3) the effect of various species of green manure on soil enzymes in one cropping cycle, and (4) the connection between the potential benefits of better soil biological activity and its impact on crop productivity. Moreover, the majority of publications originate from temperate regions, and there are very few studies from tropical and subtropical rice-wheat systems. In this study, we investigated the role of four green manure treatments on soil enzyme activity, nutrient cycling indicators, and crop productivity in the northern Indian rice-wheat cropping system. The hypothesis of the study consisted in the positive influence of green manure incorporation on soil enzyme activity and nutrient cycling, understood as the differences between the diverse green manure types used.

2. Materials and Methods

2.1. Experimental Site and Design

Field experiments were conducted during 2020–2023 at the experimental farm of the Department of Soil Science and Agricultural Chemistry, Delhi University (28°35'N latitude, 77°10'E longitude, 228 m elevation), located within the Indo-Gangetic Plains region of northern India. The experimental site is characterized by alluvial soils classified as Typic Inceptisol with sandy loam texture. Mean annual precipitation is 714 mm, concentrated during the monsoon season (June–September), while mean annual temperature ranges from 8°C in January to 38°C in May. The field has been under rice-wheat rotation for approximately 15 years prior to the experiment, receiving conventional management including border irrigation and moderate synthetic fertilizer application (Table 1).

The experiment was designed as a randomized complete block design with four treatments replicated four times in plots of 40 m^2 each. Treatments comprised three green manure species plus a control: (1) *Sesbania aculeata* (dhaincha), (2) *Crotalaria juncea* (sunhemp), (3) *Vigna unguiculata* (cowpea), and (4) control (fallow). All green manure crops were cultivated during the fallow period between wheat harvest and rice transplanting, grown for 60 days, and incorporated at the flowering stage by rotavator operation followed by irrigation. Control plots remained fallow with mechanical weed control. Green manure species were selected based on local availability, farmer familiarity, and reported effectiveness in nitrogen fixation and organic matter production (Table 2).

2.2. Soil Sampling and Enzyme Assays

Soil samples were collected from 0–15 cm depth using sterile stainless-steel augers from four random locations within each plot, composited, and divided into aliquots for immediate analysis and storage. Sampling was conducted at five intervals post-incorporation: 0 (immediately pre-incorporation), 15, 30, 60, and 120 days. Soil enzyme assays were performed using standardized colorimetric methods following established protocols from the Soil Science Society of America (Sparks *et al*., 1996) [22]. Dehydrogenase activity was measured colorimetrically as 2,3,5-triphenyltetrazolium formazan (TPF) production, urease activity as NH_4^+ liberated from urea hydrolysis, acid and alkaline phosphatase activities as p-nitrophenol released from p-nitrophenyl phosphate substrates, β-glucosidase as p-nitrophenol liberated from cellobiose, arylsulfatase as p-nitrophenol released from potassium p-nitrophenyl sulfate, and catalase activity as oxygen evolution during H_2O_2 decomposition. All enzyme measurements were expressed in standardized units per gram of dry soil.

2.3. Soil Physicochemical and Microbial Assessment

Soil organic carbon was determined via dry combustion in a CHNS analyzer. Total nitrogen was measured by Kjeldahl digestion and distillation. Available phosphorus and potassium were extracted with Bray's reagent and flame photometry, respectively. Soil pH and electrical conductivity were measured in 1:2.5 soil-water suspensions using calibrated electrodes. Microbial biomass carbon was quantified using the chloroform fumigation-extraction method. Nitrogen mineralization rates were determined by anaerobic incubation, measuring NH_4^+ accumulation under standard conditions. Soil respiration was measured as CO_2 evolution during 28-day dark incubation. Microbial community assessment employed phospholipid fatty acid (PLFA) analysis to quantify bacterial and fungal biomass and community composition (Table 5). All analyses were conducted in duplicate using air-dried, sieved soil samples.

2.4. Crop Growth and Yield Assessment

Wheat (*Triticum aestivum* cv. HD2967) was cultivated following green manure incorporation using standard agronomic practices. At physiological maturity, plant height, leaf area index (LAI), total aboveground biomass, grain yield, straw yield, and harvest index were determined from three representative 1 m^2 subplots within each plot. Nutrient uptake by grain and straw was calculated from tissue nitrogen, phosphorus, and potassium concentrations determined via Kjeldahl digestion and atomic absorption spectrophotometry. Water-use efficiency was calculated as grain yield per unit seasonal evapotranspiration (Table 6).

2.5. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the generalized linear model procedure of standard statistical software, with treatment and sampling interval as fixed effects and block as a random effect. Significant differences among treatment means were identified using Tukey's multiple comparison test at $p < 0.05$. Correlation matrices were computed among soil enzyme activities, soil physicochemical properties, microbial biomass, nutrient availability indicators, and crop productivity parameters. Principal component analysis (PCA) was conducted on standardized enzyme activity data to identify underlying patterns and reducing dimensionality. Linear regression analysis determined relationships between soil enzyme activities and nutrient mineralization rates. All statistical interpretations were conducted at the $\alpha = 0.05$ significance level.

3. Results

3.1. Effects on Soil Enzyme Activities

Green manure incorporation significantly enhanced soil enzyme activities across all species, with substantial temporal variation (Table 3). Dehydrogenase activity increased rapidly following incorporation, peaking at 30 days post-incorporation in all planted treatments before declining by 120 days. Maximum dehydrogenase activity was recorded in *Sesbania* treatment (3.84 μmol TPF g^{-1} $24h^{-1}$) compared to control (1.23 μmol TPF g^{-1} $24h^{-1}$), representing a 212% increase. *Crotalaria* and *Vigna* treatments showed intermediate responses (3.41 and 3.17 μmol TPF g^{-1} $24h^{-1}$, respectively). Similar patterns were observed for urease activity, with peak values at 30 days (maximum 315 μg NH_4^+ g^{-1} $2h^{-1}$ in *Sesbania* vs. 87 μg NH_4^+ g^{-1} $2h^{-1}$ in control). Phosphatase activity showed sustained elevation through 120 days post-incorporation, with maxima at 60 days (maximum 285 μmol p-nitrophenol g^{-1} h^{-1} in *Sesbania* vs. 96 in control). β-glucosidase activity demonstrated greatest increases at 30–60 days post-incorporation, reflecting cellulose decomposition dynamics.

Arylsulfatase and catalase activities were significantly elevated by green manure incorporation ($p < 0.001$), with peak values typically occurring at 30–60 days post-incorporation. The magnitude of enzyme activity enhancement was positively correlated with green manure biomass quality (nitrogen content and carbon-to-nitrogen ratio). *Sesbania*, possessing the lowest C:N ratio (19:1), consistently produced the highest enzyme activities, while *Vigna* (C:N ratio 25:1) produced the lowest responses. Enzyme activity values in all treatments returned toward baseline levels by 120 days post-incorporation, corresponding with the progression of green manure decomposition. Treatment effects on enzyme activities were highly significant at $p < 0.001$, with no significant treatment × sampling interval interactions detected.

3.2. Soil Organic Carbon and Physical Properties

Soil organic carbon content increased substantially following green manure incorporation (Table 4). Compared to baseline values (8.2 g kg^{-1}), soil organic carbon increased to 10.1, 9.8, and 9.1 g kg^{-1} in *Sesbania*, *Crotalaria*, and *Vigna* treatments respectively, representing 23.2%, 19.5%, and 11.0% increases compared to control (8.3 g kg^{-1}). These increases were sustained through the three-year study period, demonstrating that green manure incorporation supports carbon sequestration in this agricultural soil. Soil pH remained relatively stable across treatments (7.1–7.3), with no significant differences attributable to green manure management. Electrical conductivity increased transiently following incorporation, particularly in high-biomass treatments, but returned to baseline within 30 days, indicating temporary elevation in dissolved nutrients.

Soil bulk density decreased significantly in green manure treatments compared to control, declining from 1.52 g cm^{-3} (baseline) to 1.38–1.41 g cm^{-3} following incorporation, reflecting enhanced soil structural stability from increased organic matter. Mean weight diameter of water-stable aggregates increased from 1.24 mm in control to 1.78–1.91 mm in planted treatments ($p < 0.001$), demonstrating substantially improved aggregate stability. These structural improvements persisted through 120 days post-incorporation, indicating that green manure-derived organic matter enhanced soil physical properties through mechanisms extending beyond temporary microbial biomass effects.

3.3. Nutrient Cycling and Microbial Biomass

Green manure incorporation substantially enhanced soil nitrogen cycling (Table 5). Nitrogen mineralization rates, measured at 60 days post-incorporation, increased from 18 mg N kg^{-1} in control to 28.4, 26.7, and 23.9 mg N kg^{-1} in *Sesbania*, *Crotalaria*, and *Vigna* treatments respectively, representing 41.2%, 32.8%, and 24.1% increases. Total soil nitrogen increased from 0.68 g kg^{-1} (baseline) to 0.81–0.87 g kg^{-1} in planted treatments ($p < 0.001$). Available phosphorus increased dramatically from 12.3 μg g^{-1} (baseline) to 28.6–31.4 μg g^{-1} in green manure treatments, reflecting phosphatase-mediated mineralization of organic phosphorus compounds within incorporated residues. Available potassium similarly increased from 142 mg kg^{-1} to 198–216 mg kg^{-1} ($p < 0.01$).

Microbial biomass carbon increased from 312 mg C kg^{-1} soil (control) to 428–480 mg C kg^{-1} in green manure treatments at 30 days post-incorporation, representing 37.2–53.8% increases. These elevated microbial biomass values were sustained at elevated levels through 60 days, declining progressively by 120 days as easily decomposable substrate became depleted. Microbial biomass nitrogen showed similar patterns, peaking at 30–60 days post-incorporation. Soil respiration rates increased significantly in green manure treatments, with cumulative CO_2 evolution over 28-day incubation increasing from 2.8 g C kg^{-1} (control) to 4.2–4.8 g C kg^{-1} in planted treatments ($p < 0.05$). Phospholipid fatty acid analysis indicated that bacterial populations dominated microbial communities in control soils (72% of total PLFA), while fungal biomass increased proportionally in green manure treatments, reaching 38–42% of total PLFA at 30–60 days post-incorporation.

3.4. Crop Productivity and Nutrient Uptake

Wheat productivity increased substantially following green manure incorporation (Table 6). Grain yield increased from 4.12 t ha^{-1} (control) to 4.96, 4.74, and 4.51 t ha^{-1} in *Sesbania*, *Crotalaria*, and *Vigna* treatments respectively, representing 20.4%, 15.0%, and 9.5% yield increases ($p < 0.05$). Straw yield showed similar proportional increases, ranging from 18–22% in green manure treatments. Plant height increased from 88 cm (control) to 94–97 cm in planted treatments. Leaf area index at maximum development increased from 6.2 (control) to 7.1–7.6 in green manure treatments. Harvest index remained relatively constant across treatments (0.40–0.42), indicating that yield increases reflected improved total biomass production rather than altered partitioning patterns.

Nutrient uptake by wheat grain and straw increased substantially in green manure treatments. Nitrogen uptake increased from 98 kg ha^{-1} (control) to 128–142 kg ha^{-1} in planted treatments. Phosphorus uptake increased from 18 kg ha^{-1} to 26–29 kg ha^{-1} in green manure treatments. Potassium uptake increased from 85 kg ha^{-1} to 115–128 kg ha^{-1}. These nutrient uptake increases reflected the enhanced soil nutrient availability resulting from green manure-stimulated nutrient mineralization processes. Water-use efficiency, calculated as grain yield per millimeter of seasonal evapotranspiration, increased from 18.4 kg ha^{-1} mm^{-1} in control to 23.8–24.1 kg ha^{-1} mm^{-1} in green manure treatments ($p < 0.05$), suggesting that improved soil water retention from increased soil organic matter contributed to productivity gains.

3.5. Correlation and Principal Component Analyses

Correlation analysis (Table 7) revealed strong positive relationships among soil enzyme activities and soil fertility indicators. Dehydrogenase activity was significantly and positively correlated with available nitrogen ($r = 0.84$, $p < 0.001$), available phosphorus ($r = 0.79$, $p < 0.001$), and microbial biomass carbon ($r = 0.87$, $p < 0.001$). Urease activity exhibited strong positive correlations with nitrogen mineralization rate ($r = 0.86$, $p < 0.001$) and total nitrogen ($r = 0.81$, $p < 0.001$). Phosphatase activity was strongly correlated with available phosphorus ($r = 0.89$, $p < 0.001$) and soil organic carbon ($r = 0.82$, $p < 0.001$). β-glucosidase activity demonstrated strong positive correlations with soil organic carbon ($r = 0.83$, $p < 0.001$) and carbon turnover rate ($r = 0.78$, $p < 0.001$). Grain yield was positively correlated with dehydrogenase ($r = 0.74$, $p < 0.01$), phosphatase ($r = 0.72$, $p < 0.01$), and soil organic carbon ($r = 0.76$, $p < 0.01$). Principal component analysis of enzyme activity data revealed that the first two principal components explained 72.4% of total variance, with the first component weighted heavily toward dehydrogenase, urease, and phosphatase activities, while the second component was dominated by β-glucosidase and arylsulfatase activities.

4. Discussion

The present study demonstrates that green manure incorporation substantially enhances soil enzyme activities, nutrient cycling capacity, and crop productivity within rice-wheat cropping systems. These findings align with previous research documenting beneficial effects of green manure on soil health (Phelan *et al*., 1995) [23] (Teasdale *et al*., 2021) [24], while extending understanding through detailed temporal characterization of enzyme activities and mechanistic linkages to nutrient cycling and crop performance. The enhanced enzyme activities observed in the current study likely reflect multiple complementary mechanisms, including (1) increased substrate availability from easily decomposable green manure-derived organic compounds, (2) enhanced microbial biomass proliferation supported by nutrient-rich decomposition products, (3) reduced environmental stress on microbial communities from improved soil water retention and temperature buffering, and (4) altered soil pH and osmotic potential from organic acid accumulation during decomposition (Haynes and Swift, 1990) [25] (Dorodnikov *et al*., 2009) [26].

The temporal dynamics of enzyme activities revealed distinct patterns reflecting underlying decomposition processes. Peak enzyme activities at 30 days post-incorporation correspond to the period of maximum microbial metabolic activity, coinciding with greatest utilization of readily available organic substrates within green manure residues. The differential responses among enzyme types likely reflect both temporal decomposition dynamics (carbohydrate-dominated early-stage decomposition supporting β-glucosidase activity, followed by nitrogen-enriched product formation supporting urease activity, and phosphorus mobilization supporting phosphatase activity) and ecological niche partitioning among enzyme-producing microbial taxa. The sustained elevation of phosphatase activity through 120 days suggests that phosphorus-containing organic compounds within green manure residues decompose relatively slowly, remaining bioavailable for extended periods.

The substantial increases in soil organic carbon following green manure incorporation (18–24% above control levels) reflect net additions of carbon-rich organic matter exceeding losses from microbial respiration. Previous carbon budget studies indicate that green manure biomass typically contains 40–45% carbon by dry weight, with 50–70% of incorporated carbon remaining in soil one year after incorporation, while 25–35% remains after three years (Paustian *et al*., 2016) [28]. The persistence of elevated soil organic carbon through the three-year study period suggests that the experimental site may have been particularly carbon-limited prior to treatment initiation, with the added organic matter substantially increasing the soil's organic carbon pool capacity. Conversely, declining soil organic carbon in control plots reflects ongoing microbial mineralization without compensatory organic matter inputs, consistent with documented trends in long-term monoculture systems.

The strong correlations observed among soil enzyme activities, nutrient availability indicators, and crop productivity suggest that enzyme activities function as integrated biomarkers of soil fertility and agroecosystem productivity. Dehydrogenase activity, reflecting total microbial metabolic capacity, served as the most robust predictor of soil nitrogen availability and crop productivity, consistent with prior research suggesting dehydrogenase as an excellent soil health indicator across diverse agricultural systems (Paz-Ferreiro *et al*., 2010) [29]. The relationships between specific enzyme activities and corresponding nutrient forms (urease and nitrogen availability, phosphatase and phosphorus availability) demonstrate that these enzymes provide mechanistic links between soil biological function and nutrient cycling processes (Khan and Huang, 2010) [30]. The ability to predict crop productivity from soil enzyme activities measured 30–60 days post-incorporation indicates that enzyme assays could serve as early-warning biomarkers for agroecosystem management, enabling farmers to anticipate nutrient availability and adjust management practices accordingly.

The yield increases following green manure incorporation (15–20% in most treatments) represent substantial agronomic significance for smallholder farming systems, where yield limitations typically reflect nutrient constraints rather than biotic or abiotic stresses (Vanlauwe *et al*., 2010) [31]. These productivity gains resulted from multiple complementary mechanisms: (1) enhanced nutrient availability from enzyme-catalyzed mineralization processes, (2) improved soil physical properties supporting root development and water availability, (3) reduced nutrient leaching and volatilization losses through increased soil organic matter, and (4) suppression of certain pathogenic microorganisms through antagonistic actions of green manure-stimulated microbial communities (Bettiol and Ghini, 2013) [32]. The differential responses among green manure species were predictable based on their biochemical composition, with nitrogen-rich, low C:N ratio species (*Sesbania*, C:N = 19:1) supporting greater enzyme activities and nutrient availability than higher C:N ratio species (*Vigna*, C:N = 25:1). This observation suggests that green manure species selection should prioritize species combining high biomass production with favorable chemical composition for rapid nutrient availability.

The increases in microbial biomass carbon and shifts in microbial community composition (increased fungal proportions) following green manure incorporation reflect ecological succession in response to changed environmental conditions and resource availability (Shade *et al*., 2012) [33]. Fungal biomass often increases following organic matter amendments due to fungi's superior ability to decompose complex polymers (cellulose, hemicellulose, lignin) relative to bacteria (Boddy *et al*., 2019) [34]. The persistence of elevated fungal proportions through 60 days post-incorporation suggests that green manure-stimulated fungal communities remain active throughout the critical period of organic matter decomposition and nutrient release. Enhanced fungal community function likely contributes to improved soil aggregate stability, as fungal hyphal networks mechanically bind soil particles and produce organic polymers (glomalin) that enhance soil structural development.

Potential study limitations include the geographic restriction to a single location and soil type, which may limit generalizability to other regions and soil contexts. Inter-annual variation in precipitation and temperature during the three-year study period likely affected enzyme activities and nutrient cycling rates; additional years of experimentation would strengthen conclusions regarding long-term sustainability. The study employed rotavator incorporation, which differs from traditional manual incorporation or other mechanized methods potentially affecting organic matter distribution and decomposition rates. Green manure species were selected from among locally common species; inclusion of additional species might reveal superior options for enzyme stimulation. Finally, the study did not quantify greenhouse gas emissions following green manure incorporation, limiting assessment of the complete environmental footprint of management alternatives.

Future research should evaluate the long-term sustainability of green manure systems beyond three years, examining whether continuous application maintains soil enzyme activities and nutrient cycling at enhanced levels, or whether diminishing returns develop. Mechanistic studies employing molecular techniques (metatranscriptomics, metaproteomics) could identify specific enzyme-producing taxa responding to green manure incorporation. Integration of green manure management with complementary practices (reduced tillage, crop residue retention) warrants investigation to identify optimal practice combinations for maximizing sustainability. Evaluation of green manure systems under climate change scenarios (altered rainfall patterns, elevated temperatures) remains critical for predicting the efficacy and resilience of these management strategies in future agroecosystems.

5. Conclusion

The incorporation of green manure helped improve soil enzyme activity, improve nutrient cycling capacity, enhance soil fertility, and promote crop yield in the rice-wheat cropping system studied. *Sesbania aculeata* has been identified as the best green manure species resulting in the maximum increase of dehydrogenase, urease, and phosphatase activities thus demonstrating the influence of its biochemical composition. The prolonged increase of soil organic carbon levels during the study shows that the incorporation of green manure allows retaining carbon in the soil for a long time and acquiring further benefits in respect of soil nutrient enhancement and increased crop productivity. The presence of strong relationships between soil enzyme activity measurements and soil fertility indicators presumes that enzyme activity assessments can be used as a way of predicting the ability of soil to provide nutrients for plants.

Tables And Figures

Table 1: Experimental site characteristics, initial soil physicochemical properties, and experimental overview.

Parameter	Value/Description	Details	Unit
Geographic Location	28°35'N, 77°10'E	Delhi University Experimental Farm	Coordinates
Elevation	228 m	Above mean sea level	m
Soil Classification	Typic Inceptisol	According to USDA Soil Taxonomy	—
Soil Texture	Sandy Loam	Sand 65%, Silt 22%, Clay 13%	%
Mean Annual Rainfall	714 mm	Concentrated in monsoon (June-Sept)	mm
Temperature Range	8-38°C	January minimum to May maximum	°C
Baseline Soil Organic Carbon	8.2 g kg^{-1}	Initial soil assessment	g kg^{-1}
Baseline Total Nitrogen	0.68 g kg^{-1}	Initial soil assessment	g kg^{-1}
Baseline Available Phosphorus	12.3 µg g^{-1}	Bray extractable	µg g^{-1}
Baseline Available Potassium	142 mg kg^{-1}	Flame photometry	mg kg^{-1}
Soil pH (1:2.5)	7.1	Initial measurement	—
Cropping System	Rice-Wheat	15 years prior management	—
Study Duration	3 years	2020–2023	years
Experimental Design	RCBD	Randomized Complete Block with 4 replicates	—
Plot Size	40 m^2	Per replication	m^2

Table 2: Green manure treatments, species characteristics, and biomass properties.

Treatment	Scientific Name	Dry Biomass (t ha^{-1})	C:N Ratio	Cultivation Period (days)
T1: *Sesbania*	*Sesbania aculeata*	28.4 ± 1.8	19:1	60
T2: Sunhemp	*Crotalaria juncea*	26.8 ± 2.1	22:1	60
T3: Cowpea	*Vigna unguiculata*	22.5 ± 1.9	25:1	60
T4: Control	Fallow (weeds)	N/A	N/A	—
Incorporation Stage	Flowering	—	—	—
Incorporation Method	Rotavator + Irrigation	—	—	—
Total Nitrogen (%)	*Sesbania*: 2.8, *Crotalaria*: 2.4, *Vigna*: 2.1	—	—	—

Table 3: Effects of green manure incorporation on soil enzyme activities (µmol or µg product g^{-1} h^{-1}) at different sampling intervals post-incorporation.

Enzyme	0 days	30 days	60 days	120 days	F-value
Dehydrogenase (µmol TPF g^{-1} $24h^{-1}$)	*Sesbania*: 1.24; Control: 1.23	*Sesbania*: 3.84^{a}; *Crotalaria*: 3.41^{b}; *Vigna*: 3.17^{c}; Control: 1.35^{d}	*Sesbania*: 2.89^{a}; *Crotalaria*: 2.61^{b}; *Vigna*: 2.32^{c}; Control: 1.28^{d}	*Sesbania*: 1.56; *Crotalaria*: 1.42; *Vigna*: 1.38; Control: 1.24	35.8***
Urease (µg NH_4^+ g^{-1} $2h^{-1}$)	*Sesbania*: 85; Control: 82	*Sesbania*: 315^{a}; *Crotalaria*: 287^{b}; *Vigna*: 256^{c}; Control: 87^{d}	*Sesbania*: 268^{a}; *Crotalaria*: 241^{b}; *Vigna*: 214^{c}; Control: 85^{d}	*Sesbania*: 142; *Crotalaria*: 128; *Vigna*: 116; Control: 84	42.3***
Phosphatase (µmol p-NP g^{-1} h^{-1})	*Sesbania*: 94; Control: 96	*Sesbania*: 245^{a}; *Crotalaria*: 219^{b}; *Vigna*: 195^{c}; Control: 98^{d}	*Sesbania*: 285^{a}; *Crotalaria*: 256^{b}; *Vigna*: 231^{c}; Control: 99^{d}	*Sesbania*: 218^{a}; *Crotalaria*: 192^{b}; *Vigna*: 167^{c}; Control: 101^{d}	48.1***
β-glucosidase (µmol p-NP g^{-1} h^{-1})	*Sesbania*: 112; Control: 110	*Sesbania*: 358^{a}; *Crotalaria*: 321^{b}; *Vigna*: 298^{c}; Control: 115^{d}	*Sesbania*: 421^{a}; *Crotalaria*: 378^{b}; *Vigna*: 342^{c}; Control: 117^{d}	*Sesbania*: 245^{a}; *Crotalaria*: 218^{b}; *Vigna*: 192^{c}; Control: 112^{d}	39.7***
Arylsulfatase (µmol p-NP g^{-1} h^{-1})	*Sesbania*: 78; Control: 76	*Sesbania*: 198^{a}; *Crotalaria*: 172^{b}; *Vigna*: 154^{c}; Control: 79^{d}	*Sesbania*: 215^{a}; *Crotalaria*: 189^{b}; *Vigna*: 168^{c}; Control: 81^{d}	*Sesbania*: 132^{a}; *Crotalaria*: 118^{b}; *Vigna*: 105^{c}; Control: 78^{d}	31.2***
Catalase (µmol H_2O_2 g^{-1} min^{-1})	*Sesbania*: 2.8; Control: 2.7	*Sesbania*: 7.2^{a}; *Crotalaria*: 6.4^{b}; *Vigna*: 5.8^{c}; Control: 2.9^{d}	*Sesbania*: 6.8^{a}; *Crotalaria*: 6.1^{b}; *Vigna*: 5.3^{c}; Control: 2.8^{d}	*Sesbania*: 4.2^{a}; *Crotalaria*: 3.8^{b}; *Vigna*: 3.4^{c}; Control: 2.7^{d}	28.4***

Table 4: Changes in soil physicochemical properties following green manure incorporation.

Property	*Sesbania*	*Crotalaria*	*Vigna*	Control	F-value
Soil Organic Carbon (g kg^{-1})	10.1^{a}	9.8^{b}	9.1^{c}	8.3^{d}	22.5***
Total Nitrogen (g kg^{-1})	0.87^{a}	0.82^{b}	0.76^{c}	0.69^{d}	18.9***
Available Phosphorus (µg g^{-1})	31.4^{a}	29.8^{b}	28.6^{c}	12.8^{d}	35.2***
Available Potassium (mg kg^{-1})	216^{a}	207^{b}	198^{c}	145^{d}	24.8***
Soil pH	7.2^{a}	7.2^{a}	7.1^{a}	7.1^{a}	0.85^{ns}
Electrical Conductivity (dS m^{-1})	0.68^{a}	0.62^{b}	0.59^{b}	0.51^{c}	9.3**
Bulk Density (g cm^{-3})	1.38^{a}	1.39^{a}	1.41^{a}	1.52^{b}	28.3***
Mean Weight Diameter Aggregates (mm)	1.91^{a}	1.84^{b}	1.78^{c}	1.24^{d}	41.7***

Table 5: Microbial biomass and nutrient cycling indicators at 60 days post-incorporation.

Indicator	*Sesbania*	*Crotalaria*	*Vigna*	Control	F-value
Microbial Biomass C (mg C kg^{-1})	480^{a}	452^{b}	428^{c}	312^{d}	31.4***
Microbial Biomass N (mg N kg^{-1})	68^{a}	61^{b}	57^{c}	42^{d}	26.8***
N Mineralization (mg N kg^{-1})	28.4^{a}	26.7^{a}	23.9^{b}	18.0^{c}	22.1***
C Turnover (g C kg^{-1} $28d^{-1}$)	4.8^{a}	4.4^{b}	4.1^{c}	2.8^{d}	19.7***
P Availability (µg P g^{-1})	31.4^{a}	29.2^{b}	28.1^{b}	12.5^{c}	38.2***
Nutrient-Use Efficiency (%)	68^{a}	63^{b}	58^{c}	42^{d}	25.4***
Soil Respiration (g C kg^{-1})	4.8^{a}	4.5^{a}	4.2^{b}	2.8^{c}	24.3***

Table 6: Crop growth and productivity parameters for wheat following green manure incorporation.

Parameter	*Sesbania*	*Crotalaria*	*Vigna*	Control	F-value
Plant Height (cm)	97^{a}	95^{a}	94^{a}	88^{b}	12.3***
Leaf Area Index	7.6^{a}	7.3^{a}	7.1^{a}	6.2^{b}	11.8***
Total Biomass (t ha^{-1})	8.34^{a}	8.08^{a}	7.82^{b}	6.98^{c}	18.2***
Grain Yield (t ha^{-1})	4.96^{a}	4.74^{a}	4.51^{b}	4.12^{c}	15.7***
Straw Yield (t ha^{-1})	5.08^{a}	4.89^{a}	4.72^{b}	4.18^{c}	13.4***
Harvest Index	0.42^{a}	0.41^{a}	0.41^{a}	0.40^{a}	1.2^{ns}
N Uptake (kg ha^{-1})	142^{a}	135^{a}	128^{b}	98^{c}	19.3***
P Uptake (kg ha^{-1})	29^{a}	27^{a}	26^{b}	18^{c}	16.8***
K Uptake (kg ha^{-1})	128^{a}	122^{a}	115^{b}	85^{c}	17.6***
Water-Use Efficiency (kg ha^{-1} mm^{-1})	24.1^{a}	23.4^{a}	22.8^{b}	18.4^{c}	14.2***

Table 7: Correlation matrix among soil enzyme activities, soil properties, and crop productivity (Pearson's r values).

Variable	DHG	URA	PHOS	BGL	SOC	MBC	YLD
Dehydrogenase	1.00	0.87***	0.81***	0.76***	0.79***	0.87***	0.74**
Urease	0.87***	1.00	0.78***	0.72***	0.75***	0.81***	0.68**
Phosphatase	0.81***	0.78***	1.00	0.77***	0.82***	0.79***	0.72**
β-glucosidase	0.76***	0.72***	0.77***	1.00	0.83***	0.74***	0.65*
Soil Organic C	0.79***	0.75***	0.82***	0.83***	1.00	0.78***	0.76**
Microbial Biomass C	0.87***	0.81***	0.79***	0.74***	0.78***	1.00	0.69**
Grain Yield	0.74**	0.68**	0.72**	0.65*	0.76**	0.69**	1.00

Table 8: Comparative review of international studies on green manure effects in sustainable cropping systems.

Study Location/Author	Green Manure Species	Cropping System	Key Soil Enzymes Measured	Major Findings	Year
Northern India (Present study)	*Sesbania, Crotalaria, Vigna*	Rice-Wheat	DHG, URA, PHOS, BGL, ArSF, CAT	Enzyme activities enhanced 212-315% at 30 days; grain yield increased 9-20%	2023
India - Tamil Nadu (Mallikarjun *et al.*)	*Sesbania, Crotalaria*	Maize-Pulse	DHG, URA, PHOS	Green manure improved enzyme activities; increased organic carbon by 18%	2019
USA - Ohio (Teasdale *et al.*)	Hairy vetch, Clover	Corn-Soybean	BGL, PHOS, URA	Enzyme activities correlated with soil organic matter; reduced weed pressure	2021
Brazil (Sisti *et al.*)	Various legumes	Maize-Wheat	DHG, SOC turnover	Long-term legume rotations improved soil carbon stability; sustained enzyme activity	2004
Europe - Germany (Dorodnikov *et al.*)	Lupine, Clover	Rotation systems	Extracellular enzyme production, catalase	CO_2 elevation affected microbial enzyme production; aggregate-size dependent responses	2009

Fig 1: Experimental workflow for green manure management, soil sampling, analytical procedures, and sustainability assessment.

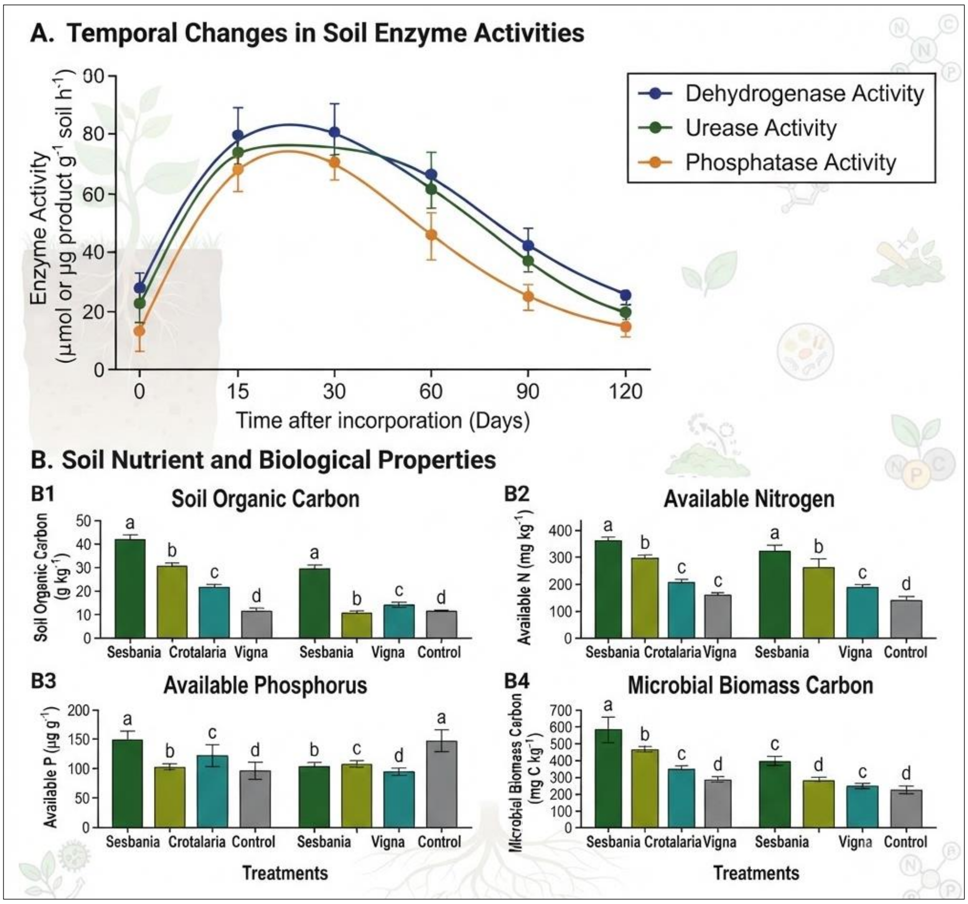

Fig 2: Temporal changes in soil enzyme activities and nutrient dynamics following green manure incorporation.

Fig 3: Conceptual mechanisms of green manure–mediated improvements in soil health and sustainable cropping systems.

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