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Influence of in-situ green manure intercropping and nitrogen levels on soil microbial dynamics and enzymatic activities in a maize-maize cropping system under the southern transition zone of Karnataka

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Abstract
A field experiment was conducted during the *Kharif* and *Rabi* seasons of 2023-24 and 2024-25 at ZAHRS, Shivamogga, to assess the influence of in-situ green manure intercrops and nitrogen (N) levels on the soil microbial population and enzymatic activities in maize-maize sequence. The experiment followed a split-plot design with three nitrogen levels (75%, 100% and 125% RDN) and four green-manure based intercropping systems: sole maize, maize + cowpea, maize + horse gram and maize + sunhemp. Data indicated that 125% RDN produced the highest grain yield in all seasons, whereas 100% RDN resulted in superior microbial population and enzyme activities. Microbial populations and enzyme activities at harvest were consistently higher under sunhemp and lowest under sole maize. The interaction effects were non-significant across seasons; however, the combination of maize + sunhemp with 100% RDN registered the most favourable balance between productivity and soil microbial activity. Overall, in-situ incorporation of green manure—particularly sunhemp—proved effective in enhancing soil biological health and improving system sustainability in humid tropical maize production systems.

Keywords: Maize, green manure, nitrogen, microbial population, enzyme activities

Introduction
Maize is a major cereal crop in the Southern Transition Zone of Karnataka and forms the backbone of several intensive cropping systems. However, continuous maize cultivation has resulted in the depletion of soil organic matter, stagnation in crop yields and an increased reliance on nitrogen (N) fertilizers. Excessive use of N not only elevates production costs but also contributes to greenhouse gas emissions. Consequently, there is a growing need for sustainable nutrient management strategies that integrate biological nitrogen inputs, improve soil health and sustain crop productivity.

Legume green manures such as cowpea, horse gram and sunhemp offer considerable potential to enhance nutrient cycling, contribute biologically fixed nitrogen and stimulate soil microbial activity. When incorporated in situ at early vegetative stages, these legumes supply readily decomposable organic matter that fosters microbial proliferation and accelerates nutrient mineralization.

Soil biological properties serve as sensitive indicators of soil health because they regulate organic matter turnover, nutrient cycling and overall ecosystem function. The addition of organic residues through green manuring substantially influences microbial biomass, activity and enzyme-mediated processes. Continuous organic matter incorporation improves soil fertility while enhancing microbial-driven nutrient transformations, which are essential for sustained soil productivity (Bastida *et al*., 2008) [1].

Microbial communities respond more rapidly to management interventions than most soil physical and chemical properties. Hence, microbiological parameters—such as microbial population and soil enzyme activities—have been widely recognized as reliable indicators of soil quality due to their sensitivity to changes in soil management. Among these, dehydrogenase activity is frequently used as a general measure of overall microbial metabolic activity. Soil enzymes play critical biochemical roles in organic matter decomposition and nutrient release, thereby reflecting shifts in soil biological functioning in response to management practices.

Despite several studies documenting the benefits of green manure incorporation on soil fertility and crop productivity, limited information exists on the combined effects of different green manure intercrops and graded nitrogen levels on soil microbial dynamics, productivity, energetics and economics under the humid tropical conditions of Karnataka. Therefore, the present study was undertaken to evaluate the influence of in-situ incorporation of legume green manure intercrops and varied nitrogen levels on yield performance, microbial population, energy use efficiency and economics in a maize-maize cropping sequence.

Materials and Methods

The field experiment was carried out over four seasons (*Kharif* and *Rabi* 2023-24 and 2024-25) at ZAHRS, Shivamogga. The soil was red sandy loam, moderately acidic, low in nitrogen and medium in phosphorus and potassium. A split-plot design was adopted with three nitrogen levels in the main plots and four green manure intercropping systems in subplots.

Treatment details

Main plot (Nitrogen levels)

N_1: 75% RDN

N_2: 100% RDN

N_3: 125% RDN

Subplot (Intercropping systems)

I_0: Sole maize

I_1: Maize + cowpea

I_2: Maize + horse gram

I_3: Maize + sunhemp

Green manure crops were incorporated at 45 DAS. No fertilizers were applied to green manure crops.

Standard agronomic practices were followed except for imposed treatments. Maize grain yield was measured at harvest at 15% moisture content for each treatment and expressed in kg ha^{-1}.

Microbial populations (bacteria, fungi, actinomycetes) were estimated using serial dilution plate count technique following Tate (1995) [22]. Soil samples (treatment-wise) were collected at harvest and analysed using the following methods

Bacteria (10^6 CFU g^{-1} soil)	36	Serial dilution plate technique (Bunt and Rovira, 1955) [4]
Fungi (10^4 CFU g^{-1} soil)	8.35	Serial dilution plate technique (Kuster and Williams, 1964) [12]
Actinomycetes (10^3 CFU g^{-1} soil)	2.65	Serial dilution plate technique (Norris, 1959) [18]
Urease activity (µg urea hydrolyzed g^{-1} hr^{-1})	10.30	Colorimetric ammonium determination (Kandeler and Gerber, 1988) [10]
Dehydrogenase activity (µg TPF g^{-1} day^{-1})	28.50	2,4,5-Tryphenyl Tetrazolium Chloride (Casida *et al*., 1964) [5]

The data recorded on yield microbial population were subjected to analysis of variance. The level of significance used in the 'F' test was at 5 per cent. Pooled analysis was carried out as per the procedure outlined by Gomez and Gomez (1984) since seasonal variation was non-significant for most parameters.

Results and Discussion

Microbial population

The results demonstrated that microbial populations in the maize rhizosphere, including bacteria, fungi and actinomycetes, were significantly influenced by green manure intercropping, whereas nitrogen levels exerted a comparatively less effect (Table 1).

Although statistically non-significant, the application of 100 per cent RDN (N_2) consistently supported relatively higher populations of all microbial groups compared to 75 per cent RDN (N_1) and 125 per cent RDN (N_3). Optimum nitrogen likely enhanced root growth and exudation, thereby creating a favourable environment for microbial proliferation. In contrast, excessive nitrogen (125% RDN) may have suppressed microbial activity by altering the soil C:N ratio, increasing osmotic stress and promoting bacterial dominance over fungi and actinomycetes (Geisseler and Scow, 2014; Singh *et al*., 2018) [7, 20].

Green manure intercrops exerted a pronounced influence on microbial populations. Among the treatments, maize intercropped with sunhemp (I_3) consistently recorded the highest populations of bacteria, fungi and actinomycetes, followed by Maize + Cowpea (I_1) and Maize + Horse gram (I_2). Sole maize (I_0) recorded the lowest microbial counts. Significantly higher bacterial populations under green manure intercropping systems can be ascribed to enhanced root exudation and rhizodeposition from leguminous intercrops provide carbonaceous substrates for microbial growth, coupled with N-rich residues (from sunhemp and cowpea) through repeated biomass incorporation, supplying easily decomposable organic matter that sustains a favorable niche for microbial proliferation across seasons. On the contrary, sole maize plots, with limited organic matter input and reduced rhizosphere interactions, harboured significantly lower microbial populations, reflecting a depleted microbial environment. These findings are consistent with Nooli *et al*. (2001) [17], Bhayal *et al*. (2018) [2] and Xu *et al*. (2023) [24] who suggested that incorporation of green manure enhances microbial activity in maize-based systems through greater organic matter returns and improved rhizosphere conditions.

The interaction between nitrogen levels and intercrops (N × I) was statistically non-significant; however, numerically, the combination of Maize + Sunhemp (N_2I_3) with 100 per cent RDN consistently supported the highest microbial populations across all groups. Conversely, 125 per cent RDN with sole maize (N_3I_0) recorded the lowest populations. These observations suggest that the synergistic effect of optimum nitrogen and legume intercropping can sustain microbial activity and improve nutrient cycling in the rhizosphere.

The findings clearly indicate that microbial abundance and activity in maize rhizosphere are predominantly governed by the type and quality of intercrops rather than nitrogen levels alone. Integration of legume-based green manures, particularly sunhemp and cowpea, significantly enhanced bacterial, fungal and actinomycetes populations, thereby promoting soil biological health, nutrient turnover and sustainability of maize-based cropping systems.

Enzymatic activity

Nitrogen levels did not significantly influence either urease or dehydrogenase activities. However, relatively higher values under 100 per cent RDN compared with 75 per cent and 125 per cent RDN indicate that an optimum supply of nitrogen creates a favorable environment for microbial proliferation and enzymatic expression while, excess nitrogen (125% RDN) possibly suppresses microbial activity through osmotic stress, altered C:N ratio or reduced dependence on microbial mineralization pathways. This observation corroborates the findings of Tejada *et al.* (2006) [23] and Mohammadi (2011) [14], who noted that balanced fertilizer use stimulates microbial activity whereas, excessive application may inhibit it.

Intercropping with green manures significantly enhanced enzymatic activity while, sole maize recorded the lowest activities (Table 2). This can be attributed to greater biomass addition, higher nitrogen content and faster decomposition rate of biomass added, which supply labile organic matter and stimulate microbial proliferation which in turn enhances the enzymatic activity. These results are in agreement with the findings of Su *et al*. (2022) [21] and Kucerik *et al*. (2024) [11], who emphasized that green manuring improves soil enzymatic activities through better organic matter supply, enhanced rhizosphere interactions and symbiotic microbial associations.

The interaction effect of nitrogen levels and intercropping was statistically non-significant for both enzymes, indicating that the beneficial influence of green manure intercrops on soil biological activity was consistent across different nitrogen regimes. Nevertheless, numerically higher values were recorded under the combination of 100 per cent RDN with Maize + Sunhemp, which highlights the synergistic role of balanced fertilization and legume incorporation in sustaining soil enzymatic functions.

The progressive increase in enzymatic activity up to 60 DAS, followed by a slight decline at harvest. This pattern aligns with the active crop growth phase, where higher root exudation, greater microbial proliferation and decomposition of green manure residues collectively enhance enzymatic synthesis. The subsequent decline at harvest may be attributed to depletion of easily decomposable substrates and senescence of microbial populations. Similar temporal dynamics of enzyme activity in cropping systems have been reported by Brzezinska *et al.* (2014) [3].

Overall, the study establishes that green manure intercropping particularly with sunhemp, plays a pivotal role in enhancing soil enzymatic activities and thereby contributes to improved soil health and nutrient cycling. Optimum nitrogen fertilization (100% RDN) further supports microbial functioning whereas, higher N levels (125% RDN) do not provide additional benefits. This emphasizes the importance of integrated nutrient management strategies that combine mineral fertilizers with legume intercrops to sustain the soil biological quality and productivity of maize-based systems.

Grain and straw yield

The grain yield in any crop is dependent on the photosynthetic source it can utilize. The economic yield is a function of various independent factors like dry matter production, efficiency to translocate photosynthates from the assimilatory area of the sink and its accumulation in different parts of the plant and ultimately leading to higher grain yield (Donald, 1962) [6].

Grain yield and straw yield was significantly increased with nitrogen level (Table 2). 125% RDN (N_3) produced the maximum grain yield (6735 kg ha^{-1}) and straw yield (8721 kg ha^{-1}), however, it was statistically on par with 100% RDN (6399 and 8313 kg ha^{-1}) and lowest was recorded under 75% RDN, with a mean increase of 11.61 per cent and 5.25 per cent in N_3 over N_1 and N_2, respectively (Table 3). N_1 (6035 and 7909 kg ha^{-1}). The overall improvement in grain yield with increasing nitrogen levels ($N_1 < N_2 < N_3$) reflects the central role of nitrogen in promoting meristematic activity, cell division and cell elongation. Nitrogen, being a structural component of chlorophyll, proteins and nucleic acids, directly enhanced photosynthetic capacity and assimilate production, which in turn accelerated vegetative growth and biomass accumulation, resulting in more translocation of dry matter to reproductive parts and grain filling. Similar positive responses of maize growth to higher nitrogen supply were reported by Geith *et al*. (2022), Mahat *et al*. (2023) [13] and Nisar *et al*. (2024) [16].

Among intercropping treatments, Maize + Sunhemp (I_3) recorded the maximum grain (6572 kg ha^{-1}) and straw yield (8540 kg ha^{-1}), statistically on par with Maize + Cowpea and the lowest values were recorded under sole maize. Compared to sole maize, sunhemp intercropping gave a yield advantage of 7.80 per cent, followed by cowpea (6.62%) and horse gram (4.80%). This can be attributed to better soil health and nitrogen availability following in-situ incorporation of the intercrop biomass at 45 DAS, which adds organic matter and facilitates nutrient mineralization during the crop's reproductive phase. Sunhemp's rapid biomass accumulation and higher N content likely supported better root proliferation and nutrient uptake by maize, as also observed by Muttanna *et al*. (2018) [15].

Cowpea intercrops (I_1) also enhanced yields, slightly less than sunhemp, likely due to moderate biomass and nutrient contribution. Horse gram (I_2), while beneficial, showed comparatively lower yield benefits, possibly due to its slower early growth and reduced canopy cover, limiting the magnitude of nutrient addition within the same growth window.

The N × I interaction was significant, with the highest grain yield (6900 kg ha^{-1}) and straw yield (8928 kg ha^{-1}) in N_3I_3 (Maize + Sunhemp with 125% RDN) and the lowest (5711 kg ha^{-1}) in N_1I_0 (sole maize with 75% RDN). The significant N × I interaction highlights that the yield advantage of intercropping is maximized under higher N supply, especially in the N_3I_3 combination. However, yield was significantly increased in green manure treatments even at 100% RDN and 75% RDN compared to their respective sole maize treatments. This synergistic effect reinforces the role of both adequate nutrient management and choice of green manure species in optimizing maize productivity under intensive double-cropping systems. These results are in conformity with Pasha *et al*. (2018) [19] and Gundur *et al*. (2015), who showed that improved nitrogen fertilizer along with sunhemp green manuring resulted in improved yield attributes and yield.

Table 1: Microbial population in rhizosphere soil of maize at harvest as influenced by in-situ green manure intercropping under varied levels of nitrogen

Treatments	Bacteria × 10^6 CFU g^{-1} of soil	Fungi × 10^4 CFU g^{-1} of soil	Actinomycetes × 10^3 CFU g^{-1} of soil
Main plot (N: Nitrogen levels)			
N_1	62.46	13.00	9.69
N_2	63.91	13.42	10.06
N_3	60.73	12.53	9.34
S. Em. (±)	0.79	0.17	0.14
C.D. @ 5%	NS	NS	NS
Subplots (I: Intercropping systems)			
I_0	48.88	8.47	4.00
I_1	67.25	14.47	11.61
I_2	62.25	12.88	9.65
I_3	71.08	16.10	13.52
S. Em. (±)	0.68	0.15	0.13
C.D. @ 5%	2.03	0.44	0.39
Interaction (N×I)			
N_1I_0	49.27	8.67	4.06
N_1I_1	67.54	14.45	11.76
N_1I_2	62.28	12.92	9.47
N_1I_3	70.74	15.96	13.48
N_2I_0	51.18	9.00	4.26
N_2I_1	68.11	14.77	11.60
N_2I_2	63.28	13.28	10.43
N_2I_3	73.07	16.62	13.96
N_3I_0	46.18	7.74	3.69
N_3I_1	66.10	14.20	11.49
N_3I_2	61.20	12.44	9.06
N_3I_3	69.44	15.73	13.11
S. Em. (±)	1.19	0.25	0.23
C.D. @ 5%	NS	NS	NS

Note: N_1 - 75% RDN; N_2 -100% RDN; N_3 - 125% RDN; I_0 - Sole maize; I_1 - Maize + cowpea; I_2 - Maize + horsegram and I_3 - Maize + sunhemp

Table 2: Enzymatic activity in rhizosphere soil of maize at harvest as influenced by in-situ green manure intercropping under varied levels of nitrogen

Treatments	Urease activity (µg urea hydrolyzed g^{-1} soil hr^{-1})	Dehydrogenase activity (µg TPF g^{-1} day^{-1})
Main plot (N: Nitrogen levels)		
N_1	19.79	40.07
N_2	20.16	40.64
N_3	19.26	38.86
S. Em. (±)	0.25	0.49
C.D. @ 5%	NS	NS
Subplots (I: Intercropping systems)		
I_0	15.11	28.30
I_1	20.64	44.25
I_2	20.40	40.10
I_3	22.79	46.78
S. Em. (±)	0.22	0.45
C.D. @ 5%	0.66	1.35
Interaction (N×I)		
N_1I_0	15.30	28.64
N_1I_1	20.75	44.30
N_1I_2	20.38	40.30
N_1I_3	22.73	47.04
N_2I_0	15.63	29.02
N_2I_1	21.06	45.09
N_2I_2	20.71	40.95
N_2I_3	23.23	47.50
N_3I_0	14.42	27.25
N_3I_1	20.10	43.36
N_3I_2	20.11	39.05
N_3I_3	22.43	45.79
S. Em. (±)	0.38	0.79
C.D. @ 5%	NS	NS

Note: N_1 - 75% RDN; N_2 -100% RDN; N_3 - 125% RDN; I_0 - Sole maize; I_1 - Maize + cowpea; I_2 - Maize + horsegram and I_3 - Maize + sunhemp

Table 3: Grain and straw yield (kg ha^{-1}) of maize as influenced by in-situ green manure intercropping under varied levels of nitrogen during different seasons

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Main plot (N: Nitrogen levels)		
N_1	5920	6090
N_2	6235	6458
N_3	6549	6797
S. Em. (±)	81	87
C.D. @ 5%	317	340
Subplots (I: Intercropping systems)		
I_0	6102	6132
I_1	6283	6563
I_2	6198	6455
I_3	6356	6643
S. Em. (±)	49	66
C.D. @ 5%	145	197
Interaction (N×I)		
N_1I_0	5800	5750
N_1I_1	5962	6213
N_1I_2	5884	6105
N_1I_3	6035	6291
N_2I_0	6075	6140
N_2I_1	6292	6572
N_2I_2	6198	6460
N_2I_3	6374	6660
N_3I_0	6430	6505
N_3I_1	6595	6905
N_3I_2	6512	6801
N_3I_3	6660	6978
S. Em. (±)	85	115
C.D. @ 5%	252	341

Note: N_1 - 75% RDN; N_2 -100% RDN; N_3 - 125% RDN; I_0 - Sole maize; I_1 - Maize + cowpea; I_2 - Maize + horsegram and I_3 - Maize + sunhemp

Conclusion

The study clearly demonstrated that green manure intercropping, particularly with sunhemp, significantly enhanced microbial populations, enzymatic activities and overall soil biological health compared with sole maize. Optimum nitrogen application (100% RDN) supported better microbial and enzymatic functioning, while higher N levels (125% RDN) mainly improved yield without proportional biological benefits. Sunhemp and cowpea intercrops contributed substantial organic matter and nitrogen, improving nutrient cycling and sustaining maize productivity. Grain and straw yields were highest under Maize + Sunhemp, with further enhancement under 125% RDN. The significant N × I interaction underscores the importance of integrated nutrient management. Overall, combining balanced nitrogen fertilization with legume green manures offers a sustainable strategy to maintain soil health and maximize yields in maize-maize systems.

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