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Assessment of Legume Crops′ Impact on Soil Microbial Populations, Enzymatic Activities and Productivity under Rice-based Cropping Systems

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ABSTRACT

Background: A long-term experiment on legume crops, integrated with various rice-based cropping systems, was initiated in the 2019-20 agricultural year under the All India Coordinated Research Project. This experiment is being conducted at the research farm of the Department of Agronomy, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Ayodhya, Uttar Pradesh, India. The present study focuses on legume crops and was carried out during the 2022 and 2023 growing seasons.

Methods: The present investigation was conducted with ten treatments: T_1-rice-wheat-fallow; T_2-rice-wheat-green gram; T_3- rice-french bean-green gram; T_4-rice-gram-cowpea; T_5-rice-mustard-green gram; T_6-rice-linseed-black gram; T_7-rice-berseem-sudan grass; T_8-rice-oat-maize+cowpea; T_9-rice-cauliflower-okra; and T_{10}-rice-potato-cowpea (vegetable).

Result: The rice-frenchbean-greengram cropping sequence consistently outperformed other sequences in terms of grain and straw yields, biological yield and soil microbial activity. The inclusion of legumes and their residues significantly enhanced both yields and soil health, leading to improved microbial populations and enzymatic activities, particularly dehydrogenase and urease.

Key words: Enzymatic activities, Legume crops, Rice based cropping system, Soil microbial population.

INTRODUCTION

The rice-wheat cropping system (RWCS) is the dominant agricultural production system in South Asia, particularly in India, where rice (*Oryza sativa* L.) is a staple food. Covering an area of 26 million hectares, RWCS plays a crucial role in food security, employment and income generation, especially in the Indo-Gangetic Plains (IGP) region. However, the continuous mono-cropping of rice in intensive systems has led to the depletion of soil fertility and a reduction in productivity over time. The reliance on inorganic fertilizers for nutrient supply has exacerbated soil degradation, increased micronutrient deficiencies, lowered the water table and contributed to excessive greenhouse gas emissions in the IGP (Kumar *et al.,* 2021). To address these challenges, integrating legume crops into rice-based systems is gaining attention for its potential to improve soil health, biological activity and overall system productivity (Kumar *et al.,* 2019). Legumes, through their symbiotic relationship with nitrogen-fixing bacteria (*Rhizobium* spp.), can significantly enhance the nitrogen economy of the system, reducing the need for synthetic fertilizers while simultaneously improving soil fertility and structure (Bhattacharyya *et al.,* 2020).

The inclusion of legumes in rice-based cropping systems has been observed to significantly alter the soil microbial population. The root exudates from legume plants provide a readily available carbon source for soil microorganisms, leading to increased microbial activity and diversity (Jaiswal *et al.,* 2020). In particular, Rhizobia, which form symbiotic relationships with legumes, contribute to increased populations of nitrogen-fixing bacteria, enhancing nitrogen availability in the soil (Patra *et al.,* 2021). This boost in microbial activity is often accompanied by an increase in other beneficial microbial groups, such as phosphate solubilizers and plant growth-promoting rhizobacteria (PGPR) (Choudhary *et al.,* 2018).

The effects of legumes on microbial populations are not limited to the immediate cropping season. Several

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studies have demonstrated that the positive effects on microbial populations and enzymatic activities can persist for multiple seasons, contributing to the long-term sustainability of rice-based cropping systems (Tiwari *et al.,* 2022). This is particularly important for regions where farmers rely on continuous cropping to meet food demands, as improved soil health can mitigate the yield declines typically associated with continuous rice cultivation. Incorporating legumes into rice-based cropping systems has also been associated with significant improvements in crop productivity. This is due to the synergistic effects of enhanced soil fertility, increased nitrogen availability and improved microbial activity (Verma *et al.,* 2020). Several studies have reported higher yields in rice crops following legume cultivation, as the residual nitrogen from legume biomass provides an essential nutrient source for the rice plants (Prasad *et al.,* 2018).

Moreover, legume crops can improve the overall efficiency of nutrient use within the cropping system. By fixing atmospheric nitrogen, legumes reduce the amount of nitrogen that needs to be applied as fertilizer, which can lower production costs and reduce the environmental impacts of agriculture (Khan *et al.,* 2019). Additionally, the increased microbial and enzymatic activities associated with legume cultivation contribute to a more efficient nutrient cycling process, ensuring that nutrients are available to plants when they are needed the most (Kumar *et al.,* 2021). The yield benefits of legume-based cropping systems are particularly evident in regions where soil fertility has been degraded due to intensive mono-cropping. In such areas, legume-rice rotations can restore soil health and lead to substantial increases in crop productivity, making them a viable option for sustainable intensification (Yadav *et al.,* 2020).

MATERIALS AND METHODS

Experimental site, climate and soil characteristics

A field experiment was conducted under the All India Coordinated Research Project (AICRP) on IFS the Agronomy Farm at Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, India, which was initiated 2019-20 and still continue using the same site and layout. The experimental soil was alkaline in reaction (pH 8.20), low in available N (166 kg/ha), medium in available P (16.67 kg/ha) and medium in available K (243 kg/ha). The experimental site falls under the subtropical zone in the Indo-Gangetic plains, which has alluvial soil and lies between 24.4^{o} and 26.5^{o} North and 82.12^{o} and 83.98^{o} East longitude, with an elevation of about 113 meters above mean sea level. The climate is topical to sub-tropical and the seasonal variation in temperature remains within a narrow range (the average minimum temperature of the coldest month is January, whereas the average maximum temperature of the warmest month is June). The experimental data of soil biological properties collected after 3 and 4-year crop cycle (2021-22 and 2022-23).

Experimental details

Ten different cropping sequences were tried in a randomized block design (RBD) with three replications. They were: T_1, rice-wheat-fallow; T_2, rice-wheat-green gram; T_3, rice-frenchbean-green gram; T_4, rice-gram-cowpea; T_5, rice-mustard-green gram; T_6, rice-linseed-black gram; T_7, rice-berseem-sudanchari; T_8, rice-oat – maize + cowpea; T_9, rice-cauliflower-okra; T_{10}, rice-potato-cowpea (veg). The varieties of different crops used were: rice 'NDR-359', wheat 'PBW-343, green gram 'NDM-1', Gram 'Aurodhi', Black gram 'NDU-1', mustard 'NDR-8501', linseed 'Garima', Sudan Chari 'MP Chari', Berseem 'Vardan', maize 'Jaunpuri Safedi', okra ′VRO-5, cauliflower 'Pusa Himjyoti' Potato 'Kufri Safed' and Cowpea (Veg) 'Kashi Kanchan'. The gross plot size was 6 m × 5 m.

During the rainy season, 25-day-old rice seedlings were transplanted manually in early July and harvested in late October. For the post-rainy season, crops were sown manually in late November, while summer crops were sown manually in March after harvesting the post-rainy season crops. To minimize water loss through deep percolation and seepage in rice, the field was puddled with 4-5 cross-cultivations in 5 cm of water, then leveled and bunded. After the rice harvest, the fields were irrigated and prepared for post-rainy season crops with two cross-cultivations, two harrowings and planking using a wooden beam. For summer crops, the field was prepared with one deep plowing, two harrowings and planking.

Fertilization followed specific recommendations for each crop: Rice (150 kg N, 60 kg P_2O_5, 60 kg K_2O), Wheat (150:60:60), Mustard (100:60:40), Chickpea (20:60:0), Frenchbean (120:60:60), Greengram (20:40:20), Blackgram (15:40:20), Gram (20:60:0), Cowpea (Veg) (20:50:50), Cowpea (Fodder) (60:40:0), Okra (150:60:60), Potato (150:60:90), Maize (150:60:60), Oat (100:40:0), Sudanchari (80:40:0), Cauliflower (120:60:40), Linseed (100:60:40) and Berseem (40:60:0). Phosphorus and potassium, along with a basal dose of nitrogen, were applied before sowing or planting. The remaining nitrogen was applied in splits. For rice, phosphorus and potassium and a basal dose of nitrogen were incorporated before puddling. Urea, single superphosphate and muriate of potash were used for nutrients. Rice received water through rainfall and irrigation, while post-rainy and summer crops were irrigated using tube well water, with each irrigation providing 5 cm of water measured by a Parshall flume. Recommended inter-culturing practices were followed for successful crop cultivation.

Soil biochemical properties

The fresh sample of soil collected for estimating Soil microbial analysis (Parmar and Schmidt, 1966), dehydrogenase activity (DHA) following TTC method (Burns, 1978), phosphatase (Tabatabai and Bermner, 1969), Soil sampling and analysis were made after the harvest of the cropping system (after 4 years) to determine the physical and chemical properties of the soil. Microbial counts were performed in fresh soil post-harvest using the serial dilution pour plate method (Parmar and Schmidt, 1966). Thornton's agar, Rose Bengal streptomycin agar and Casein starch agar were used for bacterial, fungal and actinomycetes counts, respectively, after 48 hours of incubation at 28±2°C. Dehydrogenase activity was measured spectrophotometrically at 485 nm by quantifying Tri-Phenyl Formazan (TPF) formed via TTC reduction. Urease activity was determined by incubating 5 g of moist soil with urea and KCl-Ag_2SO_4 solution, followed by distillation and titration to measure NH4-N. Phosphatase activity was assayed by incubating soil with p-nitro phenyl phosphate in a modified universal buffer, followed by the addition of $CaCl_2$ and NaOH. Absorbance was measured at 420 nm. Each experiment was conducted in triplicate and microbial counts were expressed as cfu g^{-1} soil, dehydrogenase activity as Tg TPF g^{-1} soil h^{-1} and phosphatase activity based on absorbance.

The data collected from the experiment were subjected to statistical testing by following the 'Analysis of Variance Technique' as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Yield (q/ha)

The grain and straw yields of rice fluctuated between 41.08 and 45.65 q/ha and 58.60 and 65.50 q/ha, respectively, with data on grain yield, straw yield, biological yield and harvesting index presented in Table 1. Among the different cropping sequences, the Rice-frenchbean-greengram sequence delivered the highest grain yield of 45.65 q/ha, followed closely by rice-gram-greengram, rice-linseed-blackgram and rice-mustard-greengram sequences, all of which exhibited statistically similar results. The integration of legume crop residues such as greengram, blackgram and cowpea led to a 10.01% rise in grain yield and a comparable increase in straw yield when compared to the rice-wheat-fallow (T_1) treatment. On the other hand, the rice-wheat-fallow sequence recorded the lowest grain yield of 41.08 q/ha over two years of trials and in pooled data. The straw yield followed a similar trend, with the rice-frenchbean-greengram treatment achieving the highest straw yield of 65.50 q/ha, followed by treatments T_4, T_9, T_{10} and T_8. These treatments also showed no significant differences in straw yield. The addition of legume residues further enhanced straw yields by 10.53% over the rice-wheat-fallow sequence, which posted the lowest straw yield of 58.60 q/ha. The biological yield was maximized under the rice-frenchbean-greengram sequence, which significantly outperformed other sequences, while the lowest biological yield (9.77 q/ha) was observed in the rice-oat-maize+cowpea (fodder) sequence in both individual years and pooled data.

The superior performance of the rice-frenchbean-greengram sequence is largely attributed to the sufficient supply of essential nutrients, which bolstered plant growth

Table 1: Grain and straw yield of rice influenced by the legume crops under rice-based cropping system.

Treatment	Yield (q/ha) 2021-22		2022-23		Pool		Biological Yield (t/ha)			Harvesting Index (%)		
	Grain	Straw	Grain	Straw	Grain	Straw	2021-22	2022-23	Pool	2021-22	2022-23	Pool
T_1	41.95	58.75	40.20	58.45	41.08	58.60	10.07	9.86	9.97	41.65	40.74	41.20
T_2	43.10	60.35	43.95	63.20	43.53	61.78	10.34	10.71	10.53	41.66	41.01	41.34
T_3	44.50	62.30	46.80	68.70	45.65	65.50	10.68	11.55	11.12	41.66	40.52	41.09
T_4	41.65	58.40	42.10	60.30	41.88	59.35	10.00	10.24	10.12	41.62	41.11	41.37
T_5	42.70	59.80	42.30	61.90	42.50	60.85	10.25	10.42	10.34	41.65	40.60	41.13
T_6	42.85	60.15	42.95	62.25	42.90	61.20	10.30	10.52	10.41	41.60	40.80	41.20
T_7	39.95	55.90	38.10	53.90	39.03	54.90	9.58	9.20	9.39	41.67	41.40	41.54
T_8	41.20	57.60	39.37	57.20	40.29	57.40	9.88	9.66	9.77	41.69	40.73	41.21
T_9	42.80	59.90	39.60	58.50	41.20	59.20	10.27	9.81	10.04	41.67	40.31	40.99
T_{10}	41.30	57.82	40.85	59.75	41.08	58.79	9.91	10.06	9.99	41.66	40.58	41.12
SEm±	1.49	2.09	1.08	0.83	1.29	1.46	0.36	0.18	0.27	1.48	1.45	1.47
CD (P=0.05)	4.42	6.19	3.19	2.47	3.81	4.33	1.06	0.52	0.79	4.38	4.28	4.33

and yield components, resulting in higher grain and straw production and thereby increasing overall biological yield. The harvest index across the cropping systems varied from 40.99% to 41.37%, with the rice-gram-greengram sequence achieving the highest index, while the rice-oat-maize+cowpea (fodder) sequence recorded the lowest. However, the cropping systems did not exert a significant influence on the harvest index of rice across the two experimental years.

The incorporation of legume crop residues, particularly from greengram, blackgram and cowpea, along with the application of optimal nutrient doses, proved instrumental in boosting rice yields. Notably, the rice-frenchbean-greengram sequence exhibited the most favorable results in terms of both grain and straw yields, underscoring the critical role of effective nutrient management and residue incorporation in improving crop productivity. Although the cropping sequences had minimal impact on the harvest index, further research into the interaction between rotational crops could provide valuable insights for achieving sustainable agricultural systems.

The application of recommended fertilizer doses for rice and wheat without legume residue incorporation resulted in the lowest yields due to suboptimal plant growth and metabolic activity, which hindered yield components. Similar observations were made by Jat *et al.* (2018) and Bowal *et al.* (2021). Conversely, the inclusion of legume crop residues led to increased yields, likely due to the release of micronutrients that play key roles in enzymatic reactions, growth processes, hormone synthesis and protein formation, contributing to enhanced translocation of photosynthates to reproductive organs and ultimately improving growth and yield. These findings align with earlier research by Menia *et al.* (2022) and Jain and Kushwaha (2014). Furthermore, studies by Zhao *et al.* (2020) and Gudadhe *et al.* (2020) highlight the critical importance of residue management and nutrient recycling in sustainable rice production systems.

Total microbial count (Bacteria, Actinomycetes, Fungi)

The microbial populations in soil were assessed in relation to various legume-based cropping sequences, as shown in Table 2. Significant effects of these cropping systems were observed over four years of study and in the pooled data. The highest microbial counts were recorded under treatment T_4 (Rice-gram-greengram), with bacterial populations reaching 13.28 × 10^4 cfu g^{-1} soil, actinomycetes at 8.53 × 10^6 cfu g^{-1} soil and fungal populations at 7.48 × 10^3 sfu g^{-1} soil. In contrast, the lowest microbial populations were found in treatment T_1 (Rice-Wheat-Fallow), where bacterial populations were 11.14 × 10^4 cfu g^{-1} soil, actinomycetes were 7.27 × 10^6 cfu g^{-1} soil and fungal counts were 6.03 × 10^3 sfu g^{-1} soil. These findings emphasize the positive impact of legume-based cropping systems on soil microbial communities. The inclusion of legumes in crop rotations, as seen in T_4 (Rice-gram-greengram), boosts microbial diversity and abundance. This is likely due to the

Table 2: Microbial count affected by the legume crops under rice-based cropping system.

Treatment	Bacteria (10^6 cfu g^{-1} soil)			Actinomycetes (10^4 cfu g^{-1} soil)			Fungi (10_3 sfu g^{-1} soil)		
	2021-22	2022-23	Pool	2021-22	2022-23	Pool	2021-22	2022-23	Pool
T_1	10.18	12.10	11.14	6.33	8.20	7.27	5.38	6.68	6.03
T_2	10.17	12.28	11.23	6.78	8.24	7.51	5.78	7.19	6.49
T_3	12.26	14.06	13.16	7.38	9.30	8.34	6.78	8.18	7.41
T_4	12.07	14.48	13.28	7.61	9.24	8.53	6.59	8.22	7.48
T_5	11.58	13.83	12.71	7.03	8.56	7.80	6.25	7.78	7.02
T_6	11.87	13.32	12.60	7.13 7	6.27	7.74	7.01		
T_8	11.23	13.59	12.41	6.39	8.75	7.57	6.18	7.63	6.91
T_9	11.56	13.81	12.69	7.19	9.12	8.16	6.29	7.74	7.02
T_{10}	11.86	13.88	12.87	7.18	9.20	8.19	6.38	8.06	7.22
SEm ±	0.41	0.49	0.45	0.25	0.32	0.29	0.22	0.28	0.25
CD(P=0.05)	1.22	1.44	1.33	0.74	0.94	0.84	0.66	0.82	0.74

greater variety of root exudates and organic inputs provided by legumes, which foster enhanced nutrient cycling and decomposition processes (Kumar *et al.,* 2020; Nisha *et al.,* 2019). On the other hand, continuous cropping systems like T1 (Rice-Wheat-Fallow) result in reduced microbial populations, likely due to persistent selection pressures and a lack of organic inputs during fallow periods (Sun *et al.,* 2019; Zhou *et al.,* 2021).

Soil enzymatic activities

Dehydrogenase (µg TPF g^{-1} soil day^{-1})

The soil dehydrogenase enzyme activity was significantly affected by the inclusion of legume crops within various cropping sequences, as presented in Table 3. The highest enzyme activity was recorded in the Rice-Chickpea-Cowpea sequence (T_4), reaching 178.27 µg TPF g^{-1} soil day^{-1}, while the rice-wheat-fallow sequence (T_1) had the lowest activity at 134.79 µg TPF g^{-1} soil day^{-1}. This finding emphasizes that legume-based cropping systems, such as those involving chickpea and cowpea, play a crucial role in enhancing soil enzymatic activities. This boost in dehydrogenase activity is attributed to legumes' ability to increase organic matter through biomass input and improve nitrogen levels in the soil via biological nitrogen fixation. Supporting recent research by Smith *et al.* (2018), this study underscores the positive impact of legumes on soil health. Therefore, diversifying cropping systems by integrating legumes can help maintain soil health and sustain agricultural productivity.

Urease (µg urea g^{-1} soil hr^{-1})

Urease enzyme activities ranging from 224.00 to 233.63 µg urea g^{-1} soil hr^{-1} were presented in Table 3. In particular, the Rice-Chickpea-Cowpea (T_4) cropping system exhibited significantly higher urease activity (233.63 µg urea g^{-1} soil hr^{-1}) compared to other systems followed by T_3> T_7 and T_{10}, while the lowest activity was observed under Rice-Wheat-Fallow (T_1). This investigation suggests that urease activity is influenced by soil nutrient status, particularly nitrogen levels. Higher urease activity correlates with increased nitrogen application. Recent studies support this, emphasizing the link between urease activity and soil nitrogen availability (Zhang *et al.,* 2019; Li *et al.,* 2021). These findings emphasize the importance of managing nitrogen inputs to regulate urease activity and optimize soil health.

Alkaline phosphatase (µg p-nitrophenol g^{-1} hr^{-1})

Alkaline phosphatase activity was significantly higher (183.01 µg p-nitrophenol g^{-1} hr^{-1}) in the Rice-Gram-Green gram (T_4) treatment compared to other cropping systems. conversely, the rice-wheat-fallow (T_1) treatment exhibited lower alkaline phosphatase activity (176.90 µg p-nitrophenol g^{-1} hr^{-1}). Alkaline phosphatase activity is enhanced in systems where legume crops are cultivated and then incorporated into the soil after harvesting for green manuring. Recent research supports this, highlighting the

Table 3: Enzymatic activities affected by the legume crops under rice-based cropping system.

Treatment	Dehydrogenase (µg TPF g^{-1} soil day^{-1})			Urease (µg urea g^{-1} soil hr^{-1})			Alkaline phosphatase (µg p-nitrophenol g^{-1} hr^{-1})		
	2021-22	2022-23	Pool	2021-22	2022-23	Pool	2021-22	2022-23	Pool
T_1	125.49	134.79	130.14	216.70	231.29	224.00	168.92	184.87	176.90
T_2	153.92	165.32	159.62	220.81	235.67	228.24	169.86	185.80	177.83
T_3	164.74	176.93	170.83	224.92	240.08	232.50	173.79	190.36	182.08
T_4	165.97	178.27	172.12	226.02	241.23	233.63	173.68	192.34	183.01
T_5	146.52	157.38	151.95	222.51	237.49	230.00	172.25	188.53	180.39
T_6	148.17	159.15	153.66	223.28	238.31	230.80	171.91	188.24	180.08
T_7	145.91	156.72	151.31	225.16	240.31	232.74	170.89	187.52	179.21
T_8	143.14	153.74	148.44	224.74	239.52	232.13	169.89	185.92	177.91
T_9	148.91	159.94	154.42	220.89	235.40	228.15	170.81	186.34	178.58
T_{10}	152.55	163.85	158.20	224.69	239.81	232.25	172.30	188.55	180.43
SEm±	5.41	5.81	5.61	7.93	8.46	8.20	6.09	6.68	6.39
CD (P=0.05)	16.01	17.20	16.60	23.49	25.07	24.28	18.05	19.79	18.92

role of legume residues in stimulating soil enzyme activity, including alkaline phosphatase (Singh *et al.,* 2020; Kumar *et al.,* 2022). These findings underscore the importance of crop rotation and residue management in improving soil nutrient cycling.

CONCLUSION

It could be concluded that the significant impact of integrating legume crops into rice-based cropping sequences on both crop yield and soil health. The rice-frenchbean-greengram sequence emerged as the most effective in enhancing grain and straw yields, achieving a notable 10% increase compared to the rice-wheat-fallow sequence. The inclusion of legume residues not only improved crop productivity but also boosted soil microbial populations and enzymatic activities. Particularly, the Rice-gram-greengram sequence demonstrated superior microbial diversity and enzymatic functions, such as dehydrogenase, urease and alkaline phosphatase, indicative of improved soil health and nutrient availability.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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