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RESEARCH ARTICLE

Role of soil microbial flora, enzyme activities and nitrogen levels in maintaining legume-maize crop sequence

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Abstract

The save soil campaign and the recent international summit, COP-28, highlighted the importance of soil microbial fauna in the ecosystem for the years to come, which supports soil-plant symbiosis under current dire conditions. The inclusive recycling of garbage into feasible residues is necessary to boost nitrogen (N) levels in order to combat climate change and encourage responsible production in accordance with Sustainable Development goals (SDG) . Using these residues to boost the activity of the microbial community that is present both above and below the rhizosphere including endospheric diazotrophs in the legume-maize system is a significant benefit. This study examined a legume (Groundnut, Soybean, Greengram)-Maize sequence with varying nitrogen rates (100 %, 125 %, 150 % N) in a split-plot design using different treatment combinations during the *kharif* and *rabi* seasons of successive periods. The results indicated that the sustained biomass of greengram for *rabi* maize under zero-till conditions led to a progressive improvement in bacterial consortium activity up to 60 days and enzymatic activity from one cropping cycle to the next, compared to soybean and groundnut. Furthermore, a 50 % deviation from normal nitrogen rates proved superior to 25 % and 0 % nitrogen, revealing the importance of the C:N ratio of biomass and the decomposition process in accelerating consortium activity for improved fertility. Additionally, a rotating pulse crop serves as a vital component for rhizospheric nodulation and the soil microbial community, as the continuous cultivation of greengram demonstrates elevated soil consortium activity at higher levels. Moreover, the interaction (*Legume biomass* X *Nitrogen rates*) on zero-till maize was found to be minimal.

Keywords: bacterial count; biomass retention; legume-maize sequence; nitrogen rates; phosphatase activity

Introduction

The present generation's tagline for sustainability goals is to utilize crop remains as a system in the recycling process for biodiversity, rather than demand high input from external sources. Thus, for agricultural ecosystems to remain viable, soil health is a keystone determinant of crop productivity and sustainability. Among them is the soil microbial community which plays a pivotal role in nutrient cycling, organic matter decomposition and plant growth promotion. The dynamic interactions between soil microbes and crops like legumes and cereals are due to the unique capabilities of legumes to fix atmospheric nitrogen. This symbiotic relationship anchors microbial diversity and enzymatic activities are essential for soil dynamics. Maize arose in the Andean region of Central America and is commonly known as corn as it is a grain plant cultivated in many nations across the globe.

The world produced 1.3 billion tonnes of maize on 215.43 million hectares, with an average productivity of 5.95 tonnes per hectare (1). Most of the maize varieties are made in the United States, China, Brazil, Sub-Saharan Africa and Asia. In 2024, Karnataka, Madhya Pradesh and Maharashtra will be India's top maize-producing states. Karnataka leads the country in maize production, accounting for approximately 15 % of total production due to its humid climate and fertile land. Maize is an essential diet in many nations, as well as an industrial source of carbohydrates, oil, protein and alcohol. It is consumed in the form of feed, food, fresh kernels, baby corn and popcorn. Maize is consumed worldwide for 59 % of feed, 23 % for food and 18 % for industrial processing units. The crop is cultivated in countries having average daily temperatures above 15 °C in temperate and tropical climates and is susceptible to frost, especially during their phase, yet it can withstand hot and dry climatic conditions as long as there is enough water available and temperatures are under 45 °C. Notwithstanding, the productivity of these systems of cultivation can be considerably impacted by the rates of nitrogen fertilizer applied. Although nitrogen is a paramount element for crop development, its usage needs to be monitored carefully to prevent adverse ecological effects in addition to optimising bacterial activities in the soil.

In former studies there is a direct correlation between soil health and crop yield with the amount of organic matter available (2). Soil organic matter is rapidly declining due to prolonged cereal-based cropping patterns, uneven fertilizer use and intense tillage techniques (3). A change to more sustainable farming methods is necessary to avert this decline. Maize is a thriving crop for minerals and it responds positively to rises in the rate of nitrogen fertilizers (4). Sustainable crop output and soil quality are boosted by the integration of legumes into cereal-based continuous cultivation techniques (5). So, the addition of legumes and conventional nitrogen could boost the nitrogen budget of the farming system while advancing overall crop efficiency. Retaining nutrients in the root layer by the judicious use of inorganic nitrogen in combination with natural sources is crucial to restoring soil health.

Legume leftovers are rapidly combustible due to their high nutritional value and low lignin concentration. This makes them intriguing sources for soil microorganisms, thereby enhancing nutrient availability and energy (6, 7). Although, analysing soil fertility and firmness in response to varying external variables, soil microbial diversity can be utilized as a biomarker (8). Previous studies revealed that diverse crop cycles affect the variety and composition of the soil bacteria *via*. exudates from root systems, crop debris and synergistic associations, which constitute over 89 % of the overall surface microbiome (9). Legumes serve as excellent weed inhibitors and also assist in enriching the soil by disintegrating rock phosphorus, altering the external conditions of the soil and stimulating the growth of bacteria, fungi and actinomycetes that restore humus fractions (10).

Addressing the vital roles of microbiomes and enzymes related with soil is essential particularly regarding endospheric diazotrophs and root ecotone nodulation (11, 12). These microorganisms are crucial for biochemical processes such as nitrogen cycling, the decomposition of humus components and the prevention of illnesses spread by soil (13). In this study, the microbial and biochemical reactions to nitrogen inputs in legume-maize systems will be investigated using a mix of sophisticated bacterial activity and enzymatic tests. The results are anticipated to support ecological balance and agricultural sustainability by optimizing nitrogen management techniques.

This work assists international efforts towards sustainable agricultural intensification whilst addressing an elementary issue in soil science. We aim to elucidate the methodology *via.* how different cropping sequences alter nitrogen utilization by examining the range of microbes in conjunction with substantial nitrogen-cycling enzymes under the driving force of SDG goals 12 (responsible production), 13 (climate change), 15 (life on land) to achieve 2 (zero hunger).

Materials and Methods

Description site

The trial site was set up at a college farm, AICRP-Maize, Hyderabad, India, at an elevation of 601.2 Mean Sea level, with location points of 16°92' N latitude and 69°04' E longitude, under a Semi-Arid Tropics (SAT) geographical area. The investigation was executed throughout the four seasons from 2021 to 2023. During the experimental quarter, the daily average solar radiations fluctuating between 0.7 to 8.9 hr day^{-1}, overall precipitation of 902.36 mm and cardinal degrees around 13.45 to 34.54 °C, seemed to be conducive for the pulse-cereal cycle. The treatment combination was scheduled with 3 cropping sequences (green gram, groundnut and soybean-maize) with 2 levels in *kharif* followed by 3 sub-levels (100, 125, 150 %) of Nitrogen in *rabi* in the following years. The sequence crop of Groundnut, Soybean and Green gram was sown on June 25th in advance of the *kharif* season. In both seasons, a mechanical planter was deployed for maize which was sown on Sept 25th adhering to the harvest of the greengram and further Oct 15th for Soybean and Groundnut crops in Table 1. The soil properties were analyzed with the legume-maize sequence represented in Table 2.

Statistical analysis

The observations were examined statistically utilizing the ANOVA approach related to the split block layout by IBM SPSS Statistics 28.0 (14). The treatment means were assessed using the LSD at the 5 % probability level and Fisher's exact test was performed to figure out the level of significance for the treatment values. The Principal Component Analysis (PCA) and correlation matrix based on the test were conducted using the R (doe-bioresearch) package to compare the mean.

Table 1. Recommended spacing and fertilizer application rates for various crop varieties

Crop	Variety	Spacing (cm)	Fertilizer kg N-P_2O_5-K_20 ha^{-1}	Duration (days)
Groundnut	K-8	30 x 10	20-40-50	100
Green gram	WGG-42	30 x 10	20-50-0	73
Soybean	JS-335	30 x 10	60-60-40	100
***rabi* Maize**	DHM-121	60 x 20	240-80-80	120

Table 2. Comprehensive assessment of ahysical and chemical properties of soil

S. No	Particulars	Value	Particulars	Value	Particulars	Value
	Physical properties		**Chemical properties**			
1.	Mechanical analysis Sand (%) Silt (%) Clay (%)	 11.00 36.74 53.56	Soil *pH* (1:2.5 soil: water suspension)	7.87	Available P_2O_5 (kg ha^{-1})	33.58
2.	Textural class	Sandy clay loam	Electrical conductivity (dS m^{-1})	0.41	Available K_2O (kg ha^{-1})	369.95
3.	Bulk density (g cc^{-1})	1.39	Organic carbon (%)	0.39	C:N ratio	9.4:1
4.	Soil wetting pattern (%)	21.77	Available Nitrogen (kg ha^{-1})	246.88		

Approach to identify bacterial colonies (CFU g^{-1} soil)

The bacteria's colony-forming units (X 10^6 CFU g^{-1}) were measured via plating on specific media and by periodic dilution. The replicates of the inoculated agar plates were incubated at 37 ^{0}C for two days to be screened for bacteria. A digital colony counter was utilized for recording the number of colonies per gram of soil and the number of colonies on the plates was reported. The factor for dilution compounded by the number of colonies was the formula employed to determine the colony-forming units per gram of soil.

Number of bacteria-free living in 1 g soil = No. of CFU × dilution dry weight of 1 g moist soil × aliquot taken

Methodology for Dehydrogenase (µg TPF g^{-1} soil 24 hr^{-1})

The procedure for dehydrogenase activity is based on the organism to generate energy, the respiratory chain enzyme dehydrogenase needs to be functional. This enzyme is critical for the functioning of the metabolic system. During cropping, rhizosphere processes such as mineralization, breakdown and exudate discharge from the roots may produce DHA. One gram of soil substrate, 50 mg of $CaCO_3$, 2.5 mL of distilled water and 1 ml of 3 % TTC were added into a 50 mL glass tube. The tube was swirled for just a few minutes before being incubated at 370 ^{0}C for an entire day. The TPF red precipitate was mixed in 10 mL of methanol and stirred for half an hour before the mixture was filtered into a 25 mL volumetric flask and the rest of the volume was added to the flask using methanol. With a spectrophotometer, the red colour's intensity was determined at 485 nm (15).

Urease mechanism (µg NH_4-N g^{-1} soil hr^{-1})

For measuring the amount of urease activity in the soil, the ratio of NH_4+ released during the hydrolysis of urea was illustrated in a previous study (16). An appropriate mixture was added to a 50 ml volumetric flask: 5g of soil, 0.2 mL toluene and 9 ml THAM buffer. After rapidly swirling the flask to mix the contents, 1 mL of a 0.2 M urea solution was added and the resultant mixture was shaken once again for a brief length of time. The flask was then sealed and inserted into an incubator set at 370 ^{0}C. The stopper was removed after two hours and around 35 mL of KCl-Ag_2SO_4 solution were added. Following an instant shake, the flask was allowed to stand for approximately five minutes, or until the contents had reached ambient temperature. The vessel was corked and shaken multiple times to mix the contents once the volume reached 50 mL and the KCl-Ag_2SO_4 solution was added. Pipetting out a 20 ml aliquot and distilling it with 0.2 g of MgO for 4 min allowed us to calculate the amount of NH_4-N in the final soil suspension. The enzyme urease performance assessment approach mentioned above was adopted to carry out controls, except by adding 1 mL of 0.2 M urea solution after the KCl-Ag_2SO_4 solution was introduced.

Phosphatase reactions (µg PNP g^{-1} soil)

One gram of soil from the sample was added to an acrylic cylinder for testing. In this blend, 1 mL of p-nitrophenyl phosphate (just for samples), 4 mL of pH 6.5 MUB buffer (for acid phosphatase) and 4 mL of pH 11.0 MUB buffer (for alkaline phosphatase) were added. The glass tube was stirred for a few minutes before being turned off and incubated for sixty minutes at 37 ^{0}C. After the incubated interval, 1 mL of 0.5 M $CaCl_2.2H_2O$ and 4 mL of 0.5M NaOH were included, agitated and filtered. A spectrophotometer calibrated at 420 nm was used to determine the yellow colour's intensity. (17) revealed that controls were performed concurrently employing the same approach, for the addition of 1 mL of p-nitrophenyl phosphate solution.

Results and Discussion

Soil enzymatic activity

Enzymatic metabolism is the primary driver of soil fertility and firmness and it necessitates matter as an anchor for nutrient exploration. Tables 4-7 highlight the characteristics of urease, dehydrogenase and phosphatases, detailing that greengram plant material generated at *kharif* via 100 % N dose put on *rabi* maize outperformed more dry matter and which supplement for better urease, dehydrogenase, acid and alkaline phosphatases activity over 125 and 100 % N scales. Furthermore, sub-treatments involving 150 % N-50 % more than the conventional dose exhibited a substantial rise in the activity of enzymes over 125 % and 100 % N levels in two years. Nonetheless, the relationship between N gradient dose and kharif pulse biomass was not significant from 30 to 90 development stages.

Fig. 1 represents that scatterplot matrices and correlation coefficients for the years 2021-22 and 2022-23 offer insights into the relationships between different enzymatic analysis revealed that strong positive correlations (coefficients close to 1), such as 0.87 between U_{30das} and U_{60das}, 0.93 between DHO_{30das} and DHO_{60das}, 0.91 between DH_{30das} and DH_{60das} and moderative positive ranging from 0.70 between U_{30das} and U_{90das}, 0.76 between AP_{30das} and AP_{60das}. Further weak relation was noted in 0.41 between AP_{90das} and ALP_{30das}, 0.33 between U_{60das} and DHO_{30das}. Similar trends were noted in the second year also. The following interpretation signifies that the higher biomass availability and synergistic mobility with greengram at early stages (30 and 60 days) showed higher improvement in positive mutualism and decreased subsequently lower correlation in enzymatic activity at the later phase of crop growth. This emphasizes the vital aspect of dry matter as a component in soil microbiome. The experiment was similar in various studies (18-22).

The PCA biplots for the years 2021-22 and 2022-23 are displayed in Fig. 2, which provides shared perspectives on the distribution and interactions among the individual treatments based on the principal components affirm that individuals are spread across the four quadrants with varying cos2 values, most individuals (e.g., 16, 17, 13, 18) with high cos^2 values are in the upper right quadrant, indicating strong contributions to both Dim_1 and Dim_2. Individuals 1 and 7 are near the origin with lower cos^2 values, indicating weaker contributions to the principal components. Dim_1 consistently explains a significant majority of the variance (95.8 % and 95.9 %), indicating that the primary dimension captures most of the information in the data. The figure elucidates that higher biomass/dry-matter in treatments (16-18) decipher that residual-N rates build the urease activity that degraded urea and enhanced the nitrogen mineralize and mobile in a root rhizospheric zone of legume-

Table 3. Total soil microbial bacteria count (CFU g^{-1} soil) of zero-till *rabi* maize as influenced by *kharif* legumes and nitrogen fertility levels during 2021-22 and 2022-23

Treatments	15 DAS			30 DAS			45 DAS			60 DAS		
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
***Kharif* Legumes x nitrogen levels (CXN)**												
C_1N_1: Groundnut$_{100\ \%\ RDN}$	6.00	5.01	5.51	8.05	8.37	8.21	6.68	6.71	6.70	5.74	5.86	5.80
C_1N_2: Groundnut$_{75\ \%\ RDN}$	5.63	4.53	5.08	7.45	7.78	7.62	6.36	6.46	6.41	5.42	5.47	5.45
C_2N_1: Soybean$_{100\ \%\ RDN}$	6.64	5.96	6.30	8.61	9.14	8.88	7.21	7.19	7.20	6.23	6.27	6.25
C_2N_2: Soybean$_{75\ \%\ RDN}$	6.49	5.51	6.00	8.35	8.80	8.58	7.02	7.02	7.02	6.02	6.06	6.04
C_3N_1: Greengram$_{100\ \%\ RDN}$	6.95	6.87	6.91	9.17	9.86	9.52	7.64	7.51	7.58	6.58	6.83	6.71
C_3N_2: Greengram$_{75\ \%\ RDN}$	6.80	6.42	6.61	8.91	9.53	9.22	7.42	7.34	7.38	6.41	6.58	6.50
***SEm*±**	0.05	0.13	-	0.07	0.07	-	0.05	0.04	-	0.05	0.05	-
C.D. (P=0.05)	0.14	0.42	-	0.23	0.21	-	0.17	0.13	-	0.15	0.16	-
***Rabi* maize with varied nitrogen fertility levels (F)**												
F_1: 100 % RDN	6.25	6.14	6.20	8.68	8.64	8.66	6.96	6.83	6.90	5.89	5.78	5.84
F_2: 125 % RDN	6.52	6.46	6.49	8.90	8.83	8.87	7.21	7.07	7.14	6.14	6.01	6.08
F_3: 150 % RDN	6.69	6.58	6.64	9.11	8.95	9.03	7.39	7.25	7.32	6.28	6.15	6.22
***SEm*±**	0.05	0.07	-	0.06	0.06	-	0.04	0.03	-	0.03	0.03	-
C.D. (P=0.05)	0.15	0.19	-	0.18	0.17	-	0.12	0.09	-	0.10	0.09	-
Interaction												
Sub treatments at the same level as Main treatments F X (C x N)												
***SEm*±**	0.10	0.13	-	0.12	0.10	-	0.09	0.06	-	0.06	0.07	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-	NS	NS	-
Main treatments at same/different levels of sub-treatments (C X N) X F												
***SEm*±**	0.12	0.16	-	0.15	0.14	-	0.1	0.08	-	0.08	0.08	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-	NS	NS	-

Table 4. Urease activity (µg NH_4-N g^{-1} soil hr^{-1}) of zero-till *rabi* maize as influenced by *kharif* legumes and nitrogen fertility levels during 2021-22 and 2022-23

Treatments	30 DAS			60 DAS			90 DAS		
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
***Kharif* Legumes x nitrogen levels (C X N)**									
C_1N_1: Groundnut$_{100\ \%\ RDN}$	51.42	48.61	50.02	130.42	126.36	128.39	106.33	104.02	105.18
C_1N_2: Groundnut$_{75\ \%\ RDN}$	42.92	39.97	41.45	111.25	107.65	109.45	91.33	98.78	95.06
C_2N_1: Soybean$_{100\ \%\ RDN}$	77.75	74.80	76.28	146.75	143.17	144.96	132.33	129.78	131.06
C_2N_2: Soybean$_{75\ \%\ RDN}$	71.33	68.58	69.96	140.58	137.87	139.23	123.33	120.19	121.76
C_3N_1: Greengram$_{100\ \%\ RDN}$	97.67	95.23	96.45	175.83	172.70	174.27	154.67	151.80	153.24
C_3N_2: Greengram$_{75\ \%\ RDN}$	87.83	84.77	86.30	169.42	166.16	167.79	147.33	144.89	146.11
***SEm*±**	2.02	2.28	-	1.87	1.48	-	1.82	1.15	-
C.D. (P=0.05)	5.87	6.64	-	5.89	4.65	-	5.25	3.64	-
***Rabi* maize with varied nitrogen fertility levels (F)**									
F_1: 100 % RDN	65.08	62.32	63.70	135.50	132.00	133.75	117.00	123.28	120.14
F_2: 125 % RDN	70.75	68.27	69.51	146.13	143.06	144.60	125.00	129.64	127.32
F_3: 150 % RDN	78.63	75.39	77.01	155.50	151.90	153.70	135.67	137.31	136.49
***SEm*±**	1.84	1.98	-	1.35	1.49	-	1.23	1.83	-
C.D. (P=0.05)	5.34	5.76	-	3.94	4.36	-	3.58	5.35	-
Interaction									
Sub treatments at the same level as Main treatments F X (C X N)									
***SEm*±**	7.63	7.38	-	2.98	3.25	-	2.87	4.12	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-
Main treatments at same/different levels of sub-treatments (C X N) X F									
***SEm*±**	8.17	9.13	-	3.31	3.66	-	3.00	4.49	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

Table 5. Dehydrogenase (µg TPF g^{-1} soil 24 hr^{-1}) of zero-till *rabi* maize as influenced by *kharif* legumes and nitrogen fertility levels during 2021-22 and 2022-23

Treatments	30 DAS			60 DAS			90 DAS		
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
***Kharif* Legumes x nitrogen levels (C X N)**									
C_1N_1: Groundnut$_{100\ \%\ RDN}$	42.02	40.18	41.10	31.28	34.59	32.94	27.40	25.10	26.25
C_1N_2: Groundnut$_{75\ \%\ RDN}$	36.16	33.19	34.68	26.09	24.47	25.28	22.25	21.25	21.75
C_2N_1: Soybean$_{100\ \%\ RDN}$	55.41	53.17	54.29	40.24	37.31	38.78	34.23	30.83	32.53
C_2N_2: Soybean$_{75\ \%\ RDN}$	49.70	47.07	48.39	36.09	30.27	33.18	30.74	28.11	29.43
C_3N_1: Greengram$_{100\ \%\ RDN}$	66.85	67.71	67.28	49.02	50.95	49.99	39.83	39.83	39.83
C_3N_2: Greengram$_{75\ \%\ RDN}$	60.54	60.27	60.41	44.45	44.26	44.36	37.08	35.08	36.08
***SEm*±**	1.60	1.92	-	1.36	1.72	-	0.80	0.70	-
C.D. (P=0.05)	5.05	6.06	-	3.97	5.42	-	2.52	2.03	-
***Rabi* maize with varied nitrogen fertility levels (F)**									
F_1: 100 % RDN	47.54	45.48	46.51	35.60	32.84	34.22	28.75	28.75	28.75
F_2: 125 % RDN	51.95	49.43	50.69	39.99	37.41	38.70	32.37	32.37	32.37
F_3: 150 % RDN	56.40	53.69	55.05	44.34	41.18	42.76	36.44	36.44	36.44
***SEm*±**	1.08	0.93	-	0.93	0.85	-	0.89	0.76	-
C.D. (P=0.05)	3.17	2.72	-	2.70	2.47	-	2.59	2.26	-
Interaction									
Sub treatments at the same level as Main treatments F X (C x N)									
***SEm*±**	2.39	2.02	-	1.89	1.96	-	2.03	2.07	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-
Main treatments at same/different levels of sub-treatments (C X N) X F									
***SEm*±**	2.66	2.29	-	2.04	2.07	-	2.18	2.18	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

Table 6. Acid phosphatase (µg PNP g^{-1} soil) of zero-till *rabi* maize as influenced by *kharif* legumes and nitrogen fertility levels during 2021-22 and 2022-23

Treatments	30 DAS			60 DAS			90 DAS		
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
***Kharif* Legumes x nitrogen levels (C X N)**									
C_1N_1: Groundnut$_{100\ \%\ RDN}$	48.02	41.49	44.76	69.80	67.01	68.41	62.81	59.88	61.35
C_1N_2: Groundnut$_{75\ \%\ RDN}$	40.98	37.10	39.04	62.03	58.94	60.49	52.46	50.32	51.39
C_2N_1: Soybean$_{100\ \%\ RDN}$	54.43	51.52	52.98	81.21	78.26	79.74	72.41	70.01	71.21
C_2N_2: Soybean$_{75\ \%\ RDN}$	51.49	46.80	49.15	76.12	73.82	74.97	66.20	64.86	65.53
C_3N_1: Greengram$_{100\ \%\ RDN}$	63.31	60.95	62.13	92.99	90.82	91.91	82.49	79.57	81.03
C_3N_2: Greengram$_{75\ \%\ RDN}$	57.48	56.45	56.97	88.63	86.90	87.77	76.82	74.63	75.73
***SEm*±**	0.61	1.22	-	0.70	1.36	-	1.50	1.61	-
C.D. (P=0.05)	1.92	3.84	-	2.21	3.91	-	4.36	4.59	-
***Rabi* maize with varied nitrogen fertility levels (F)**									
F_1: 100 % RDN	47.79	45.75	46.77	73.53	71.22	72.38	63.53	61.00	62.27
F_2: 125 % RDN	53.06	50.92	51.99	78.48	76.19	77.34	69.20	66.43	67.82
F_3: 150 % RDN	58.01	55.49	56.75	83.38	80.49	81.94	73.90	71.89	72.90
***SEm*±**	0.56	0.90	-	0.75	1.31	-	1.31	1.49	-
C.D. (P=0.05)	1.62	2.64	-	2.18	3.81	-	3.83	4.33	-
Interaction									
Sub treatments at the same level as Main treatments F X (C x N)									
***SEm*±**	1.31	2.01	-	1.69	2.97	-	3.09	3.47	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-
Main treatments at same/different levels of sub-treatments (C X N) X F									
***SEm*±**	1.36	2.22	-	1.83	3.20	-	3.22	3.64	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

Table 7. Alkaline phosphatase (µg PNP g^{-1} soil) of zero-till *rabi* maize as influenced by *kharif* legumes and nitrogen fertility levels during 2021-22 and 2022-23

Treatments	30 DAS			60 DAS			90 DAS		
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
***Kharif* Legumes x nitrogen levels (C X N)**									
C_1N_1: Groundnut$_{100\ \%\ RDN}$	27.59	28.88	28.24	51.75	49.67	50.71	40.78	36.82	38.80
C_1N_2: Groundnut$_{75\ \%\ RDN}$	24.85	23.64	24.25	46.14	43.58	44.86	36.34	31.77	34.06
C_2N_1: Soybean$_{100\ \%\ RDN}$	35.29	39.39	37.34	63.32	63.95	63.64	50.93	48.41	49.67
C_2N_2: Soybean$_{75\ \%\ RDN}$	30.07	35.86	32.97	58.37	55.99	57.18	45.37	42.17	43.77
C_3N_1: Greengram$_{100\ \%\ RDN}$	49.81	47.17	48.49	73.62	71.04	72.33	60.92	58.50	59.71
C_3N_2: Greengram$_{75\ \%\ RDN}$	46.99	44.51	45.75	68.54	66.87	67.71	55.29	53.09	54.19
***SEm*±**	1.20	0.90	-	1.48	1.30	-	1.20	1.44	-
C.D. (P=0.05)	3.50	2.56	-	4.31	4.10	-	3.78	4.53	-
***Rabi* maize with varied nitrogen fertility levels (F)**									
F_1: 100 % RDN	32.08	30.34	31.21	55.89	53.67	54.78	46.49	43.79	45.14
F_2: 125 % RDN	36.35	34.23	35.29	60.69	58.38	59.54	51.48	48.32	49.90
F_3: 150 % RDN	39.87	36.66	38.27	65.29	63.00	64.15	56.84	53.52	55.18
***SEm*±**	1.10	0.67	-	1.07	0.81	-	1.00	1.10	-
C.D. (P=0.05)	3.21	1.94	-	3.11	2.38	-	2.90	3.21	-
Interaction									
Sub treatments at same level as Main treatments F X (C X N)									
***SEm*±**	2.58	1.42	-	2.39	1.92	-	2.27	2.43	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-
Main treatments at same/different levels of sub-treatments (C X N) X F									
***SEm*±**	2.70	1.54	-	2.53	2.00	-	2.44	2.70	-
C.D. (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

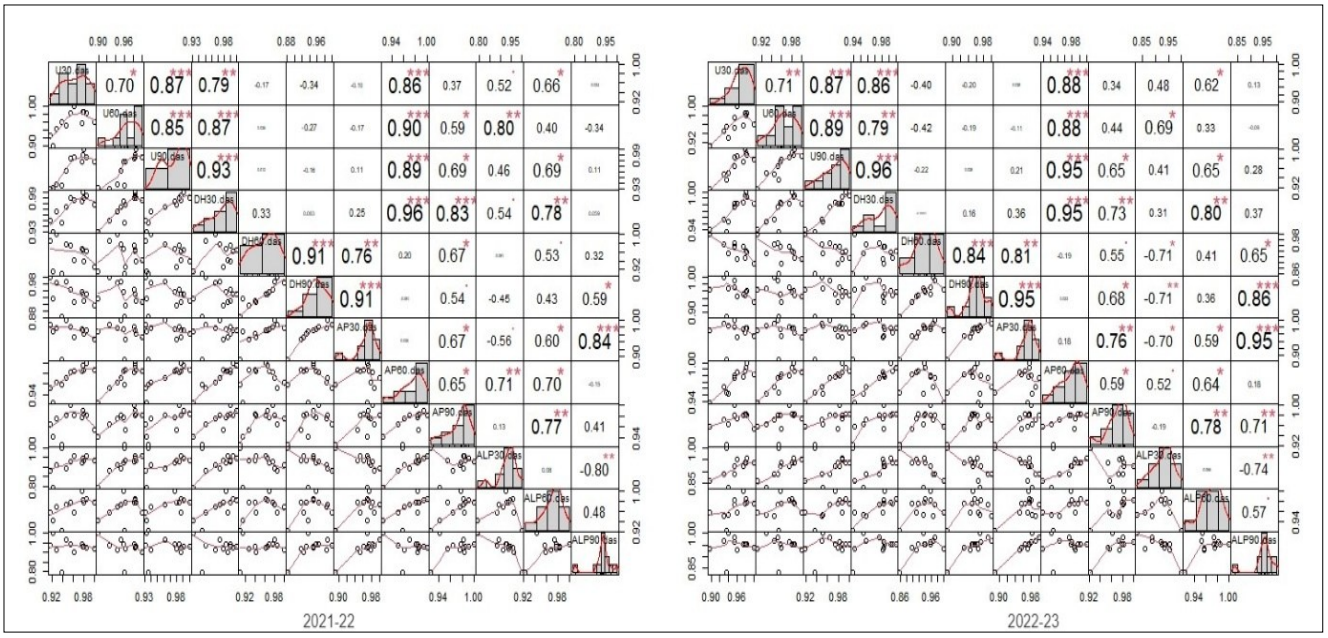

Fig. 1. Data representation in correlation by using R-software of soil enzymatic activity at the crop stages of *rabi* maize with *kharif* legumes of 2021-22 and 2022-23.

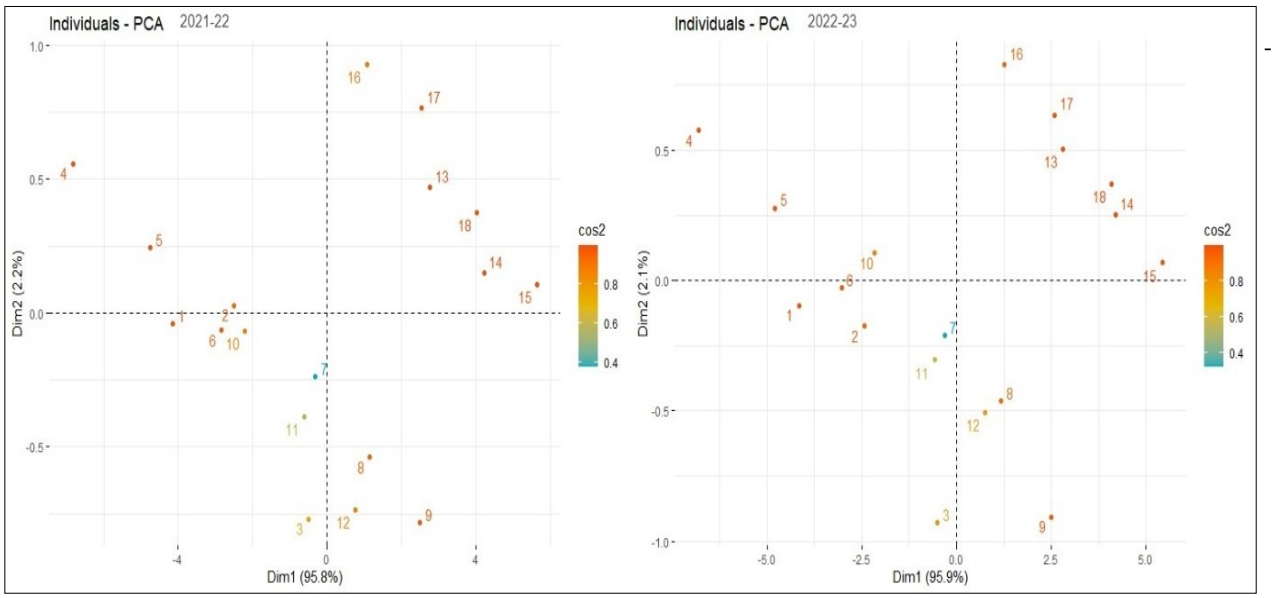

Fig. 2. Treatment comparison analysis of the residues of legumes in rabi maize in 2021-22 and 2022-23 by using PCA in R-software. Note: "cos2 (squared cosine)" value represents the quality of representation of a variable on a principal component, "Dim2" would refer to the second principal component, representing the second-highest direction of variance.

maize sequence. Besides, a further indirect measure of soil microbial biomass is dehydrogenase activity, a member of the class of intracellular enzymes that actively catalyze the oxidation of organic compounds present in microorganism cells, it was illustrated that the growth of microbial activity was dependent on residual retention; the addition of nitrogen initially favoured the activity and it decreased until harvest. The previous findings support for this study (23, 24) .

Soil microbial properties of zero-till maize

The trial outcomes in Table 3 depict that the activity of higher biomass accumulation synchronized for higher bacterial build-up reflected in green gram with optimum N rates (6.91, 9.52, 7.58, 6.71 CFU g^{-1} soil) followed by soybean (6.00, 8.58, 7.02, 6.04 CFU g^{-1} soil) and least was seen in groundnut-maize rotation (5.51, 8.21, 6.70, 5.80 CFU g^{-1} soil) at 15, 30, 45 and 60 DAS respectively.

Likewise, a 50 % more N rate deviation from normal out turn (6.64, 9.03, 7.32, 6.22 CFU g^{-1} soil) in significantly greater bacterial colonies than a 25 % deviation (6.49, 8.87, 7.14, 6.08 CFU g^{-1} soil) and a 0 % deviation (6.20, 8.66, 6.90, 5.84 CFU g^{-1} soil) in nitrogen alone until 60 growing days. However, the correlation across biomass pulse with N gradient rates proved to be minimal over time.

Further, fig. 3 depicts that PCA biplots of bacterial count and CN values for the two years interpreted a consistent structure in the data, with Dim1 explaining most of the variance (around 84.6 %-84.7 %) and Dim2 explaining a smaller portion (around 12.7 %-12.9 %). The variables grouped by treatment conditions (BC and CN) exhibit strong correlations along phenophase stages and have a substantial influence on the primary principal component (Dim_1) suggesting that the primary factors influencing the variance in the data remain stable over the two periods. Among BC (bacterial count) Variables: BC_{15das} (days after sowing), BC_{30das}, BC_{45das} and BC_{60das} are variables that cluster closely and show a moderate

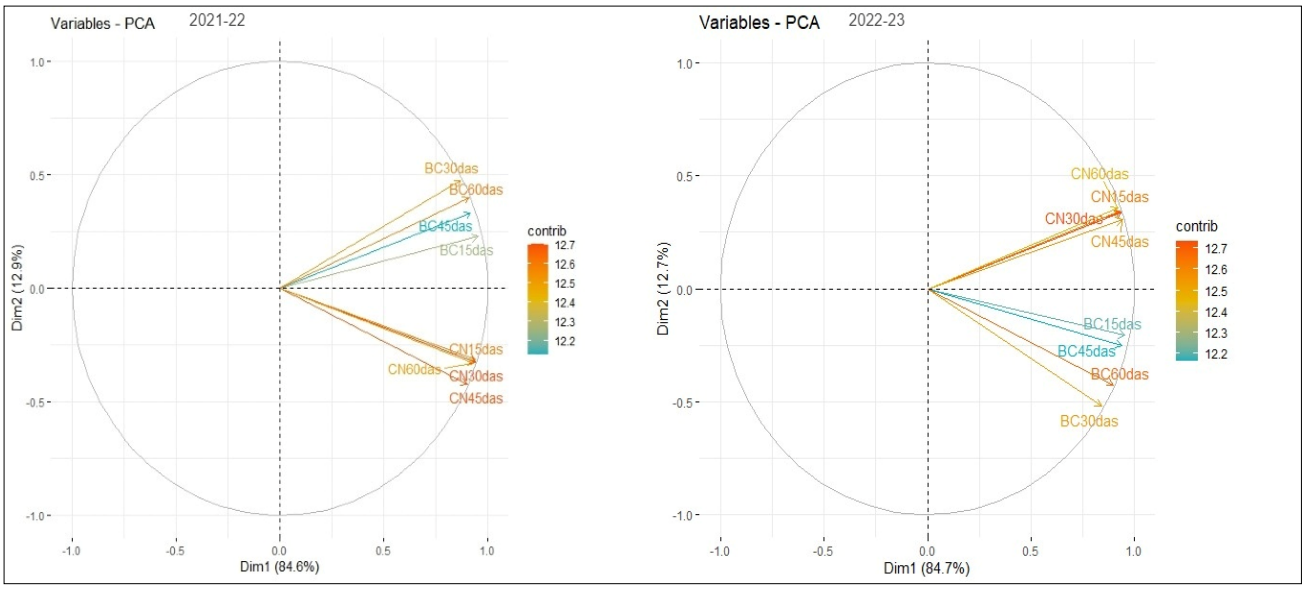

Fig. 3. Performing a PCA in R to compare the bacterial count and the C: N within the legume-maize system across the years 2021-22 and 2022-23. Note: "Dim1" refers to the first principal component, which represents the direction in a dataset with the highest variance. It's the axis along which the data points are most spread out.

contribution to Dim_1, suggesting they are strongly correlated with each other and primarily influence Dim_1. Further, CN (carbon and nitrogen) Variables: CN_{15das}, CN_{30das}, CN_{45das} and CN_{60das} represent similar measures, also cluster and have a strong contribution to Dim1, identical to the BC variables in their contributions as indicated by the colour gradient. This reciprocates the systematic availability of residual shoots and leaves having a C:N ratio for soil microbial activity enhanced by green gram as a component in increasing yields above and activity of soil below the biosphere. Further, the overall variation in field was represented in fig. 4. Previous researcher's mentioned comparable studies with the current trial (25).

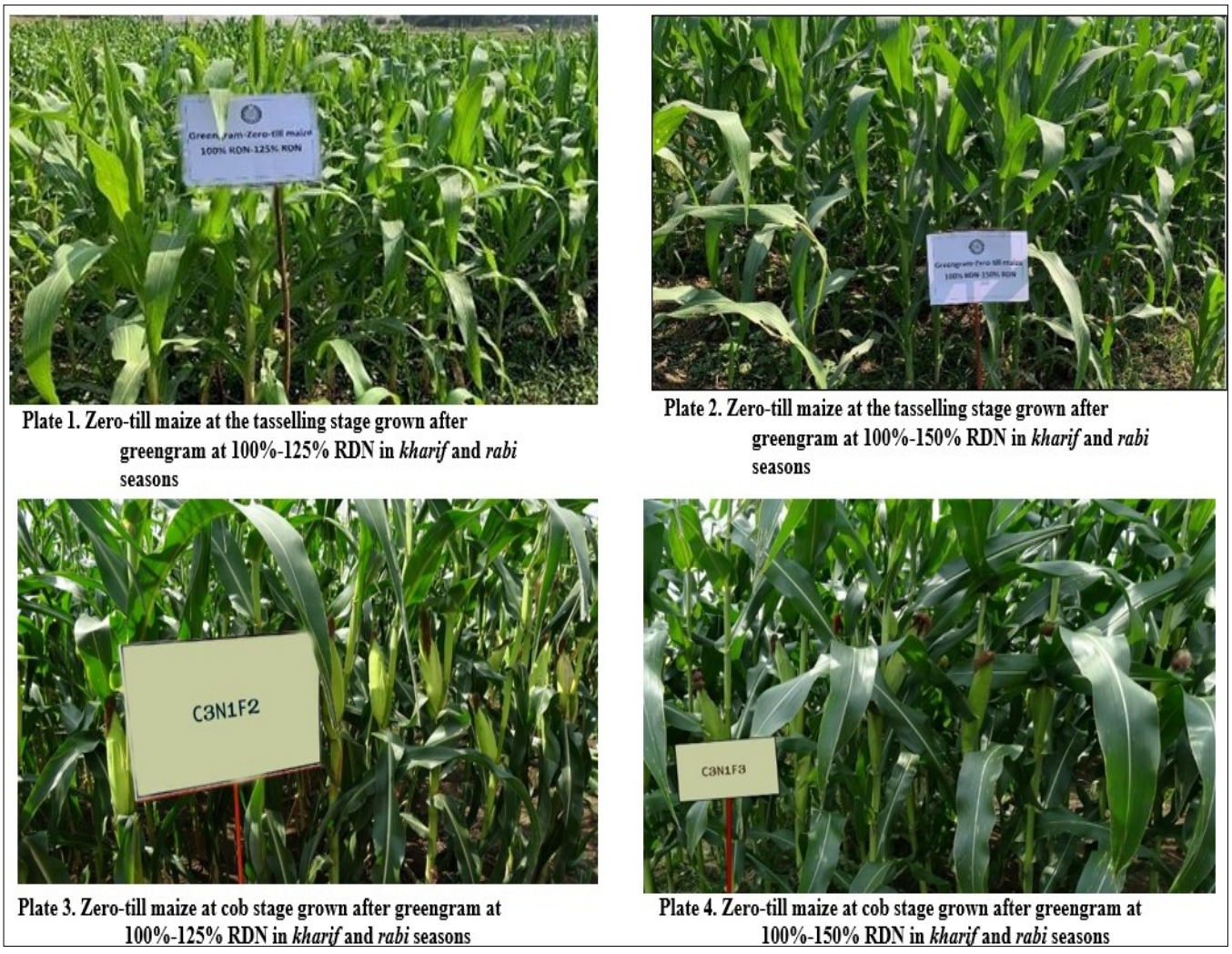

Fig. 4. Comparative study of the critical stage of greengram followed by maize with 125 and 150 % RDN.

Conclusion

The research conclusively indicated that the greengram–maize cropping sequence produced more biomass when it was treated with a full dose of nitrogen during the *kharif* season and 150 % nitrogen during the *rabi* season over a period of years. This nutritional approach boosted microbial populations, promoted enhanced microbial activity and sped up residue breakdown favours the increased soil nutrient availability, which raised crop yields. Although, this methodology promotes the effective conversion of crop waste into enhanced organic matter that stimulates sustainable approach to nitrogen management that is in line with cropping system regenerative practices.

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Authors' contributions

SKA carried out the experiment and wrote the original draft. SD supervision, validation, reviewed and edited the manuscript. MRM performed data validation. SKT analysis and interpretation of results. NKMV supplied the necessary resources. TSK and SKA reviewed and edited the manuscript. SKA analysis and interpretation of results. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The writers affirm that neither of the works disclosed in this publication may have been influenced by any known competing financial interests or personal claims.

Ethical issues: None

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