



Cajanus cajan enhances nutrient cycling and supports *Eragrostis curvula* growth

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Abstract

Cajanus cajan (Pigeon pea) enhances soil nutrition by increasing bacterial communities and their associated enzyme activities, creating optimal conditions for the growth of succeeding crops like *Eragrostis curvula* (Weeping Love Grass). This study explored the viability of rotating *C. cajan* and *E. curvula* and assessed their effects on soil nutrient cycling. *Cajanus cajan* and *E. curvula* were monocropped and rotated in soils collected from Jameson Park, Kaydale, and Rensburg, Gauteng, South Africa. Pre-planting and post-harvest soil analyses were done to determine the nutrient concentrations, pH, total cation concentrations, exchange acidity, enzyme activities, and bacterial identification. Furthermore, the growth kinetics and nitrogen (N) source reliance of *C. cajan* and *E. curvula* were determined. *Cajanus cajan* and *E. curvula* selected for different bacterial genera in their rhizosphere soils. *Cajanus cajan* selected for *Pseudomonas*, *Novosphingobium*, *Paraburkholderia*, *Burkholderia*, and *Croceicoccus* genera, while *E. curvula* selected for *Pseudomonas*, *Flavobacterium*, *Pedobacter*, *Arthrobacter*, *Pseudoarthrobacter*, and *Luteimonas* species. The rotation of *C. cajan* and *E. curvula* selected for *Pseudomonas*, *Caulobacter*, and *Pedobacter* genera. Monocropped *C. cajan* significantly increased nitrate reductase, β -glucosidase, and acid and alkaline phosphatase activities compared to rotation treatments and *E. curvula* monocropping. *Cajanus cajan* primarily derived N from the atmosphere (NDFA), with 62.67%, 63.38%, and 62.04% in Jameson Park, Kaydale, and Rensburg soils, respectively. Rotated *E. curvula* showed higher reliance on NDFA and had higher plant N and P concentrations than in monocropping. This study highlights the role of *C. cajan* in enhancing soil nutrient dynamics, suggesting its rotation with *E. curvula* can yield multiple benefits for subsistence farmers in impoverished communities.

Keywords Nutrient cycling · Crop rotation · Pigeon pea · Weeping love grass

1 Introduction

By 2050, an estimated 130 million people could face climate-related poverty as climate change continues to pose a significant threat to global food security, particularly impacting vulnerable communities (World Economic Forum

2023). The Food and Agriculture Organisation (2016) reported that extreme weather events driven by climate change are expected to push millions into acute food insecurity across various regions. Furthermore, climate change has been reported to exacerbate soil degradation, reducing soil fertility and agricultural productivity (Lal et al. 2011). The impacts of soil degradation have been reported to cause salinisation, reduce organic matter, lower pH, and alter microbial profiles (Kopittke et al. 2019; Drinkwater and Snapp 2022), further threatening food security.

Human activities, combined with the impacts of climate change, have a compounding effect on soil degradation, altering soil properties and consequently disrupting microbial communities (Dixit et al. 2024). These disturbances often lead to reduced microbial diversity, essential for maintaining soil health and supporting nutrient cycling (Dixit et al. 2024). Silva et al. (2024) reported that degraded soils had significantly lower bacterial diversity and richness,

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highlighting the impact of soil degradation on soil ecosystem functioning. Soil microbes play a critical role in nutrient cycling by producing extracellular enzymes that mineralise nutrients (Adetunji et al. 2017), helping sustain plant growth and soil fertility. Diversified cropping systems such as crop rotations have been reported to optimise soil health by 45% (Yang et al. 2024), reducing reliance on inorganic fertiliser and improving agricultural productivity (Zotarelli et al. 2007; Siddique et al. 2012). Rotations involving pulse crops such as *Cajanus cajan* have been shown to improve soil organic carbon, fertility, and microbial activity, which in turn enhances nutrient cycling (Govaerts et al. 2007; Kamanga et al. 2014).

The soil nutrient enhancements are due to crop residuals such as leaf litter and leftover roots during harvest that increase the decomposition rate, consequently enhancing soil nutrient cycling and making nutrients available for uptake by succeeding crops (Govaerts et al. 2007; Venkatesh et al. 2017). *Cajanus cajan* is an underutilised legume that plays a role in soil fertility restoration, as it improves soil pH, organic carbon, and nitrogen levels (Mamuye et al. 2020) and has been shown to increase microbial carbon levels, supporting its use in soil quality improvements (Bicalho et al. 2024). Aihou et al. (2006) reported that *C. cajan* is primarily used in cropping systems to improve soil fertility, potentially influencing the nutrition of succeeding crops such as *Eragrostis curvula* in crop rotations. *Eragrostis curvula*, primarily used as pasture in South Africa, has potential as a food crop, particularly for its gluten-free and nutrient-dense grain (Ghebrehewot et al. 2016). Its use in rotation systems with legumes like *C. cajan* offers a promising solution to soil degradation and food security issues in sub-Saharan Africa.

This study investigated the viability of a rotation system incorporating *E. curvula* and *C. cajan*, exploring its impacts on soil nutrition, enzyme activities, bacterial composition, and the growth of *E. curvula* in both monocropping and rotation systems. The objectives of this study include: (1) assessing the soil nutrient concentrations in the *E. curvula* monocropping, *C. cajan* monocropping, and *C. cajan-E. curvula* rotation treatment, (2) assaying the soil extracellular enzyme activities in *E. curvula* monocropping, *C. cajan* monocropping, and *C. cajan-E. curvula* rotation treatment, (3) isolating and identifying soil bacteria from the *E. curvula* monocropping, *C. cajan* monocropping, and *C. cajan-E. curvula* rotation treatment, (4) isolating and identifying bacteria in *C. cajan* nodules, and (5) determining the nitrogen (N) reliance of *E. curvula* and *C. cajan* in the monocropping and rotation treatments.

2 Materials and methods

2.1 Soil collection

Experimental soil samples were collected at three geographical sites in Heidelberg, Gauteng, South Africa. These sites included Jameson Park (26°26'31.7 "S; 28°26'01.4 "E), Kaydale (26°29'12.4 "S; 28°23'02.1 "E), and Rensburg (26°30'16.0"S; 28°26'11.3"E). From each site, 30 soil samples spaced 2 m apart were combined to create composite samples. Five 500 g subsamples from each site were sent to the Analytical Services Unit of the KwaZulu-Natal Department of Agriculture and Rural Development in Cedara, South Africa, for analysis of soil characteristics, including nutrient and total cation concentrations, exchange acidity, and pH. The remaining soil was utilised for plant growth experiments, bacterial extraction and identification, and extracellular enzyme activity assays.

2.2 *Cajanus cajan-Eragrostis curvula* pot trials

The experiment included three treatments per collection site: *C. cajan* monocrop, *E. curvula* monocrop, and *C. cajan-E. curvula* rotation, each with 18 replicates. *Cajanus cajan* seeds from AGT Foods Africa were soaked overnight in deionised water to break dormancy, then placed in sterile Petri dishes with filter paper and kept moist until radicle emergence. Seedlings were transplanted into 18 cm pots and grown under greenhouse conditions with temperatures ranging from 22 to 37 °C during the day and 12–17 °C at night. Treatments were watered every two days and harvested 57 days post-emergence, with half of the pots harvested 22 days after planting. Plants were separated into leaves, stems, roots, and nodules for bacterial extraction. The plant material was oven-dried at 65 °C to constant weight.

Soils collected after harvesting *C. cajan* were mixed into composite samples and decanted into clean 18 cm diameter pots with 15 replicates per site. The remaining soils were sent for nutrient analysis and stored for bacterial extraction, identification, and soil enzyme assays. *Eragrostis curvula* seeds from Agricol, South Africa, were planted at 1–1.5 cm depths, with three seeds per pot. The same planting method was used for the monocropping system. Pots were arranged in a randomised block design in the greenhouse and irrigated every two days. Both monocropped and rotated *E. curvula* plants were harvested 57 days post-emergence, oven-dried at 65 °C until a constant weight was achieved. Soils from the *E. curvula* treatments were stored at 4 °C for nutrient and acidity analysis, bacterial extraction, and enzyme activity assays. Dried plant material from all treatments and collection sites was weighed, ground into powder, and sent for

nitrogen isotope and phosphorus analysis at the University of Cape Town and Stellenbosch University, respectively.

2.3 Nodule content and soil bacterial extraction and identification

Cajanus cajan nodules were harvested and surface sterilised by immersing them in 70% ethanol (v/v) for 30 s, followed by a 3-minute treatment with 3.5% sodium hypochlorite solution (v/v), and rinsing thoroughly with sterile distilled water 10 times, as outlined by Somasegaran and Hoben (1994). The nodules were crushed in 100 μ L of 15% glycerol using sterile tips. A 10 μ L aliquot of the resulting suspension was cultured on yeast mannitol agar in sterile Petri dishes and incubated at 30 °C for five days. Pure bacterial colonies were isolated through repeated streaking and further amplified via polymerase chain reaction (PCR) using the 16 S primers 63 F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGTGTGTACAA-3'). PCR amplification was conducted with EmeraldAmp GT Master Mix (Takara Bio Inc., distributed by Separations, South Africa) under the following conditions: an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. Amplified PCR products were separated on a 1% agarose gel and visualised under UV light to confirm correct product size before being sent for sequencing (Inqaba Biotechnical Industries, South Africa). The resulting DNA sequences were compared to known bacterial nucleotide sequences in the GenBank database (National Centre for Biotechnology Information, NCBI) using the BLAST tool (<https://www.ncbi.nlm.nih.gov>).

Fifty grams of composite soil samples from *C. cajan* monocropping, *E. curvula* monocropping, and *C. cajan-E. curvula* rotation systems were subjected to serial dilutions. Three rounds of dilutions were performed on each 10 g soil sample, and 100 μ L from each dilution was cultured in sterile Petri dishes containing selective media. Pikovskaya's medium with tricalcium phosphate (TCP) was used for phosphorus-solubilising bacteria, Simmons citrate agar for nitrogen-cycling bacteria, and Jensen's agar for nitrogen-fixing bacteria, following Ndabankulu et al. (2022). Each medium was plated in triplicate and incubated at 30 °C for five days. Pure bacterial colonies were isolated through repeated streaking, and a portion of these colonies was amplified via PCR using the protocol described above.

2.4 Soil extracellular enzyme activity analysis

Soils collected from each harvest and treatment were analysed for enzymatic activities, including β -glucosidase, acid

phosphatase, nitrate reductase, and alkaline phosphatase. The activities of β -glucosidase, acid phosphatase, and alkaline phosphatase were quantified using a fluorescence-based method adapted from Jackson et al. (2013) and expressed as $\text{nmol h}^{-1} \text{g}^{-1}$. In summary, 10 g of soil was homogenised in 100 mL of autoclaved distilled water at medium speed for two hours, followed by overnight storage at 4 °C. The supernatants were then transferred to black 96-well microplates, and their respective substrates were added. The assay included several controls: reference standards (200 μ L bicarbonate buffer+50 μ L standard), quench standards (200 μ L soil aliquot+50 μ L standard), sample controls (200 μ L soil aliquot+50 μ L buffer), negative controls (200 μ L buffer+50 μ L substrate), and blanks (250 μ L buffer). The plates were incubated at 25 °C for two hours, and the reaction was terminated by adding 5 μ L of 0.5 M NaOH to each well. Fluorescence was measured at 450 nm using a Glomax Multi Plus microplate reader. Acid phosphatase activity was determined under adjusted conditions, with the buffer and standard maintained at pH 5.

Nitrate reductase activity was assessed following a modified protocol by Kandeler (1995). A foil-covered volumetric flask was prepared with 1 mL of 25 mM KNO_3 , 4 mL of 0.9 mM 2,4-dinitrophenol, and 5 mL of Milli-Q distilled water. Five grams of soil were added to the mixture, sealed with foil, shaken, and incubated at 30 °C for 24 h. After incubation, 10 mL of 4 M KCl was added, mixed thoroughly, and filtered through Whatman No. 1 filter paper. The enzymatic reaction was initiated by combining 2 mL of the filtrate with 1.2 mL of 0.19 M ammonium chloride buffer (pH 8.5) and 0.8 mL of a colour reagent containing 1% sulfanilamide, 1 N HCl, and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). This solution was incubated at 30 °C for 30 min. Absorbance was measured at 520 nm using an 1800 UV spectrophotometer, and the nitrite (NO_2^-) released was calculated using a standard curve prepared with KNO_3 . Nitrate reductase activity was expressed as $0.1 \mu\text{mol h}^{-1} \text{g}^{-1}$.

2.5 Soil nutrient and relative acidity analysis

Three 500 g soil samples were collected following the harvests of *C. cajan* monocropping, *E. curvula* monocropping, and *C. cajan-E. curvula* rotations and sent to the Analytical Services Unit at the KwaZulu Natal Department of Agriculture and Rural Development, Cedara, South Africa, for soil characteristic analyses. The analyses, including nutrient concentrations, total cation concentrations, exchange acidity, and pH, were conducted according to the protocols described by Manson and Roberts (2000).

Phosphorus, potassium, zinc, copper, and manganese concentrations were extracted using an Ambic-2 solution containing 0.25 M NH_4CO_3 , 0.01 M Na_2EDTA , 0.01 M

NH₄F, and 0.05 g/L superfloc (N100), which was adjusted to pH 8 with concentrated ammonia. The extracts were filtered using Whatman No. 1 paper, and aliquots of the filtrate were used for analysis. Phosphorus concentration was measured using a modified version of Murphy and Riley's (1962) molybdenum blue method. Potassium concentration was determined by diluting a 5 mL aliquot of the filtrate with 20 mL deionised water, followed by atomic absorption spectroscopy. The undiluted filtrate was used to measure zinc, copper, and manganese concentrations, also via atomic absorption.

For magnesium and calcium analysis, 25 mL of soil sample was mixed with 25 mL of 1 M KCl solution and stirred at 400 rpm for 10 min in a multiple stirrer. The mixture was filtered through Whatman No. 1 paper, and 5 mL of the filtrate was diluted with 20 mL of 0.0356 M SrCl₂. Magnesium and calcium concentrations were then determined using atomic absorption spectroscopy.

Nitrogen concentration in the soil was measured using the Automated Dumas dry combustion method with a LECO CNS 2000 analyser (Leco Corporation, USA). Soil samples were weighed into ceramic crucibles, and 0.5 g of vanadium pentoxide was added as a combustion catalyst. The samples were combusted in a horizontal furnace at 1350 °C under a stream of oxygen, and nitrogen content was quantified as N₂ using a thermal conductivity cell.

Soil pH was measured by mixing 10 mL of soil sample with 25 mL of 1 M KCl solution, followed by stirring at 400 rpm for 5 min. After allowing the suspension to rest for 30 min, the pH was recorded using a gel-filled combination glass electrode while the mixture was stirred.

2.6 Plant growth kinetics

Relative growth rate and root-to-shoot ratio.

The relative growth rate (RGR) and root-to-shoot ratio of *C. cajan* and *E. curvula* under all treatments were determined following the method described by Agren and Franklin (2003).

The RGR was calculated using the formula:

$$\text{RGR} = [(\ln W_2 - \ln W_1) / t_2 - t_1]$$

W₁ and W₂ represent the plant weights at the initial and final harvests, respectively, and t₁ and t₂ denote the time points corresponding to the two harvests.

Root-to-Shoot ratio was calculated as the proportion of dry root mass over shoot mass (WR/WS).

Specific nitrogen/phosphorus absorption rate (SNAR/SPAR) and specific nitrogen/phosphorus utilisation rate (SNUR/SPUR).

The total nitrogen (N) and phosphorus (P) concentrations of plants across all treatments and sites were utilised to determine the specific nitrogen/phosphorus absorption rate and the specific nitrogen/phosphorus utilisation rate, as described by Nielsen et al. (2001).

$$\text{SNAR} = (L_2 - L_1/t_2 - t_1) \times [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SPAR} = (M_2 - M_1/t_2 - t_1) \times [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SNUR} = (W_2 - W_1/t_2 - t_1) \times [(\log_e L_2 - \log_e L_1) / (L_2 - L_1)]$$

$$\text{SPUR} = (W_2 - W_1/t_2 - t_1) \times [(\log_e M_2 - \log_e M_1) / (M_2 - M_1)]$$

L and M represent the plant's total nitrogen (N) and phosphorus (P) contents, respectively. T denotes the duration of plant growth between the two harvests, and R corresponds to the root dry weight.

Percentage nitrogen derived from the atmosphere (%NDFA).

The percentage of nitrogen derived from the atmosphere (%NDFA) was determined following the method of Shearer and Kohl (1986), using the formula $\delta = 1000 \times (R_{\text{sample}} / R_{\text{standard}})$, where R is the molar ratio of ¹⁵N to ¹⁴N in the samples and standards. Sample preparation and analysis were performed as outlined by Matiwané et al. (2019).

$$\% \text{NDFA} = 100 \left(\frac{(\delta^{15}\text{N}_{\text{referenceplant}} - \delta^{15}\text{N}_{\text{legume/grass}})}{(\delta^{15}\text{N}_{\text{referenceplant}} - \delta)} \right)$$

The β value represents the δ¹⁵N natural abundance of the N derived from the biological N₂ fixation of *C. cajan* and *E. curvula*. The B values were estimated from plants grown in plain sand with no nutrients added and under the same conditions as the experimental plants. The total amount of N in the plant derived from N₂ fixation (Nfx) was determined by: Nfx = Ndfa × N content.

2.7 Statistical analysis

The statistical software R (version 3.6.2) was used to test for differences in the nutrient concentrations, pH, exchange acidity, soil enzyme activity, and total cation concentrations in soil samples collected after the *E. curvula* monocropping, *C. cajan* monocropping, and *C. cajan*-*E. curvula* rotation harvest using One-way ANOVA. Where the ANOVA showed significant differences among the treatments, a

Tukey post hoc test was used to separate the means. A One-way ANOVA was used to test for differences in the growth kinetics of *C. cajan* growing in Jameson Park, Kaydale, and Rensburg soils. Where the ANOVA showed significant differences among the treatments, a Tukey post hoc test was used to separate the means. An independent samples t-test was used to assess differences in the growth parameters and plant nutrition of monocropped and rotated *E. curvula*. The assumptions of normality and homogeneity of variances were evaluated using the Shapiro-Wilk test and Levene's test, respectively. When these assumptions were not met, the Wilcoxon rank-sum test, a non-parametric alternative, was applied.

3 Results

3.1 Nutrient concentrations, relative acidity and bacterial composition of pre-planting soils

The nutrient concentrations, relative acidity and bacterial composition of pre-planting soils is represented in Supplementary Table 1. Soils collected from all sites were acidic, with pH ranging from 4.72 to 5.06. The pH, total cation, Mg, Ca, and Cu concentrations of Rensburg soils were significantly higher than those of Jameson Park and Kaydale soils. Jameson Park soils had significantly lower pH, total cation, N, Mn, Zn, Mg, and Ca concentrations than Kaydale and Rensburg soils. The phosphorus concentration was the lowest macronutrient concentration, followed by N for all three sites. The bacteria isolated from Jameson Park soils belonged to the *Bacillus* and *Flavobacterium* genera.

3.2 Cajanus cajan growth kinetics

The growth kinetics of *C. cajan* in soils collected from Jameson Park, Kaydale, and Rensburg, Heidelberg, are presented in Table 1. *Cajanus cajan* plants grown in Jameson Park, Kaydale, and Rensburg soils showed no significant differences in root, shoot, leaf, and total biomass. Growth parameters, including the root-to-shoot ratio, RGR, SNAR, SNUR, SPAR, SPUR, and concentrations of P and N in *C. cajan* plants, were also not significantly different across the study sites. The *C. cajan* plants across all collection sites relied more on NDFA (> 60%).

3.3 Eragrostis curvula growth kinetics

The aboveground, belowground, and total plant biomass of rotated *E. curvula* was significantly higher than that of monocropped *E. curvula* across all soil collection sites (Table 2). In Jameson Park soils, rotated *E. curvula* showed significantly higher root-to-shoot ratio, RGR, SNAR, SNUR, NDFA, and plant P and N concentrations, with no significant differences in SPAR, SPUR, and NDFS compared to monocropping. In Kaydale soils, rotated *E. curvula* showed significantly higher RGR, SNAR, SNUR, plant P, and N concentrations, while root-to-shoot ratio, SPAR, SPUR, NDFA, and NDFS showed no significant differences. In Rensburg soils, rotated *E. curvula* showed significantly higher RGR, SNAR, SNUR, NDFA, and plant P and N concentrations than monocropped plants, with no significant differences in root-to-shoot ratio, SPAR, and SPUR. Across all sites, rotated *E. curvula* relied more on NDFA, with %NDFA consistently above 50, than on NDFS.

Table 1 Plant growth kinetics of *Cajanus cajan* growing in soils collected in Jameson park, Kaydale and Rensburg soils, Heidelberg, Gauteng. Values mean $\pm$ sd, $n = 54$

	Jameson Park	Kaydale	Rensburg
Biomass (g)			
Roots	0.412 $\pm$ 0.022 ^a	0.401 $\pm$ 0.025 ^a	0.336 $\pm$ 0.014 ^a
Shoot	0.645 $\pm$ 0.069 ^a	0.584 $\pm$ 0.045 ^a	0.516 $\pm$ 0.007 ^a
Leaves	0.411 $\pm$ 0.023 ^a	0.368 $\pm$ 0.026 ^a	0.329 $\pm$ 0.015 ^a
Total plant biomass	1.083 $\pm$ 0.046 ^a	0.981 $\pm$ 0.028 ^a	0.864 $\pm$ 0.005 ^a
Plant growth kinetics			
Root: Shoot	0.623 $\pm$ 0.047 ^a	0.691 $\pm$ 0.029 ^a	0.648 $\pm$ 0.021 ^a
RGR (g day ⁻¹)	0.022 $\pm$ 0.002 ^a	0.027 $\pm$ 0.005 ^a	0.038 $\pm$ 0.004 ^a
SNAR (mg N g ⁻¹ root DW day ⁻¹)	2.83 $\pm$ 0.44 ^a	2.97 $\pm$ 0.39 ^a	3.59 $\pm$ 0.97 ^a
SPAR (mg P g ⁻¹ root DW day ⁻¹)	0.257 $\pm$ 0.040 ^a	0.322 $\pm$ 0.145 ^a	0.214 $\pm$ 0.011 ^a
SNUR (mg N g ⁻¹ DW day ⁻¹)	1.86 $\pm$ 0.09 ^a	1.65 $\pm$ 0.03 ^a	1.64 $\pm$ 0.02 ^a
SPUR (mg P g ⁻¹ DW day ⁻¹)	1.081 $\pm$ 0.000 ^a	0.978 $\pm$ 0.001 ^a	0.862 $\pm$ 0.002 ^a
%NDFA	62.67 $\pm$ 2.13 ^a	63.38 $\pm$ 1.98 ^a	62.04 $\pm$ 2.87 ^a
Nitrogen derived from the atmosphere (mmol N g ⁻¹)	1.12 $\pm$ 0.17 ^a	1.17 $\pm$ 0.15 ^a	1.42 $\pm$ 0.39 ^a
Nitrogen derived from the soil (mmol N g ⁻¹)	0.69 $\pm$ 0.11 ^a	0.73 $\pm$ 0.10 ^a	0.88 $\pm$ 0.24 ^a
Plant nutrition			
Nitrogen (mmol g ⁻¹)	1.81 $\pm$ 0.31 ^a	1.94 $\pm$ 0.32 ^a	2.39 $\pm$ 0.53 ^a
Phosphorus (μ mol g ⁻¹)	63.05 $\pm$ 14.27 ^a	62.29 $\pm$ 15.62 ^a	75.11 $\pm$ 7.32 ^a

Table 2 Plant growth kinetics of *Eragrostis curvula* monocrop and rotation growing in soils collected from Jameson park, Kaydale, and Rensburg, Heidelberg, Gauteng. Values represent mean $\pm$ se, $n=108$. Different letters represent significant differences after an independent samples Ttest ($p \leq 0.05$)

Parameter	Jameson Park		Kaydale		Rensburg	
	<i>Eragrostis curvula</i> monocrop	<i>Eragrostis curvula</i> rotation	<i>Eragrostis curvula</i> monocrop	<i>Eragrostis curvula</i> rotation	<i>Eragrostis curvula</i> monocrop	<i>Eragrostis curvula</i> rotation
Biomass (g)						
Aboveground biomass	0.001 $\pm$ 0.0003 ^a	0.05 $\pm$ 0.003 ^b	0.002 $\pm$ 0.0001 ^c	0.06 $\pm$ 0.003 ^d	0.007 $\pm$ 0.0004 ^e	0.101 $\pm$ 0.001 ^f
Belowground biomass	0.002 $\pm$ 0.0001 ^g	0.04 $\pm$ 0.0001 ^h	0.003 $\pm$ 0.000 ⁱ	0.05 $\pm$ 0.0003 ^j	0.005 $\pm$ 0.0002 ^k	0.09 $\pm$ 0.002 ^l
Total plant biomass	0.006 $\pm$ 0.001 ^m	0.09 $\pm$ 0.003 ⁿ	0.012 $\pm$ 0.001 ^o	0.08 $\pm$ 0.002 ^p	0.005 $\pm$ 0.0004 ^q	0.10 $\pm$ 0.010 ^r
Growth kinetics						
Root: Shoot	1.26 $\pm$ 0.23 ^s	0.68 $\pm$ 0.02 ^t	1.20 $\pm$ 0.37 ^u	0.91 $\pm$ 0.04 ^u	1.00 $\pm$ 0.13 ^w	0.89 $\pm$ 0.04 ^w
RGR (g day ⁻¹)	0.027 $\pm$ 0.005 ^y	0.049 $\pm$ 0.001 ^z	0.031 $\pm$ 0.006 ^{aa}	0.096 $\pm$ 0.019 ^{ab}	0.033 $\pm$ 0.001 ^{ac}	0.063 $\pm$ 0.010 ^{ad}
SPAR (mg P g ⁻¹ root DW day ⁻¹)	0.083 $\pm$ 0.002 ^{ae}	0.080 $\pm$ 0.001 ^{ae}	0.087 $\pm$ 0.001 ^{ae}	0.087 $\pm$ 0.003 ^{ae}	0.039 $\pm$ 0.0004 ^{ae}	0.036 $\pm$ 0.008 ^{ae}
SPUR (mg P g ⁻¹ plant DW day ⁻¹)	0.242 $\pm$ 0.005 ^{af}	0.217 $\pm$ 0.004 ^{af}	0.165 $\pm$ 0.002 ^{af}	0.167 $\pm$ 0.045 ^{af}	0.072 $\pm$ 0.001 ^{ag}	0.067 $\pm$ 0.002 ^{ag}
SNAR (mg N g ⁻¹ root DW day ⁻¹)	0.395 $\pm$ 0.103 ^{ah}	9.769 $\pm$ 1.341 ^{ai}	2.373 $\pm$ 0.331 ^{aj}	7.918 $\pm$ 3.091 ^{ak}	1.628 $\pm$ 0.817 ^{al}	8.652 $\pm$ 0.019 ^{am}
SNUR (mg N g ⁻¹ plant DW day ⁻¹)	0.129 $\pm$ 0.090 ^{an}	1.039 $\pm$ 0.215 ^{ao}	0.065 $\pm$ 0.002 ^{ap}	1.859 $\pm$ 0.012 ^{aq}	0.078 $\pm$ 0.003 ^{ar}	1.915 $\pm$ 0.649 ^{as}
%Ndfa	32.460 $\pm$ 4.037 ^{at}	51.063 $\pm$ 2.687 ^{au}	36.217 $\pm$ 7.022 ^{av}	51.694 $\pm$ 5.575 ^{aw}	35.936 $\pm$ 4.911 ^{ax}	55.145 $\pm$ 6.723 ^{ay}
Nitrogen derived from the atmosphere (mmol N g ⁻¹)	1.300 $\pm$ 0.147 ^{az}	3.198 $\pm$ 0.070 ^{ba}	3.607 $\pm$ 1.310 ^{bb}	2.696 $\pm$ 0.143 ^{bb}	1.732 $\pm$ 0.641 ^{bc}	2.939 $\pm$ 0.061 ^{bd}
Nitrogen derived from the soil (mmol N g ⁻¹)	3.541 $\pm$ 0.112 ^{be}	3.065 $\pm$ 0.067 ^{be}	3.521 $\pm$ 0.471 ^{bf}	2.381 $\pm$ 0.150 ^{bf}	2.663 $\pm$ 0.819 ^{bg}	2.607 $\pm$ 0.355 ^{bg}
Plant nutrition						
Phosphorus (μ mol g ⁻¹)	51.493 $\pm$ 1.871 ^{bh}	72.974 $\pm$ 2.738 ^{bi}	53.767 $\pm$ 0.798 ^{bj}	78.850 $\pm$ 11.407 ^{bk}	51.110 $\pm$ 4.827 ^{bl}	74.707 $\pm$ 9.510 ^{bm}
Nitrogen (mmol g ⁻¹)	3.097 $\pm$ 0.914 ^{bn}	6.614 $\pm$ 0.138 ^{bo}	3.921 $\pm$ 0.723 ^{bp}	5.076 $\pm$ 0.008 ^{bq}	3.665 $\pm$ 0.113 ^{br}	5.546 $\pm$ 0.416 ^{bs}

3.4 Bacterial identification

The bacterial identification of isolates from pre-planting soils is represented in Supplementary Table 2. The *Pedobacter*, *Flavobacterium* and *Lysobacter* genera were isolated from Kaydale soils. Rensburg soils had bacteria belonging to the *Enterobacter*, *Pedobacter*, and *Bacillus* genera. The bacterial identification of isolates from post harvest soils and *C. cajan* nodules is represented in Table 3. The bacteria isolated from the nodules of *C. cajan* grown in Jameson Park soils belonged to the *Bacillus*, *Rhizobium*, and *Pseudomonas* genera. Bacteria belonging to the *Paenibacillus*, *Rhizobium*, and *Pseudomonas* genera were isolated from the nodules of *C. cajan* from Kaydale soils. The *C. cajan* nodules in Rensburg soils had bacteria from the *Rhizobium* and *Pseudomonas* genera. The *E. curvula* monocropping treatment in Jameson Park soils had bacteria from the *Flavobacterium* and *Pseudomonas* genera. In Kaydale soils, the *E. curvula* monocropping treatment had bacterial isolates from the *Arthrobacter* and *Pedobacter* genera. The *E. curvula* monocropping treatment in Rensburg soils

had bacteria from the *Luteimonas*, *Pseudoarthrobacter*, and *Flavobacterium* genera. In Jameson Park soils, the *C. cajan* monocropping treatment had bacteria belonging to the *Croceicoccus*, *Novosphingobium*, *Paraburkholderia*, *Burkholderia*, and *Pseudomonas* genera. The *C. cajan* monocropping treatment in Kaydale and Rensburg soils had bacterial isolates belonging to the *Pseudomonas* genus. The rotation treatment in Jameson Park soils had bacteria from the *Pseudomonas*, *Caulobacter*, and *Pedobacter* genera. In Kaydale soils, the bacteria isolated from the rotation treatment belonged to the *Pseudomonas* genus. The bacteria isolated from the rotation treatment in Rensburg soils belonged to the *Pseudomonas* and *Pedobacter* genera (Table 3).

The rotation treatment in Jameson Park had the highest number of bacterial isolates compared to other treatments in different sites (Fig. 1). The *C. cajan* monocropping treatment in Jameson Park was the most diverse treatment, with bacterial isolates belonging to many species. The *C. cajan* monocropping treatment in Rensburg and Kaydale was the least diverse treatment, with bacterial isolates only from the *Pseudomonas* genera. The *Pseudomonas* genus is the most

Table 3 Molecular identification of bacterial strains isolated from Cajanus cajan nodules and soils post Cajanus cajan and Eragrostis curvula treatment harvests

Treatment	Strain	Accession number	Similarity (%)
Jameson Park			
<i>Cajanus cajan</i> nodules	<i>Bacillus zanthoxyli</i> strain 1433	NR_164882.1	95.69
	<i>Rhizobium deserti</i> strain SPY-1	NR_165733.1	100.00
	<i>Pseudomonas batumici</i> strain UCM B-321	NR_118125.1	100.00
<i>Eragrostis curvula</i> monocropping	<i>Flavobacterium tistranum</i> strain GB 56.1	NR_149282.1	98.57
	<i>Pseudomonas aylmerensis</i> strain S1E40	NR_169460.1	100.00
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	99.01
<i>Cajanus cajan</i> monocropping	<i>Croceicoccus sediminis</i> strain S2-4-2	NR_169491.1	100.00
	<i>Novosphingobium fuchskuhlense</i> strain FNE08-7	NR_118270.1	99.00
	<i>Paraburkholderia caribensis</i> strain MWAP64	NR_026462.1	97.73
	<i>Burkholderia guangdongensis</i> strain DHOM02	NR_178811.1	95.58
	<i>Pseudomonas silesiensis</i> strain A3	NR_156815.1	100.00
<i>Cajanus cajan</i> - <i>Eragrostis curvula</i> rotation	<i>Pseudomonas neuropathica</i> strain P155	NR_179483.1	100.00
	<i>Pseudomonas paralactis</i> strain DSM 29,164	NR_156987.1	99.41
	<i>Pseudomonas pratensis</i> strain MHJ-10 J	NR_181265.1	100.00
	<i>Caulobacter hibisci</i> strain THG-AG3.4	NR_157984.1	98.99
	<i>Caulobacter segnis</i> strain ATCC 21,756	NR_074208.1	98.62
	<i>Caulobacter endophyticus</i> strain 774	NR_179936.1	94.57
	<i>Pedobacter ghigonis</i> strain Marseille-Q2390	NR_179485.1	99.69
Kaydale			
<i>Cajanus cajan</i> nodules	<i>Paenibacillus polymyxa</i> strain DSM 36	NR_117733.2	97.64
	<i>Rhizobium pongamiae</i> strain VKLR-01	NR_178395.1	100.00
	<i>Rhizobium zea</i> strain CRZM18R	NR_157662.1	100.00
	<i>Pseudomonas veronii</i> strain CIP 104,663	NR_028706.1	100.00
	<i>Pseudomonas arenae</i> strain VK110	NR_181728.1	100.00
<i>Eragrostis curvula</i> monocropping	<i>Arthrobacter pokkalii</i> strain P3B162	NR_149802.1	100.00
	<i>Pedobacter frigiditerrae</i> strain RP-1-13	NR_173500.1	98.92
	<i>Pedobacter chitinilyticus</i> strain CM134L-2	NR_180020.1	98.16
<i>Cajanus cajan</i> monocropping	<i>Pseudomonas khazarica</i> strain TBZ2	NR_169334.1	100.00
	<i>Pseudomonas germanica</i> strain FIT28	NR_181838.1	99.68
<i>Cajanus cajan</i> - <i>Eragrostis curvula</i> rotation	<i>Pseudomonas protegens</i> strain CHA0	NR_114749.1	96.12
	<i>Pseudomonas fildesensis</i> strain KG01	NR_170438.1	97.43
	<i>Pseudomonas koreensis</i> strain Ps 9-14	NR_025228.1	100.00
	<i>Pseudomonas viciae</i> strain 11K1	NR_180948.1	99.38
Rensburg			
<i>Cajanus cajan</i> nodules	<i>Rhizobium panacihumi</i> strain DCY116	NR_179174.1	99.08
	<i>Pseudomonas chlororaphis</i> subsp. <i>aurantiaca</i> strain NCIB 10,068	NR_164626.1	100.00
		NR_181891.1	99.79
	<i>Pseudomonas soyae</i> strain JL117		
<i>Eragrostis curvula</i> monocropping	<i>Luteimonas lumbrici</i> strain 1.1416	NR_170461.1	97.91
	<i>Pseudoarthrobacter phenanthrenivorans</i> Sphe3	NR_074770.2	99.10
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	100.00
	<i>Flavobacterium amnigenum</i> strain I3-3	NR_169462.1	100.00
<i>Cajanus cajan</i> monocropping	<i>Pseudomonas lactis</i> strain DSM 29,167	NR_156986.1	100.00
	<i>Pseudomonas kribbensis</i> strain 46-2	NR_178854.1	100.00
	<i>Pseudomonas meliae</i> strain LMG 2220	NR_178761.1	100.00
	<i>Pseudomonas glycinis</i> strain PI111	NR_181729.1	100.00
	<i>Pseudomonas asturiensis</i> strain LPPA 221	NR_108461.1	98.68
<i>Cajanus cajan</i> - <i>Eragrostis curvula</i> rotation	<i>Pseudomonas fulva</i> strain NRIC 0180	NR_104280.1	99.69
	<i>Pseudomonas lundensis</i> strain ATCC 49,968	NR_024704.1	100.00
	<i>Pedobacter terrae</i> strain DS-57	NR_044005.1	94.92

dominant among treatments and collection sites. In Jameson Park, the *C. cajan* monocropping treatment had more bacterial isolates, followed by the rotation and *E. curvula* monocropping treatments. The *E. curvula* monocropping treatment is the most diverse in Kaydale and Rensburg soils. *Cajanus cajan* nodules from Kaydale soils had many

bacterial isolates compared to nodules from Rensburg and Jameson Park soils (Fig. 1).

3.5 Soil extracellular enzymes

In Jameson Park, Kaydale, and Rensburg soils, the nitrate reductase, β -glucosidase, acid, and alkaline phosphatase

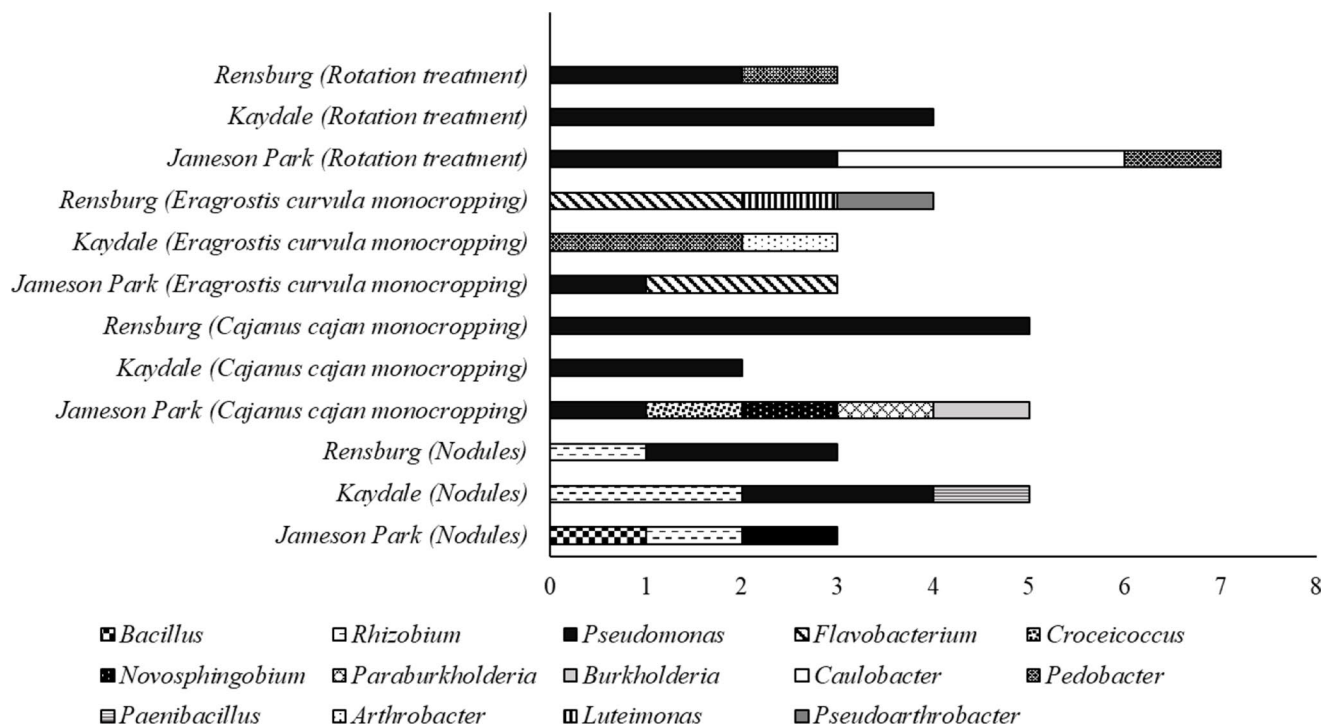


Fig. 1 Number of bacterial isolates from *Cajanus cajan* monocropping, *Eragrostis curvula* monocropping and *Cajanus cajan-Eragrostis curvula* rotation post-harvest soils collected from Jameson Park, Kaydale, and Rensburg

activities of the *C. cajan* monocropping were significantly higher than *E. curvula* monocropping and *C. cajan-E. curvula* rotation. There were no significant differences in the β -glucosidase, acid phosphatase, and alkaline phosphatase activity of *E. curvula* monocropping and *C. cajan-E. curvula* rotation in Jameson Park, Kaydale, and Rensburg soils (Fig. 2).

3.6 Post-harvest soil nutrient analysis and relative acidity

The soil nutrient concentrations and relative acidity post *E. curvula*, *C. cajan*, and *C. cajan-E. curvula* harvest is represented in Table 4. In Jameson Park soils, no significant differences were observed across treatments for pH, P, Ca, K, Mg, exchange acidity, and total cation concentrations. Manganese and N concentrations were significantly higher in monocropping treatments, and Zn concentrations were significantly higher in both the *E. curvula* monocropping and rotation treatments than in *C. cajan* monocropping. In Kaydale soils, no significant differences were observed across treatments for exchange acidity, pH, K, and Mg, while *C. cajan* monocropping had significantly higher N, P, Ca, Mn, Zn, and total cation concentrations.

In Rensburg soils, no significant differences existed across treatments in exchange acidity, pH, P, Ca, K, Mg, Mn, and Zn concentrations.

4 Discussion

This study evaluated the benefits of the *Cajanus cajan-Eragrostis curvula* rotation system for enhancing soil nutrient cycling and improving the nutritional quality of *E. curvula* for human and livestock consumption. Soils from Jameson Park, Kaydale, and Rensburg exhibited distinct nutrient concentrations and pH levels, which influenced microbial composition, soil fertility, and plant growth. Rensburg soils, with relatively higher pH (4.72 to 5.06) and increased concentrations of total cations, magnesium (Mg), calcium (Ca), and copper (Cu), demonstrated better buffering capacity and nutrient availability. Higher pH likely enhanced nutrient accessibility, as soil acidity affects the solubility of essential nutrients like Ca, Mg, and phosphorus (P) (Price 2006). However, P was the most limited macronutrient, particularly in acidic soils such as those from Jameson Park, where it likely formed insoluble complexes with aluminium and iron (Adnan et al. 2003). This P limitation may have hindered processes like root development and energy transfer (Ha and Tran 2013).

Crop rotation treatments, especially at Jameson Park, promoted microbial diversity by introducing varied root exudates that influence rhizosphere microbial communities. Root exudates are critical in shaping soil microbial populations, as they provide organic compounds that regulate microbial activity and composition (Mayerhofer et al.

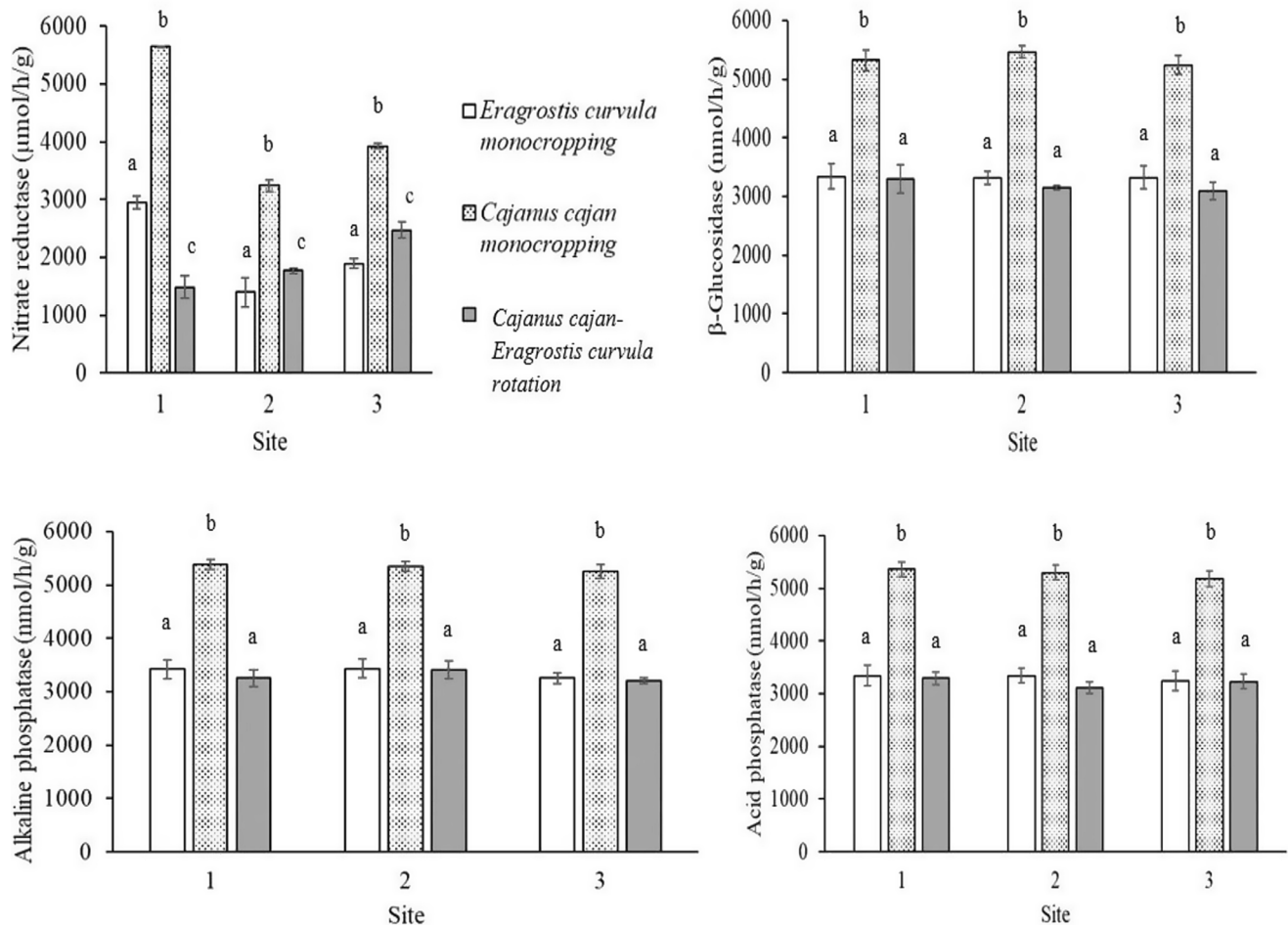


Fig. 2 Nitrate reductase ($\mu\text{mol/h/g}$), acid phosphatase (nmol/h/g), β -glucosidase (nmol/h/g), and alkaline phosphatase (nmol/h/g) activity of *Eragrostis curvula* monocropping, *Cajanus cajan* monocropping, and *Cajanus cajan-Eragrostis curvula* rotation. Values represent

mean $\pm$ SE, different letters denote statistical differences after a One-Way ANOVA. Site 1 represents Jameson Park soils, site 2 represents Kaydale soils and site 3 represents Rensburg soils

2021). In this study, *C. cajan* selected for bacterial genera such as *Novosphingobium*, *Croceicoccus*, *Paraburkholderia*, and *Pseudomonas*, while *E. curvula* favoured *Pedobacter*, *Arthrobacter*, and *Flavobacterium*. These microbial communities play essential roles in P solubilisation, nitrogen (N) fixation, and siderophore production, which enhance soil nutrient characteristics and plant growth (Ghosh et al. 2016; Sah et al. 2021). The abundance of P-cycling bacteria in *C. cajan* post-harvest soils increased phosphatase activity, mineralising P and enhancing its bioavailability (Adetunji et al. 2017).

This improved P availability supports biological nitrogen fixation (BNF), an energy-intensive process requiring P for ATP generation (Magadlela et al. 2016). Rhizobial and non-rhizobial bacteria associated with *C. cajan* enhanced atmospheric N fixation (Martínez-Hidalgo and Hirsch 2017), contributing to a high reliance on nitrogen derived from the atmosphere (Ndfa). Post-harvest, Ndfa likely “spilled over” into the soil (Fornara and Tilman 2008), increasing

soil N concentrations and nitrate reductase activity, particularly in Jameson Park soils. The transfer of N from *C. cajan* to the soil enhanced N availability for *E. curvula*, supporting higher nitrogen uptake and utilisation efficiency. This aligns with findings by Kumar Rao et al. (1987) and Oswald and Ransom (2001), who reported similar benefits of legume rotations on succeeding crops. While the rotation treatments showed minimal effects on overall soil nutrient profiles, this study highlights the potential for longer rotation periods or additional crop cycles to induce more substantial changes in soil chemistry.

The *C. cajan-E. curvula* rotation also improved P and N uptake in *E. curvula*, driven by microbial activity and P-cycling enzyme activity associated with *C. cajan*. These improvements reduce reliance on synthetic fertilizers and enhance *E. curvula*’s nutritional quality, which could translate into greater productivity and enriched seed nutrient concentrations. Such findings support the incorporation of *E. curvula* into diversified food systems to address food

Table 4 Soil characteristics of Jameson park, Kaydale, and Rensburg soils collected from Heidelberg, Gauteng, after the harvest of *Cajanus cajan*, *Eragrostis curvula*, and *Cajanus cajan-Eragrostis curvula*. Values represent mean $\pm$ se, $n = 45$. Significant differences are represented by different letters after a one-way ANOVA analysis ($p \leq 0.05$)

Nutrient concentrations (mg/kg)	Jameson Park			Kaydale			Rensburg		
	Cajanus cajan	Eragrostis curvula	Cajanus cajan-Eragrostis curvula	Cajanus cajan	Eragrostis curvula	Cajanus cajan-Eragrostis curvula	Cajanus cajan	Eragrostis curvula	Cajanus cajan-Eragrostis curvula
P	14.63 $\pm$ 4.71 ^a	12.83 $\pm$ 3.45 ^a	12.50 $\pm$ 2.04 ^a	13.69 $\pm$ 2.12 ^b	4.97 $\pm$ 1.25 ^c	3.50 $\pm$ 0.71 ^c	12.00 $\pm$ 1.41 ^d	10.16 $\pm$ 1.40 ^d	11.50 $\pm$ 1.71 ^d
Ca	1027.50 $\pm$ 229.81 ^e	1006.43 $\pm$ 12.35 ^e	1077.5 $\pm$ 74.25 ^e	1072.51 $\pm$ 184.55 ^f	526.33 $\pm$ 24.11 ^g	603.51 $\pm$ 7.78 ^g	671.00 $\pm$ 36.77 ^h	590.67 $\pm$ 6.81 ^h	737.00 $\pm$ 41.01 ^h
K	223.00 $\pm$ 18.38 ⁱ	206.33 $\pm$ 0.58 ⁱ	186.50 $\pm$ 31.82 ⁱ	226.00 $\pm$ 11.31 ^j	188.05 $\pm$ 1.02 ^j	205.48 $\pm$ 13.44 ^j	187.49 $\pm$ 3.54 ^k	153.41 $\pm$ 4.37 ^k	169.50 $\pm$ 7.78 ^k
Mg	306.50 $\pm$ 57.28 ^l	295.70 $\pm$ 4.50 ^l	289.00 $\pm$ 19.81 ^l	293.00 $\pm$ 52.33 ^m	175.45 $\pm$ 11.24 ^m	170.92 $\pm$ 7.73 ^m	157.50 $\pm$ 2.12 ⁿ	160.58 $\pm$ 0.71 ⁿ	182.00 $\pm$ 15.66 ⁿ
Mn	252.45 $\pm$ 3.54 ^o	106.67 $\pm$ 16.26 ^o	85.51 $\pm$ 7.78 ^p	258.18 $\pm$ 2.83 ^q	57.66 $\pm$ 1.16 ^r	28.10 $\pm$ 1.43 ^r	58.91 $\pm$ 5.66 ^s	32.33 $\pm$ 0.58 ^s	40.81 $\pm$ 4.24 ^s
Cu	4.80 $\pm$ 0.99 ^t	11.35 $\pm$ 0.45 ^u	12.26 $\pm$ 1.35 ^u	13.90 $\pm$ 2.54 ^v	4.01 $\pm$ 0.19 ^w	4.65 $\pm$ 0.21 ^w	6.25 $\pm$ 0.49 ^x	3.89 $\pm$ 0.12 ^x	7.15 $\pm$ 0.49 ^x
Zn	17.15 $\pm$ 0.92 ^y	14.70 $\pm$ 0.56 ^y	14.50 $\pm$ 0.71 ^y	18.85 $\pm$ 0.49 ^z	6.23 $\pm$ 0.21 ^{aa}	7.70 $\pm$ 2.14 ^{aa}	9.90 $\pm$ 1.85 ^{ab}	7.30 $\pm$ 0.18 ^{ab}	10.95 $\pm$ 0.21 ^{ab}
N	1703.53 $\pm$ 173.24 ^{ac}	1488.67 $\pm$ 95.04 ^{ac}	632.50 $\pm$ 17.68 ^{ad}	1992.00 $\pm$ 149.91 ^{ae}	720.47 $\pm$ 17.56 ^{af}	650.95 $\pm$ 112.23 ^{af}	1413.50 $\pm$ 371.23 ^{ag}	870.43 $\pm$ 44.21 ^{ah}	696.58 $\pm$ 47.38 ^{ah}
Relative acidity									
Exchange acidity (cmol/L)	0.10 $\pm$ 0.01 ^{a.i.}	0.07 $\pm$ 0.001 ^{a.i.}	0.06 $\pm$ 0.01 ^{a.i.}	0.06 $\pm$ 0.01 ^{aj}	0.06 $\pm$ 0.01 ^{aj}	0.05 $\pm$ 0.01 ^{aj}	0.07 $\pm$ 0.00 ^{ak}	0.05 $\pm$ 0.01 ^{ak}	0.06 $\pm$ 0.01 ^{ak}
Total cations (cmol/L)	8.16 $\pm$ 1.89 ^{al}	8.09 $\pm$ 0.11 ^{al}	8.31 $\pm$ 0.62 ^{al}	9.72 $\pm$ 0.47 ^{am}	4.64 $\pm$ 0.35 ^{an}	4.99 $\pm$ 0.08 ^{an}	5.20 $\pm$ 0.21 ^{ao}	4.79 $\pm$ 0.19 ^{ao}	5.67 $\pm$ 0.28 ^{ao}
pH	4.96 $\pm$ 0.16 ^{ap}	5.18 $\pm$ 0.12 ^{ap}	5.03 $\pm$ 0.11 ^{ap}	4.95 $\pm$ 0.17 ^{ar}	4.94 $\pm$ 0.06 ^{ar}	4.84 $\pm$ 0.01 ^{ar}	5.25 $\pm$ 0.18 ^{as}	5.24 $\pm$ 0.21 ^{as}	5.23 $\pm$ 1.01 ^{as}

security challenges. Future studies can investigate the long-term effects of rotation systems on seed nutrient composition, soil organic matter dynamics, soil health, nutrient cycling, and crop productivity and assess the sustainability of these systems under diverse environmental conditions to optimise sustainable agricultural practices.

5 Conclusions

This study illustrates how soil type, microbial composition, and cropping treatments influence soil nutrient dynamics and plant growth. *Cajanus cajan* monocropping enhanced soil N and enzyme activity, underscoring the benefits of legumes for soil fertility, while rotation with *E. curvula* improved plant biomass and nutrient concentrations. These findings indicate that integrating legumes into crop rotations can enhance soil nutrient availability, promote microbial diversity, and support food and nutritional security, especially in nutrient-poor, acidic soils.

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Data availability The data and material can be made available upon request.

Declarations

Conflict of interest We declare no known competing financial and non-financial interests with regards to the current research.

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