



Legume-grass intercropping systems improve soil health and plant nutrition

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Abstract

Background and aims Intercropping legumes and grasses presents a sustainable strategy to improve soil fertility, enhance crop productivity, and strengthen food security in low-input agricultural systems. This study investigated the effects of intercropping *Vigna unguiculata* with *Eragrostis curvula* on soil bacterial communities, extracellular enzyme activities, nutrient dynamics, and plant growth kinetics and nutrition.

Methods Pre- and post-harvest soil samples were analysed for chemical and biological properties, and plant samples were assessed for growth and nutrient uptake.

Results Intercropping resulted in a shift in soil bacterial composition, favouring phosphorus (P)-solubilising taxa such as *Burkholderia* and *Pseudomonas*,

while reducing soil enzymes activities, including N-acetylglucosaminidase, β -glucosidase, nitrate reductase, and acid and alkaline phosphatases. Despite lower enzymatic activity, intercropped soils showed increased P and nitrogen (N) concentrations. *Vigna unguiculata* belowground biomass increased and established associations with P-solubilising bacteria from the *Pseudomonas* and *Paenibacillus* genera, which enhanced plant P concentrations, and P use efficiency. *Eragrostis curvula* showed variable growth across soil types but demonstrated increased reliance on atmospheric N, higher plant N concentrations, and improved uptake of soil-derived N. Intercropped *V. unguiculata*, increased reliance on atmospheric N, nodule weight, plant N concentrations, and specific N utilisation rates.

Conclusions These findings highlight the potential of *V. unguiculata* and *E. curvula* intercropping to improve soil health, boost crop nutrition, and reduce dependency on external inputs, offering a viable solution for sustainable agriculture in resource-constrained environments facing challenges of climate change and soil degradation.

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Introduction

Global hunger remains a critical challenge, with forty-two countries currently experiencing severe or alarming levels of food insecurity (Global Hunger Index 2024). To meet the demands of a growing global population, the United States Agency for International Development (2024) estimates that food production must increase by 60% by 2050. However, soil degradation, one of the major drivers of declining agricultural productivity, poses a significant threat to global food security by reducing crop yields (Vlek et al. 2010). Soil acidity is among the key factors contributing to soil degradation and represents a widespread and persistent concern (Agegnehu et al. 2021). In acidic soils, phosphorus (P) becomes unavailable as it binds to sesquioxides in the soil matrix, resulting in P deficiency (Saleque et al. 2004) and subsequent declines in the diversity and abundance of beneficial soil microbes (Patra et al. 2021). Collectively, these pressures underscore the urgent need to transition towards more resilient, diverse, and sustainable food systems which mitigate soil degradation while addressing food insecurity. Incorporating neglected and underutilised species (NUS) into agricultural systems offers a promising strategy for addressing food insecurity and soil degradation.

These “future-smart” crops are well-adapted to marginal and stress-prone environments, supporting the development of climate-resilient and nutritionally diverse food systems (Padulosi et al. 2013; Chivenge et al. 2015; Hunter et al. 2019). *Eragrostis curvula*, an underutilised grass species, has shown considerable potential to diversify food systems and contributes to soil health by supporting nutrient cycling (Ghebrehiwot et al. 2016; Motsomane et al. 2025; Motsomane and Magadlela 2025). The benefits of *E. curvula* can be complemented by *Vigna unguiculata*, a multipurpose crop that provides protein-rich grain, edible leaves, and livestock fodder (Devi et al. 2015; Da Silva et al. 2018; Ongom et al. 2023). Through its ability to form symbiotic associations with plant growth promoting bacteria, *V. unguiculata* can contribute to soil N availability and promote the mobilisation of P (Oruru et al. 2018; Lo Presti et al. 2021). In this context, intercropping *E. curvula* with *V. unguiculata* may serve as a multifunctional strategy to improve soil fertility and the productivity of underutilised crops in low-input systems.

Intercropping systems modify root exudation profiles, influencing the composition and functional activity of soil microbial communities involved in nutrient cycling (Lami et al. 2020). These interactions may lead to enhanced activities of key soil enzymes that mediate N and P turnover (Xiao et al. 2023). Increases in acid phosphatase activity were reported in legume-based intercropping systems (Lo Presti et al. 2021), improving P availability. Although legume–grass combinations are widely studied, the interaction between underutilised legumes and grasses remains understudied. This study evaluates the ecological and agronomic effects of intercropping *V. unguiculata* and *E. curvula* on soil chemical properties, bacterial communities, enzyme activities, and plant growth and nutrition. The study species were grown in monocropping and intercropping treatments. At harvest, soil and plant samples were analysed to assess changes in soil chemical properties, identify associated bacterial species, and assay enzyme activities. Plant materials were used to analyse the growth performance and nutrient concentrations. We tested the following hypotheses:

Hypothesis 1: Intercropping *V. unguiculata* with *E. curvula* will improve soil chemical properties. We predicted that intercropping would enhance the abundance of nutrient cycling bacteria and associated enzyme activities, leading to improved soil chemical properties.

Hypothesis 2: Intercropping will enhance the relative abundance of beneficial soil bacterial communities. We predicted that root exudates from both species would promote the selection and proliferation of a more diverse and beneficial bacterial community (Backer et al. 2018).

Hypothesis 3: Soil enzyme activities involved in nutrient cycling will be higher in the intercropping treatments. The increase in nutrient cycling bacterial communities in the intercropping would result in greater enzyme activities.

Hypothesis 4: Intercropping will enhance the reliance of *V. unguiculata* on N derived from the atmosphere compared to monocropping, reducing its competition with *E. curvula* for soil N (Neumann et al. 2009). This increased biological N fixation will be supported by the association of *V. unguiculata* with P-solubilising bacteria to meet the energy demands of N fixation, while *E. cur-*

vula will benefit from improved soil N availability facilitated by this interaction (Fornara and Tilman 2008).

Hypothesis 5: Intercropping will enhance growth and biomass accumulation in both species, leading to higher N and P concentrations. Intercropping will improve the growth performance and biomass accumulation of both species. We predicted that enhanced biological N fixation and increased nutrient assimilation would support greater biomass accumulation (Matiwane et al. 2019) and improved plant nutrient profiles.

Methods and materials

Soil collection

Soil samples were collected from 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) in Heidelberg, Gauteng, South Africa, and were used as growth substrates for the greenhouse trials. Heidelberg is under the Lesedi local municipality, which plays a significant role in food production in Gauteng, with 60% of the area having agricultural potential (Lesedi Municipality 2020). Furthermore, being situated in the South-west of Johannesburg and traversed by two national roads, N17 and N3, creates potential for future economic development (Lesedi Municipality 2020). The Lesedi Local Municipality and the Department of Agriculture and Land Reform face challenges aligning food security and small-scale farming development initiatives to economic development (Lesedi Municipality 2020). Encouraging the adoption of low-input cropping systems can have positive implications for small-scale farmers, as they have diverse benefits such as food and animal feed, and can increase productivity, subsistence income, and food security. At each site, thirty soil samples were collected from randomly selected points 2 m apart. The soils collected from each site were mixed to form composite soils. Five kilograms from each composite sample were stored in a refrigerator (2–4 °C) for analysis of soil chemical properties and bacterial extraction and identification for pre-planting soils. The remaining soil was distributed into pots for plant growth trials.

Plant growth trials

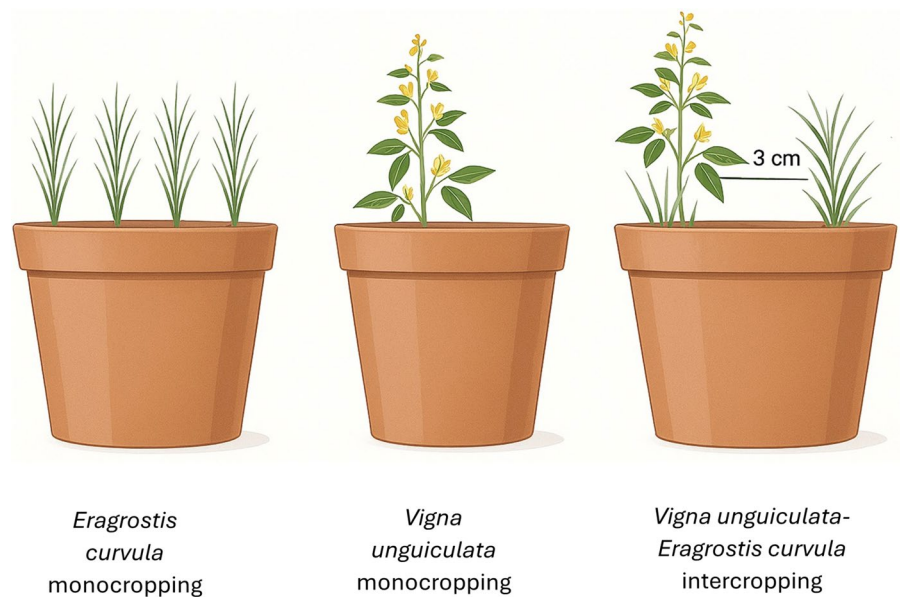
Vigna unguiculata (Betchwhite cultivar) and *E. curvula* (Agpal cultivar) seeds were sourced from AGT Foods (Krugersdorp, South Africa) and Agri-col (Howick, South Africa), respectively. The seeds were planted composite in soils collected from Jameson Park, Kaydale, and Rensburg. The seeds were planted in 15 cm diameter pots at 1–1.5 cm depths following a randomised block design consisting of three treatments (twelve replicates each) per site (*V. unguiculata* monocrop, *E. curvula* monocrop, and *V. unguiculata*-*E. curvula* intercrop). For the *E. curvula* monocrop treatment, four seeds per pot were directly sown into the soil, while *V. unguiculata* seeds for the monocrop and intercrop treatments were germinated in sterile petri dishes with germination paper and irrigated with deionised water. Seven-day-old seedlings were transplanted into their respective pots. For the intercropping treatment, *V. unguiculata* seedlings were allowed to grow for an additional seven days before *E. curvula* seeds were sown approximately 3 cm away from each *V. unguiculata* plant, as illustrated in Fig. 1. The growth trials were conducted in the greenhouse at the University of KwaZulu Natal, Westville Campus, South Africa. The treatments were irrigated every two days, and the initial harvest took place after 28 days, and the final harvest 97 days post seedling emergence. *Vigna unguiculata* and *E. curvula* plants were harvested from all treatments, oven dried at 65 °C until a constant weight was maintained, and ground using an industrial blender. The ground powder was stored in Eppendorf tubes and sent for analysis. The samples were sent to the Archaeometry department, University of Cape Town, South Africa, for N isotope analysis. The remaining samples were sent to the Central Analytical Facilities (CAF) at Stellenbosch University, South Africa for P analysis using inductively coupled plasma mass spectrometry (ICP-MS).

Plant growth kinetics

Relative growth rate and root-to-shoot ratio

A methodology adopted from Agren and Franklin (2003) was used to calculate the relative growth rate (RGR) and root-to-shoot ratio (R:S) for the *V.*

Fig. 1 Schematic representation of monocropping and intercropping treatments involving *Vigna unguiculata* and *Eragrostis curvula*. In the intercropping treatment, *E. curvula* seeds were sown 3 cm from each *V. unguiculata* plant. Created in BioRender (<https://biorender.com>)



unguiculata monocrop, *E. curvula* monocrop, and *V. unguiculata*-*E. curvula* intercrop treatments.

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

W is the plant's weight, with W_1 representing the initial harvest and W_2 representing the final harvest (W_2). t is the time for plant growth.

$$\text{Root} - \text{to} - \text{Shoot ratio} = W_R / W_S$$

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

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

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

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

The plant's total N and P were represented using L and M, respectively. The duration of plant growth and root dry weights were represented using T and R, respectively.

Percentage nitrogen derived from the atmosphere (%Ndfa)

W is the dry weight of the roots (W_R) and shoots (W_S)

Specific N/P absorption rate and specific N/P utilisation rates

Following the methodology outlined by Nielsen et al. (2001), the total N and P of *E. curvula* and *V. unguiculata* growing in their respective treatments were analysed to determine the specific N and P absorption and utilisation rates.

A formula described by Shearer and Kohl (1986) was used to determine the percentage of nitrogen derived from the atmosphere for all treatments: $\delta = 1000 \times 3411 (R_{\text{sample}} / R_{\text{standard}})$, where R denotes

the molar ratio of ^{15}N to ^{14}N in the samples and

standards. The samples were prepared and analysed as per the protocols of Matiwane et al. (2019).

$$\text{Percentage N derived from the atmosphere} = 100((\delta^{15}\text{N}_{\text{referenceplant}} - ^{15}\text{N}_{\text{legume/grass}})/(\delta^{15}\text{N}_{\text{referenceplant}} - \delta))$$

The β value represented the $\delta^{15}\text{N}$ natural abundance of the N derived from biological N fixation by *V. unguiculata* and *E. curvula*. *Vigna unguiculata* and *E. curvula* plants were planted in plain sand with no nutrients added and under the same conditions as the experimental plants and used to estimate the β values. The $\text{Nfx} = \text{Ndfa} \times \text{N content}$ was used to determine the total amount of N in the plant derived from biological N fixation.

Bacterial extraction and identification

Nodules from *V. unguiculata* plants were surface sterilised by rinsing with autoclaved water, 70% ethanol for 30 s, 3.5% sodium hypochlorite solution for 3 min, and lastly rinsing with autoclaved water (Somasegaran and Hoben 1994). Following surface sterilisation, the nodules were crushed and mixed with 100 μL of 15% glycerol, and 10 μL of the solution was plated on yeast mannitol agar plates. Each treatment was replicated four times and cultured at 30 °C for five days. The colonies from each plate were repeatedly streaked to get pure colonies. Small portions of the pure colonies were amplified using polymerase chain reaction (PCR) with 16S primers 63F (5' CAG GCCTAACACATGCAAGTC 3') and 1387R (5' GGGCGGTGTGTACAAGGC 3') (Marchesi et al. 1998). The EmeraldAmp GT Master Mix (Takara Bio Inc, supplied by Separations, South Africa) was used for PCR using the following procedure: 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, extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. Gel electrophoresis using 1% agarose gel was used to separate the PCR product, and UV light was used to confirm the amplification of the correct product size. The amplicons were sent to Inqaba Biotechnical Industries, South Africa, for sequencing, and DNA sequences were compared to known bacterial sequences in the GenBank database using BLAST (<https://www.ncbi.nlm.nih.gov>).

Pre-planting and post-harvest soils from all treatments were used for serial dilutions, and 100 μL of

each dilution was cultured in sterile Petri dishes with selective media (Jensen's media agar for N-fixing bacteria, Simmons citrate agar for N-cycling bacteria, and Pikovskaya's agar for P-solubilising bacteria) to isolate specific bacterial groups (Ndabankulu et al. 2022). Selective media plates were replicated three times, and cultures were incubated at 30 °C for a maximum of five days. Media plates were repeatedly streaked to obtain pure colonies, and a small portion of each colony was subjected to PCR amplification using the abovementioned protocol.

Extracellular enzyme activities

Pre-planting and post-harvest soils from all treatments were assayed for N and P cycling enzyme activities. Enzyme activities for β -glucosidase, N-acetylglucosaminidase, acid, and alkaline phosphatase were determined per the protocol described by Jackson et al. (2013) and reported as $\text{nmol h}^{-1} \text{g}^{-1}$. To prepare samples, 10 g of soil was mixed in 100 ml of autoclaved distilled water, homogenised on a shaker for two hours, and stored overnight at 4 °C. The supernatants were pipetted into black 96-well microplates, then their respective substrates were added. Assay components included soil aliquots (200 μL) mixed with substrates (50 μL), reference standards, quench standards, sample controls, negative controls, and blanks. Plates were incubated at 25 °C for two hours before stopping the reaction by adding five μL of 0.5 M NaOH to each well. Fluorescence readings were recorded at 450 nm using a Glomax Multi Plus microplate reader. Acid phosphatase activity was assayed with a buffer adjusted to pH 5.

Nitrate reductase activity was determined through a modified method based on Kandeler (1995). One millilitre of 25 mM KNO_3 , 4 ml of 0.9 mM 2,4-dinitrophenol, and 5 ml of milliQ water were mixed in a volumetric flask wrapped in foil. To this solution, 5 g of soil was added, and the mixture was sealed,

shaken, and incubated at 30°C for 24 h. At the end of the incubation period, 10 ml of 4 M KCl was added to the solution, thoroughly mixed, and filtered through Whatman no. 1 filter paper. A colour reagent containing 1% sulfanilamide, 1N HCl, and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) was prepared, and 0.8 ml of the reagent was mixed with 2 ml of the filtrate and 1.2 ml of 0.19 M ammonium chloride buffer (pH 8.5). The solution was incubated at 30°C for 30 min, and absorbance was measured at 520 nm using a 1800 UV spectrophotometer. Nitrite (NO_2^-) concentrations were calculated from a KNO_3 standard curve, and nitrate reductase activity was expressed as $\mu\text{mol h}^{-1} \text{g}^{-1}$.

Soil chemical properties

Five subsamples of 500 g each of pre-planting and post-harvest soils were sent to the Analytical Services Unit, KwaZulu-Natal Department of Agriculture and Rural Development, Cedara, South Africa. The characteristic soil analysis was performed per the protocols Manson and Roberts (2000) explained. Ambic-2 solution containing 0.25 M NH_4CO_3 , 0.01 M Na_2EDTA , 0.01 M NH_4F , and 0.05 g/L superfloc (N100) was adjusted to pH 8 using concentrated ammonia solution and used to extract available P. The extracts were filtered using Whatman no.1, and a 2 ml filtrate aliquot was used to determine the P concentration using a modified protocol of Murphy and Riley's (1962) molybdenum blue procedure. Total N content was measured using the Automated Dumas dry combustion method with a LECO CNS 2000 (Leco Corporation, USA). Soil samples were weighed in

a ceramic crucible, and 0.5 g vanadium pentoxide was used as a combustion catalyst. The crucible was placed in a horizontal furnace and burned in a stream of oxygen at 1350 °C, and soil N was measured as N_2 in a thermal conductivity cell. Soil pH was determined by mixing 10 ml of soil sample and 25 ml of 1 M KCl in sample cups and stirring in a multiple stirrer at 400 rpm for 5 min. The suspension was left to rest for 30 min, and the pH was measured using a gel-filled combination glass electrode while stirring. The N, P and pH of pre-planting soils is represented in Supplementary Table 1.

Statistical analysis

The statistical software R (version 3.6.2) was used to test for differences in plant growth kinetics and nutrition between monocropped and intercropped *V. unguiculata* and *E. curvula* using an independent samples T-test. Normality was assessed using the Shapiro–Wilk test from the stats package, and homogeneity of variances was tested with Levene's test from the car package (Fox and Weisberg 2019). When assumptions were not met, data were log-transformed to improve normality and homogeneity of variance. A One-Way ANOVA from the stats package was employed to compare differences in extracellular enzyme activities and soil chemical properties following the *V. unguiculata* monocrop, *E. curvula* monocrop, and *V. unguiculata*–*E. curvula* intercrop treatments. Where the ANOVA indicated significant differences, a Tukey post-hoc test from the stats package separated the means (R Team Core, 2023).

Table 1 Nitrogen, phosphorus, and pH of postharvest soils. Values represent means $\pm$ SE ($n=45$)

		Jameson Park	Kaydale	Rensburg
Nitrogen (mg/kg)	<i>Vigna unguiculata</i>	760.00 $\pm$ 22.43 ^a	607.53 $\pm$ 31.11 ^a	632.50 $\pm$ 17.68 ^a
	<i>Eragrostis curvula</i>	1488.67 $\pm$ 95.04 ^b	720.47 $\pm$ 17.56 ^a	870.43 $\pm$ 44.21 ^a
	Intercropping	1027.00 $\pm$ 1.41 ^c	1937.92 $\pm$ 110.31 ^b	1151.61 $\pm$ 15.56 ^b
Phosphorus (mg/kg)	<i>Vigna unguiculata</i>	10.00 $\pm$ 2.83 ^a	4.00 $\pm$ 10.01 ^a	13.00 $\pm$ 0.01 ^a
	<i>Eragrostis curvula</i>	12.83 $\pm$ 3.45 ^a	4.97 $\pm$ 1.25 ^a	10.16 $\pm$ 1.40 ^a
	Intercropping	17.53 $\pm$ 0.71 ^b	7.00 $\pm$ 0.03 ^b	15.00 $\pm$ 0.01 ^b
pH	<i>Vigna unguiculata</i>	5.28 $\pm$ 0.81 ^a	4.76 $\pm$ 0.02 ^a	4.89 $\pm$ 0.01 ^a
	<i>Eragrostis curvula</i>	5.18 $\pm$ 0.12 ^a	4.94 $\pm$ 0.06 ^a	5.24 $\pm$ 0.21 ^b
	Intercropping	5.17 $\pm$ 0.01 ^a	5.02 $\pm$ 0.84 ^a	5.45 $\pm$ 0.02 ^b

Significant differences among treatments are indicated by different letters according to one-way ANOVA ($p \leq 0.05$)

Results

Hypothesis 1: Intercropping V. unguiculata with E. curvula will improve soil chemical properties.

Soil chemical properties

The nutrient concentrations and pH of post-harvest soils were determined to test the first hypothesis, as represented in Table 1. In Jameson Park soils, N concentration in the intercropping treatment was 35% higher than in *E. curvula* monocropping but 31% lower than in *V. unguiculata* monocropping. The P concentration in intercropping was 42–70% higher than in the monocropping treatments, while pH was slightly lower, by 0.2–2%. In Kaydale soils, the intercropping treatment increased N, P, and pH by 169–219%, 41–75%, and 2–5%, respectively, compared to the monocropping treatments. In Rensburg soils, intercropping increased N, P, and pH by 32–82%, 15–50%, and 4–11%, respectively, relative to the monocropping treatments.

Hypothesis 2: Intercropping will enhance the relative abundance of beneficial soil bacterial communities.

Soil bacterial communities

Bacteria isolated from post-harvest soils were extracted and identified to test the second hypothesis, as represented in Fig. 2. In Jameson Park soils, the abundance of *Pseudomonas* species increased from 33.3% in *E. curvula* monocropping to 80% in the intercropping treatment. *Vigna unguiculata* selected for *Bacillus* species in the monocropping treatment. *Eragrostis curvula* monocropped in Kaydale soils selected for *Pseudoarthrobacter* and *Caballeronia* species. The relative abundance of the *Pseudomonas* genus increased from 50% in *V. unguiculata* monocropping to 75% in the intercropping treatment in Kaydale soils. Monocropped *E. curvula* in Rensburg soils selected for *Pedobacter* species while *V. unguiculata* selected for *Chryseobacterium*, *Pedobacter*, and *Pseudomonas* genera. The intercropping treatment in Rensburg soils selected for *Burkholderia*, *Variovorax*, *Flavobacterium* and *Pseudomonas* species, the

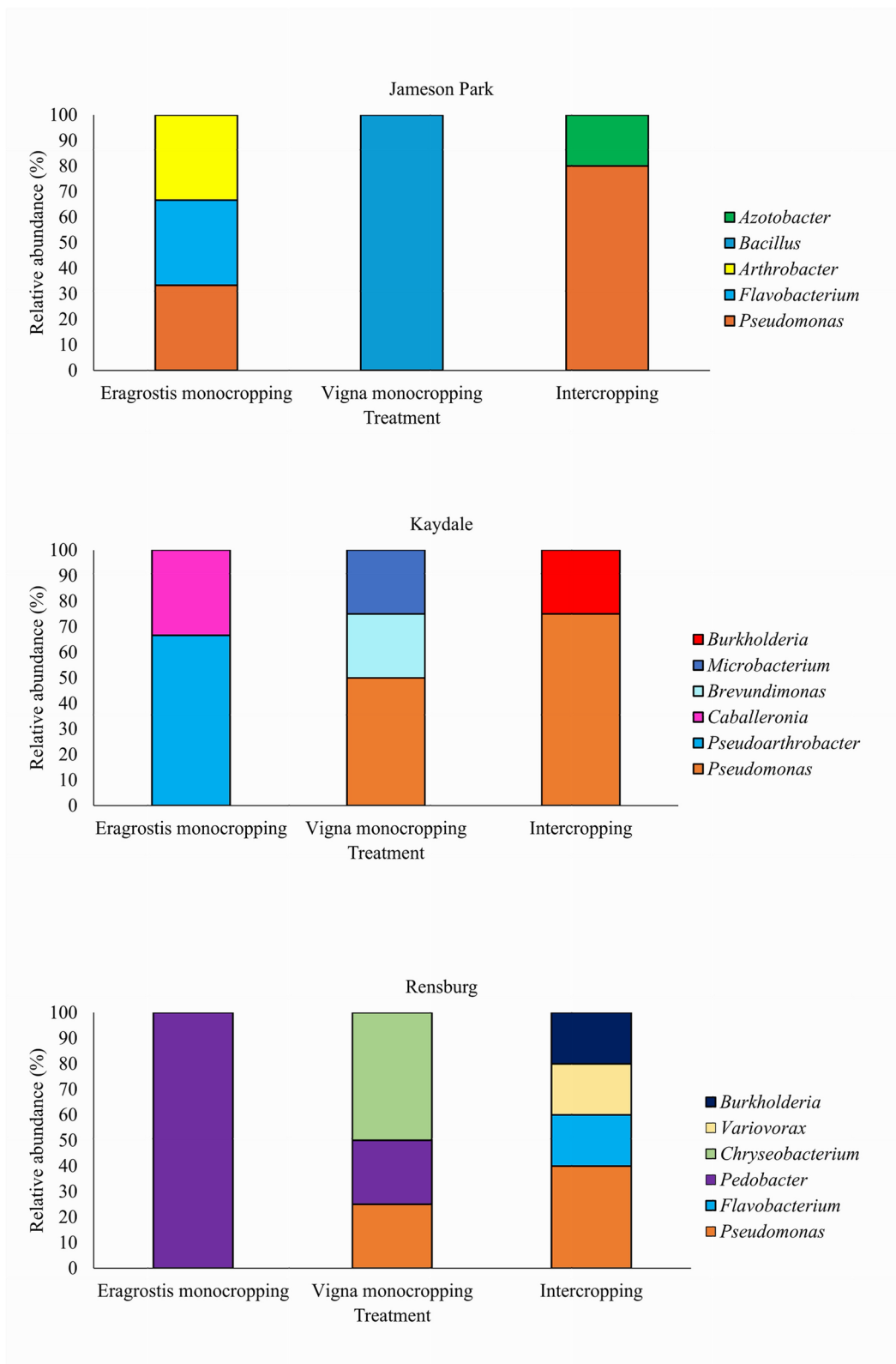
relative abundance of *Pseudomonas* increased from 25% in *V. unguiculata* monocropping to 40% in the intercropping treatment.

Hypothesis 3: Soil enzyme activities involved in nutrient cycling will be higher in the intercropping treatments.

Extracellular enzyme activities

The nitrate reductase, N-acetylglucosaminidase, acid phosphatase, alkaline phosphatase, and β -Glucosidase activities of post-harvest soils were assayed to test hypothesis three, as represented in Table 2. In Jameson Park soils, activities of nitrate reductase, N-acetylglucosaminidase, alkaline phosphatase, acid phosphatase, and glucosidase in the intercropping treatment were 69–85%, 41–45%, 1–40%, 5–38%, and 3–43% lower, respectively, than in the monocropping treatments. In Kaydale soils, the intercropping treatment had 51–61%, 11–47%, 1–45%, and 3–45% lower nitrate reductase, alkaline phosphatase, acid phosphatase, and glucosidase activities, respectively, compared to the monocropping treatments. The N-acetylglucosaminidase activity did not differ significantly between the monocropping treatments; however, the intercropping treatment was 43% lower than both monocrops. In Rensburg soils, nitrate reductase, N-acetylglucosaminidase, and acid phosphatase activities in the intercropping treatment were 71–89%, 45–47%, and 5–44% lower, respectively, than in the monocropping treatments. Alkaline phosphatase and glucosidase activities did not differ significantly between the *E. curvula* monocropping and intercropping treatments; however, the intercropping treatment was 44% and 43% lower, respectively, than the *V. unguiculata* monocropping treatment.

Hypothesis 4: Intercropping will enhance the reliance of V. unguiculata on N derived from the atmosphere compared to monocropping. This increased biological N fixation will be supported by the association of V. unguiculata with P-solubilising bacteria to meet the energy demands of fixation, while E. curvula will benefit from improved soil N availability facilitated by this interaction.



◀**Fig. 2** Relative abundance of bacterial genera isolated from the post-harvest soils

Plant bacterial associations and N reliance

Vigna unguiculata bacterial associations

Bacteria in *V. unguiculata* nodules were extracted and identified to test for the fourth hypothesis and presented in Fig. 3. The abundance of the *Pseudomonas* genus in monocropped and intercropped *V. unguiculata* in Jameson Park soils was the same. In Kaydale soils, intercropped *V. unguiculata* selected for the *Pseudomonas* genus. The *Rhizobium* genus was isolated in monocropped *V. unguiculata* in all soil collection sites, while the *Pseudomonas* genus was only isolated in intercropped *V. unguiculata*.

Plant N reliance

To test the fourth hypothesis, intercropped and monocropped *V. unguiculata* and *E. curvula* plant material were subjected to N isotope analysis to determine the N source reliance, as represented in Table 3. The concentration of N derived from the atmosphere in intercropped *V. unguiculata* was 67–173% higher than in monocropped plants across all soil types. In contrast, the concentration of N derived from the soil in intercropped *V. unguiculata* was 70–89% lower than in monocropped plants. The percentage N derived from the atmosphere in intercropped *V. unguiculata* was 34–87% higher than in the monocropped treatment. Intercropped *E. curvula* plants had N derived from the atmosphere concentrations that were 39–311% higher than monocropped plants across soil collection sites. The N derived from the soil concentration in intercropped *E. curvula* was 6–67% higher than in monocropped plants. Lastly, the percentage N derived from the atmosphere in intercropped *E. curvula* was 30–63% higher than in monocropped plants.

Hypothesis 5: Intercropping will improve the growth performance and biomass accumulation of both species, leading to higher N and P concentrations.

Plant growth, biomass and nutrition

To test the fifth hypothesis, biomass, nutrient uptake and utilization, and plant nutritional status were measured for monocropped and intercropped *V. unguiculata* and *E. curvula* plants, as shown in Table 4–6. Intercropped *V. unguiculata* had significantly higher specific N and P utilisation and absorption rates, nodule weight, and belowground biomass compared to monocropped plants across all soil collection sites. In some soils, intercropping also increased aboveground biomass, nodule number, root-to-shoot ratio, and specific N absorption rate. However, monocropped *V. unguiculata* had a higher relative growth rate in Rensburg soils. (Table 4). Monocropped *E. curvula* had higher root-to-shoot ratios across all soil collection sites, while aboveground and belowground biomass, relative growth rates, and specific N and P utilisation and absorption rates varied across sites. Intercropped *E. curvula* had higher specific N absorption rates in Jameson Park and Rensburg soils (Table 5). Intercropped *V. unguiculata* had significantly higher N and P concentrations than monocropped plants across all soil collection sites. Intercropped *E. curvula* had higher N concentrations across all soil collection sites, while P concentrations varied by site (Table 6).

Discussion

Intercropping *V. unguiculata* and *E. curvula* improved soil bacterial community composition, improved soil nutrient status, and increased plant productivity, suggesting a biologically sustainable approach to improving soil function and crop performance. The observed enrichment of functionally relevant bacterial genera indicates that intercropping alters rhizosphere conditions in ways that support beneficial microbial activity, leading to increased nutrient bioavailability and uptake. By promoting biological N fixation, this intercropping combination reduces reliance on synthetic fertilisers, providing a practical approach to addressing both food insecurity and soil degradation in acidic and nutrient poor soils.

Table 2 Extracellular enzyme activities of postharvest soils. Values represent means $\pm$ SE ($n = 36$)

Site	Enzyme activities	<i>Vigna unguiculata</i>	<i>Eragrostis curvula</i>	<i>Vigna unguiculata</i> - <i>Eragrostis curvula</i>
Jameson Park	Nitrate reductase ($\mu\text{mol/h/g}$)	3377.67 $\pm$ 431.27 ^a	1650.17 $\pm$ 16.19 ^b	509.33 $\pm$ 34.91 ^c
	N-acetylglucosaminidase (nmol/h/g)	5688.23 $\pm$ 92.87 ^a	6135.00 $\pm$ 118.18 ^b	3361.45 $\pm$ 179.21 ^c
	Acid phosphatase (nmol/h/g)	5541.40 $\pm$ 57.57 ^a	3313.04 $\pm$ 130.32 ^b	3349.45 $\pm$ 97.36 ^b
	Alkaline phosphatase (nmol/h/g)	5592.50 $\pm$ 48.56 ^a	3290.79 $\pm$ 161.67 ^b	3457.72 $\pm$ 99.66 ^b
	β -Glucosidase activity (nmol/h/g)	6123.82 $\pm$ 100.00 ^a	3367.80 $\pm$ 94.18 ^b	3484.11 $\pm$ 117.01 ^b
Kaydale	Nitrate reductase ($\mu\text{mol/h/g}$)	966.56 $\pm$ 213.47 ^a	1201.00 $\pm$ 98.99 ^b	471.83 $\pm$ 13.44 ^c
	N-acetylglucosaminidase (nmol/h/g)	5855.11 $\pm$ 123.24 ^a	5832.50 $\pm$ 117.86 ^a	3353.17 $\pm$ 152.71 ^b
	Acid phosphatase (nmol/h/g)	5838.54 $\pm$ 111.31 ^a	3469.49 $\pm$ 64.77 ^b	3079.18 $\pm$ 124.03 ^c
	Alkaline phosphatase (nmol/h/g)	5873.03 $\pm$ 94.83 ^a	3238.74 $\pm$ 107.01 ^b	3218.23 $\pm$ 73.50 ^b
	β -Glucosidase activity (nmol/h/g)	6009.17 $\pm$ 107.37 ^a	3430.77 $\pm$ 128.09 ^b	3302.40 $\pm$ 86.10 ^b
Rensburg	Nitrate reductase ($\mu\text{mol/h/g}$)	5716.56 $\pm$ 213.47 ^a	2249.33 $\pm$ 12.62 ^b	651.83 $\pm$ 30.96 ^c
	N-acetylglucosaminidase (nmol/h/g)	5813.61 $\pm$ 157.57 ^a	5942.50 $\pm$ 32.02 ^a	3174.94 $\pm$ 69.26 ^b
	Acid phosphatase (nmol/h/g)	5823.51 $\pm$ 102.96 ^a	3444.92 $\pm$ 82.33 ^b	3266.60 $\pm$ 60.32 ^c
	Alkaline phosphatase (nmol/h/g)	5943.65 $\pm$ 108.95 ^a	3294.88 $\pm$ 166.70 ^b	3303.84 $\pm$ 23.82 ^b
	β -Glucosidase activity (nmol/h/g)	5892.58 $\pm$ 107.37 ^a	3369.53 $\pm$ 124.54 ^b	3356.84 $\pm$ 111.27 ^b

Significant differences among treatments are indicated by different letters according to one-way ANOVA ($p \leq 0.05$)

Hypothesis 1: Intercropping V. unguiculata with E. curvula will improve soil chemical properties.

Intercropping increased post-harvest soil N, P, and pH supporting the first hypothesis. The Increase in soil N concentrations suggests that *E. curvula* and *V. unguiculata* employed complementary N acquisition strategies that enhanced overall N retention (Cong et al. 2015). This finding aligns with studies on faba bean, chickpea, soybean, and oilseed rape intercropping systems, which reported 4–6% higher soil N compared to monocropping (Xing et al. 2023), and with tea–soybean systems that similarly demonstrated the benefits of legume–non-legume interactions (Duan et al. 2024). Such effects may be mediated by shifts in rhizosphere microbial communities, as intercropping has been shown to promote N-cycling taxa (Chen et al. 2023), potentially contributing to the observed N enrichment. In parallel, the intercropping treatment also had higher soil P, consistent with patterns observed in maize–soybean systems (Wang et al. 2017). Interestingly, while this study recorded an increase in soil pH, systems involving *Glycine max*, *Populus cathayana*, and *P. canadensis* cv. Xinlin No.1 showed increased soil N and P but a reduction in pH (Liu et al. 2024), suggesting that the direction of pH change may depend on specific plant–soil–microbe interactions. Collectively, the concurrent increases in soil N, P, and pH indicate that intercropping fosters synergistic plant–microbial

interactions in the rhizosphere, enhancing nutrient retention and availability, consistent with evidence that soil pH, N, and P jointly influence bacterial community composition (Duan et al. 2024).

Hypothesis 2: Intercropping will enhance the relative abundance of beneficial soil bacterial communities.

The intercropping treatment harboured distinct bacterial genera, including *Burkholderia*, *Variovorax*, and *Azotobacter*, which were absent in monocropping treatments, supporting the second hypothesis. Similar findings were reported by Taschen et al. (2017), who identified unique bacterial communities in wheat–pea intercropping soils compared to monocropping systems. The coexistence of the two plant species in the present study likely promoted the secretion of diverse root exudates (Baudoin et al. 2003), which may have stimulated metabolically inactive or dormant bacterial populations (van Vliet 2015). Intercropping likely mediated the restructuring of bacterial communities, promoting diversification and enhancing the abundance of *Pseudomonas* species. These findings align with reports of enhanced relative abundance of beneficial bacteria in tea–soybean intercropping systems (Sun et al. 2022). Similarly, Liu et al. (2024) observed higher relative abundances of dominant bacterial phyla under intercropping, which may also account for the enrichment of *Pseudomonas* species in the present study.

Hypothesis 3: Soil enzyme activities involved in nutrient cycling will be higher in the intercropping treatments.

The third hypothesis is not supported, as intercropping treatments had lower enzyme activities in post-harvest soils. This contrasts with findings by Chen et al. (2023) and Nasar et al. (2024), who reported increased activities of N-targeting enzymes and acid phosphatase in intercropping systems, indicating that enhanced enzyme activity is generally the norm. In the present study, however, declines in enzyme activities under intercropping suggest a different regulatory mechanism. Extracellular enzyme production is typically upregulated under nutrient-limiting conditions (Sinsabaugh and Moorhead 1994); thus, the higher P availability observed in intercropped soils may have reduced microbial demand for P-cycling enzymes. Similarly, despite elevated N concentrations, activities of nitrate reductase and N-acetylglucosaminidase were lowest under intercropping, consistent with the resource allocation model proposed by Sinsabaugh and Moorhead (1994). Phosphorus and N-cycling enzyme activities likely peaked during active plant growth and declined toward maturity as nutrient uptake stabilised. This pattern is consistent with observations by Motsomane and Magadlela (2025), who reported temporal variation in enzyme activities across *E. curvula* cultivars, indicating that microbial enzyme production decreases when nutrient availability is high and enzyme-mediated cycling becomes less critical.

Hypothesis 4: Intercropping will enhance the reliance of V. unguiculata on N derived from the atmosphere compared to monocropping, reducing its competition with E. curvula for soil N. This increased biological N fixation will be supported by the association of V. unguiculata with P-solubilising bacteria to meet the energy demands of N fixation, while E. curvula will benefit from improved soil N availability facilitated by this interaction.

The results support the fourth hypothesis, indicating that intercropping *V. unguiculata* and *E. curvula* increases reliance on N derived from the atmosphere in both species. Legumes intercropped with cereals generally exhibit higher atmospheric N fixation than monocrops, with Toleikiene et al.

(2025) reporting that soybean intercropped with wheat relied on twice as much atmospheric N as monocropped soybean. Similarly, Génard et al. (2017) reported that lupine, clover, and vetch significantly increased their reliance on atmospheric N when intercropped with rapeseed, consistent with the findings of the present study. In the intercropping treatment, *V. unguiculata* hosted bacterial isolates with P-solubilising functions, likely meeting P demands and promoting enhanced N fixation (Amri et al. 2023). The increased reliance on atmospheric N by *V. unguiculata* also suggests reduced competition for soil N with *E. curvula*, aligning with previous reports on nutrient complementarity in legume–grass intercropping systems (Nielsen et al. 2001; Neumann et al. 2009). Notably, *E. curvula* also showed increased reliance on atmospheric N, potentially due to N transfer from *V. unguiculata* (Wei et al. 2022) or the activity of free-living N-fixing bacteria, with the presence of N-fixing species in rhizosphere soils further facilitating atmospheric N assimilation even under conditions of sufficient soil N (Lu et al. 2017).

Hypothesis 5: Intercropping will enhance the growth and biomass accumulation of both species, leading to higher N and P concentrations.

The findings of this study partially support the fifth hypothesis, as intercropped *V. unguiculata* plants demonstrated higher N and P assimilation and utilisation rates, along with increased N and P concentrations. However, these effects were not consistently observed across all soil collection sites for intercropped *E. curvula*. These results align with Nasar et al. (2024), who reported enhanced nutrient uptake in maize–soybean intercropping, and Chi et al. (2023), who observed increased nutrient acquisition in cotton–peanut systems. Intercropped *V. unguiculata* had a higher root biomass, suggesting vertical differentiation in nutrient acquisition and reduced interspecific competition with *E. curvula* (Homulle et al. 2022). This likely expanded root surface area, enhancing P uptake efficiency, specific P absorption and utilisation rates, and ultimately plant P concentrations (Liang et al. 2022). Previous studies similarly show that intercropping improves legume performance, with clover and lotus exhibiting higher yields and N and P concentrations when intercropped with ryegrass and

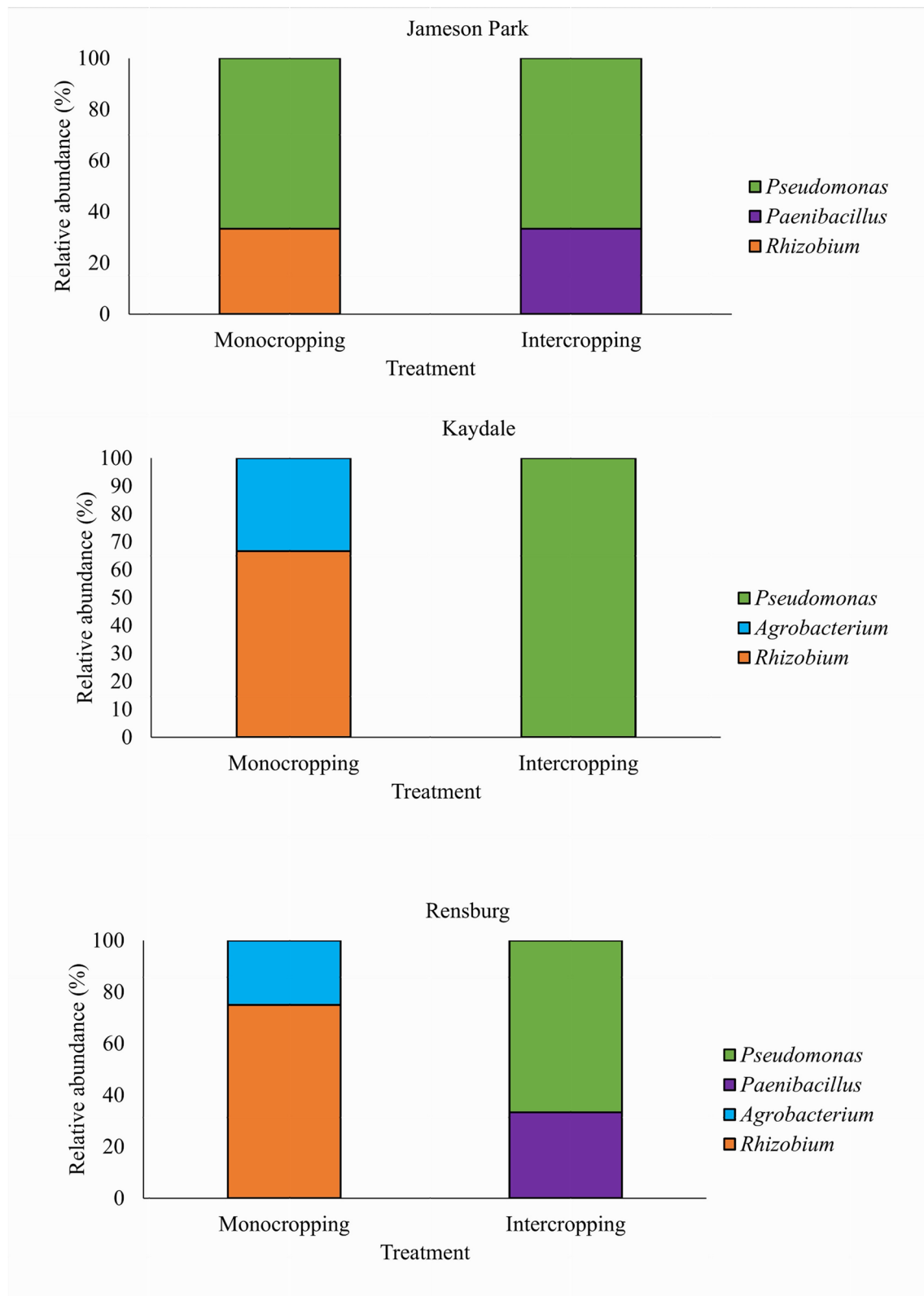


Fig. 3 Relative abundance of bacterial genera isolated from the nodules of monocropped and intercropped *Vigna unguiculata*

cocksfoot (Wei et al. 2022). Higher N absorption rates in intercropped *V. unguiculata* indicate that,

in addition to atmospheric N fixation, soil-derived N contributed to growth, consistent with findings in *Vigna radiata* (Matiwane et al. 2019). Compared to *V. unguiculata*, the growth of *E. curvula* varied across sites, reflecting strong dependence on local soil conditions. The increases in N concentration,

Table 3 Nitrogen source reliance of monocropped and intercropped *Vigna unguiculata* and *Eragrostis curvula* (mean $\pm$ SE, $n = 36$)

Parameter	Treatment	Jameson Park	Kaydale	Rensburg
<i>Vigna unguiculata</i>				
Nitrogen derived from the atmosphere (mmol nitrogen g ⁻¹)	Monocropping	1.011 $\pm$ 0.119 ^a	1.003 $\pm$ 0.06 ^a	2.293 $\pm$ 0.638 ^a
	Intercropping	2.170 $\pm$ 0.046 ^b	2.743 $\pm$ 0.248 ^b	3.839 $\pm$ 0.275 ^b
Nitrogen derived from the soil (mmol nitrogen g ⁻¹)	Monocropping	2.943 $\pm$ 0.116 ^a	4.442 $\pm$ 0.090 ^a	3.391 $\pm$ 0.055 ^a
	Intercropping	0.313 $\pm$ 0.012 ^b	1.031 $\pm$ 0.488 ^b	1.029 $\pm$ 0.309 ^b
Nitrogen derived from the atmosphere (%)	Monocropping	42.202 $\pm$ 10.091 ^a	40.337 $\pm$ 7.083 ^a	50.569 $\pm$ 0.265 ^a
	Intercropping	78.915 $\pm$ 0.625 ^b	69.447 $\pm$ 0.127 ^b	67.953 $\pm$ 3.167 ^b
<i>Eragrostis curvula</i>				
Nitrogen derived from the atmosphere (mmol nitrogen g ⁻¹)	Monocropping	1.265 $\pm$ 0.237 ^a	2.238 $\pm$ 0.248 ^a	1.571 $\pm$ 0.312 ^a
	Intercropping	5.205 $\pm$ 0.012 ^b	3.106 $\pm$ 0.687 ^b	2.793 $\pm$ 1.106 ^b
Nitrogen derived from the soil (mmol nitrogen g ⁻¹)	Monocropping	2.329 $\pm$ 0.430 ^a	3.086 $\pm$ 0.200 ^a	2.379 $\pm$ 0.301 ^a
	Intercropping	3.886 $\pm$ 0.019 ^b	3.267 $\pm$ 0.118 ^a	3.527 $\pm$ 0.209 ^b
Nitrogen derived from the atmosphere (%)	Monocropping	35.200 $\pm$ 3.052 ^a	31.323 $\pm$ 0.764 ^a	36.758 $\pm$ 1.718 ^a
	Intercropping	57.251 $\pm$ 2.725 ^b	49.860 $\pm$ 2.357 ^b	48.050 $\pm$ 9.261 ^b

Different letters indicate significant differences between treatments (t-test, $p \leq 0.05$)

Table 4 Plant growth kinetics of monocropped and intercropped *Vigna unguiculata*

Parameter	Treatment	Jameson Park	Kaydale	Rensburg
Aboveground biomass (g)	Monocropping	0.13 $\pm$ 0.05 ^a	0.10 $\pm$ 0.02 ^a	0.14 $\pm$ 0.03 ^a
	Intercropping	1.26 $\pm$ 0.08 ^b	0.04 $\pm$ 0.01 ^a	0.62 $\pm$ 0.06 ^b
Belowground biomass (g)	Monocropping	0.30 $\pm$ 0.05 ^a	0.30 $\pm$ 0.04 ^a	0.39 $\pm$ 0.09 ^a
	Intercropping	3.80 $\pm$ 0.35 ^b	1.30 $\pm$ 0.20 ^b	1.58 $\pm$ 0.10 ^b
Number of nodules	Monocropping	12.00 $\pm$ 3.00 ^a	8.00 $\pm$ 2.00 ^a	14.00 $\pm$ 4.00 ^a
	Intercropping	36.00 $\pm$ 4.00 ^b	18.00 $\pm$ 3.00 ^b	16.00 $\pm$ 2.00 ^a
Nodule weight (g)	Monocropping	0.85 $\pm$ 0.30 ^a	0.81 $\pm$ 0.17 ^a	0.82 $\pm$ 0.10 ^a
	Intercropping	1.70 $\pm$ 0.53 ^b	1.25 $\pm$ 0.24 ^b	1.29 $\pm$ 0.13 ^b
Root-to-shoot ratio	Monocropping	0.47 $\pm$ 0.04 ^a	0.28 $\pm$ 0.14 ^a	0.34 $\pm$ 0.18 ^a
	Intercropping	0.50 $\pm$ 0.04 ^a	0.47 $\pm$ 0.07 ^b	0.60 $\pm$ 0.05 ^b
Specific P absorption rate (mg P g ⁻¹ root DW day ⁻¹)	Monocropping	0.26 $\pm$ 0.01 ^a	0.30 $\pm$ 0.03 ^a	0.33 $\pm$ 0.01 ^a
	Intercropping	2.480 $\pm$ 0.05 ^b	1.16 $\pm$ 0.19 ^b	1.37 $\pm$ 0.15 ^b
Specific N absorption rate (mg N g ⁻¹ root DW day ⁻¹)	Monocropping	1.94 $\pm$ 0.07 ^a	3.63 $\pm$ 0.33 ^a	4.37 $\pm$ 0.54 ^a
	Intercropping	2.26 $\pm$ 0.09 ^a	5.93 $\pm$ 1.18 ^b	6.56 $\pm$ 0.63 ^b
Specific P utilisation rate (mg P g ⁻¹ plant DW day ⁻¹)	Monocropping	0.05 $\pm$ 0.01 ^a	0.04 $\pm$ 0.00 ^a	0.05 $\pm$ 0.00 ^a
	Intercropping	0.42 $\pm$ 0.01 ^b	0.21 $\pm$ 0.03 ^b	0.27 $\pm$ 0.03 ^b
Specific N utilisation rate (mg N g ⁻¹ plant DW day ⁻¹)	Monocropping	4.60 $\pm$ 0.54 ^a	7.34 $\pm$ 0.70 ^a	5.73 $\pm$ 0.95 ^a
	Intercropping	14.53 $\pm$ 3.54 ^b	16.80 $\pm$ 3.97 ^b	17.07 $\pm$ 1.32 ^b
Relative growth rate (g day ⁻¹)	Monocropping	0.013 $\pm$ 0.01 ^a	0.02 $\pm$ 0.00 ^a	0.02 $\pm$ 0.01 ^a
	Intercropping	0.02 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^b

Values represent mean $\pm$ SE, $n = 36$. Significant differences among treatments are indicated by different letters according to an Independent Samples T-test ($p \leq 0.05$)

Table 5 Plant growth kinetics of monocropped and intercropped *Eragrostis curvula*

Parameter	Treatment	Jameson Park	Kaydale	Rensburg
Aboveground biomass (g)	Monocropping	0.002 ± 0.001 ^a	0.010 ± 0.002 ^a	0.010 ± 0.002 ^a
	Intercropping	0.002 ± 0.001 ^a	0.004 ± 0.002 ^b	0.003 ± 0.001 ^b
Belowground biomass (g)	Monocropping	0.001 ± 0.0003 ^a	0.001 ± 0.0003 ^a	0.010 ± 0.002 ^a
	Intercropping	0.004 ± 0.0003 ^b	0.001 ± 0.0003 ^a	0.011 ± 0.002 ^a
Root-to-shoot ratio	Monocropping	1.411 ± 0.360 ^a	6.882 ± 0.901 ^a	1.062 ± 0.111 ^a
	Intercropping	0.310 ± 0.144 ^b	0.620 ± 0.010 ^b	0.273 ± 0.094 ^b
Specific P absorption rate (mg P g ⁻¹ root DW day ⁻¹)	Monocropping	0.0013 ± 0.0001 ^a	0.0041 ± 0.000 ^a	0.006 ± 0.000 ^a
	Intercropping	0.002 ± 0.0001 ^b	0.004 ± 0.000 ^a	0.006 ± 0.000 ^a
Specific N absorption rate (mg N g ⁻¹ root DW day ⁻¹)	Monocropping	0.524 ± 0.000 ^a	2.001 ± 0.184 ^a	1.320 ± 0.542 ^a
	Intercropping	1.337 ± 0.003 ^b	2.396 ± 0.057 ^a	2.057 ± 0.441 ^b
Specific P utilisation rate (mg P g ⁻¹ plant DW day ⁻¹)	Monocropping	0.001 ± 0.000 ^a	0.004 ± 0.000 ^a	0.003 ± 0.000 ^a
	Intercropping	0.0010 ± 0.000 ^a	0.001 ± 0.000 ^a	0.001 ± 0.000 ^b
Specific N utilisation rate (mg N g ⁻¹ plant DW day ⁻¹)	Monocropping	0.095 ± 0.006 ^a	0.076 ± 0.001 ^a	0.082 ± 0.007 ^a
	Intercropping	0.081 ± 0.004 ^b	0.074 ± 0.003 ^a	0.075 ± 0.003 ^a
Relative growth rate (g day ⁻¹)	Monocropping	0.022 ± 0.005 ^a	0.017 ± 0.002 ^a	0.055 ± 0.006 ^a
	Intercropping	0.022 ± 0.004 ^a	0.049 ± 0.012 ^a	0.032 ± 0.002 ^b

Values represent mean ± SE, $n = 36$. Significant differences among treatments are indicated by different letters according to an Independent Samples T-test ($p \leq 0.05$)

reliance on atmospheric N and variable patterns of P assimilation, utilisation, and plant P concentration in intercropped *E. curvula*, suggest that N availability plays a greater role than P in influencing *E. curvula* nutrition. While increased P availability under intercropping enhanced P assimilation and utilisation in *V. unguiculata*, *E. curvula* showed no such response, likely due to its lower P requirement for growth (Halsted and Lynch 1996). Overall, these findings highlight that nutrient uptake differs between species and that intercropping benefits are greater for N than for P in grass–legume systems.

Conclusions

The findings of this study demonstrate the potential of underutilised legume and grass intercropping systems to enhance nutrient use efficiency, particularly through increased biological N fixation and improved P uptake. These interactions indicate that legume–grass intercropping can contribute to restoring soil health, reducing reliance on synthetic fertilisers, and supporting biomass production in resource-constrained agricultural systems. The complementary use of belowground resources

Table 6 Phosphorus and nitrogen concentrations of monocropped and intercropped *Vigna unguiculata* and *Eragrostis curvula*

Values represent mean ± SE, $n = 36$. Significant differences among treatments are indicated by different letters according to an Independent Samples T-test ($p \leq 0.05$)

Parameter	Treatment	Jameson Park	Kaydale	Rensburg
<i>Vigna unguiculata</i>				
Phosphorus (mmol g ⁻¹)	Monocropping	47.73 ± 5.10 ^a	38.86 ± 6.14 ^a	61.24 ± 15.43 ^a
	Intercropping	62.02 ± 12.95 ^b	51.00 ± 9.01 ^b	45.96 ± 9.72 ^b
Nitrogen (mmol g ⁻¹)	Monocropping	5.95 ± 0.15 ^a	3.45 ± 1.09 ^a	2.68 ± 0.24 ^a
	Intercropping	10.48 ± 2.06 ^b	8.77 ± 2.74 ^b	6.87 ± 2.33 ^b
<i>Eragrostis curvula</i>				
Phosphorus (mmol g ⁻¹)	Monocropping	46.01 ± 7.94 ^a	44.96 ± 8.51 ^a	53.47 ± 12.81 ^a
	Intercropping	51.00 ± 9.01 ^b	45.99 ± 14.86 ^a	50.62 ± 11.52 ^b
Nitrogen (mmol g ⁻¹)	Monocropping	3.59 ± 0.66 ^a	4.32 ± 0.49 ^a	3.95 ± 1.00 ^a
	Intercropping	9.09 ± 0.21 ^b	6.37 ± 1.52 ^b	6.15 ± 2.32 ^b

between species suggests functional niche differentiation, which can improve system resilience and long-term productivity. However, total N measurements include both organic and inorganic forms and may not fully represent the N immediately available to plants. In this study, total N was used as a broad indicator of soil N status, and it is possible that actual plant-available N differed locally, which could influence the observed patterns of N assimilation. Moreover, the controlled greenhouse conditions used here limit the generalisability of the results, highlighting the need for long-term field-based studies to assess the consistency of these outcomes under diverse soil, climate, and management conditions and to determine the practical applicability of this intercropping system across agroecological contexts.

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Author contributions A.M was involved in the study conception and design. Material preparation, data collection and analysis were performed by N.M. The first draft of the manuscript was written by N.M and A.M commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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