

Vicia sativa and Vicia villosa enhance soil microbial composition, enzyme activities, and chemical properties in nutrient-deficient small-scale sugarcane plantation soils

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Research Article

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Abstract

Vicia sativa and *Vicia villosa* are nitrogen (N) fixing legumes commonly used as forage and cover crops due to their ability to enhance N fixation, soil N contributions, and enzyme activities in nutrient-deficient soils. Using *V. sativa* and *V. villosa* as cover crops can potentially improve nutrient cycling in nutrient-deficient sugarcane plantations owned by small-scale growers (SSGs) in KwaZulu-Natal, South Africa. This study investigated the chemical and biological inputs of *V. sativa* and *V. villosa* in nutrient-deficient sugarcane plantation soils. The nutrient concentration, N and phosphorus (P) cycling bacteria, and extracellular enzyme activities of soils collected from five small-scale sugarcane plantations were determined pre-planting and post-*V. sativa* and *V. villosa* harvest. Post-*V. sativa* and *V. villosa* soils had higher pH levels than pre-planting soils across all plantation soils. The number of plant growth-promoting rhizobacteria (PGPR) isolated from soils post-*V. sativa* and *V. villosa* harvest increased across all plantation soils. The *Arthrobacter*, *Burkholderia* and *Paraburkholderia Pseudomonas* were the most dominant genera isolated from post-harvest soils. The number of P-solubilising bacteria increased, increasing acid phosphatase activities. The findings of this study reveal that *V. sativa* and *V. villosa* increase PGPR, pH and enzyme activities in soils, making them sustainable options as cover crops for nutrient-deficient sugarcane plantation soils owned by SSGs.

1 Introduction

Arable land availability is one of the main factors affecting crop productivity worldwide [1]. According to [2], arable land per person in 2050 is expected to decrease by 33% from what it was in 1970. Reduced arable land coupled with the ever-increasing world population raises concerns about food security [3]. In recent years, food insecurity levels have increased steadily in southern Africa and remain a significant developmental problem in the sub-region [4]. [5] reported increases in food insecurity rates in most South African households, with food insecurity and poverty being some of the dominant socioeconomic issues in the country [6]. In 2021, 3.7 million out of 17.9 million households were reported to be food insecure [7], with food insecurity affecting most small-scale farming homesteads [8, 9]. Food insecurity is exacerbated by the declining soil fertility and low crop yield experienced by many SSGs in the country [10]. Approximately 70% of South African SSGs residing in rural areas rely on subsistence farming as their source of livelihood [11].

Sugarcane is the main cash crop cultivated by SSGs in KwaZulu-Natal [12]. The sugar industry, primarily rural-based, plays a crucial role in supporting these SSGs by generating income and aiding in poverty alleviation and food security [12]. However, SSGs have been experiencing a decline in productivity [12], which could result from the intensive farming practices employed for cane production [13]. Small-scale growers practice monoculture because of land limitations. However, it has been reported to lead to land degradation and declining soil health and fertility [13]. This is due to the loss of the soil's organic matter, breakdown of the soil structure, soil acidification and nutrient leaching [14]. Furthermore, high-yield intensive farming systems require extensive amounts of chemical fertilisers, which are detrimental to the environment and are too costly for poor, resource-limited SSGs [10]. Increased fertiliser inputs do not guarantee fertility and productivity, as a significant portion of the fertiliser is lost through leaching and runoff [15, 16]. Therefore, soil fertility management practices that sustainably improve soil health and increase production by SSGs are required to mitigate food insecurity and poverty.

Vicia species have been reported to be a green manure alternatives in sustainable agricultural systems [17, 18]. *Vicia* species can effectively enhance soil fertility when used as cover crops by fixing atmospheric N and returning it to the soil through N spillover [19]. The success of *Vicia* species can be attributed to their association with diverse microorganisms that benefit the plants' development [20]. [21] suggested that *Vicia* species foster a substantial community of rhizobial bacteria, promoting the ecosystem function of the bacteria as plant growth promoters. *Vicia villosa* has been reported to improve acid phosphatase activities and promote soil physical and chemical properties, thereby increasing crop yields and quality [22].

Incorporating *V. sativa* and *V. villosa* as green manure between sugarcane plant cycles can help alleviate the long-term effects of sugarcane monoculture by reducing the need for N fertiliser for subsequent crops, offering a cheaper alternative to synthetic fertilisers [23]. Moreover, adding *V. sativa* and *V. villosa* in between the crop cycles increases crop diversity [24], reducing the population of detrimental soil microbes [25] and diversifying the root exudates released by the plant roots into the soil [26]. The function of plant growth-promoting rhizobacteria (PGPR) can be limited by several environmental conditions, including decreases in soil pH and reduced nutrient availability. However, root exudates can increase their plant growth-promoting activities by

influencing the physiochemical properties of the soil [27] which influences the soil enzyme activities [26]. The decomposition of roots from the cover crop residue can release the stored N into the soil [28], introducing organic matter, increasing resistance to biological degradation, facilitating a longer and deeper carbon for the next crop [29].

Although the utilisation of *V. sativa* and *V. villosa* as green manure and cover crop is a growing practice in many agroecosystems [30, 31], its biological and chemical contribution to sugarcane farming systems in South Africa is unknown. Therefore, this study aims to investigate the chemical and biological contributions of *V. sativa* and *V. villosa* to small-scale KwaZulu-Natal sugarcane soils. Additionally, the study seeks to examine how the soil conditions influence the growth physiology and nutrient assimilation rates of *V. sativa* and *V. villosa* cover crops. The objectives of the study are to isolate and identify nutrient-cycling bacteria, their associated enzymes and soil chemical characteristics in sugarcane soils collected from five different sugarcane plantation sites pre-planting and post-*V. sativa* and *villosa* harvest. Understanding the growth physiology of *V. sativa* and *V. villosa* in the different plantation soils will help quantify the N and P accumulated by the legume, which will facilitate the biomass residue management of the crop.

2 Materials and Methods

2.1 Soil Collection Sites

Soil samples were collected from five small-scale sugarcane plantations at different geographical locations in the north and south coast of KwaZulu-Natal (KZN). The northern KZN plantation sites included Mvutshini (28° 50' 52.9" S 32°00'09.0" E), Mpembeni (28°51' 25.7" S 31°58'19.4" E), and Gingindlovu (28°56'29.3"S 31°34'58.9"E). The southern KZN plantations, Hibberdene (30°35'37.1214"S,30°31'30.2916"E) and Mzinto (30°16'41.0262"S,30°39'47.9658"E), were located in the South Coast. Northern KZN has a subtropical climate that is warm and humid but is characterised by heavy rainfalls throughout the year. The South coast of KZN is characterised by a subtropical climate, with warm temperatures, mild winters, high humidity, and generous summer rainfall. From each site, the experimental soils, classified as sandy soils, were collected from 20 random points of the plantation at a depth of 10–20 cm, where microbial nutrient cycling activities occur, using a Dutch auger. Pre-planting, the soils for enzyme activity assays, bacterial extraction and identification experiments were collected and packaged into sterile plastic bags. The soils were then immediately placed on ice during transportation and stored at 4°C before analysis.

2.2 Experimental setup, Growth conditions, and Soil Chemical Analysis

Vicia sativa and *V. villosa* seeds used in this study were obtained from AGT Foods Africa, Marji Mizuri farm, KwaZulu-Natal. The seeds were handled as per Plant Improvement Act 53 of 1976, Regulations Relations To Establishment, Varieties, Plant And Propagation Material. The seeds were germinated by soaking in warm water overnight to stimulate germination, and then transferred into petri dishes lined with moist, sterile filter paper and stored in the dark for four days at room temperature. After germination, the seeds were planted at 1–2 cm depths in 10 cm diameter pots filled with soil from the different plantations. The experiment was a completely randomised design with the plantation sites as treatments with 10 replications per site per species, resulting in a total of 100 experimental pots. The experimental trials were conducted in the greenhouse at the University of KwaZulu-Natal, Westville campus, School of Life Sciences building, South Africa. The average night and day temperatures in the greenhouse ranged from 11–15°C and 30–35°C, respectively. Initial and final harvesting were conducted 14 and 45 days after seedling emergence. The initial and final harvesting was required for the growth, nutrient assimilation, and utilisation calculations. Post-harvest, the plants were rinsed with distilled H₂O, separated into roots, stems and leaves, and dried at 65°C until constant weight was reached. The plant dry weights (DW) were recorded, and dried material was ground using a mortar and pestle.

The ground plant material was then sent for carbon (C) and N isotope analysis at the Archaeometry Department at the University of Cape Town, South Africa, and P analysis at the Central Analytical Facilities of Stellenbosch University, South Africa. Post final harvest, the soils were transferred into sterile plastic bags and stored at 4°C to be used for nutrient analysis, enzyme activity assays, and bacterial extraction and identification. For total soil nutrient analysis, three kilogram (kg) soil samples from each plantation (initial and post-harvest) were air-dried, sieved through a 2 mm sieve, and sent to the South African Sugarcane Research Institute (SASRI) Fertiliser Advisory Services (FAS) for pH, exchange acidity, total cations, primary, secondary and micronutrients analysis.

2.3 Soil Bacterial Extraction

Three-fold soil serial dilutions were conducted where 10 g of soil sample were diluted in 100 ml of autoclaved distilled water under sterile conditions. The dilutions were then transferred into selective media plates by inoculating each plate with 100 µl of the dilution. Jensen media plates were used to grow N-fixing bacteria, while tricalcium phosphate and Simmons citrate plates were used to grow P-solubilising and N-cycling bacteria, respectively. The media plates were incubated at 30°C and allowed to grow for 3 to 7 days. Thereafter, single colonies were distinguished according to colour and size and sub-cultured into sterile, separate media plates to form pure colonies/cultures.

2.4 Bacterial Amplification and Identification

Pure colonies/cultures obtained from repeated streaking were amplified using a colony PCR where pure bacterial colonies were amplified using the 63F (5'- CAGGCCTAACACATGCAAGTC - 3') and 1387R (5'- GGGCGGTGTGTACAAGGC - 3') primers. A T100 Thermal Cycler (Bio-Rad, USA) was used for amplification with the initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 92°C for 30 sec, annealing at 56°C for 45 sec and elongation at 75°C for 45 sec. with the final elongation at 75°C for 10 min. The PCR products were resolved on 1.0% (w/v) agarose gels (Seakem) and visualised after staining. Positive amplicons were sent for sequencing at Inqaba Biotech Pty. Ltd., Pretoria, South Africa. Thereafter, the sequences were edited and compared against the GenBank database. Homologues were identified using the BLASTN program at the National Center for Biotechnology Information (NCBI) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (accessed 27 December 2023).

2.5 Soil Enzyme Activity Analysis

2.5.1 β-Glucosidase and acid phosphatase activity

The soil β-Glucosidase and acid phosphatase activity ($\text{nmol h}^{-1} \text{g}^{-1}$) was determined using the fluorescence-based method adapted from [32]. Briefly, 10 g of soil sample were homogenised in 100 ml of dH_2O at low speed for 2 hrs. An appropriate MUB substrate, bicarbonate buffer (100 mM) and a 4-Methylumbelliferone standard (100 µM) were prepared. The different solutions were then transferred into 96-well microplates and incubated for 1hr at 30°C; 0.5 M of NaOH was used to stop the reaction. MUB-phosphatase substrate and 4-MUB-glucopyranoside substrates were used for acid phosphatase and β-Glucosidase activities, respectively. The buffer and standard had their pH adjusted to 5 for the acid phosphatase activity. The fluorescent absorbance was measured at 450 nm using an Apex Scientific microplate reader (Durban, South Africa).

2.5.2 Nitrate Reductase Activity

The nitrate reductase activity was determined using the method adapted from [33]. Briefly, 5 g of soil sample were mixed with 4 ml of 0.9 mM 2,4 DNP, 1 ml of 25 mM KNO_3 and 5ml of autoclaved distilled water in a conical flask wrapped with foil to prevent light penetration. The mixture was mixed vigorously before incubation for 24 hrs at 30°C. After incubation, 10ml of 4 M KCl was added to each sample, mixed, and passed through Whatman no. 1 filter paper. The enzyme activity was initiated by adding 2 ml of the filtrate into 1.2 ml of 0.19 M of ammonium chloride (pH ~ 8.5) and 0.8ml of the colour reagent (1% sulphanilamide in 1 N HCl and 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The sample was then incubated at 30°C for 30 mins. After that, the absorbance was measured at 520 nm using an Agilent Cary 60UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The amount of nitrite released into the medium was expressed as $0.1 \mu\text{mol h}^{-1} \text{g}^{-1}$.

2.6 Plant Nutrition

2.6.1 Calculation of Percentage Nitrogen Derived from the Atmosphere (%Ndfa)

Nitrogen isotope analyses were conducted at the Archeometry Department, University of Cape Town, South Africa. The isotopic ratio of N was calculated as $\delta = 1000\text{‰}$ ($R_{\text{sample}}/R_{\text{standard}}$), where R is the molar ratio of the heavier to the lighter isotope of the samples and standards as described by [34]. Between 2.100 and 2.200 mg of each milled sample were weighed into 8 mm x 5-mm tin capsules (Elemental Micro-analysis, Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons Instruments SpA, Milan, Italy). The N isotope values for the

N gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyser by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift, namely, two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(\text{NH}_4)_2\text{SO}_4$.

%NDFA was calculated according to [35], as follows:

$$\% \text{NDFA} = 100 \left(\frac{\delta^{15} N_{\text{reference plant}} - \delta^{15} N_{\text{legume}}}{\delta^{15} N_{\text{reference plant}} - B} \right);$$

Where the reference plant was non-nodulated *V. sativa* or *V. villosa*, planted 2 weeks later than the experimental plants and grown under the same glasshouse conditions using 500 mM N in a Long Ashton nutrient solution (25% strength). The B value is the $\delta^{15}\text{N}$ natural abundance of the N derived exclusively from biological N-fixation of nodulated *V. sativa* or *V. villosa*. The seeds were germinated in the natural inoculum, and thereafter, the seedlings were grown with N-free 25% strength Long Ashton nutrient solution in sterile-sand culture. The B-value of *V. sativa* was determined as - 2.58.

2.6.2 Specific N/P Absorption Rate (SNAR/SPAR)

The net N/P absorption rate per unit root DW was calculated according to [36] using the plant's total N/P content.

$$\text{SNAR} = (N_2 - N_1 / t_2 - t_1) \times ((\log_e R_2 - \log_e R_1) / (R_2 - R_1))$$

$$\text{SPAR} = (P_2 - P_1 / t_2 - t_1) \times ((\log_e R_2 - \log_e R_1) / (R_2 - R_1))$$

Where N_2 and N_1 are the final and initial N, respectively; P_2 and P_1 are the P content, t is the duration of plant growth, and R is the root dry weight (mg N g^{-1} root DW day^{-1})

2.6.3 Specific N/P utilisation rates (SNUR/SPUR)

The total N/P were used to calculate the specific N/P utilisation rate ($\text{g DW mg}^{-1} \text{N/P day}^{-1}$) according to [36].

$$\text{SNUR} = (W_2 - W_1 / t_2 - t_1) \times ((\log_e N_2 - \log_e N_1) / (N_2 - N_1))$$

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

Where W , N , P and t represent the plant DW, total N content, total P content and the duration of the plant growth, respectively.

2.6.5 Relative Growth Rate (RGR)

The relative growth rate was calculated using the method by [37]:

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

Where W denotes the dry plant weights accumulated from the initial (W_1) and final (W_2) harvest and t is the time for plant growth.

2.6.6 Root: shoot ratio

The root: shoot ratio was calculated according to [37] using the root biomass per shoot biomass of the plant.

$$\text{Root: shoot} = D_R / D_S$$

Where D_R is the dry weight of the root while D_S is the dry weight of the shoot.

2.7 Statistical Analysis

R-studio (R version 4.3.1) was used for all analyses where soil nutrition (pre- and post-planting and harvest), soil enzyme activities (pre- and post-harvest), growth kinetics, plant biomass and plant mineral nutrition of *V. sativa* and *V. villosa* were examined using the analysis of variance (One-way ANOVA). A Tukey multiple comparisons post hoc test was conducted when significant results were observed in the ANOVA ($p < 0.05$). The assumptions for normal distribution were tested using the Shapiro-Wilk test, while the

assumption for homogeneity of variance was tested using Levene's test (library car package). A non-parametric (Kruskal-Wallis's test) alternative was used when the assumptions were not met.

3 Results

3.1 Soil Chemical Analysis

Post-harvesting, there was a significant decrease in soil N concentrations in Mpembeni, Mzinto, and Gingindlovu under both species (Table 1). The soil P concentrations decreased significantly in Mvutshini, Mzinto and Hibberdene under *V. sativa* treatment, while a significant decrease was observed under both species treatments in Gingindlovu (Table 1). The potassium (K) concentrations post-harvesting showed a significant increase in Mpembeni and a decrease in Hibberdene under both species treatments (Table 1). Calcium (Ca) concentrations significantly increased across all sites, while magnesium (Mg) concentrations increased significantly in Mpembeni, Mzinto, and Hibberdene. The soil pH increased significantly across all sites under both species except for Mvutshini- *V. sativa*, which showed no significant differences (4.67 to 5.04). The exchange acidity increased significantly in Mvutshini, Mpembeni, and Gingindlovu post-harvesting soils.

Table 1

The chemical parameters of pre-planting and post-*Vicia sativa* and *Vicia villosa* harvest in soils collected from Mvutshini, Mpembeni, Mzinto, Hibberdene and Gingindlovu. The different values are presented as mean $\pm$ SE. Different letters represent significant differences after One-Way ANOVA

		Nitrogen (mg/L)	Phosphorus (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	pH	Exchange Acidity (Al + H)
Mvutshini	Pre	0.085 $\pm$ 0.00 ^{ab}	3.70 $\pm$ 0.12 ^a	33.00 $\pm$ 0.00 ^a	196.00 $\pm$ 13.86 ^a	72.50 $\pm$ 8.37 ^a	4.67 $\pm$ 0.03 ^a	0.50 $\pm$ 0.15 ^a
	<i>Vicia sativa</i>	0.070 $\pm$ 0.01 ^a	2.85 $\pm$ 0.14 ^b	40.50 $\pm$ 4.91 ^a	400.00 $\pm$ 54.80 ^b	134.50 $\pm$ 22.80 ^b	4.75 $\pm$ 0.16 ^a	0.36 $\pm$ 0.03 ^b
	<i>Vicia villosa</i>	0.090 $\pm$ 0.00 ^b	3.60 $\pm$ 0.00 ^a	43.00 $\pm$ 0.00 ^a	391.00 $\pm$ 3.46 ^b	119.00 $\pm$ 1.15 ^{ab}	5.04 $\pm$ 0.05 ^b	0.33 $\pm$ 0.00 ^b
Mpembeni	Pre	0.055 $\pm$ 0.00 ^a	3.30 $\pm$ 0.00 ^a	18.50 $\pm$ 0.29 ^a	150.50 $\pm$ 0.29 ^a	46.00 $\pm$ 0.00 ^a	4.64 $\pm$ 0.02 ^a	0.27 $\pm$ 0.02 ^a
	<i>Vicia sativa</i>	0.025 $\pm$ 0.00 ^b	3.15 $\pm$ 0.14 ^a	26.50 $\pm$ 0.29 ^b	270.00 $\pm$ 2.89 ^b	79.50 $\pm$ 0.87 ^b	5.99 $\pm$ 0.05 ^b	0.05 $\pm$ 0.00 ^b
	<i>Vicia villosa</i>	0.020 $\pm$ 0.00 ^b	3.00 $\pm$ 0.06 ^a	22.00 $\pm$ 0.58 ^c	219.00 $\pm$ 4.04 ^c	58.50 $\pm$ 3.75 ^c	5.49 $\pm$ 0.26 ^b	0.08 $\pm$ 0.01 ^b
Mzinto	Pre	0.070 $\pm$ 0.00 ^a	10.70 $\pm$ 0.29 ^a	61.00 $\pm$ 1.73 ^a	726.00 $\pm$ 53.12 ^a	151.00 $\pm$ 12.12 ^a	5.71 $\pm$ 0.04 ^a	0.05 $\pm$ 0.00 ^a
	<i>Vicia sativa</i>	0.035 $\pm$ 0.00 ^b	9.75 $\pm$ 0.03 ^b	72.00 $\pm$ 1.73 ^a	923.00 $\pm$ 7.51 ^b	219.00 $\pm$ 2.89 ^b	6.40 $\pm$ 0.02 ^b	0.05 $\pm$ 0.00 ^a
	<i>Vicia villosa</i>	0.040 $\pm$ 0.00 ^b	10.10 $\pm$ 0.23 ^{ab}	68.50 $\pm$ 3.75 ^a	930.50 $\pm$ 68.99 ^b	222.00 $\pm$ 22.52 ^b	6.38 $\pm$ 0.01 ^b	0.05 $\pm$ 0.00 ^a
Hibberdene	Pre	0.060 $\pm$ 0.00 ^a	11.40 $\pm$ 0.63 ^a	100.50 $\pm$ 2.60 ^a	563.00 $\pm$ 3.46 ^a	156.00 $\pm$ 5.77 ^a	5.53 $\pm$ 0.13 ^a	0.06 $\pm$ 0.01 ^a
	<i>Vicia sativa</i>	0.045 $\pm$ 0.00 ^a	6.60 $\pm$ 0.64 ^b	73.00 $\pm$ 0.00 ^b	726.00 $\pm$ 6.06 ^b	204.00 $\pm$ 0.58 ^b	6.39 $\pm$ 0.06 ^b	0.05 $\pm$ 0.00 ^a
	<i>Vicia villosa</i>	0.050 $\pm$ 0.01 ^a	8.70 $\pm$ 1.10 ^{ab}	64.50 $\pm$ 3.75 ^b	694.50 $\pm$ 1.44 ^c	202.50 $\pm$ 3.75 ^b	6.39 $\pm$ 0.01 ^b	0.05 $\pm$ 0.00 ^a
Gingindlovu	Pre	0.055 $\pm$ 0.00 ^a	5.90 $\pm$ 0.46 ^a	55.00 $\pm$ 1.73 ^a	221.00 $\pm$ 0.58 ^a	128.50 $\pm$ 1.44 ^a	4.64 $\pm$ 0.04 ^a	0.30 $\pm$ 0.01 ^a
	<i>Vicia sativa</i>	0.030 $\pm$ 0.00 ^b	2.70 $\pm$ 0.06 ^b	60.50 $\pm$ 5.48 ^a	337.00 $\pm$ 31.70 ^b	158.00 $\pm$ 18.48 ^a	5.83 $\pm$ 0.01 ^b	0.05 $\pm$ 0.00 ^b
	<i>Vicia villosa</i>	0.035 $\pm$ 0.00 ^b	3.00 $\pm$ 0.06 ^b	51.00 $\pm$ 1.73 ^a	338.00 $\pm$ 6.93 ^b	152.50 $\pm$ 3.18 ^a	5.83 $\pm$ 0.01 ^b	0.05 $\pm$ 0.00 ^b

3.2 Bacterial identification and abundance

A total of 16 bacterial strains were isolated pre-planting, compared to 31 isolated post-*V. sativa* harvest and 27 post-*V. villosa* harvesting (Fig. 1, Table 2). Pre-planting, six bacterial strains were isolated from Mvutshini (1 N-fixing; 1 phosphate solubilising and N-cycling; 1 N-cycling; 3 N-fixing and phosphate solubilising). The number of bacterial strains increased to seven post-*V. sativa* harvest (1 N-cycling; 4 N-fixing and phosphate solubilising; 2 N-fixing, phosphate solubilising and N-cycling) and five post-*V. villosa* harvesting (3 N-fixing and phosphate solubilising, and 2 phosphate solubilising, N-fixing and N-cycling). In Mpembeni, five bacterial strains were isolated pre-planting (1 phosphate solubilising; 3 N-fixing and phosphate solubilising; 1 phosphate solubilising, N-fixing and N-cycling). The number of isolated strains increased to eight (2 N-fixing, phosphate solubilising and N-cycling, and 6 N-fixing and phosphate solubilising) post-*V. sativa* harvest and eight N-fixing and phosphate solubilising strains

post-*V. villosa* harvest. Two N-fixing, phosphate solubilising and N-cycling bacterial strains were isolated from Mzinto pre-planting, which increased to six (2 phosphate solubilising, N- fixing and N- cycling; 1 phosphate solubilising and N cycling; 3 N- fixing & phosphate solubilising) post-*V. sativa* harvest and four (1 phosphate solubilising, N- fixing and N- cycling, and 3 N- fixing and phosphate solubilising) bacterial strains post-*V. villosa* harvest. Pre-planting, two bacterial strains (1 phosphate solubilising and 1 N- fixing and phosphate solubilising) were isolated from Hibberdene, which increased to five (2 phosphate solubilising and N- fixing; 1 N-cycling and phosphate solubilising; 1 N-cycling; 1 phosphate solubilising) bacterial strains post-*V. sativa* and harvesting and five phosphate solubilising, N-fixing and N-cycling bacterial strains isolated post-*V. villosa* harvest. One phosphate solubilising and N-cycling bacterial strain was isolated from Gingindlovu pre-planting compared to five (2 phosphate solubilising, N-fixing and N-cycling; 1 N-fixing; 2 N-fixing and phosphate solubilising) isolated post-*V. sativa* harvest and five (1 N-fixing, phosphate solubilising and N-cycling, and 4 N-fixing and phosphate solubilising) bacterial strains isolated post-*V. villosa* harvest.

Table 2

Nutrient cycling bacteria (accession no. and percentage identity) isolated from Mvutshini, Mpembeni, Mzinto, Hibberdene and Gingindlovu plantation soil, pre and post-*Vicia villosa* planting and harvest

Pre-planting	<i>Vicia sativa</i>			<i>Vicia villosa</i>		
Site	Species	Functional traits	Species	Functional traits	Species	Functional traits
Mvutshini	<i>Paraburkholderia phymatum</i> (Acc: HE864336.1,%ID:100)	N-fixing	<i>Arthrobacter</i> sp. (MT826384.1, %ID:97.2)	N-fixing, P-solubilising, N-cycling	<i>Lycinibacillus sphaericus</i> (Acc: MH260970.1, %ID: 96.46)	N-fixing, P-solubilising, N-cycling
	<i>Paraburkholderia sabiae</i> (Acc:MK139731.1, %ID:99.8)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: MN305768.1, %ID: 972.6)	N-fixing, P-solubilising	<i>Burkholderia</i> sp. (Acc: LC661717.1, %ID: 99.11)	N-fixing, P-solubilising
	<i>Pseudomonas koreensis</i> (Acc:ON428965.1, %ID:100)	N-fixing, P-solubilising	<i>Arthrobacter</i> sp. (AF409020.1, %ID94.2)	N-fixing, P-solubilising, N-cycling	<i>Burkholderia</i> sp. (Acc: KY022417.1, %ID: 98.17)	N-fixing, P-solubilising
	<i>Burkholderia</i> sp. (Acc:MK612762.1, %ID: 100)	N-fixing, P-solubilising	<i>Bacillus</i> sp. (Acc: OP890999.1, %ID: 94.2)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: CP117460.1, %ID: 98.59)	N-fixing, P-solubilising
	<i>Chromobacterium piscinae</i> (Acc: LR634122.1, %ID: 99.9)	P-solubilising, N-cycling	<i>Burkholderia</i> sp. (Acc: AB911043.1, %ID: 99.3)	N-fixing, P-solubilising	<i>Pseudomonas bengalensis</i> (Acc: MT912698.2, %ID: 96.79)	N-cycling, N-fixing, P-solubilising
	<i>Pseudomonas nitroreducens</i> (Acc: KY038284.1, %ID: 99.6)	N-cycling	<i>Burkholderia</i> sp. (Acc: AB299574.1, %ID: 99.2)	N-fixing, P-solubilising		
			<i>Pseudomonas saccharophila</i> (Acc: AF368755.1, %ID: 83.5)	N-cycling		
Mpembeni	<i>Burkholderia cepacian</i> (Acc: MN691121.1, %ID: 99.4)	N-fixing, P-solubilising, N-cycling	<i>Pseudomonas</i> sp. (Acc: CP117460.1, %ID: 98.5)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: EU449118.2, %ID: 97.65)	N-fixing, P-solubilising
	<i>Burkholderia</i> sp. (Acc: KT390908.1, %ID: 98.3)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: HQ403189.1, %ID: 97.8)	N-fixing, P-solubilising	<i>Pseudomonas moraviensis</i> (Acc: LR027434.1, %ID: 98.26)	N-fixing, P-solubilising
	<i>Burkholderia</i> sp. (Acc: MN594801.1, %ID: 99.7)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: 117452.1, %ID: 97.8)	N-fixing, P-solubilising	<i>Pseudomonas koreensis</i> (Acc: KJ567119.2, %ID: 97.56)	N-fixing, P-solubilising
	<i>Pseudomonas protegens</i> (Acc: MT505104.1, %ID: 100)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: AF368755.1, %ID: 96.4)	N-fixing, P-solubilising	<i>Bacillus</i> sp. (Acc: KY618763.1, %ID: 98.11)	N-fixing, P-solubilising
	<i>Burkholderia cenocepacia</i> (Acc: MT001454.1, %ID: 99.4)	P-solubilising	<i>Caulobacter rhizosphaerae</i> (Acc: CF048815.1, %ID: 97.86)	N-cycling, N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: MN305768.1, %ID: 97.47)	N-fixing, P-solubilising

Pre-planting			<i>Vicia sativa</i>		<i>Vicia villosa</i>	
Site	Species	Functional traits	Species	Functional traits	Species	Functional traits
			<i>Pseudomonas koreensis</i> (Acc: MT998061.1, %ID: 99)	N-fixing, P-solubilising, N-cycling	<i>Pseudomonas</i> sp. (Acc: OR131167.1, %ID: 97.81)	N-fixing, P-solubilising
			<i>Paraburkholderia</i> sp. (Acc: MH118322.1, %ID: 98.7)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: OP990591.1, %ID: 97.71)	N-fixing, P-solubilising
			<i>Pseudomonas koreensis</i> (Acc: MH17853.1, %ID: 99.3)	N-fixing, P-solubilising	<i>Pseudomonas koreensis</i> (Acc: MH725617.1, %ID: 98.25)	N-fixing, P-solubilising
Mzinto	<i>Arthrobacter</i> sp. (Acc: MN081011.1, %ID: 100)	N-cycling, N-fixing, P-solubilising	<i>Arthrobacter</i> sp. (Acc: MH669311.1, %ID: 98.95)	N-cycling, N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: JN411662.1, %ID: 99.18)	N-fixing, P-solubilising
	<i>Pseudomonas</i> sp. (Acc: MT354507.1, %ID: 99.9)	N-cycling, N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: HQ403180.1, %ID: 97.42)	N-fixing, P-solubilising	<i>Caulobacter</i> sp. (Acc: MH899441.1, %ID: 97.47)	N-fixing, P-solubilising, N-cycling
			<i>Caulobacter rhizosphaerae</i> (Acc: CP048815.1, %ID: 97.86)	N-fixing, P-solubilising, N-cycling	<i>Pseudomonas umsongensis</i> (Acc: KP409809.1, %ID: 98.28)	N-fixing, P-solubilising
			<i>Burkholderia</i> sp. (Acc: AB982940.1, %ID: 98.37)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: FJ605433.1, %ID: 97.78)	N-fixing, P-solubilising
			<i>Burkholderia</i> sp. (Acc: MH337960.1, %ID: 99.69)	N-fixing, P-solubilising		
			<i>Pseudomonas frederiksbergensis</i> (Acc: KT369914.1, %ID: 97.38)	N-cycling, P-solubilising		
Hibberdene	<i>Pseudomonas poae</i> (Acc: OR122193.1, %ID: 99.9)	P-solubilising	<i>Pseudomonas</i> sp. (Acc: MK602511.1, %ID: 98.54)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: MT354507.1, %ID: 97.99)	N-fixing, P-solubilising
	<i>Pseudomonas</i> sp. (Acc: MT354507.1, %ID: 99.3)	N-fixing, P-solubilising	<i>Pseudoarthrobacter chlorophenolicus</i> (Acc: MT192880.1, %ID: 98.85)	P-solubilising, N-cycling	<i>Pseudomonas</i> sp. (Acc: MZ571910.1, %ID: 98.14)	N-fixing, P-solubilising
			<i>Cupriavidus yeoncheonensis</i> (Acc: MK824648.1, %ID: 97.13)	P-solubilising	<i>Pseudomonas vancouverensis</i> (Acc: MT409572.1, %ID: 98.80)	N-fixing, P-solubilising
			<i>Pseudomonas</i> sp. (Acc: LC474074.1, %ID: 99.57)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: OR131117.1, %ID: 97.79)	N-fixing, P-solubilising

Pre-planting			<i>Vicia sativa</i>		<i>Vicia villosa</i>	
Site	Species	Functional traits	Species	Functional traits	Species	Functional traits
			<i>Caulobacter</i> sp. (Acc: MN181102.1, %ID: 98.09)	N-cycling	<i>Pseudomonas</i> sp. (Acc: KU738933.1, %ID: 99.04)	N-fixing, P-solubilising
Gingindlovu	<i>Pseudomonas frederiksbergensis</i> (Acc: MT378522.1, %ID: 99.8)	P-solubilising, N-cycling	<i>Caulobacter</i> sp. (Acc: MN181102.1, %ID: 98.09)	N-fixing, P-solubilising, N-cycling	<i>Lysinibacillus</i> sp. (Acc: MN335310.1, %ID: 98.12)	N-fixing, N-cycling, P-solubilising
			<i>Pseudomonas</i> sp. (Acc: CP117460.1, %ID: 98.71)	N-fixing, P-solubilising	<i>Pseudomonas</i> sp. (Acc: MN559094.1, %ID: 96.95)	N-fixing, P-solubilising
			<i>Pseudomonas</i> sp. (Acc: CP117460.1, %ID: 98.71)	N-fixing, P-solubilising	<i>Burkholderia</i> sp. (Acc: AB911072.1, %ID: 98.18)	N-fixing, P-solubilising
			<i>Rhodobacter</i> sp. (Acc: MH136844.1, %ID: 97.69)	N-fixing, P-solubilising, N-cycling	<i>Paraburkholderia</i> sp. (Acc: OR191068.1, %ID: 98.58)	N-fixing, P-solubilising
			<i>Xanthobacter</i> sp. (Acc: KF560403.1, %ID: 99.13)	N-fixing	<i>Cupriavidus</i> sp. (Acc: HQ443228.1, %ID: 98.14)	N-fixing, P-solubilising

3.3 Enzyme activities

The soil enzyme activities varied across all sites and treatments (Fig. 2). Nitrate reductase activities increased significantly in Mvutshini post-*V. sativa* harvest, increased in Mzinto post-*V. sativa* and *V. villosa* harvest and decreased significantly in Hibberdene and Gingindlovu post-*V. villosa* harvests (Fig. 2A). Acid phosphatase activities increased significantly in Gingindlovu and Hibberdene post-*V. villosa* harvest, and also increased in Mvutshini soils post-*V. sativa* and *V. villosa* harvest (Fig. 2B). The glucosidase activities increased significantly across all sites and treatments (Fig. 2C).

3.4 Plant Biomass and Mineral Nutrition

Vicia sativa accumulated significantly higher root biomass in Mzinto and Gingindlovu, while shoot and total biomass were significantly higher in Mzinto plantation soils (Table 3). Gingindlovu-grown *V. villosa* plants accumulated more biomass, with Gingindlovu showing a significantly higher root: shoot ratio (Table 3).

Table 3

The relative growth rate (g/day) of *Vicia sativa* and *Vicia villosa* plants grown in Mvutshini, Mpembeni, Mzinto, Hibberdene and Gingindlovu plantation soils for 45 days. The different values are presented as mean $\pm$ SE. The different letters represent significant differences between the sites after a One-Way ANOVA

	RGR (g/day)	Root RGR (g/day)	Shoot RGR (g/day)	Root: Shoot RGR
<i>Vicia sativa</i>				
Mvutshini	0.009 $\pm$ 0.00 ^a	0.006 $\pm$ 0.01 ^a	0.010 $\pm$ 0.00 ^a	0.608 $\pm$ 0.06 ^a
Mpembeni	0.016 $\pm$ 0.00 ^a	0.013 $\pm$ 0.01 ^{ac}	0.018 $\pm$ 0.00 ^a	0.635 $\pm$ 0.10 ^a
Mzinto	0.036 $\pm$ 0.00 ^b	0.041 $\pm$ 0.00 ^b	0.033 $\pm$ 0.00 ^b	0.861 $\pm$ 0.03 ^a
Hibberdene	0.012 $\pm$ 0.00 ^a	0.014 $\pm$ 0.00 ^{ac}	0.011 $\pm$ 0.00 ^a	0.671 $\pm$ 0.06 ^a
Gingindlovu	0.029 $\pm$ 0.00 ^b	0.023 $\pm$ 0.00 ^c	0.033 $\pm$ 0.00 ^b	0.671 $\pm$ 0.06 ^a
<i>Vicia villosa</i>				
Mvutshini	0.021 $\pm$ 0.001 ^{ab}	0.022 $\pm$ 0.000 ^a	0.020 $\pm$ 0.001 ^a	0.667 $\pm$ 0.052 ^a
Mpembeni	0.027 $\pm$ 0.002 ^{ab}	0.022 $\pm$ 0.006 ^a	0.029 $\pm$ 0.001 ^a	0.667 $\pm$ 0.052 ^a
Mzinto	0.014 $\pm$ 0.003 ^a	0.021 $\pm$ 0.002 ^a	0.007 $\pm$ 0.004 ^b	1.156 $\pm$ 0.14 ^a
Hibberdene	-	-	-	-
Gingindlovu	0.028 $\pm$ 0.004 ^b	0.038 $\pm$ 0.005 ^a	0.019 $\pm$ 0.002 ^{ab}	1.459 $\pm$ 0.198 ^b

Vicia villosa grown in Hibberdene died before the final harvest date was reached.

Vicia sativa cultivated in Mzinto, Hibberdene, and Gingindlovu had the highest specific N absorption rates compared to Mvutshini and Mpembeni (Fig. 3A). Mzinto showed the highest N utilisation rate, followed by Gingindlovu, while Hibberdene showed a decrease in N utilisation (Fig. 3B). Mpembeni and Gingindlovu showed increased P absorption rates, while Hibberdene showed a decrease in P absorption rates (Fig. 3C). Hibberdene showed the highest P utilisation rate (Fig. 3D).

Vicia villosa grown in Mpembeni had the highest specific N assimilation rate, while Mzinto and Gingindlovu had the lowest (Fig. 4A). Gingindlovu had the highest specific N utilisation rate, while Mzinto had the lowest (Fig. 4B). The specific P assimilation rate was highest in Gingindlovu, while the other sites didn't show significant differences (Fig. 4C). Lastly, the specific P utilisation rate was highest in Mpembeni and lowest in Mzinto (Fig. 4D).

Mzinto had the highest %N derived from the atmosphere (55%), while Mpembeni had the lowest (20%) (Fig. 5A). Total plant N content was highest in Mzinto and lowest in Hibberdene (Fig. 5B). Plants from all the sites derived most of their N from the soil, except for Mzinto, which derived most of its N from the atmosphere (Fig. 5C). Total plant P content was the lowest in Hibberdene (Fig. 5D). Mzinto had the highest %NDFA (41%), while Gingindlovu had the lowest (5%) (Fig. 6A). Mpembeni-grown *V. villosa* had the highest plant N, followed by Gingindlovu (Fig. 6B). All the plants from the different plantation sites derived most of their N from the soil (Fig. 6C). *Vicia villosa* cultivated in Gingindlovu had the highest total P content, while Mzinto had the lowest (Fig. 6D).

4 Discussion

The study investigated the effects of planting *V. sativa* and *V. villosa* as cover crops in nutrient-deficient KZN small-scale sugarcane plantation soils. Post-harvest soil analysis showed that both species increased the presence of PGPR, with N-fixing, N-cycling, and P-solubilising functions in the genus *Arthrobacter*, *Bacillus*, *Burkholderia*, *Caulobacter* and *Pseudomonas*. The presence of these bacterial species post-harvest can be attributed to the release of different compounds, such as organic acids by the legumes, increasing bacterial activity during nutrient deficiency [27]. The composition and function of rhizospheric bacteria can be influenced by the composition and quantity of root exudates released by the plants [27]. During nutrient deficiency, plant

roots can modulate the chemical composition of their exudates according to the plants' requirements [27]. This influences the composition of the rhizospheric microbial community and forms the basis for the attraction and repulsion of certain bacterial species [38–40]. The experimental soils in this study were N and P deficient; this may have resulted in an increase in N-fixing and P-solubilising activities and efficiency to compensate for the soil deficient nutrients for plant use. This suggests an increase in the root exudates signals of *V. sativa* and *V. villosa* roots for N and P-cycling bacteria under nutrient-deficient conditions [27]. Similar results were obtained by [41] who reported increases in bacterial diversity under soybean soils compared to sugarcane monoculture. [42] reported that cover crop treatments increased the relative abundance of *Streptomyces*, *Arthrobacter* and *Bacillus* sp. compared to no cover crop treatments. Therefore, it would be expected that in a sugarcane-*V. sativa* or *V. villosa* rotation system, nutrient cycling microbes and associated enzymes would increase activities, increasing nutrient fixing and cycling of deficient soil nutrients in sugarcane plantation soils. Bacteria from these genera have been reported to mineralise phosphates by secreting acid phosphatases and phytases [43]. In this study, Mvutshini and Mzinto recorded increased acid phosphatase activities post-harvest, which could be attributed to the co-occurrence of *Burkholderia* and *Pseudomonas* in Mzinto and *Pseudomonas*, *Bacillus* and *Burkholderia* in Mvutshini. Mixed cultures of P-solubilising bacteria are more effective in mineralising organic phosphates [44]. [45] found that the co-inoculation of *Burkholderia anthina* and *Enterobacter aerogenes* released the highest soluble P content as compared to single inoculations. The presence of these P-solubilising bacteria makes available the cation bound P, increasing P availability for plant assimilation, and this is shown by the increased P assimilation rates in this study across all sites [46, 47].

Post-harvest, soil acidity was slightly decreased to less acidic, and similar results were reported by [42] and [48], who reported increases in pH levels (4 to 6) under cover crop systems. [47] reported contradicting results as legume cover crops (white clover and orchard grass) in their study decreased pH levels, which was attributed to the secretion of organic acids and protons by the legume roots. Soil acidity is a key constraint associated with poor root health and nutrient availability [49]. Under acidic conditions, nutrients such as P form insoluble complexes with cations, rendering them unavailable for plant uptake [50]. Since P is an important macronutrient required for metabolic processes in plants and is a significant constituent of ATP, its deficiency can decrease the photosynthetic rates [51] and N fixation in legumes [52]. The decreased biomass and %NDFA across all plantation soil treatments in this study can be linked to *V. sativa* and *V. villosa* using the available P sparingly to maintain growth function. This is shown by the low P utilisation rates across all soil treatments. Legumes switch their N sources during nutritional stress, favouring soil N to reserve energy [53]. Biological N fixation (BNF) is a high-energy process and requires 16 ATP molecules to fix one molecule of atmospheric N₂ to NH₃ [54]. *Vicia sativa* and *V. villosa* growing in Mvutshini, Mpembeni, and Gingindlovu plantation soils showed a reduced %NDFA and increased their reliance on soil N. This shows that the plants were able to use the deficient soil P sparingly, reducing the ATP-demanding BNF to maintain their growth and function. These findings are similar to findings reported by [55], [56], and [57] who reported decreased N₂ fixation rates under low P, which were reversed when P inputs were increased. Interestingly, Hibberdene-grown *V. villosa* died before harvest, while *V. sativa* persisted. This could be attributed to the documented persistence of *V. sativa* in diverse environments where other legumes would be poorly suited [58].

Mzinto-grown plants showed increased %NDFA values, and the soil had high P concentrations and decreased acidity. Even though the plants in this study did not nodulate, the plants were able to reduce atmospheric N, suggesting a symbiosis with non-nodulating endophytic N₂ fixing rhizobacteria [53]. Similar results were recorded by [53], who reported high %NDFA in non-nodulated *Leucaena leucocephala* plants growing in grassland ecosystem soils. The high *V. sativa* N concentrations, despite low soil N concentrations in Mzinto plantation soils, can indicate the high efficiency of the free-living N-fixing bacteria and P-solubilising bacteria isolated from the rhizosphere of these plantation soils. This is further supported by the increased nitrate reductase and acid phosphatase activities in Mzinto plantation soils post-*V. sativa* harvest. Soil N and P concentrations decreased post-harvest, possibly due to the uptake and use of nutrients by the legumes during growth [59]. These results are consistent with those reported by [60] and [59], who reported decreases in soil nutrient contents under cover crop treatments. However, the scavenged nutrients can be made available for the subsequent crop through the decomposition and mineralisation of *V. sativa* and *V. villosa* when it is incorporated into the soil as legume biomass [61]. In a study by [18], vetch ecotypes contributed up to 211 kg N ha⁻¹ from aboveground biomass. This serves as a sustainable form of N and P feedback as the nutrients released from the slow decomposition of the legume biomass reduce contaminant leaching from the application of chemical fertilisers [23], and provide a steady amount of N throughout the growing season [62]. Furthermore, the organic matter present from the rotation and buried legume biomass may be resistant to biological degradation, fostering a longer and deeper carbon stock in the soil. This

extended carbon stock is beneficial to the subsequent plant [29]. Soil rehabilitation processes occur over an extended period. Therefore, field trials over a prolonged experimental duration might elicit different responses as plant-microbe interactions are sensitive to environmental conditions, which might affect their associated enzyme activities and nutritional contributions. Future studies should identify and quantify the root exudates released by *V. sativa* and *V. villosa* to better understand the interactions between plant roots and soil ecosystems. Additionally, future studies should use next-generation sequencing to identify all the microbial populations present, which will better estimate the microbial diversity and the effects that the legume species had on the soil microbiota.

5 Conclusion

In this study, *V. sativa* and *V. villosa* increased the bacterial composition, acid phosphatase, and glucosidase activities and reduced the exchange acidity in the sugarcane plantation soils, and these are an indicator of improved soil health. Even so, allowing their biomass to decompose and release stored nutrients into the soil would contribute to nutrient cycling. Therefore, integrating *V. sativa* and *V. villosa* as cover crops is a promising strategy to enhance soil biological health and promote sustainable agricultural practices in sugarcane plantations.

Declarations

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Declarations

Conflicts of Interest: We declare no known competing financial and non-financial interests with regard to the current research. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability: The datasets generated and/or analysed during the current study will be stored and available in ResearchGate corresponding author (Anathi Magadlela) account and tagged to the manuscript: ***Vicia sativa* and *Vicia villosa* enhance soil microbial composition, enzyme activities, and chemical properties in nutrient-deficient small-scale sugarcane plantation soils**. Also, all raw data can be requested from the corresponding author, Prof. Anathi Magadlela at anathimagadlela@icloud.com.

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Figures

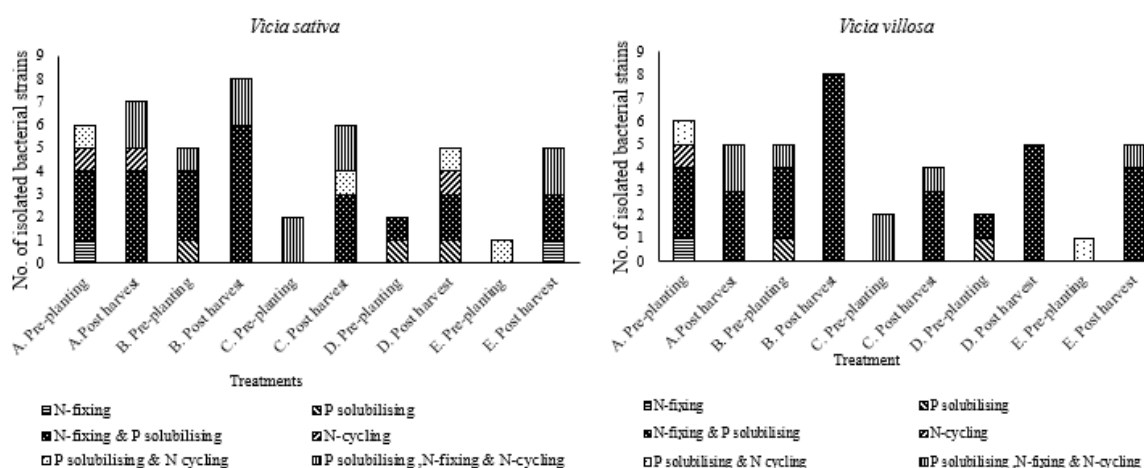


Figure 1

The abundance of bacteria isolated from A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene, and E. Gingindlovu pre-planting and post-harvesting *Vicia sativa* and *Vicia villosa*

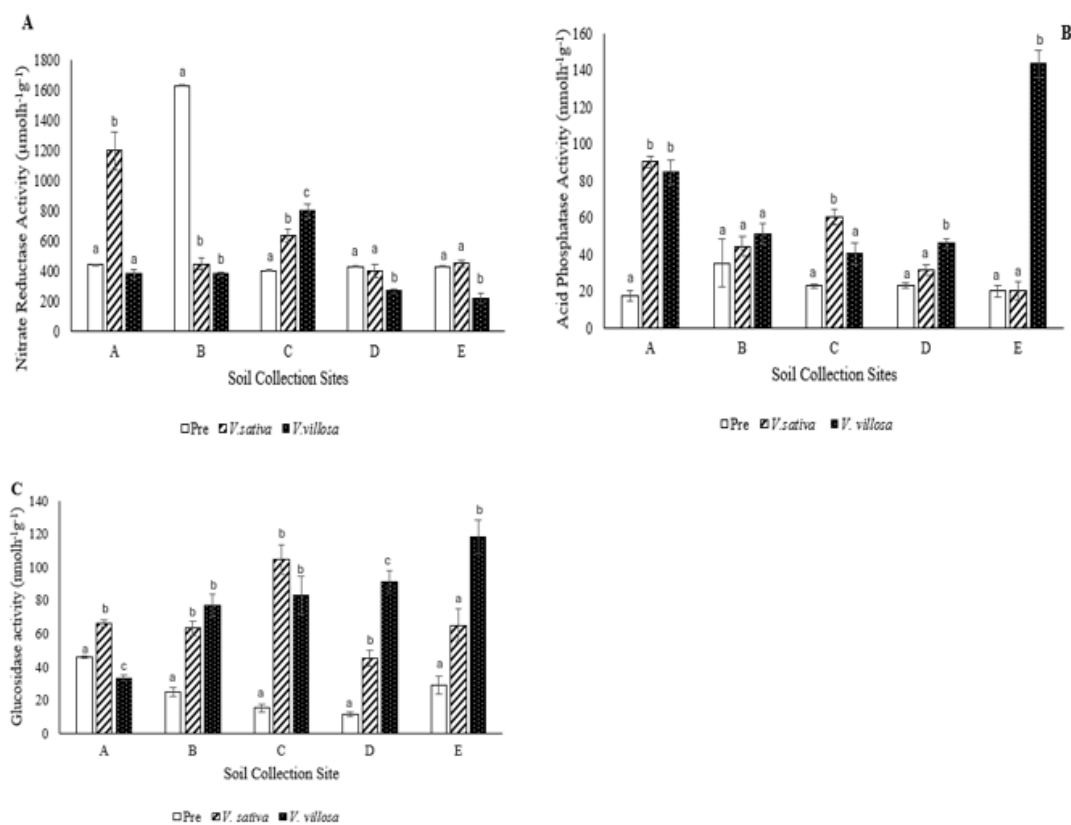


Figure 2

The enzyme activities of soil collected from A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene and E. Gingindlovu pre-planting and post-*Vicia sativa* and *Vicia villosa* harvest. The different values are presented as mean $\pm$ SE. Different letters show significant differences between treatments after One-Way ANOVA

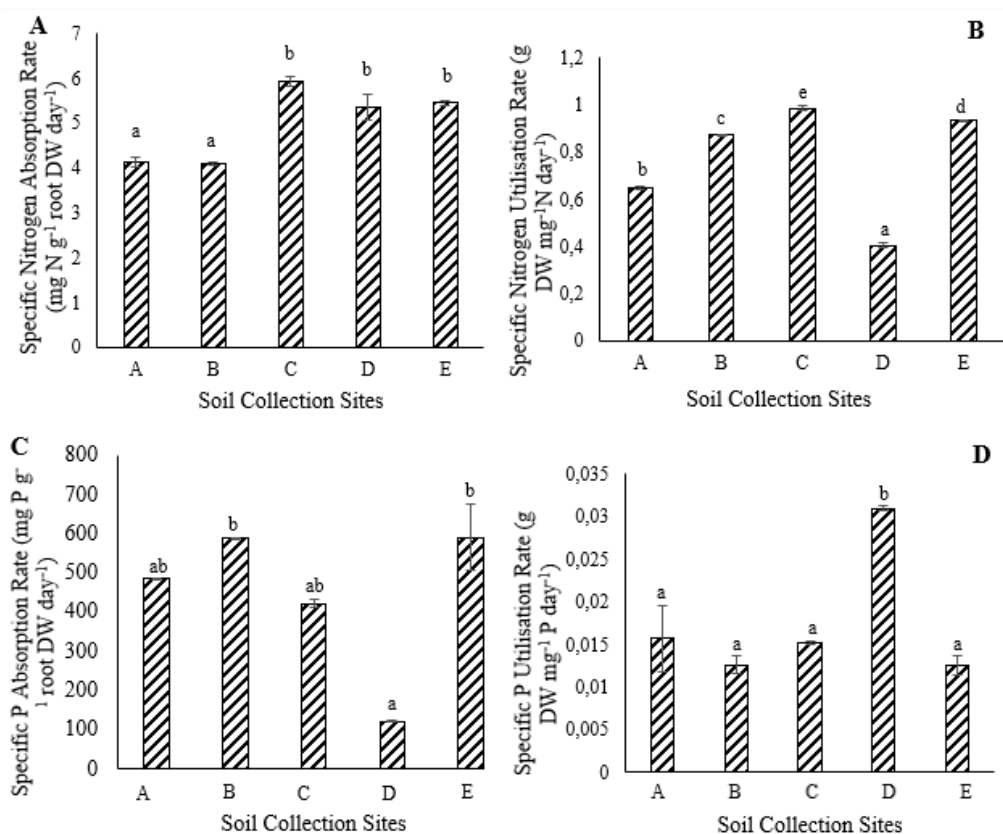


Figure 3

The **A.** Specific N Absorption Rate, **B.** Specific N Utilisation Rate, **C.** Specific P Absorption Rate and **D.** Specific P Utilisation Rate of *Vicia sativa* plants grown in A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene and E. Gingindlovu plantation soil for 45 days. The different values are presented as mean $\pm$ SE. The different letters show significant differences between the treatments after One-Way ANOVA.

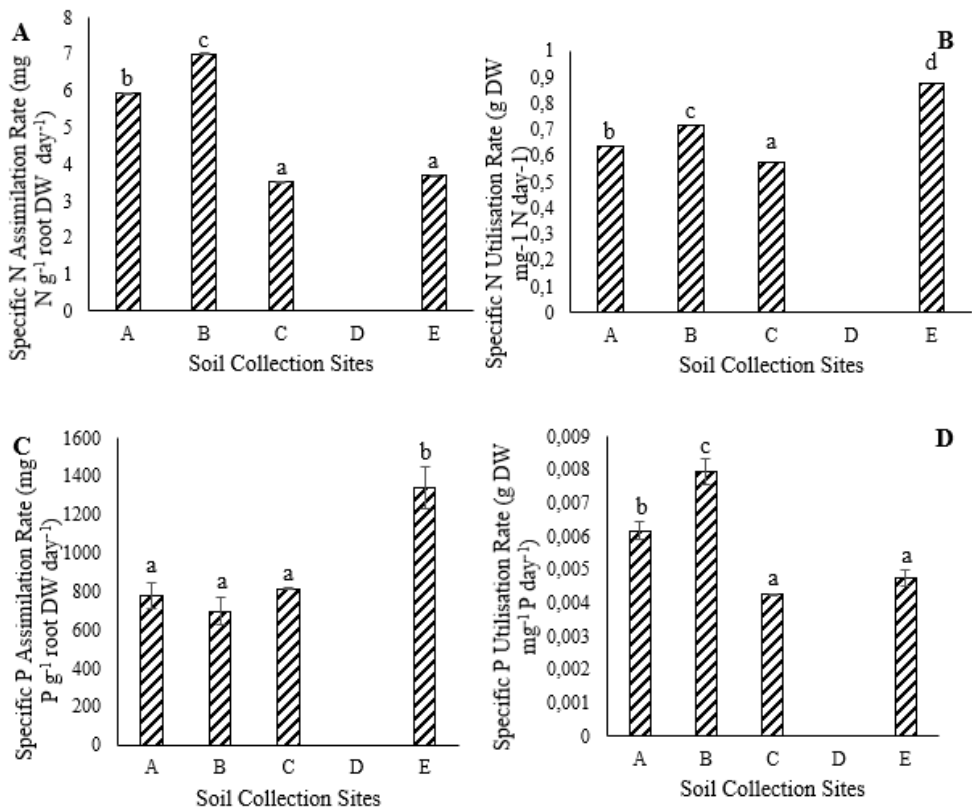


Figure 4

The **A.** Specific N Utilisation Rate, **B.** Specific N Assimilation Rate, **C.** Specific P Assimilation Rate and **D.** Specific P Utilisation Rate of *Vicia villosa* plants grown in A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene and E. Gingindlovu plantation soil for 45 days. The different values are presented as mean $\pm$ SE. The different letters show significant differences between the treatments after One-Way ANOVA

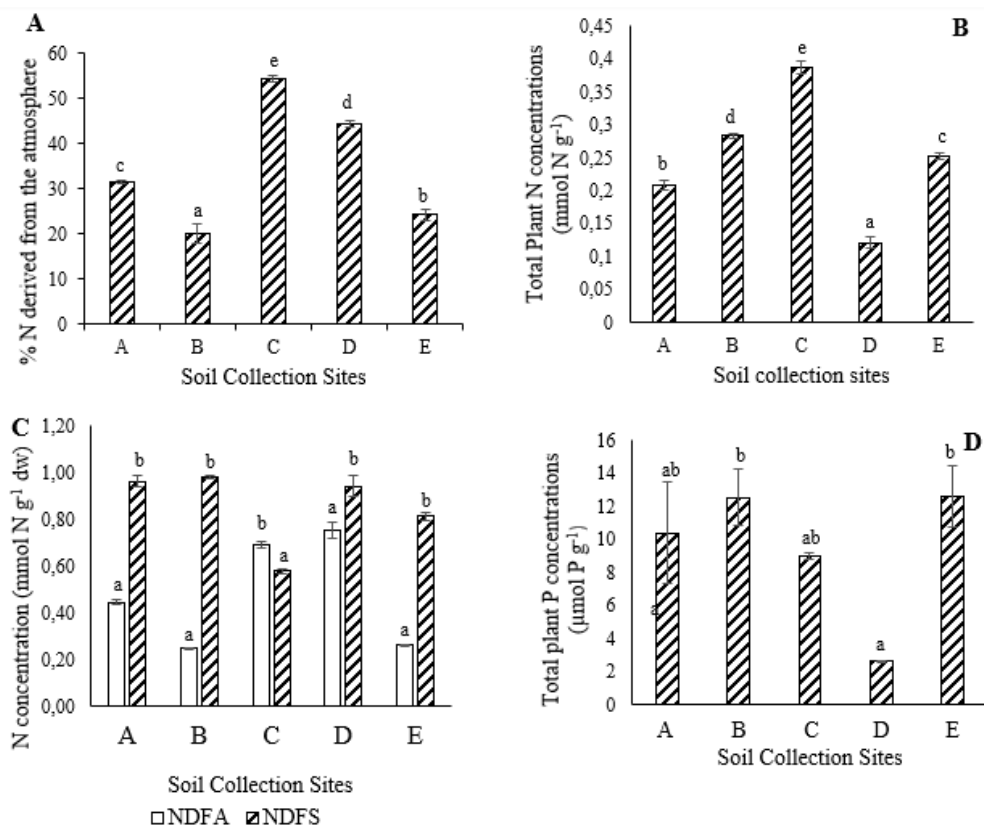


Figure 5

The **A**. % N derived from the atmosphere, **B**. Total Plant N concentrations **C**. Total N concentrations derived from the atmosphere vs N derived from the soil and **D**. Total Plant P concentrations in *Vicia sativa* plants grown in A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene and E. Gingindlovu plantation soil for 45 days. The values are presented as mean ± SE. The different letters show significant differences after One-Way ANOVA

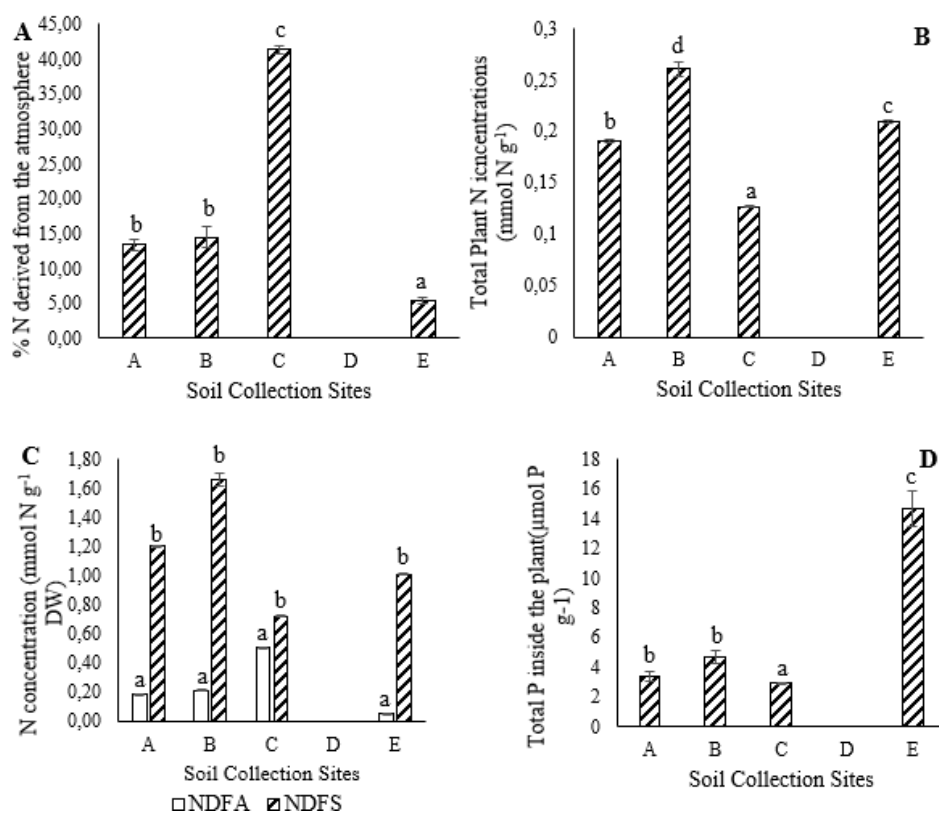


Figure 6

The **A.** %N derived from the atmosphere, **B.** Total Plant N concentrations **C.** N concentrations derived from the atmosphere vs soil and **D.** Total Plant P concentrations of *Vicia villosa* plants grown in A. Mvutshini, B. Mpembeni, C. Mzinto, D. Hibberdene and E. Gingindlovu plantation soil for 45 days. The different values are presented as mean $\pm$ SE. The different letters show significant differences between the treatments after One-Way ANOVA