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Eragrostis curvula cultivars improve soil bacterial diversity, extracellular enzyme activities, and nutrition in grassland ecosystem soils

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Research on *Eragrostis curvula* has focused primarily on its value for pastures and as a potential food source, while the contribution of its cultivars to soil nutrient cycling in nutrient-poor grassland ecosystems is still poorly understood. In this study, we assessed the effects of *E. curvula* cultivars on soil bacterial communities, enzyme activities, and soil properties in grassland soils. Soil samples were analysed for nutrient concentrations, pH, and enzyme activity. Pot trials with *E. curvula* Ermelo and Agpal cultivars were conducted over four months in a greenhouse. Pre- and post-harvest soils were assessed for changes in nutrient profiles, enzyme activities, and bacterial communities. There was an increase in the bacteria isolated from post-harvest soils compared to pre-planting soils. Soil growing the cultivars showed a significant increase in the nitrate reductase activity across all study sites. Soil N concentrations and pH increased in all post-harvest soils. The pearson correlation coefficients between soil enzymes and nutrients showed that alkaline phosphatase, acid phosphatase and glucosidase were moderately positively correlated with phosphorus ($r = 0.41$, 0.40, and 0.40 respectively) and negatively correlated with pH (r= -0.33, -0.32 and – 0.34 respectively). A weak positive correlation was observed between nitrate reductase and soil nitrogen ($r = 0.22$). These findings highlight how *E. curvula* cultivars shift the microbial profile over time while increasing N and pH in grassland ecosystem soils.

Keywords Nutrient cycling, Bacterial communities, Nutrient-poor soils, Cultivar effects

Eragrostis curvula, a C4 perennial grass species native to southern Africa[1,2], is widely distributed in natural and agricultural grasslands[3]. The wide distribution of *E. curvula* in grasslands is attributed to its ability to tolerate a wide range of environmental conditions[4], fast germination[5,6], and water use efficiency[7]. While considered an invasive weed that threatens natural ecosystems in countries such as Chile, Europe, and Asia[6,8], in native southern Africa, *E. curvula* holds potential for diversifying food systems[9] and is cultivated for pasture[4]. The benefits of utilising *E. curvula* as a food and for pasture extend beyond its forage value and food but may be linked to nutrient cycling. Brevedan et al.[10] studied nitrogen (N) cycling in an ecosystem with *E. curvula* and reported that microbes might play a role in the immobilisation of N from dead *E. curvula* roots- showcasing an intricate interplay between the plant, soil microbiome, and nutrient cycling in ecosystems where *E. curvula* thrives.

Soil microorganisms and their associated enzyme activities play a significant role in the geochemical cycling of elements and their subsequent conversion into organic compounds[11]. These microorganisms and their associated enzyme activities are influenced by environmental factors such as soil properties and other abiotic factors, as per the filter theory explained by Motsomane et al.[12]. Plants influence soil microbial communities through root exudates, which supply energy and facilitate colonisation of the rhizosphere[13–16]. The composition of these exudates varies across species and cultivars[17], thereby shaping the microbial assemblages that establish in the rhizosphere and producing plant-specific community profiles[18]. This plant–microbe association is consistent with the hologenome theory, which proposes that a host and its microbiota constitute a single evolutionary unit—the holobiont, that collectively contributes to ecological fitness and niche adaptation[12,19].

While the influence of plants, microbial communities, and their associated enzymes on ecosystem functioning is widely studied in leguminous plants and economically significant annual plants, there is a notable

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knowledge gap regarding perennial and native plants such as *E. curvula*[18]. Investigating nutrient cycling in *E. curvula* ecosystems and assessing how different cultivars improve nutrient cycling will fill the knowledge gap and provide insights into the hologenome dynamics of *E. curvula* cultivars in South African grasslands. The most prevalent *E. curvula* cultivar is the Ermelo cultivar, as Grunow et al.[20] reported that a high percentage of *E. curvula* pastures in South Africa are the Ermelo cultivar. In contrast to the Ermelo cultivar, the Agpal cultivar is a newer cultivar that is rarely reported in literature[21]. The lack of knowledge on the Agpal cultivar underscores the need for a comprehensive study on these cultivars' nutrient cycling roles and hologenome dynamics.

This study aims to determine the effects of *E. curvula* on soil bacterial communities, associated extracellular enzyme activities, and soil chemical properties. Also, this study aims to determine if these effects differ between the Ermelo and Agpal cultivars. The objectives of this study include (1) determining the soil characteristics (pH, exchange acidity, total cation, and nutrient concentrations) of soils collected at three sites in Heidelberg, pre-planting and post-harvest of *E. curvula* cultivars grown for over four months, (2) Identifying the nitrogen (N) fixing, phosphorus (P) solubilising, and N cycling bacteria found in the collected soils pre-planting and post-harvest of *E. curvula* cultivars, and (3) assaying the soil nutrient (N, carbon (C), and P) cycling enzyme activities in collected soils pre-planting and post-harvest of *E. curvula* cultivars.

Materials and methods

Study sites and soil collection

Soil samples were collected from three geographical sites in Heidelberg, Gauteng, South Africa. These sites included Jameson Park (26°26'31.7 "S; 28°26'01.4"E), Kaydale (26°29'12.4"S; 28°23'02.1"E), and Rensburg (26°30'16.0"S; 28°26'11.3"E). Heidelberg is in Gauteng, which consists of savanna and grassland ecosystems[22]. In Gauteng, Heidelberg experiences summer rainfall followed by dry winters[22]. Annual temperatures range from 3 to 25 °C, and precipitation ranges from 600 to 700 mm per annum[22,23]. The soil clay percentage for Jameson Park, Kaydale, and Rensburg soils was 29.25%, 29%, and 27.5%, respectively. The clay content of the study sites may be influenced by the study sites being near the Tsakane Clay grassland. From each site, 30 soil samples collected 2 m apart were mixed to form composite soils as per Magadlela et al.[24].

Pot trials

Eragrostis curvula, Ermelo and Agpal cultivars, seeds were sourced from AGT Foods, South Africa, and Agricol seeds, South Africa, respectively. Forty pots (25 cm diameter) were used; each pot contained four seeds. This randomized block experimental design was used for each site and cultivar. Seed germination and plant growth trials were conducted under ambient conditions in the greenhouse at the University of KwaZulu Natal, Westville Campus, South Africa. The greenhouse day temperatures were 22–37 °C and 12–17 °C at night. The pots were irrigated on alternative days. Every month, five pots per site for each cultivar were harvested. The soils collected from each replicate were mixed to form composite soil samples, three 500 g samples from each composite mix were sent for soil characteristic analysis at the Analytical Services Unit, KwaZulu Natal Department of Agriculture and Rural Development, Cedara, South Africa, and the remainder was stored at 4 °C for extracellular enzyme activity and bacterial extraction and identification. Chaparro et al.[25] reported that plant age influences root secretions, thus affecting microbial communities in rhizosphere soils. Alagbo and Chauhan[26] reported that *E. curvula* matures after four months. Thus, the study was conducted over four months (late Autumn, April-May, and early Winter, June-July).

Extracellular enzyme activities

Soil samples collected after each harvest were assayed for β-glucosidase, N-acetylglucosaminidase, acid phosphatase, and alkaline phosphatase activities (expressed as nmol $h^{-1}g^{-1}$) using the fluorescence-based method described by Jackson et al.[27] and Zungu et al.[28]. Briefly, soil samples (10 g soil/100 ml autoclaved dH_2O) were homogenised at medium speed in a shaker for two hours and stored overnight at 4˚C. The supernatants were transferred into black 96-well microplates before adding their respective substrates. The sample run consisted of 200 µl soil aliquot and 50 µl substrate, alongside reference standards (200 µl bicarbonate buffer + 50 µl standard), quench standard (200 µl soil aliquot + 50 µl standard), sample control (200 µl soil aliquot + 50 µl buffer), negative controls (200 µl buffer + 50 µl substrate), and blanks (250 µl buffer). The 96-well plate was incubated at 25˚C for 2 h. Thereafter, the reaction was stopped by adding 5 µl of 0.5 M NaOH to each well. The fluorescence was measured at 450 nm on a Glomax Multi Plus microplate reader. The buffer and standard were adjusted to pH five before determining acid phosphatase activity.

Nitrate reductase activity (expressed as 0.1 µmol $h^{-1}g^{-1}$) assays were done using a modified protocol described by Kandeler[29] and Ndabankulu et al.[30]. A volumetric flask wrapped in foil was filled with 1 ml of 25 mM KNO_3, 4 ml of 0.9 mM 2,4-dinitrophenol, and 5 ml of milliQ dH_2O. After that, 5 g of soil was added to the solution, and the flask was sealed with foil, shaken, and incubated in an oven at 30˚C for 24 h. After incubation, 10 ml of 4 M KCl was added to the soil mixture, succinctly mixed, and filtered using filter paper (Whatman number 1, Sigma-Aldrich, Darmstadt, Germany). The enzymatic reaction was initiated by adding 2 ml of the filtrate to 1.2 ml of 0.19 M ammonium chloride buffer (pH 8.5) and 0.8 ml of a colour reagent consisting of 1% sulfanilamide, 1 N HCl, and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The solution was incubated at 30˚C for 30 min. The absorbance was measured at 520 nm using an 1800 UV spectrophotometer. The nitrite (NO_2^-) liberated into the medium was extrapolated from a prepared standard curve with KNO_3.

Soil bacterial identification

To examine the effects of *E. curvula* cultivars on soil bacterial communities, experimental soils sampled before and after each harvest of *E. curvula* growth period over four months were used for bacterial extraction and identification as per protocols by Ndabankulu et al.[30]. The soil samples were subjected to serial dilutions, and

50 µL of each serial dilution were cultured in sterile Petri plates containing selective media (Pikovskaya's plate containing tricalcium phosphate (TCP) for P-solubilizing bacteria, Simmons citrate agar for N-cycling bacteria, and Jensen's media agar for N-fixing bacteria). Each selective media was replicated three times and incubated at 30˚C for five days. Pure bacterial colonies were obtained by repeated streaking/subculturing. A small portion of the pure bacterial colonies was amplified through polymerase chain reaction (PCR) using the 16S ribosomal RNA gene primers: 63 F (5' CAGGCCTAACACATGCAAGTC 3') and 1387R (5' GGGCGGTGTGTACAA GGC 3') from Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). The PCR amplification was performed using an EmaraldAmp GT Master Mix with the following conditions: Initial denaturation at 94˚C for 5 min, followed by 30 cycles of denaturation at 94˚C for 30 s, annealing at 55˚C for 30 s and extension at 72˚C for 2 min, with additional extension at 72˚C for 10 min. The PCR products were separated by electrophoresis on 1% (w/v) agarose gel and visualized under UV light to determine the correct product size amplification. The amplicons were sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. The DNA sequences were edited and compared to the nucleotide sequences of known bacteria in the GenBank database of the National Centre for Biotechnology Information (NCBI) by using the Basic Local Aligned Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov, 19/12/23).

Soil chemical properties determination

Three subsamples of 500 g from pre-planting and post harvest soils were sent to the Analytical Services Unit, KwaZulu Natal Department of Agriculture and Rural Development, Cedara, South Africa, for soil characteristic analysis (nutrient concentrations, total cation concentration, exchange acidity, and pH). The characteristic soil analysis was performed per protocols Manson and Roberts[31] 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 P, potassium (K), zinc (Zn), and copper (Cu)[31]. 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[32] molybdenum blue procedure. The K concentration was determined by diluting 5 ml aliquot of the filtrate with 20 ml de-ionised water using atomic absorption, and the remaining undiluted filtrate was used to determine the zinc, copper, and manganese concentration using atomic absorption spectroscopy[31]. The magnesium (Mg) and calcium (Ca) concentrations were determined by stirring sample cups containing 25 ml of soil sample and 25 ml of 1 M KCl solution in a multiple stirrer (400 rpm) for 10 min[31]. The stirred mixture was filtered with Whatman no.1 paper. Five millilitres of the filtrate was diluted with 20 ml 0.0356 M $SrCl_2$, and Ca concentrations were determined using atomic absorption[31]. Soil nitrogen concentration 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 pentaoxide was used as a combustion catalyst[31]. The crucible was placed in a horizontal furnace and burned in a stream of oxygen at 1350 °C, and soil nitrogen was measured as N_2 in a thermal conductivity cell[31]. 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[31].

Statistical analysis

The statistical software R (version 3.6.2) was used for all analyses. A two-way ANOVA was conducted separately for each site to assess the effects of cultivar (Ermelo and Agpal) and month of harvest (April, May, June, and July) on soil enzyme activities and soil characteristics. The stats package was used for core functions, and the car package was used for Levene's test of homogeneity. Assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's test, respectively[33]. Where ANOVA results indicated significant effects, Tukey's HSD post hoc test was performed to separate the means. In cases where assumptions of normality or homogeneity of variance were violated, the data were log-transformed to stabilise variances and improve normality prior to analysis. Assumptions were re-assessed following transformation, and the two-way ANOVA was conducted on the transformed data where appropriate. Furthermore, relationships between soil nutrients and enzyme activities were determined using principal component analysis (PCA). The PCA was performed using the prcomp() function from base R, and visualisation was done using the ggplot2 package[34]. The heatmap was generated in R using the ggplot2 package[34].

Results

Soil bacterial identification

Pre-planting soils from all sites had 2–3 bacterial isolates from *Bacillus*, *Flavobacterium*, and *Pedobacter* genera (Table 1). Post-harvest, bacterial diversity increased across all sites, with more than four isolates from Ermelo and Agpal soils, including genera such as *Arthrobacter*, *Pseudomonas*, and *Achromobacter* (Table 1).

Rensburg soils had the highest bacterial diversity post-harvest, and overall, both cultivars increased the bacterial diversity and abundance compared to pre-planting soils (Fig. 1).

Effects of month of harvest and cultivar on extracellular enzyme activities

The effects of *E. curvula* cultivars and month of harvest on soil extracellular enzyme activity over four months are represented in Table 2. In Jameson Park, the cultivar and month of harvest significantly affected β-glucosidase, alkaline phosphatase, acid phosphatase, N-acetylglucosaminidase, and nitrate reductase activities, with significant interaction effects observed for all enzymes. In Kaydale, the month of harvest significantly influenced β-glucosidase, acid phosphatase, alkaline phosphatase, and N-acetylglucosaminidase activities, while cultivar–month interactions were significant for β-glucosidase, acid phosphatase, and nitrate reductase. In Rensburg, harvest month had a significant main effect on β-glucosidase, acid phosphatase, alkaline phosphatase,

Treatment	Strain	Accession number	Similarity (%)
Jameson Park			
Pre-planting soils	*Bacillus zanthoxyli* strain 1433 *Flavobacterium anhuiense* strain D3	NR_164882.1 NR_044388.1	98.67 93.01
Ermelo	*Arthrobacter nitrophenolicus* strain SJCon *Paraburkholderia guartelaensis* strain CNPSo 3008 *Extensimonas perlucida* strain HX2-24 *Mycolicibacter virginiensis* strain MO-233 *Flavobacterium anhuiense* strain D3	NR_178397.1 NR_169402.1 NR_169362.1 NR_149186.1 NR_044388.1	100.00 99.93 98.27 99.67 99.09
Agpal	*Pantoea ananatis* strain LMG 2665 *Pantoea stewartii subsp. indologenes* strain CIP 104,006 *Pseudomonas piscis* strain CMAA1215 *Flavobacterium tistrianum* strain GB 56.1 *Pseudomonas aylmerensis* strain S1E40 *Flavobacterium anhuiense* strain D3	NR_119362.1 NR_104928.1 NR_169429.1 NR_149282.1 NR_169460.1 NR_044388.1	99.50 99.07 99.92 96.32 91.00 99.58
Kaydale			
Pre-planting	*Flavobacterium anhuiense* strain D3 *Pedobacter chitinilyticus* strain CM134L-2 *Lysobacter prati* strain SYSU H10001	NR_044388.1 NR_180020.1 NR_180692.1	99.90 98.41 97.38
Ermelo	*Caballeronia catudaia* strain LMG 29,318 *Achromobacter marplatensis* strain R-46,660 *Arthrobacter pokkalii* strain P3B162 *Flavobacterium anhuiense* strain D3 *Pedobacter frigiditerrae* strain RP-1-13 *Pedobacter chitinilyticus* strain CM134L-2 *Pedobacter pollutisoli* strain TBBPA-24	NR_145605.1 NR_117614.1 NR_149802.1 NR_044388.1 NR_173500.1 NR_180020.1 NR_165750.1	98.92 99.15 99.96 98.48 89.92 99.05 99.29
Agpal	*Flavobacterium anhuiense* strain D3 *Luteimonas lumbrici* strain 1.1416 *Flavobacterium amnigenum* strain I3-3 *Flavobacterium tistrianum* strain GB 56.1 16 S *Flavobacterium fluviatile* strain TAPY14	NR_044388.1 NR_170461.1 NR_169462.1 NR_149282.1 NR_163630.1	100 99.73 99.12 99.89 100.00
Rensburg			
Pre-planting	*Enterobacter ludwigii* strain EN-119 *Pedobacter chitinilyticus* strain CM134L-2 *Bacillus subtilis subsp. subtilis* strain 168	NR_042349.1 NR_180020.1 NR_102783.2	98.65 99.37 96.92
Ermelo	*Acidovorax delafieldii* strain 133 *Simplicispira soli* strain CA-16 *Pseudarthrobacter enclensis* strain NIO-1008 *Pseudarthrobacter niigatensis* strain LC4 *Bacillus nakamurai strain* NRRL B-41,091 *Erwinia phyllosphaerae* strain CMYE1 *Providencia huaxiensis* strain WCHPr000369	NR_028714.1 NR_159921.1 NR_134699.1 NR_041400.1 NR_151897.1 NR_181782.1 NR_174258.1	99.57 99.98 97.52 92.59 93.91 95.96 98.03
Agpal	*Croceibacterium ferulae* strain SX2RGS8 *Rhodocyclus tenius* strain 2761 *Comamonas terrae* strain A3-3 *Pseudarthrobacter siccitolerans* strain 4J27 *Bacillus subtilis subsp. subtilis* strain 168 *Arthrobacter woluwensis* strain 1551 *Arthrobacter alkaliphilus* strain LC6 *Pseudomonas aylmerensis* strain S1E40	NR_165000.1 NR_025839.1 NR_108609.1 NR_108849.1 NR_102783.2 NR_044894.1 NR_041401.1 NR_169460.1	97.88 99.65 97.83 99.73 98.51 99.30 96.28 97.32

Table 1. Molecular identification of bacterial isolated from pre-planting, post Ermelo and post Agpal cultivar harvest from Jameson park, Kaydale and Rensburg soils collected from heidelburg, gauteng.

and N-acetylglucosaminidase, with cultivar–month interactions significantly affecting β-glucosidase, acid phosphatase, and nitrate reductase. Across all sites, enzyme activities were consistently higher in post-planting than pre-planting soils.

Extracellular enzyme activities of Ermelo and Agpal post-harvest soils

The extracellular enzyme activities of Ermelo and Agpal post-harvest soils across April, May, June, and July are presented in Table 3. In Jameson Park, nitrate reductase activity increased over time, peaking in July for both cultivars. N-acetylglucosaminidase and β-glucosidase activities decreased over the months. Alkaline phosphatase decreased in Ermelo soils, while Agpal showed a decrease in May and June followed by an increase in July. Acid phosphatase decreased in both cultivars, with Agpal showing a slight increase in July. In Kaydale, nitrate reductase increased monthly in Ermelo soils, while Agpal peaked in June and decreased in July. N-acetylglucosaminidase, acid phosphatase, and β-glucosidase activities decreased in June and July across both cultivars. In Rensburg, nitrate reductase decreased in Ermelo in May and June but increased in July; Agpal increased until June, then decreased. N-acetylglucosaminidase, acid phosphatase, and β-glucosidase activities decreased over time in both cultivars. Alkaline phosphatase decreased in Ermelo, with Agpal showing a decrease in May and June followed by an increase in July. Across all sites, enzyme activities were higher in post-planting compared to pre-planting soils.

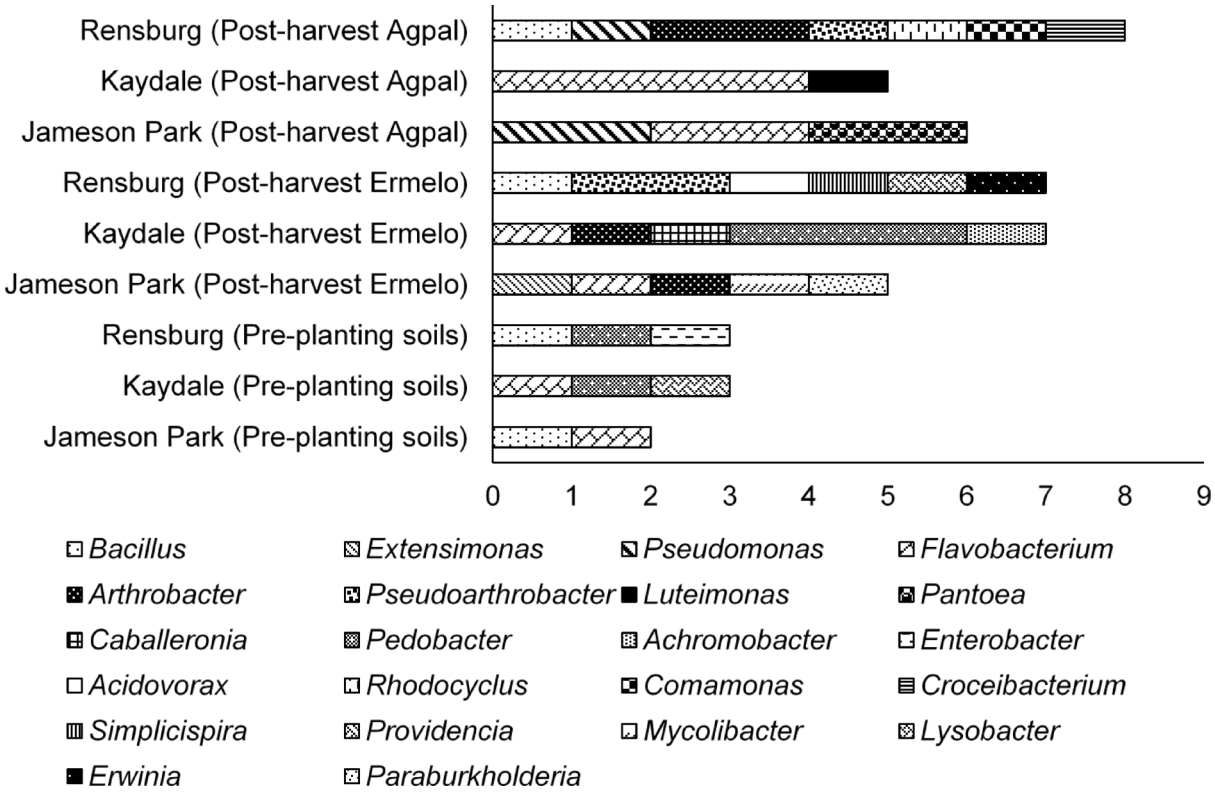

Fig. 1. Species diversity and abundance of bacteria isolated from pre-planting and post Ermelo and Agpal harvest in soils collected from Jameson Park, Kaydale, and Rensburg soil, Heidelberg, Gauteng.

Effects of month of harvest and cultivar on soil nutrient concentrations and pH

The characteristics of soils collected in Jameson Park, Kaydale, and Rensburg, Heidelburg pre- and post *Eragrostis curvula* harvest are represented in Table 4. In Jameson Park soils, the month of harvest and cultivar used had significant main effects on the P concentrations. The month of harvest had a significant main effect on the N concentrations, while cultivar and month of harvest had significant interaction effects on the N concentrations. The month of harvest and cultivar used had significant main and interaction effects on the Mg concentrations and pH in Jameson Park soils. In Kaydale soils, the month of harvest had a significant main effect on the P concentrations. Month of harvest and cultivar had a significant interaction effects on the P concentrations in Kaydale soils. The month of harvest and cultivar used had a significant interaction effect on the N concentrations in Kaydale soils. The month of harvest and cultivar used had significant main and interaction effects on the Mg concentrations and pH in Kaydale soils. In Rensburg soils, The month of harvest and cultivar used had significant main and interaction effects on the P, N, and Mg concentrations in Rensburg soils. Month of harvest had a significant main effect on the pH in Rensburg soils, and there was a significant interaction effect between cultivar used and month of harvest for the pH in Rensburg soils.

Soil nutrient concentrations and pH of Ermelo and Agpal post harvest soils

The P, N, Mg and pH of Ermelo and Agpal post harvest soils is in Table 5. In Jameson Park, N concentrations were high in April and July for both cultivars. Phosphorus concentrations showed no significant changes in Agpal but decreased significantly in Ermelo in June and July. Magnesium concentrations in Ermelo soils were highest in April and declined over time, while Agpal soils decreased in May and June before increasing in July. Soil pH increased over time for both cultivars, peaking in May. In Kaydale, N concentrations increased in Ermelo soils, peaking in June, while Agpal soils showed a decrease with highest N in May and lowest in June. Phosphorus concentrations were lowest in July for Ermelo and in June for Agpal. Magnesium concentrations decreased in April and increased afterward in both cultivars. Post-harvest soil pH was higher than pre-planting, with highest values in May for both cultivars. In Rensburg, N concentrations peaked in July for Ermelo and in May for Agpal. Phosphorus concentrations declined over time, reaching their lowest in June (Ermelo) and July (Agpal). Magnesium concentrations were highest in July (Ermelo) and May (Agpal). Soil pH increased in Ermelo soils in May, then decreased in June and July. For Agpal, pH was highest in April and lowest in July.

Correlations between enzyme activities, soil nutrients and pH

Figure 2 displays Pearson correlation coefficients between enzyme activities and soil nutrient parameters. Alkaline phosphatase showed a moderate positive correlation with phosphorus ($r = 0.41$), while acid phosphatase and glucosidase also correlated positively with phosphorus ($r = 0.40$ and $r = 0.40$, respectively). Nitrate reductase

Site			DF	F Value	*p* value
Jameson Park	Nitrate reductase (µmol/h/g)	Cultivar	1	19.77	< 0.001
		Month of harvest	3	91.76	< 0.001
		Cultivar: Month of harvest	3	21.72	< 0.001
	N-acetylglucosaminidase (nmol/h/g)	Cultivar	1	8.776	0.005
		Month of harvest	3	19.633	<0.001
		Cultivar: Month of harvest	3	4.792	0.002
	Alkaline Phosphatase (nmol/h/g)	Cultivar	1	0.415	0.522
		Month of harvest	3	398.402	<0.001
		Cultivar: Month of harvest	3	275.019	<0.001
	Acid phosphatase (nmol/h/g)	Cultivar	1	4.085	0.0331
		Month of harvest	3	544.854	<0.001
		Cultivar: Month of harvest	3	362.618	<0.001
	β-Glucosidase (nmol/h/g)	Cultivar	1	3.808	0.0566
		Month of harvest	3	586.038	< 0.001
		Cultivar: Month of harvest	3	442.015	< 0.001
Kaydale	Nitrate reductase (µmol/h/g)	Cultivar	1	16.23	< 0.001
		Month of harvest	3	440.19	< 0.001
		Cultivar: Month of harvest	3	99.68	< 0.001
	N-acetylglucosaminidase (nmol/h/g)	Cultivar	1	0.168	0.683
		Month of harvest	3	4.537	0.003
		Cultivar: Month of harvest	3	0.360	0.836
	Alkaline phosphatase (nmol/h/g)	Cultivar	1	3.52	0.067
		Month of harvest	3	988.66	< 0.001
		Cultivar: Month of harvest	3	604.13	< 0.001
	Acid phosphatase (nmol/h/g)	Cultivar	1	0.636	0.429
		Month of harvest	3	837.111	< 0.001
		Cultivar: Month of harvest	3	527.209	< 0.001
	β-Glucosidase (nmol/h/g)	Cultivar	1	0.154	0.696
		Month of harvest	3	324.161	< 0.001
		Cultivar: Month of harvest	3	246.546	< 0.001
Rensburg	Nitrate reductase (µmol/h/g)	Cultivar	1	19.77	< 0.001
		Month of harvest	3	91.76	< 0.001
		Cultivar: Month of harvest	3	21.72	< 0.001
	N-acetylglucosaminidase (nmol/h/g)	Cultivar	1	0.988	0.325
		Month of harvest	3	3.627	0.011
		Cultivar: Month of harvest	3	0.230	0.920
	Alkaline Phosphatase (nmol/h/g)	Cultivar	1	1.342	0.252
		Month of harvest	3	699.285	< 0.001
		Cultivar: Month of harvest	3	382.480	< 0.001
	Acid Phosphatase (nmol/h/g)	Cultivar	1	0.074	0.789
		Month of harvest	3	663.924	< 0.001
		Cultivar: Month of harvest	3	420.542	< 0.001
	β-Glucosidase (nmol/h/g)	Cultivar	1	0.342	0.561
		Month of harvest	3	397.053	< 0.001
		Cultivar: Month of harvest	3	257.729	< 0.001

Table 2. Two-way ANOVA results showing the effects of the cultivar used and month of harvest and their interaction on soil enzyme activities.

showed a weak positive correlation with nitrogen ($r = 0.22$). Negative correlations were observed between enzyme activities and soil pH. Specifically, glucosidase ($r = -0.34$), alkaline phosphatase ($r = -0.33$), and acid phosphatase ($r = -0.32$) were negatively correlated with pH. A weak negative correlation was also noted between alkaline phosphatase and magnesium ($r = -0.12$). Overall, enzyme activities displayed stronger correlations with phosphorus and pH than with other soil nutrients.

Enzyme	Cultivar	Pre-planting	April	May	June	July
Jameson Park						
Nitrate reductase (µmol/h/g)	Ermelo	80 ± 11^{a}	773 ± 37^{b}	1080 ± 111^{c}	1279 ± 23^{d}	2257 ± 7^{e}
	Agpal		888 ± 95^{b}	1650 ± 16^{c}	2951 ± 118^{d}	2007 ± 681^{e}
N-acetylglucosaminidase (nmol/h/g)	Ermelo	5930 ± 43^{a}	5988 ± 198^{a}	5772 ± 136^{a}	5222 ± 215^{a}	5566 ± 73^{a}
	Agpal		6020 ± 203^{a}	5913 ± 130^{a}	5515 ± 64^{b}	5972 ± 159^{c}
Alkaline phosphatase (nmol/h/g)	Ermelo	5967 ± 197^{a}	6039 ± 157^{a}	5772 ± 136^{b}	3335 ± 176^{c}	3322 ± 203^{c}
	Agpal		5750 ± 122^{a}	3263 ± 288^{b}	3420 ± 175^{b}	5878 ± 176^{c}
Acid phosphatase (nmol/h/g)	Ermelo	5930 ± 43^{a}	5988 ± 198^{a}	5772 ± 136^{a}	3356 ± 142^{b}	3063 ± 118^{c}
	Agpal		6020 ± 203^{a}	3228 ± 242^{b}	3344 ± 220^{b}	5790 ± 106^{c}
Glucosidase (nmol/h/g	Ermelo	6041 ± 80^{a}	6058 ± 60^{a}	5885 ± 144^{a}	3491 ± 159^{b}	3368 ± 158^{b}
	Agpal		6058 ± 60^{a}	5885 ± 144^{a}	3491 ± 159^{b}	3368 ± 178^{b}
Kaydale						
Nitrate reductase (µmol/h/g)	Ermelo	47 ± 3^{a}	139 ± 38^{b}	751 ± 71^{c}	1830 ± 69^{d}	2060 ± 46^{e}
	Agpal		603 ± 44^{b}	1201 ± 99^{c}	1394 ± 5^{d}	1073 ± 186^{e}
N-acetylglucosaminidase (nmol/h/g)	Ermelo	6005 ± 117^{a}	5990 ± 93^{a}	5803 ± 117^{a}	5755 ± 137^{b}	5525 ± 95^{b}
	Agpal		5953 ± 210^{a}	5953 ± 184^{a}	5564 ± 73^{b}	5404 ± 1107^{b}
Alkaline phosphatase (nmol/h/g)	Ermelo	6067 ± 137^{a}	6061 ± 157^{a}	5803 ± 117^{b}	3134 ± 144^{c}	3479 ± 119^{c}
	Agpal		5650 ± 105^{a}	3239 ± 107^{b}	3435 ± 94^{b}	5842 ± 109^{c}
Acid phosphatase (nmol/h/g)	Ermelo	6049 ± 90^{a}	6043 ± 125^{a}	5795 ± 172^{b}	3440 ± 110^{c}	3368 ± 177^{c}
	Agpal		6043 ± 34^{a}	5671 ± 172^{b}	3459 ± 110^{c}	3391 ± 177^{c}
Glucosidase (nmol/h/g	Ermelo	6005 ± 117^{a}	5990 ± 93^{a}	5803 ± 117^{a}	3568 ± 387^{b}	3172 ± 293^{b}
	Agpal		5953 ± 210^{a}	3343 ± 243^{b}	3314 ± 180^{b}	6028 ± 116^{c}
Rensburg						
Nitrate reductase (µmol/h/g)	Ermelo	11 ± 1^{a}	962 ± 35^{b}	404 ± 39^{c}	665 ± 21^{d}	4002 ± 55^{e}
	Agpal		468 ± 57^{b}	2249 ± 13^{c}	3921 ± 47^{d}	940 ± 114^{e}
N-acetylglucosaminidase (nmol/h/g)	Ermelo	6012 ± 149^{a}	6050 ± 137^{a}	5730 ± 135^{b}	5697 ± 117^{b}	5595 ± 115^{b}
	Agpal		5870 ± 119^{a}	5723 ± 163^{a}	5458 ± 143^{b}	5526 ± 1137^{b}
Alkaline phosphatase (nmol/h/g)	Ermelo	6083 ± 98^{a}	6029 ± 141^{a}	5730 ± 135^{b}	3342 ± 138^{c}	3423 ± 204^{c}
	Agpal		5817 ± 172^{a}	3388 ± 196^{b}	3253 ± 159^{b}	5848 ± 106^{c}
Acid phosphatase (nmol/h/g)	Ermelo	5986 ± 141^{a}	5964 ± 125^{a}	5770 ± 186^{a}	3355 ± 168^{b}	3342 ± 138^{b}
	Agpal		5964 ± 123^{a}	5770 ± 186^{a}	3354 ± 168^{b}	3342 ± 138^{b}
Glucosidase (nmol/h/g	Ermelo	6012 ± 149^{a}	6047 ± 137^{a}	5730 ± 135^{a}	3478 ± 138^{b}	3217 ± 371^{b}
	Agpal		5870 ± 119^{a}	3311 ± 181^{b}	3320 ± 246^{b}	5826 ± 157^{c}

Table 3. Extracellular enzyme activities of *Eragrostis curvula* Ermelo and Agpal cultivars growing in soils collected from Jameson park, kaydale, and rensburg, heidelberg, gauteng. Values represent mean ± se, different letters denote statistical differences after a two-way ANOVA test.

Discussion

Over time, *E. curvula* cultivars shift the soil microbial profile while increasing the pH of South African grassland ecosystem soils. The root exudates of the respective cultivars may be responsible for the microbial profile shift and, ultimately, changes in soil characteristics. According to Walker[16] and Sasse and Martinoia[35], various species and cultivars produce distinct root exudates that attract diverse microbes and enable plants to thrive in different environments[36]. Differences in the root exudates from the Ermelo and Agpal cultivars may have increased the diversity of culturable bacteria isolated in Jameson Park, Kaydale, and Rensburg soils (Fig. 1). Root exudates provide carbon for microorganisms[37], which may have led metabolically inactive/dormant bacteria to "wake" up[38], leading to the increased diversity in the bacterial isolates, as reported in Fig. 1. Additionally, Ermelo and Agpal associated soils showed a diversity of bacterial isolates, indicating that the cultivars may have secreted exudates that attracted different bacterial species. These findings coincide with Bulgarelli et al.[39], who reported that host-microbe interactions affected the microbial diversity of wild and domesticated *Hordeum vulgare* rhizosphere soils. According to Hinsinger et al.[40], root exudates shape the plant microbiome consisting of plant growth-promoting rhizobacteria, beneficial microbes, and biocontrol agents, as illustrated by the diversity of bacterial isolates reported for Ermelo and Agpal associated soils. Ermelo associated soils from all sites had bacterial isolates belonging to the *Erwinia*, *Pedobacter*, *Bacillus*, *Paraburkholderia*, *Achromobacter*, *Acidovorax*, and *Providencia* genera which have been reported to play a role in N-fixation, P and K solubilisation, indole-3-acetic acid (IAA) production, and chitin degradation[41–44]. The bacteria isolated from Agpal soils from all sites belonged to the *Pantoea*, *Flavobacterium*, *Arthrobacter*, *Comamonas*, *Pseudoarthrobacter*, and *Bacillus* genera, which have been reported to play a role in N cycling, N-fixation, IAA production, and P solubilisation[45–47].

Site	Soil nutrients		DF	F Value	*p* value
Jameson Park	Phosphorus (mg/kg)	Cultivar	1	45.71	0.026
		Month of harvest	3	33.68	0.019
		Cultivar: Month of harvest	3	4.80	0.607
	Nitrogen (mg/kg)	Cultivar	1	0.052	0.822
		Month of harvest	3	53.760	<0.001
		Cultivar: Month of harvest	3	6.688	0.004
	Magnesium (mg/kg)	Cultivar	1	711.9	<0.001
		Month of harvest	3	114.0	<0.001
		Cultivar: Month of harvest	3	138.6	<0.001
	pH	Cultivar	1	0.476	0.001
		Month of harvest	3	0.873	< 0.001
		Cultivar: Month of harvest	3	0.0314	< 0.001
Kaydale	Phosphorus (mg/kg)	Cultivar	1	1.890	0.188
		Month of harvest	3	4.600	0.017
		Cultivar: Month of harvest	3	6.987	0.003
	Nitrogen (mg/kg)	Cultivar	1	0.082	0.778
		Month of harvest	3	0.629	0.607
		Cultivar: Month of harvest	3	19.342	< 0.001
	Magnesium (mg/kg)	Cultivar	1	3.52	0.067
		Month of harvest	3	988.66	< 0.001
		Cultivar: Month of harvest	3	604.13	< 0.001
	pH	Cultivar	1	20.07	< 0.001
		Month of harvest	3	51.37	< 0.001
		Cultivar: Month of harvest	3	15.27	< 0.001
Rensburg	Phosphorus (mg/kg)	Cultivar	1	4.635	0.047
		Month of harvest	3	7.288	0.003
		Cultivar: Month of harvest	3	4.773	0.015
	Nitrogen (mg/kg)	Cultivar	1	34.71	< 0.001
		Month of harvest	3	41.18	< 0.001
		Cultivar: Month of harvest	3	25.14	< 0.001
	Magnesium (mg/kg)	Cultivar	1	44.57	< 0.001
		Month of harvest	3	56.47	< 0.001
		Cultivar: Month of harvest	3	48.77	< 0.001
	pH	Cultivar	1	0.054	0.819
		Month of harvest	3	5.220	0.011
		Cultivar: Month of harvest	3	10.045	< 0.001

Table 4. Two-way ANOVA results showing the effects of the cultivar used and month of harvest and their interaction on soil nutrient concentrations and pH.

Plant growth-promoting rhizobacteria enhance nutrient acquisition by secreting extracellular enzymes that play a role in soil nutrient cycling[48]. Extracellular enzymes such as β-glucosidase, N-acetylglucosaminidase, nitrate reductase, and acid and alkaline phosphatase play a significant role in soil C, N and P cycling[49]. The Ermelo and Agpal cultivars increased the nitrate reductase activity in all study soil sites, and this may be attributed to dead roots increasing soil N causing it to be immobilised through N cycling[10]. According to Brevedan et al.[10], *E. curvula* dead roots have a higher N concentration than live roots at all soil depths. Thus, the decomposition of these root residues increases N cycling, leading to a higher nitrate reductase activity. Eissenstat and Yanai[50] reported that the roots of perennial plants could last a few weeks to 35 weeks. Thus, the increased nitrate reductase activity of Ermelo and Agpal soils over the growth period may be associated with age-related root senescence, supported by the significant independent effect of the month of harvest on the nitrate reductase activity. Though an increase was observed in the nitrate reductase activity of soils associated with the Ermelo and Agpal cultivars, there were variations in the influence of the cultivars on the β-glucosidase and alkaline phosphatase activities. The increased β-glucosidase and alkaline phosphatase activity of Agpal soils in July may be attributed to a higher root senescence rate than the Ermelo cultivar. According to Martinez and Tabatabai[51], β-glucosidase plays a role in the degradation of glucosides present in plant debris, which may have prompted an increased mobilisation of mineral elements[52]. An increase in nutrient mobilisation may have led to an increase in the β-glucosidase activity and influenced the enhanced total N concentration and alkaline phosphatase activity of Agpal associated soils. Magalef et al.[53] reported that N fertilisation increases phosphatase activity, thus, the increase in soil N may have led to an increase in the alkaline phosphatase activity.

Soil characteristics	Cultivar	Pre-planting	April	May	June	July
Jameson Park						
Nitrogen (mg/kg)	Ermelo	5.00 ± 0.17^{a}	2017.33 ± 48.60^{b}	1105.33 ± 167.44^{c}	1177.33 ± 287.46^{c}	2033.00 ± 36.37^{d}
	Agpal		1596 ± 112.65^{b}	1145 ± 134.89^{c}	1488.67 ± 95.04^{d}	2158.32 ± 140.58^{e}
Phosphorus (mg/kg)	Ermelo	16.67 ± 0.58^{a}	13.97 ± 1.05^{a}	15.03 ± 3.15^{a}	8.59 ± 2.42^{b}	9.40 ± 2.42^{b}
	Agpal		15.00 ± 2.65^{a}	16.43 ± 4.17^{a}	12.83 ± 3.45^{a}	13.76 ± 1.76^{a}
Magnesium (mg/kg)	Ermelo	120.00 ± 6.08^{a}	306.00 ± 5.57^{b}	213.63 ± 15.06^{c}	111.00 ± 10.54^{d}	127.13 ± 1.86^{d}
	Agpal		308.73 ± 9.72^{b}	267.60 ± 18.40^{c}	295.70 ± 4.56^{c}	329.67 ± 2.08^{d}
pH	Ermelo	4.72 ± 0.04^{a}	5.01 ± 0.02^{a}	6.01 ± 0.04^{b}	5.89 ± 0.35^{b}	5.73 ± 0.06^{b}
	Agpal		5.26 ± 0.13^{a}	6.06 ± 0.30^{b}	5.18 ± 0.12^{c}	5.02 ± 0.07^{c}
Kaydale						
Nitrogen (mg/kg)	Ermelo	4.30 ± 0.30^{a}	735 ± 24.27^{b}	924.00 ± 40.45^{b}	1437.33 ± 186.95^{c}	1023.67 ± 81.00^{d}
	Agpal		837.30 ± 32.40^{b}	1238 ± 256.38^{c}	720.47 ± 17.56^{c}	993.67 ± 58.43^{d}
Phosphorus (mg/kg)	Ermelo	16.67 ± 2.08^{a}	4.90 ± 1.15^{b}	8.97 ± 2.40^{b}	12.25 ± 2.63^{c}	6.37 ± 1.08^{d}
	Agpal		7.67 ± 3.74^{b}	10.02 ± 0.05^{c}	4.97 ± 1.25^{d}	5.27 ± 0.83^{d}
Magnesium (mg/kg)	Ermelo	216.67 ± 18.50^{a}	191.33 ± 8.50^{b}	257.33 ± 23.03^{c}	286.67 ± 10.50^{c}	210.70 ± 10.45^{d}
	Agpal		196.00 ± 12.53^{ch}	224.09 ± 7.23^{ch}	175.45 ± 11.24^{ci}	195.02 ± 0.05^{ci}
pH	Ermelo	4.88 ± 0.01^{a}	4.82 ± 0.07^{a}	5.46 ± 0.03^{b}	5.19 ± 0.10^{b}	5.44 ± 0.06^{b}
	Agpal		4.98 ± 0.07^{a}	5.40 ± 0.08^{b}	4.93 ± 0.06^{c}	5.02 ± 0.10^{c}
Rensburg						
Nitrogen (mg/kg)	Ermelo	11.77 ± 0.58^{a}	563.67 ± 62.16^{b}	984.67 ± 33.50^{c}	763 ± 65.60^{d}	1404.33 ± 188.70^{e}
	Agpal		659.71 ± 47.29^{b}	870.43 ± 44.21^{c}	675.30 ± 22.50^{d}	746.73 ± 20.18^{d}
Phosphorus (mg/kg)	Ermelo	18.33 ± 0.58^{a}	12.03 ± 3.75^{a}	15.93 ± 2.44^{a}	11.53 ± 0.57^{b}	13.43 ± 2.68^{a}
	Agpal		13.40 ± 3.82^{a}	15.87 ± 4.46^{a}	10.16 ± 1.40^{b}	3.50 ± 0.95^{c}
Magnesium (mg/kg)	Ermelo	432.67 ± 6.03^{a}	175.67 ± 12.22^{b}	224.50 ± 11.17^{c}	154.00 ± 23.52^{d}	337.30 ± 23.07^{e}
	Agpal		177.00 ± 23.07^{b}	221.92 ± 4.31^{c}	160.58 ± 0.71^{d}	173.58 ± 0.71^{d}
pH	Ermelo	5.06 ± 0.02^{a}	5.03 ± 0.19^{a}	5.60 ± 0.06^{b}	5.42 ± 0.16^{b}	5.41 ± 0.36^{b}
	Agpal		5.73 ± 0.23^{b}	5.62 ± 0.07^{b}	5.24 ± 0.21^{c}	4.94 ± 0.05^{c}

Table 5. Nitrogen, phosphorus, magnesium and pH of *Eragrostis curvula* Ermelo and Agpal post-harvest soils collected from Jameson park, kaydale, and rensburg, heidelberg, gauteng. Values represent mean ± se, different letters denote statistical differences after a two-way ANOVA test.

According to Li et al.[54], soil N concentration may have a dominant effect on the alkaline phosphatase activity due to the role of N in the synthesis of acid and alkaline phosphatases[55]. Furthermore, the genes encoding alkaline phosphatase activity regulate P starvation, making alkaline phosphatase dependent on soil N and P[54]. The lower soil P concentrations observed in Agpal soils collected in July from all study sites, combined with an increase in soil N, may have triggered the secretion of alkaline phosphatases. The moderately positive correlation of soil P and alkaline phosphatase activity in Fig. 2 is supported by the resource allocation model for extracellular enzymes, which suggests that microbes invest resources to synthesise enzymes that acquire deficient nutrients[56]. Though the alkaline phosphatase activity has been reported to be more active in pH 9–11[57], Bergkemper et al.[58] reported that acidic soils increase the abundance of genes encoding alkaline phosphatase, thus supporting the high activity of alkaline phosphatase in Agpal soils. The P concentration of Agpal soils in all sites was relatively lower than that of Ermelo associated soils, which may be attributed to a lower soil pH. Phosphorus forms insoluble complexes with iron and aluminium in acidic soils, rendering P unavailable for uptake[59]. The slightly higher pH of Ermelo associated soils may have led to a higher soil P concentration and, consequently, lower acid and alkaline phosphatase activities, as supported by the negative correlations between acid and alkaline phosphatase and pH in Fig. 2. Philippot et al.[60] reported that plant secondary metabolites alter soil pH. Thus, we can deduce that the Ermelo cultivar secreted higher metabolite concentrations, leading to an increased soil pH. Furthermore, an increase in pH has been linked to higher absorption and uptake of cations[61], which could have influenced the reduction of Mg concentrations in Ermelo associated soils compared to Agpal soils.

Cultivars used had significant effects on the soil enzyme activities and soil nutrients which may indicate that the exudates produced by the different cultivars had varying selection effects on the bacterial diversity and consequently affected the associated extracellular enzyme activities and soil characteristics. According to Motsomane et al.[12], environmental filters such as soil properties and abiotic factors play a role in the selection of bacterial communities. In addition, Hargreaves et al.[62] reported that soil properties associated with topographic position significantly influenced the microbial composition more than the plant species in a corn-based annual cropping system and perennial switchgrass cropping system across three topographic positions. We can deduce that environmental factors influenced the soil characteristics, bacterial diversity, and soil nutrition for both cultivars. Differences in the influence of the Ermelo and Agpal cultivars on bacterial diversity, extracellular enzyme activities, and soil characteristics may have been attributed to differences in the timing of rhizodeposition. In a study on the rhizodeposition of maize, Pausch et al.[63] reported that 62% of total rhizodeposition was mineralised

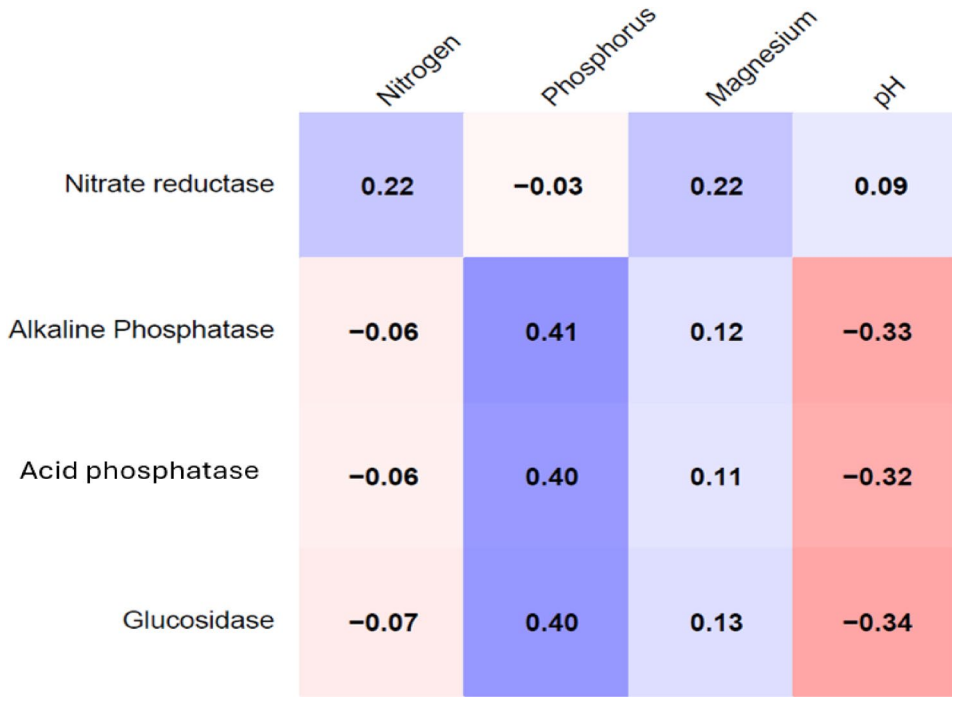

Fig. 2. Heatmap showing Pearson correlation coefficients between soil enzyme activities, soil nutrients and pH of Ermelo and Agpal post harvest soils. Positive correlations are indicated in purple, while negative correlations are shown in peach. The strength of the correlation is represented by both the colour gradient and the numerical values within each cell. The heatmap was generated in R (version 3.6.2; R Core Team, https://www.r-project.org/) using the ggplot2 package.

in 16 days, with 31% in the soil and 7% in microbial biomass. Thus, the timing of rhizodeposition may have influenced the enzyme activity and soil nutrient analysis.

Engedal et al.[64] reported that root morphology plays a significant role in rhizodeposition. The lack of literature reporting on the growth physiology of the Agpal cultivar has made it difficult to compare how the growth physiology of these two cultivars could have influenced their role in soil nutrient cycling. While this study provides valuable insights into how *E. curvula* cultivars influence soil nutrient cycling, there are some limitations to consider. The study did not isolate or identify specific metabolites in root exudates, which limits our understanding of the precise mechanisms driving microbial shifts and enzyme activity changes. Additionally, the lack of detailed information on the growth physiology of the Agpal cultivar constrained comparative analyses between cultivars. Environmental variables, such as soil heterogeneity and topographic influences, may have also impacted results but were not fully controlled. Future studies incorporating metabolomic analyses of root exudates and more detailed physiological characterisation of cultivars would strengthen the understanding of these interactions.

Data availability

The datasets generated and/or analysed during the current study will be stored and available to the public in ResearchGate corresponding author (Anathi Magadlela) account and tagged to the manuscript once published. Also, all raw data can be requested from the corresponding author, Prof. Anathi Magadlela at [anathimagadlela@icloud.com](mailto: anathimagadlela@icloud.com).

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Author contributions

Conceptualisation and Funding, A. M; methodology, N. M and A. M; validation, N. M and A. M; formal analysis, N. M; writing-original draft preparation, N. M; writing-review and editing, A. M; supervision, A. M; project administration, A. M and N. M; funding acquisition, A. M. Both authors reviewed the manuscript before submission for publication.

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Declarations

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The authors declare no competing interests.

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No approval from research ethics committees was necessary for this study's objectives.

Informed consent

All individual participants included in this article provided informed consent for their identifying information.

Consent to publish

The participant has granted consent for the submission of the case report to the journal.

Additional information

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