

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

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

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Abstract

Eragrostis curvula has been studied extensively due to its primary use in pastures and potential utilisation as a food source, however the role of *E. curvula* cultivars in soil nutrient cycling in nutrient-poor grassland ecosystems is understudied. This study investigated the effects of *E. curvula* cultivars on soil bacterial communities, associated enzyme activities, and soil characteristics in soils collected in grassland ecosystems in Heidelberg, Gauteng, South Africa. Soil bacterial communities were isolated and identified in pre-planting soils and post *E. curvula* harvest. Over four months, soil characteristics and enzyme activities were determined for pre-planting and post *E. curvula* harvest. There was an increase in the bacteria isolated from post-harvest soils compared to pre-planting soils for both cultivars in all study sites. Soil growing the cultivars showed a significant increase in the nitrate reductase activity across all study sites. An increase in β -glucosidase in July was only observed in Agpal soils across all study sites. Soil N concentrations and pH increased in all post-harvest soils. Potassium and P concentrations in post-harvest soils were lower than in pre-planting soils for both cultivars. The principal component analysis revealed that the Ermelo cultivar uniformly influences the soil characteristics and extracellular enzyme activities in all study sites, while the Agpal cultivar had varying responses. This study's findings highlight how *E. curvula* cultivars shift the microbial profile over time while increasing N and pH in grassland ecosystem soils.

Keywords: Nutrient cycling, Enzymes, Cultivars, Bacteria, *Eragrostis*, lovegrass1.

Introduction

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], water conservation [7,8], and tolerance to grazing mismanagement. While considered an invasive weed that threatens natural ecosystems in countries such as Chile, Europe, and Asia [6,9], in native southern Africa, *E. curvula* holds potential for diversifying food systems [10] and is cultivated for pasture [4]. The benefits of utilising *E. curvula* as a food and for pasture extend beyond mere forage value and food but may be linked to nutrient cycling. Brevedan et al [11] 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 [12]. 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 [13]. Soil microbial communities are influenced by plants through root secretions that provide microbes with energy, thus supporting rhizosphere colonisation [14-17]. Root secretions differ between species and cultivars [18] and influence the selection of microbial communities in rhizosphere soils, resulting in rhizosphere microbial communities unique to the plant [19]. The uniqueness of rhizosphere microbial communities and their respective plants aligns with the hologenome theory, which states that the genetic material of a plant and its associated microbial communities should be regarded as a single unit, a holobiont, [20] that influences ecological fitness and adaptation to different niches [13].

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 knowledge gap regarding perennial and native plants such as *E. curvula* [19]. 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 [21] 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 [22]. 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 nutrition. 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. We hypothesized that *E. curvula* cultivars would have varying effects on bacterial diversity, associated enzyme activities, and soil nutrition.

2. Materials and methods

2.1. 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 located in Gauteng, which consists of savanna and grassland ecosystems [23]. In Gauteng, Heidelberg experiences summer rainfall followed by dry winters [24]. Annual temperatures range from 3-25°C, and precipitation ranges from 600-700 mm per annum [24,25]. 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. Three subsamples of 500g from each site 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 [26] explained. Ambic-2 solution containing 0.25M NH_4CO_3 , 0.01M Na_2EDTA , 0.01M NH_4F , and 0.05g/L superfloc (N100) was adjusted to pH 8 using concentrated ammonia solution and used to extract P, potassium (K), zinc (Zn), and copper (Cu). 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 [27] molybdenum blue procedure. The K concentration was determined by diluting 5ml aliquot of the filtrate with 20ml de-ionised water using atomic absorption, and the remaining undiluted filtrate was used to determine the zinc, copper, and manganese concentration using atomic absorption. The magnesium (Mg) and calcium (Ca) concentrations were determined by stirring sample cups containing 25 ml of soil sample and 25 ml of 1M KCl solution in a multiple stirrer (400 rpm) for 10 minutes. The stirred mixture was filtered with Whatman no.1 paper. Five millilitres of the filtrate was diluted with 20 ml 0.0356M SrCl_2 , and Ca concentrations were determined using atomic absorption. 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. The crucible was placed in a horizontal furnace and burned in a stream of oxygen at 1350°C, and soil nitrogen was measured as N₂ in a thermal conductivity cell. Soil pH was determined by mixing 10 ml of soil sample and 25 ml of 1M KCl in sample cups and stirring in a multiple stirrer at 400rpm for 5 minutes. The suspension was left to rest for 30 minutes, and the pH was measured using a gel-filled combination glass electrode while stirring.

2.2. Pot trials

Eragrostis curvula Ermelo and Agpal cultivars were sourced from AGT Foods, Africa, and Agricol, 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. After 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 500g 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 [28] reported that plant age influences root secretions, thus affecting microbial communities in rhizosphere soils. Alagbo and Chauhan [29] 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).

2.3. Extracellular enzyme activities

Soil samples collected after each harvest were assayed for β -glucosidase, N-acetylglucosaminidase, acid phosphatase, and alkaline phosphatase activities using the fluorescence-based method described by [30] and expressed as nmol h⁻¹g⁻¹. Briefly, soil samples (10 g soil/100 ml autoclaved dH₂O) 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 μ l soil

aliquot and 50µl substrate, alongside reference standards (200µl bicarbonate buffer + 50µl 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 hours. Thereafter, the reaction was stopped by adding 5 µl of 0.5M 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 assays were done using a modified protocol described by [31]. A volumetric flask wrapped in foil was filled with 1 ml of 25 mM KNO₃, 4 ml of 0.9 mM 2,4-dinitrophenol, and 5 ml of milliQ dH₂O. 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 hours. After incubation, 10 ml of 4M KCl was added to the soil mixture, succinctly mixed, and filtered using filter paper (Whatman number 1). The enzymatic reaction was initiated by adding 2 ml of the filtrate to 1.2 ml of 0.19M ammonium chloride buffer (pH 8.5) and 0.8ml of a colour reagent consisting of 1% sulfanilamide, 1N HCl, and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The solution was incubated at 30°C for 30 minutes. The absorbance was measured at 520nm using an 1800 UV spectrophotometer. The nitrite (NO₂⁻) liberated into the medium was extrapolated from a prepared standard curve with KNO₃. The nitrate reductase activity was expressed as 0.1 µmol h⁻¹g⁻¹.

2.4. 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. 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: 63F (5' CAGGCCTAACACATGCAAGTC 3') and 1387R (5' GGGCGGTGTGTACAAGGC 3') from Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). The PCR amplification was performed using an EmeraldAmp GT Master Mix with the following conditions: Initial denaturation at 94°C for 5 minutes, followed by 30 cycles

of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 2 minutes, with additional extension at 72°C for 10 minutes. 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). A phylogenetic approach was used to determine the evolutionary relationship between bacterial nucleotide sequences. The nucleotide alignment was done using the MUSCLE tool in MEGA 11 and checked manually before constructing the phylogenetic tree using the neighbour-joining likelihood tree approach. Also, a bootstrap resampling was performed with 1000 replicates following the procedure by [32] (Supplementary figure 1).

2.5. Statistical analysis

The statistical software/program R (version 3.6.2) was used to test for differences in the nutrient concentrations, pH, exchange acidity, soil enzyme activity, and total cation concentrations in soil samples collected pre and post *E. curvula* cultivars harvest using One-way ANOVA. Where the ANOVA showed significant differences among the treatments, a Tukey post hoc test was used to separate the means. A three-way ANOVA was used to determine the effects of the site, month of harvest, and cultivar used on the extracellular enzyme activity and soil characteristics. Furthermore, relations between soil nutrients and enzyme activities were determined using principal component analysis. The principal component analysis was performed using the ggplot statistical package and pr comp function on R (version 3.6.2).

3. Results

3.1. Soil bacterial identification

The bacteria isolated from Jameson Park, Kaydale, and Rensburg soils pre and post *E. curvula* Ermelo and Agpal harvest is represented in Table 1. Pre-planting soils from Jameson Park, two bacterial isolates belonging to the *Bacillus* and *Flavobacterium* genera were identified. After planting the Ermelo cultivar, Jameson Park soils, five bacterial isolates belonging to the *Arthrobacter*, *Extensimonas*, *Mycolibacter*, *Flavobacterium*, and *Paraburkholderia* genera

196 were identified. Jameson Park soils, six bacterial isolates belonging to the *Pantoea*,
197 *Pseudomonas*, and *Flavobacterium* genera were identified after harvesting the Agpal cultivar.
198 In Kaydale pre-planting soils, three bacterial isolates belonging to the *Pedobacter*, *Lysobacter*,
199 and *Flavobacterium* genera were identified. After planting the Ermelo cultivar, Kaydale soils,
200 seven bacterial isolates belonging to the *Pedobacter*, *Arthrobacter*, *Caballeronia*,
201 *Flavobacterium*, and *Achromobacter* genera were identified. After planting the Agpal cultivar
202 in Kaydale soils, five bacterial isolates belonging to the *Flavobacterium* and *Luteimonas* genera
203 were identified. In Rensburg pre-planting soils, three bacterial isolates belonging to the
204 *Bacillus*, *Enterobacter*, and *Pedobacter* genera were identified. After planting the Ermelo
205 cultivar, seven bacterial isolates belonging to the *Erwinia*, *Bacillus*, *Pseudoarthrobacter*,
206 *Simplisora*, *Providencia*, and *Acidovorax* genera were identified. After planting the Agpal
207 cultivar in Rensburg soils, eight bacterial isolates belonging to the *Arthrobacter*, *Comamonas*,
208 *Pseudoarthrobacter*, *Rhodocyclus*, *Croceibacterium*, *Pseudomonas*, and *Bacillus* genera were
209 identified. The diversity of culturable bacteria isolated from pre-planting and post Ermelo and
210 Agpal cultivars in all study sites is represented in Figure 1. Agpal cultivar soils from Jameson
211 Park and Rensburg had a higher bacterial diversity than the Ermelo cultivar. Ermelo and Agpal
212 cultivar soils had a higher bacterial diversity compared to pre-planting soils.

213 Table 1: Molecular identification of bacterial isolated from pre-planting, post Ermelo and post
 214 Agpal cultivar harvest from Jameson Park, Kaydale and Rensburg soils collected from
 215 Heidelberg, Gauteng.

	Strain	Accession number	Similarity (%)
Jameson Park			
Pre-planting soils	<i>Bacillus zanthoxyli</i> strain 1433	NR_164882.1	98.67
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	93.01
Ermelo	<i>Arthrobacter nitrophenolicus</i> strain SJCon	NR_178397.1	100.00
	<i>Paraburkholderia guartelaensis</i> strain CNPSo 3008	NR_169402.1	89.93
	<i>Extensimonas perlucida</i> strain HX2-24	NR_169362.1	98.27
	<i>Mycolicibacter virginiensis</i> strain MO-233	NR_149186.1	99.67
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	99.09
Agpal	<i>Pantoea ananatis</i> strain LMG 2665	NR_119362.1	99.50
	<i>Pantoea stewartii</i> subsp. <i>indologenes</i> strain CIP 104006	NR_104928.1	99.07
	<i>Pseudomonas piscis</i> strain CMAA1215	NR_169429.1	99.92
	<i>Flavobacterium tistranum</i> strain GB 56.1	NR_149282.1	96.32
	<i>Pseudomonas aylmerensis</i> strain S1E40	NR_169460.1	91.00
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	99.58
Kaydale			
Pre-planting	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	99.90
	<i>Pedobacter chitinilyticus</i> strain CM134L-2	NR_180020.1	98.41
	<i>Lysobacter prati</i> strain SYSU H10001	NR_180692.1	97.38
Ermelo	<i>Caballeronia catudaia</i> strain LMG 29318	NR_145605.1	98.92
	<i>Achromobacter marplatensis</i> strain R-46660	NR_117614.1	99.15
	<i>Arthrobacter pokkalii</i> strain P3B162	NR_149802.1	99.96
	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	98.48
	<i>Pedobacter frigiditerrae</i> strain RP-1-13	NR_173500.1	89.92
	<i>Pedobacter chitinilyticus</i> strain CM134L-2	NR_180020.1	99.05
	<i>Pedobacter pollutisoli</i> strain TBBPA-24	NR_165750.1	99.29

Agpal	<i>Flavobacterium anhuiense</i> strain D3	NR_044388.1	100
	<i>Luteimonas lumbrici</i> strain 1.1416	NR_170461.1	99.73
	<i>Flavobacterium amnigenum</i> strain I3-3	NR_169462.1	99.12
	<i>Flavobacterium tistranum</i> strain GB 56.1 16S	NR_149282.1	99.89
	<i>Flavobacterium fluviatile</i> strain TAPY14	NR_163630.1	100.00
Rensburg			
Pre-planting	<i>Enterobacter ludwigii</i> strain EN-119	NR_042349.1	98.65
	<i>Pedobacter chitinilyticus</i> strain CM134L-2	NR_180020.1	99.37
	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> strain 168	NR_102783.2	96.92
Ermelo	<i>Acidovorax delafieldii</i> strain 133	NR_028714.1	99.57
	<i>Simplicispira soli</i> strain CA-16	NR_159921.1	99.98
	<i>Pseudarthrobacter enclensis</i> strain NIO-1008	NR_134699.1	97.52
	<i>Pseudarthrobacter niigatensis</i> strain LC4	NR_041400.1	92.59
	<i>Bacillus nakamurai</i> strain NRRL B-41091	NR_151897.1	93.91
	<i>Erwinia phyllosphaerae</i> strain CMYE1	NR_181782.1	95.96
	<i>Providencia huaxiensis</i> strain WCHPr000369	NR_174258.1	98.03
Agpal	<i>Croceibacterium ferulae</i> strain SX2RGS8	NR_165000.1	97.88
	<i>Rhodocyclus tenius</i> strain 2761	NR_025839.1	99.65
	<i>Comamonas terrae</i> strain A3-3	NR_108609.1	97.83
	<i>Pseudarthrobacter siccitolerans</i> strain 4J27	NR_108849.1	99.73
	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> strain 168	NR_102783.2	98.51
	<i>Arthrobacter woluwensis</i> strain 1551	NR_044894.1	99.30
	<i>Arthrobacter alkaliphilus</i> strain LC6	NR_041401.1	96.28
	<i>Pseudomonas aylmerensis</i> strain S1E40	NR_169460.1	97.32

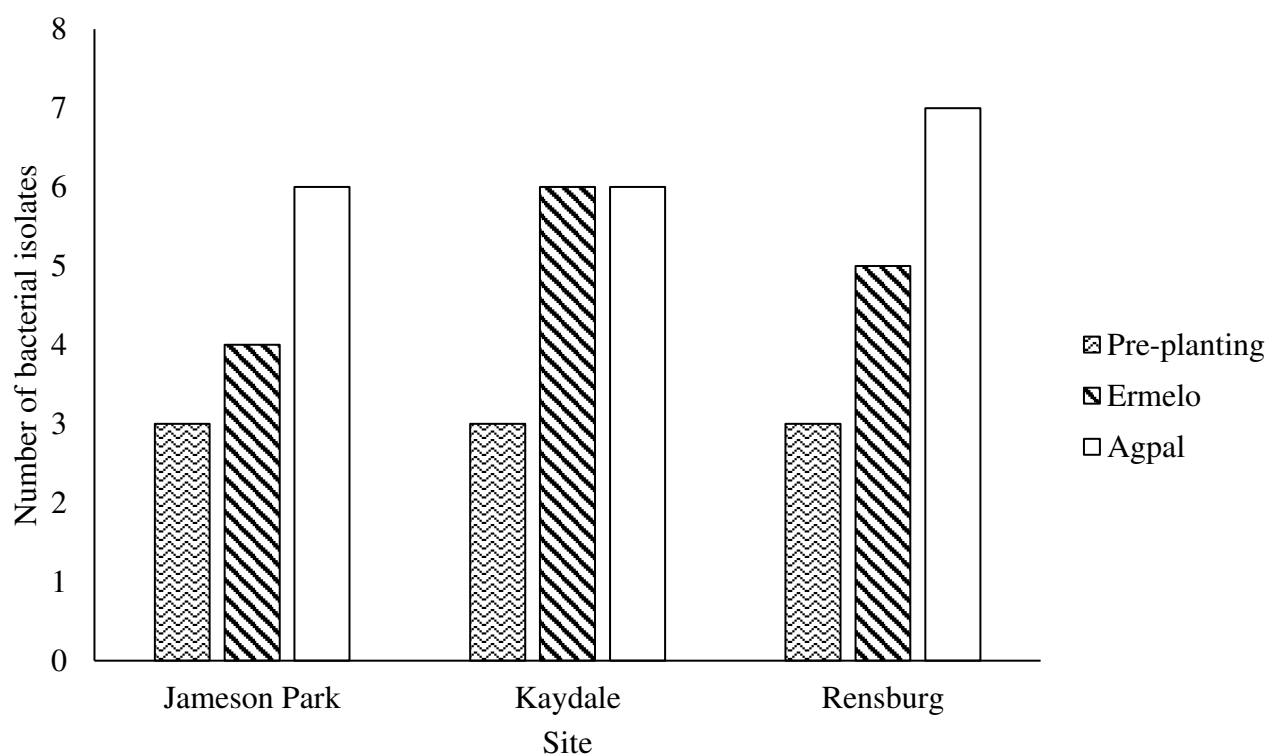


Figure 1: Number of bacterial isolates from pre-planting, Ermelo, and Agpal soils in Jameson Park, Kaydale, and Rensburg soils collected from Heidelberg, Gauteng.

3.2. Extracellular enzyme activities

The effects of *E. curvula* cultivars on the soil extracellular enzyme activity over four months are represented in Table 2. For the nitrate reductase activity, a significant independent effect was observed on the month of harvest ($Df= 1$, $F= 40,393$, $p< 0,05$), and a significant interaction effect was observed between the cultivar used and the month of harvest ($Df= 1$, $F= 7,510$, $p= 0,00743$). There was a significant increase in the nitrate reductase activity of the soils collected from all sites throughout the four months growth of both cultivars, and the alkaline and acid phosphatase activity, only the month of harvest had a significant independent effect on the activity ($Df= 1$, $F= 45,192$, $p<0,05$ and $Df= 1$, $F=48, 582$, $p<0,05$ respectively). The month and cultivar had a significant interaction effect on the alkaline and acid phosphatase activity ($Df= 1$, $F= 53,103$, $p<0,05$ and $Df= 1$, $F=48,832$, $p<0,05$ respectively). A significant decrease in the acid and alkaline phosphatase activity was observed in May and June for all cultivars in all three sites. The harvest month significantly affected the N-acetylglucosaminidase activity ($Df= 1$, $F= 27,637$, $p<0,05$). While a significant decrease was observed in the acid phosphatase activity in both cultivars in all three sites, the alkaline phosphatase, β -glucosidase, and N-

235 acetylglucosaminidase activity showed a significant increase in activity in both cultivars in
236 July. There was a significant increase in the alkaline phosphatase activity in July in Kaydale
237 (Ermelo cultivar) and all three sites (Agpal cultivar). A significant increase in the N-
238 acetylglucosaminidase activity in July was observed in Jameson Park for both cultivars. The
239 month of harvest had a significant independent effect on the β -glucosidase activity ($Df= 1$, $F=$
240 $27,637$, $p<0,05$), and the interaction effect was observed in the cultivar and month of harvest
241 ($Df= 1$, $F= 50,109$, $p< 0,05$). A significant increase in the β -glucosidase activity was observed
242 in July in all sites for the Agpal cultivar. For both cultivars, the nitrate reductase activity of
243 each monthly harvest is significantly higher than pre-planting soils in all study sites.

244 Table 2: Extracellular enzyme activities of *Eragrostis curvula* Ermelo and Agpal cultivars growing in Heidelberg soils for four months. Where site
245 1 represents Jameson Park, site 2 represents Kaydale and site 3 represents Rensburg. Values represent mean±SE, different letters denote statistical
246 differences after a one-way ANOVA test.

		Initial	Ermelo cultivar				Agpal cultivar			
Enzyme activity	Site		April	May	June	July	April	May	June	July
Nitrate reductase (μmol/h/g)	1	80±11 ^a	773±37 ^b	1080±111 ^c	1279±23 ^d	2257±7 ^e	888±95 ^f	1650±16 ^g	2951±118 ^h	2007±681 ⁱ
	2	47±3 ^j	139±38 ^k	751±71 ^l	1830±69 ^m	2060±46 ⁿ	603±44 ^o	1201±99 ^p	1394±5 ^p	1073±186 ^q
	3	11±1 ^r	962±35 ^s	404±39 ^t	665±21 ^u	4002±55 ^v	468±57 ^w	2249±13 ^x	3921±47 ^y	940±114 ^z
N- acetylglucosaminidase (nmol/h/g)	1	5930±43 ^{ab}	5988±198 ^{ab}	5772±136 ^{ab}	5222±215 ^{ac}	5566±73 ^{ad}	6020±203 ^{ab}	5913±130 ^{ac}	5515±64 ^{af}	5972±159 ^{ag}
	2	6005±117 ^{ah}	5990±93 ^{ah}	5803±117 ^{ai}	5755±137 ^{ai}	5525±95 ^{aj}	5953±210 ^{ah}	5953±184 ^{al}	5564±73 ^{am}	5404±1107 ^{an}
	3	6012±149 ^{ao}	6050±137 ^{ao}	5730±135 ^{ap}	5697±117 ^{ap}	5595±115 ^{ap}	5870±119 ^{ao}	5723±163 ^{aq}	5458±143 ^{aq}	5526±1137 ^{aq}
Alkaline phosphatase (nmol/h/g)	1	5967±197 ^{ar}	6039±157 ^{ar}	5772±136 ^{as}	3335±176 ^{at}	3322±203 ^{at}	5750±122 ^{ar}	3263±288 ^{av}	3420±175 ^{av}	5878±176 ^{aw}
	2	6067±137 ^{ax}	6061±157 ^{ax}	5803±117 ^{ay}	3134±144 ^{az}	3479±119 ^{ba}	5650±105 ^{bb}	3239±107 ^{bc}	3435±94 ^{bc}	5842±109 ^{bd}
	3	6083±98 ^{be}	6029±141 ^{be}	5730±135 ^{bf}	3342±138 ^{bg}	3423±204 ^{bg}	5817±172 ^{bh}	3388±196 ^{bi}	3253±159 ^{bi}	5848±106 ^{bj}
Acid phosphatase (nmol/h/g)	1	6041±80 ^{bk}	6058±60 ^{bk}	5885±144 ^{bk}	3491±159 ^{bl}	3368±158 ^{bl}	6058±60 ^{bk}	5885±144 ^{bn}	3491±159 ^{bo}	3368±178 ^{bo}
	2	6049±90 ^{bp}	6043±125 ^{bp}	5795±172 ^{bq}	3440±110 ^{br}	3368±177 ^{br}	6043±34 ^{bp}	5795±172 ^{bt}	3440±110 ^{bu}	3369±177 ^{bu}
	3	5986±141 ^{bv}	5964±125 ^{bv}	5770±186 ^{bv}	3355±168 ^{bw}	3342±138 ^{bw}	5964±123 ^{bv}	5770±186 ^{bv}	3354±168 ^{bx}	3342±138 ^{bx}
Glucosidase (nmol/h/g)	1	5930±43 ^{by}	5988±198 ^{by}	5772±136 ^{by}	3356±142 ^{bz}	3063±118 ^{ca}	6020±203 ^{by}	3228±242 ^{cb}	3344±220 ^{cc}	5790±106 ^{cd}
	2	6005±117 ^{ce}	5990±93 ^{ce}	5803±117 ^{ce}	3568±387 ^{cf}	3172±293 ^{cg}	5953±210 ^{ce}	3343±243 ^{ch}	3314±180 ^{ch}	6028±116 ^{ci}
	3	6012±149 ^{cj}	6047±137 ^{cj}	5730±135 ^{ck}	3478±138 ^{cl}	3217±371 ^{cl}	5870±119 ^{cj}	3311±181 ^{cm}	3320±246 ^{cm}	5826±157 ^{cn}

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3.3. Soil nutrient concentrations and relative acidity

The characteristics (nutrient concentrations and relative acidity) of soils collected in Jameson Park, Kaydale, and Rensburg, Heidelberg pre- and post *Eragrostis curvula* harvest are represented in Table 3. The month of harvest, cultivar, and site had a significant interaction effect on the soil P concentration (Df= 1, F= 5.907, p= 0.0176). In Jameson Park, the Ermelo cultivar harvested after April, May, and July had no significant effect on the P concentration. The soil P concentration of the Ermelo cultivar harvested in June was significantly lower than the initial soil P concentration. No significant differences were observed in the P concentration of Jameson Park soils before and after each subsequent harvest of the Agpal cultivar. In Kaydale, April and July, soils from the Ermelo cultivar were significantly lower than pre-planting soil P concentration. For the Agpal cultivar, there was a significant decrease in the P concentrations of soils collected after each subsequent harvest and pre-planting levels. There were no significant differences between the Ermelo harvest soils in April, May, and July and pre-planting soils in Rensburg soils. In June, the P concentration was significantly lower than in the pre-planting soils. For the Agpal cultivar, soils collected in June and July had a significantly lower P concentration than pre-planting soils. The month of harvest, cultivar, and site had a significant interaction effect on the K concentration (Df= 1, F= 6.044, p= 0.016396). In Jameson Park, the K concentration was significantly higher than pre-planting soils for April of the Ermelo cultivar. However, from May to July, the K concentration was significantly lower than that of pre-planting soils. For the Agpal cultivar, there were significant differences in the K concentration of each subsequent harvest; April, June, and July soils were significantly higher than pre-planting soils. In Kaydale, there was no significant difference in the K concentrations of April-May and June-July soils; however, each harvest was significantly lower than the K concentration of pre-planting soils for the Ermelo and Agpal cultivars. In Rensburg, soils collected from the Ermelo cultivar in April, June, and July were significantly lower than pre-planting soils, and there was no significant difference in the K concentration of soils collected in May harvest and pre-planting soils. For the Agpal cultivar, there was no significant difference in the K concentration of soils collected in April and May, as well as soils collected in May and pre-planting. In Rensburg soils, the N concentration peaked in July for the Ermelo cultivar and in May for the Agpal cultivar. Month of harvest, cultivar, and site had a significant interaction effect on the N concentration (Df= 1, F= 6.509, P= 0.01313). The N concentration of Ermelo and Agpal soils from April to July was significantly higher than the initial N

concentrations from Jameson Park, Kaydale, and Rensburg soils. In Jameson Park soils, there were significant differences in the Ermelo cultivar's N concentrations from April to July. Soil N concentration showed no significant differences in the Agpal soils collected in May and June. In Kaydale soils, a significant increase in the N concentration of Ermelo soils in June was observed, while a decrease was observed for Agpal soils.

Month of harvest, cultivar, and site had a significant interaction effect on the Ca concentration ($Df= 1$, $F= 24.399$, $P<0.0001$). In Jameson Park, the Ermelo cultivar harvested in April and May had a significantly higher Ca concentration than pre-planting soils. Additionally, there was no significant difference in the Ca concentration of Ermelo soils in June and July compared to pre-planting soils. There was no significant difference in the Ca concentration of soils collected after the harvest of the Agpal cultivar from April to July. However, the Ca from each subsequent harvest was significantly higher than pre-planting soils. In Kaydale, soils collected after one month of planting the Ermelo cultivar had a significantly lower Ca concentration than pre-planting soils. Soils collected from May to July from the Ermelo cultivar differed significantly from pre-planting soils. In Rensburg soils, soil collected after the harvest of the Ermelo and Agpal cultivars from April to July was significantly lower than pre-planting soils.

Month, cultivar, and site had a significant interaction effect on the Mg concentration ($Df= 1$, $F= 33.382$, $P<0.0001$). In Jameson Park, the Mg concentration of Ermelo soils collected in April and May was significantly higher than pre-planting soils. However, no significant differences were observed in the Mg concentrations of Ermelo soils in June and July and pre-planting soils. For the Agpal cultivar, soils collected from April to July had a significantly higher Mg concentration than pre-planting soils. There were no significant differences in Ermelo soils collected from April, May, and July and pre-planting soils from Kaydale. Ermelo soils harvested in June had a significantly higher Mg concentration than pre-planting soils from Kaydale. Agpal soils collected in April, May, and July did not differ significantly from pre-planting soils. However, soils in June had significantly lower Mg concentrations than pre-planting soils. In Rensburg soils, Ermelo and Agpal soils collected from April to July had a significantly lower Mg concentration than pre-planting soils. In Jameson Park, no significant differences were observed in the Zn concentrations of Ermelo and Agpal soils collected in June and July. The Zn concentration of Agpal soils collected from April, June, and July was significantly higher than pre-planting soils. However, for the Ermelo cultivar, the Zn concentration in April was significantly higher than in pre-planting soils, and soils collected in

June and July were significantly lower than in pre-planting soils. In Kaydale soils, soils collected from April to July of the Ermelo and Agpal harvest had a significantly lower Zn concentration than pre-planting soils. In Rensburg soils, no significant differences were observed in the Zn concentration of soils collected in April, June, and July; however, each subsequent harvest was significantly lower than before planting the Ermelo cultivar. For the Agpal cultivar, there were no significant differences in the Zn concentration of soils collected in April, May, June, and July. Additionally, the soils collected from April to July, after the harvest of the Agpal cultivar, were significantly lower than pre-planting soils.

Month, cultivar, and site had a significant interaction effect on the Cu concentration ($Df= 1$, $F= 20.576$, $p<0.0001$). In Jameson Park, the Cu concentration of soils collected after one month (April) of planting the Ermelo cultivar was significantly higher than in May to July and pre-planting soils. Ermelo soils collected from May to July were significantly lower than pre-planting soils. For the Agpal cultivar, the Cu concentration of soils collected from April to July was significantly higher than pre-planting soils. In Kaydale, soils collected after planting the Ermelo cultivar for one month had a significantly higher Cu concentration than pre-planting soils, while soils collected from May to July had a significantly lower concentration than pre-planting soils. No significant differences were observed in the Cu concentration of Agpal soils collected from April to July and pre-planting soils. In Rensburg, there was no significant difference in the Cu concentration of Ermelo soils collected in April, May, and July. The Cu concentration from April to July was significantly lower than pre-planting soils. Month and site had a significant interaction effect on pH ($Df= 1$, $F= 4.771$, $p= 0.03225$). In Jameson Park soils, the pH of Ermelo soils collected from May to July were significantly higher than pre-planting soils. For the Agpal cultivar, only soils collected from April to June had a significantly higher pH than pre-planting soils. In Kaydale, Ermelo soils collected from May to July were significantly higher than pre-planting soils. Significant differences were observed in the pH of Agpal soils collected from April and May. However, there were no significant differences in the pH of soils collected from April, May, and July and pre-planting soils. In Rensburg, no significant differences were observed in Ermelo soils collected from April, June, and July, as well as pre-planting soils. Agpal soils collected in April and May had a significantly higher pH than pre-planting soils. However, no significant differences were observed in soils collected in June and July or in pre-planting soils.

343 Month, site, and cultivar significantly affected the total cation concentration ($Df= 1$, $F= 33.382$,
344 $p<0.0001$). In Jameson Park, soils collected after the harvest of the Ermelo cultivar from April
345 and May were significantly higher than pre-planting soils. However, no significant differences
346 were observed in the cation concentration from June and July and the pre-planting soils. The
347 total cation concentration of soils collected from April to July after the harvest of the Agpal
348 cultivar was significantly higher than pre-planting soils. In Kaydale, there was no significant
349 difference in the cation concentration of soils collected after the harvest of the Ermelo cultivar
350 in May to July and pre-planting soils. Agpal soils harvested from April, June, and July had
351 significantly lower total cation concentrations than pre-planting soils. In Rensburg, the soils
352 collected after each subsequent harvest differed significantly from each other and were
353 significantly lower than pre-planting soils. Agpal soils collected from April to July had a
354 significantly lower total cation concentration than pre-planting soils. Month, site, and cultivar
355 had a significant interaction effect on exchange acidity ($Df= 1$, $F= 8.818$, $p= 0.00407$). In
356 Jameson Park, there were no significant differences in the exchange acidity of Ermelo soils
357 collected in April and June and pre-planting soils. Agpal soils collected in May and June did
358 not differ significantly from pre-planting soils. In Kaydale, Ermelo, and Agpal soils from April
359 to July, the exchange acidity did not differ significantly from pre-planting soils. In Rensburg,
360 the exchange acidity of Ermelo soils from April to July did not differ significantly from pre-
361 planting soils. Soils collected in July after harvesting the Agpal cultivar had a significantly
362 higher exchange acidity than pre-planting soils.

Table 3: Soil characteristics of *Eragrostis curvula* Ermelo and Agpal cultivars grown in soils collected from Heidelberg soils for four months. Where site 1 represents Jameson Park, site 2 represents Kaydale and site 3 represents Rensburg. Values represent mean±SE, different letters denote statistical differences after a one-way ANOVA test.

			Ermelo cultivar				Agpal cultivar			
Nutrient concentrations (mg/L)	Site	Initial	April	May	June	July	April	May	June	July
Phosphorus	1 2 3	16.67±0.58 ^a 16.67 ± 2.08 ^a 18.33 ± 0.58 ^a	13.97±1.05 ^a 4.90±1.15 ^c 12.03±3.75 ^a	15.03±3.15 ^a 8.97±2.40 ^a 15.93±2.44 ^a	8.59±2.42 ^b 12.25±2.63 ^a 11.53±0.57 ⁱ	9.40±2.42 ^a 6.37±1.08 ^d 13.43±2.68 ^a	15.00±2.65 ^a 7.67±3.74 ^e 13.40±3.82 ^a	16.43±4.17 ^a 10.02±0.05 ^f 15.87±4.46 ^a	12.83±3.45 ^a 4.97±1.25 ^g 10.16±1.40 ^j	13.76±1.76 ^a 5.27±0.83 ^h 3.50±0.95 ^k
Potassium	1 2 3	161.33±4.04 ^a 305.33±42.67 ^t 231.33±13.58 ^y	245.99±1.74 ^l 239.00±11.14 ^u 186.02±2.22 ^z	143.80±1.59 ^m 225.98±0.44 ^u 217.30±0.67 ^{ab}	71.67±1.53 ⁿ 189.02±0.05 ^v 167.87±0.97 ^{ac}	80.97±1.53 ^o 184.96±1.02 ^v 166.24±0.49 ^{ac}	228.33±3.06 ^p 230.95±1.48 ^w 195.80±3.13 ^{ad}	161.00±2.00 ^q 242.89±2.09 ^w 204.84±3.84 ^{ad}	206.33±0.58 ^r 188.05±1.02 ^x 153.41±4.37 ^{ac}	208.65±1.17 ^s 196.32±3.55 ^x 214.07±13.93 ^{af}
Nitrogen	1 2 3	5.00±0.17 ^{ag} 4.30±0.30 ^{an} 11.77±0.58 ^{au}	2017.33±48.60 ^{ah} 735±24.27 ^{ao} 563.67±62.16 ^{av}	1105.33±167.44 ^{ai} 924.00±40.45 ^{ao} 984.67±33.50 ^{aw}	1177.33±287.46 ^{ai} 1437.33±186.95 ^{ap} 763±65.60 ^{aw}	2033.00±36.37 ^{aj} 1023.67±81.00 ^{aq} 1404.33±188.70 ^{ax}	1596±112.65 ^{ak} 837.30±32.40 ^{ar} 659.71±47.29 ^{ay}	1145±134.89 ^{al} 1238±256.38 ^{as} 870.43±44.21 ^{az}	1488.67±95.04 ^{am} 720.47±17.56 ^{at} 675.30±22.50 ^{ba}	2158.32±140.58 ^{an} 993.67±58.43 ^{at} 746.73±20.18 ^{ba}
Calcium	1 2 3	540.67±23.50 ^{bd} 882.22±60.35 ^{bh} 1353.33±31.56 ^{bn}	1048.63±43.90 ^{be} 610.00±66.56 ^{bi} 682.96±19.08 ^{bo}	944.14±57.69 ^{be} 863.20±54.23 ^{bj} 833.63±4.99 ^{bp}	522.39±40.47 ^{bf} 927.93±48.71 ^{bj} 572.67±12.10 ^{bq}	543.97±12.83 ^{bf} 861.27±45.19 ^{bj} 1112.33±118.02 ^{br}	1052.17±45.47 ^{bg} 607.97±16.05 ^{bk} 668.00±19.00 ^{bs}	1031.40±7.41 ^{bg} 781.33±21.36 ^{bl} 721.33±20.31 ^{bs}	1006.43±12.35 ^{bg} 526.33±24.11 ^{bm} 590.67±6.81 ^{bt}	1051.33±81.75 ^{bg} 569.67±37.50 ^{bm} 552.00±41.73 ^{bt}
Magnesium	1 2 3	120.00±6.08 ^{bu} 216.67±18.50 ^{cd} 432.67±6.03 ^{cj}	306.00±5.57 ^{bv} 191.33±8.50 ^{ce} 175.67±12.22 ^{ck}	213.63±15.06 ^{bw} 257.33±23.03 ^{cf} 224.50±11.17 ^{cl}	111.00±10.54 ^{bx} 286.67±10.50 ^{cf} 154.00±23.52 ^{cm}	127.13±1.86 ^{bx} 210.70±10.45 ^{cg} 337.30±23.07 ^{cn}	308.73±9.72 ^{bz} 196.00±12.53 ^{ch} 177.00±23.07 ^{co}	267.60±18.40 ^{ca} 224.09±7.23 ^{ch} 221.92±4.31 ^{cp}	295.70±4.56 ^{cb} 175.45±11.24 ^{ci} 160.58±0.71 ^{cq}	329.67±2.08 ^{cc} 195.02±0.05 ^{ci} 173.58±0.71 ^{cq}
Zinc	1 2 3	9.23±0.21 ^{cr} 20.27±1.58 ^{cz} 12.33±0.96 ^{df}	15.70±0.10 ^{cs} 6.23±0.21 ^{da} 8.42±0.62 ^{dg}	9.27±0.25 ^{ct} 11.89±0.16 ^{db} 9.53±0.57 ^{dg}	6.86±0.31 ^{cu} 13.53±0.50 ^{db} 8.07±0.21 ^{dg}	7.23±0.33 ^{cu} 14.06±0.47 ^{db} 10.36±0.62 ^{dh}	13.70±0.66 ^{cv} 9.10±0.36 ^{dc} 7.93±0.25 ^{di}	10.47±0.50 ^{cw} 11.53±0.50 ^{dd} 9.06±0.06 ^{di}	14.70±0.56 ^{cx} 6.23±0.21 ^{de} 7.30±0.18 ^{dj}	15.07±0.31 ^{cy} 7.67±0.35 ^{de} 6.26±0.23 ^{di}
Copper	1 2 3	5.00±0.17 ^{dj} 4.30±0.30 ^{dq} 11.77±0.58 ^{dv}	14.40±0.40 ^{dk} 4.23±0.20 ^{dq} 4.78±0.71 ^{dw}	5.85±0.15 ^{dl} 3.43±0.40 ^{dr} 5.36±0.12 ^{dw}	3.90±0.30 ^{dm} 9.88±0.16 ^{ds} 4.50±0.64 ^{dw}	4.01±0.01 ^{dm} 3.69±0.16 ^{dt} 8.72±0.76 ^{dx}	7.00±0.01 ^{dn} 4.53±0.41 ^{du} 3.85±0.45 ^{dz}	11.00±0.30 ^{do} 3.88±0.14 ^{du} 4.90±0.33 ^{ea}	11.35±0.45 ^{do} 4.01±0.19 ^{du} 3.89±0.12 ^{eb}	12.45±0.55 ^{dp} 3.47±0.45 ^{du} 3.96±0.12 ^{eb}
Relative acidity										

pH	1 2 3	4.72±0.04 ^{ec} 4.88±0.01 ^{eh} 5.06±0.02 ^{eo}	5.01±0.02 ^{ec} 4.82±0.07 ^{eh} 5.03±0.19 ^{eo}	6.01±0.04 ^{ed} 5.46±0.03 ^{ei} 5.60±0.06 ^{ep}	5.89±0.35 ^{ed} 5.19±0.10 ^{ej} 5.42±0.16 ^{ep}	5.73±0.06 ^{ed} 5.44±0.06 ^{ek} 5.41±0.36 ^{ep}	5.26±0.13 ^{ee} 4.98±0.07 ^{el} 5.73±0.23 ^{eq}	6.06±0.30 ^{ef} 5.40±0.08 ^{em} 5.62±0.07 ^{eq}	5.18±0.12 ^{eg} 4.93±0.06 ^{en} 5.24±0.21 ^{eq}	5.02±0.07 ^{eg} 5.02±0.10 ^{en} 4.94±0.05 ^{eq}
Total cations concentration	1 2 3	4.18±0.16 ^{er} 7.05±0.54 ^{ew} 10.98±0.19 ^{fd}	8.65±0.65 ^{es} 5.33±0.09 ^{ex} 4.86±0.71 ^{fe}	6.76±1.10 ^{et} 7.19±0.20 ^{ey} 6.64±0.25 ^{ff}	3.65±0.37 ^{eu} 7.70±0.10 ^{ey} 4.51±0.48 ^{fg}	4.05±0.25 ^{eu} 6.72±0.24 ^{ez} 8.72±0.66 ^{fh}	8.37±0.35 ^{ev} 5.22±0.05 ^{fa} 5.45±0.25 ^{fi}	7.82±0.91 ^{ev} 6.43±0.45 ^{fb} 6.16±0.34 ^{fi}	8.09±0.11 ^{ev} 4.64±0.35 ^{fc} 4.79±0.19 ^{fk}	8.59±1.00 ^{ev} 5.08±0.11 ^{fc} 5.00±0.01 ^{fk}
Exchange acidity	1 2 3	0.08±0.01 ^{fl} 0.09±0.01 ^{fq} 0.07±0.03 ^{fs}	0.08±0.01 ^{fl} 0.09±0.01 ^{fq} 0.03±0.01 ^{fs}	0.04±0.02 ^{fm} 0.06±0.01 ^{fq} 0.06±0.02 ^{fs}	0.04±0.01 ^{fm} 0.10±0.02 ^{fq} 0.07±0.01 ^{fs}	0.07±0.01 ^{fm} 0.07±0.02 ^{fq} 0.04±0.01 ^{fs}	0.06±0.01 ^{fo} 0.08±0.02 ^{fr} 0.04±0.005 ^{ft}	0.075±0.01 ^{fp} 0.08±0.02 ^{fr} 0.05±0.01 ^{ft}	0.08±0.001 ^{fp} 0.06±0.01 ^{fr} 0.05±0.01 ^{ft}	0.06±0.003 ^{fp} 0.04±0.03 ^{fr} 0.23±0.03 ^{fu}

3.4. Correlations between soil characteristics, extracellular enzymes, and soil nutrients

The correlations between soil characteristics and extracellular enzymes of Ermelo and Agpal associated soils from Jameson Park, Kaydale, and Rensburg are represented in Figure 2. The first PCA explained 25.3% of the total variation, while PCA2 explained 31.9% of the total variation. There was a separation of clusters for Agpal-Jameson Park and Agpal-Kaydale and Agpal-Jameson Park and Agpal-Rensburg, indicating that the Agpal growing in Jameson Park soils had a varying effect on soil characteristics and extracellular enzymes compared to Agpal growing in Kaydale and Rensburg soils. There were overlapping clusters for the Ermelo cultivar across all study sites, indicating that the cultivar had the same influence on the soil characteristics and extracellular enzymes. The groups closer together showed high correlations.

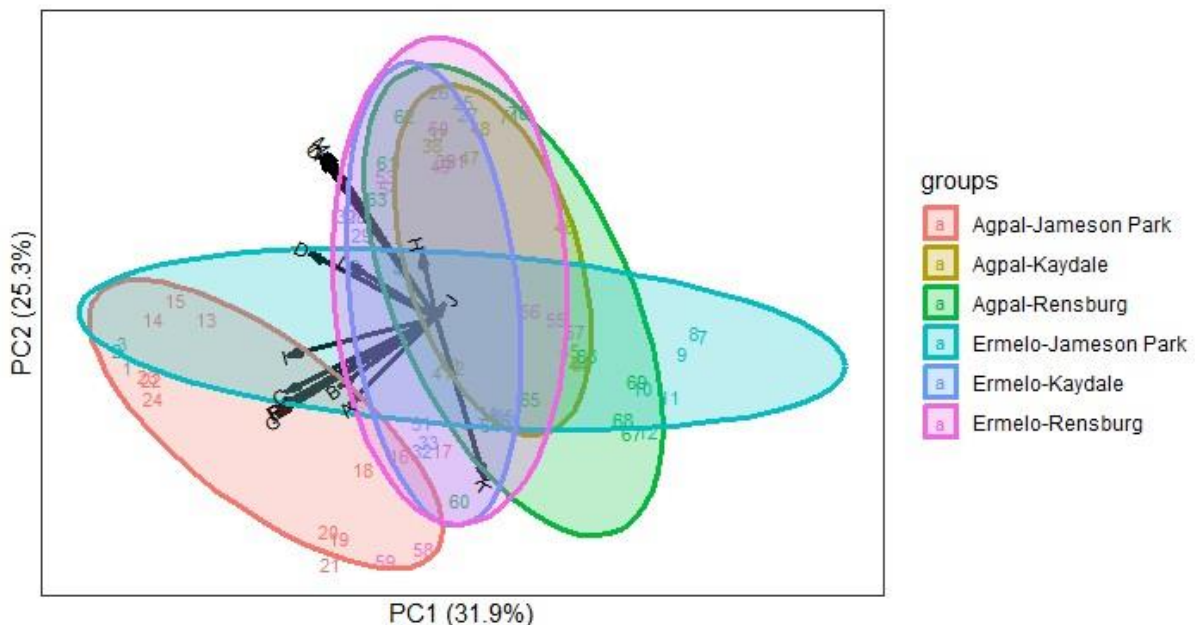


Figure 2: Correlations between the soil characteristics and extracellular enzyme activities in Agpal and Ermelo soils from Jameson Park, Kaydale, and Rensburg, Heidelberg, Gauteng. Different letters represent the following: A= nitrogen concentration, B= phosphorus concentration, C= magnesium concentration, D= potassium concentration, E= zinc concentration, F= copper concentration, G= calcium concentration, H= pH, I= total cation concentration, J= exchange acidity, K= nitrate reductase activity, L= N-acetylglucosaminidase activity, M= Acid phosphatase activity, N= alkaline phosphatase activity, and O= β -glucosidase activity. Principal component analysis (PCA)

4. 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 [15] and [33], various species and cultivars produce distinct root exudates that attract diverse microbes and enable plants to thrive in different environments [34]. 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 (Figure 1). Root exudates provide carbon for microorganisms [35], which may have led metabolically inactive/dormant bacteria to “wake” up [36], leading to the increased diversity in the bacterial isolates, as reported in Figure 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 [37], who reported that host-microbe interactions affected the microbial diversity of wild and domesticated *Hordeum vulgare* rhizosphere soils. According to [38], 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 [39-42]. 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 [43-45].

Plant growth-promoting rhizobacteria enhance nutrient acquisition by secreting extracellular enzymes that play a role in soil nutrient cycling [46]. Extracellular enzymes such as β -glucosidase, N-acetylglucosaminidase, nitrate reductase, and acid and alkaline phosphatase play a significant role in soil carbon (C), N and P cycling [47]. 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 [11]. According to [11], *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 [48] 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 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 was a difference 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 [49], β -glucosidase plays a role in the degradation of glucosides present in plant debris, which may have prompted an increased mobilisation of mineral elements [50]. 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 [51] reported that N fertilisation increases phosphatase activity, thus, the increase in soil N may have led to an increase in the alkaline phosphatase activity.

According to [52], 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 [53]. Furthermore, the genes encoding alkaline phosphatase activity regulate P starvation, making alkaline phosphatase dependent on soil N and P [52]. 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 correlation of soil P and alkaline phosphatase activity is supported by the resource allocation model for extracellular enzymes, which suggests that microbes invest resources to synthesise enzymes that acquire deficient nutrients [54]. Li et al [52] supported this inverse correlation and reported a higher acid and alkaline phosphatase activity in soils with a low P concentration. Though the alkaline phosphatase activity has been reported to be more active in pH 9-11 [55], Bergkemper et al [56] 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 [57]. 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. Philippot et al [58] 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 [59], which could have influenced the reduction of total cation, Zn, Cu, Mg, and Ca concentrations in Ermelo associated soils compared to Agpal soils.

The principal component analysis shows how the Ermelo cultivar uniformly influenced the soil characteristics and extracellular enzyme activities across study sites. This may indicate that the exudates produced by this cultivar had a more substantial selection effect on the bacterial diversity and consequently affected the associated extracellular enzyme activities and soil characteristics. The Agpal cultivar growing in Jameson Park, Kaydale, and Rensburg had varying influences on the soil characteristics and extracellular enzymes, which may suggest that both plant exudates and site played a role in the selection of bacterial communities, which consequently affected the enzyme activities and soil nutrition. According to [13], environmental filters such as soil properties and abiotic factors play a role in the selection of bacterial communities. In addition, [60] 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 Agpal cultivar's soil characteristics, bacterial diversity, and soil nutrition. 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, [61] reported that 62% of total rhizodeposition was mineralised 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 [62] 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. However, supplementary figure 2 shows how *E. curvula* roots grow deep within soils and penetrate different soil depths. Being a perennial plant, *E. curvula* can improve soil properties due to fibrous and extensive root systems [63]. Thus, we can deduce the deep fibrous root systems of the Ermelo and Agpal cultivars influenced rhizodeposition,

consequently affecting the bacterial diversity, associated enzyme activities, and soil characteristics. While the findings of this study provide insights into the influence of *E. curvula* on soil nutrient cycling, the isolation and identification of the metabolite in root exudates would provide further insights into the effects of rhizodeposition on bacterial diversity, enzyme activities, and soil characteristics. Thus, future studies can investigate the effects of *E. curvula* rhizodeposition on soil nutrient inputs. The findings of this study highlight how different cultivars affect microbial community structure and show how the wide distribution and growth of *E. curvula* in diverse environmental conditions may be linked to the role of bacterial communities and associated enzyme activities in nutrient cycling.

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Declarations

Ethics approval and consent to participate 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.

Competing interests The authors declare no competing interests.

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: ***Eragrostis curvula* cultivars improve bacterial diversity, extracellular enzyme activities, and nutrition in grassland ecosystem soils**. Also, all raw data can be requested from the corresponding author, Prof. Anathi Magadlela at anathimagadlela@icloud.com.

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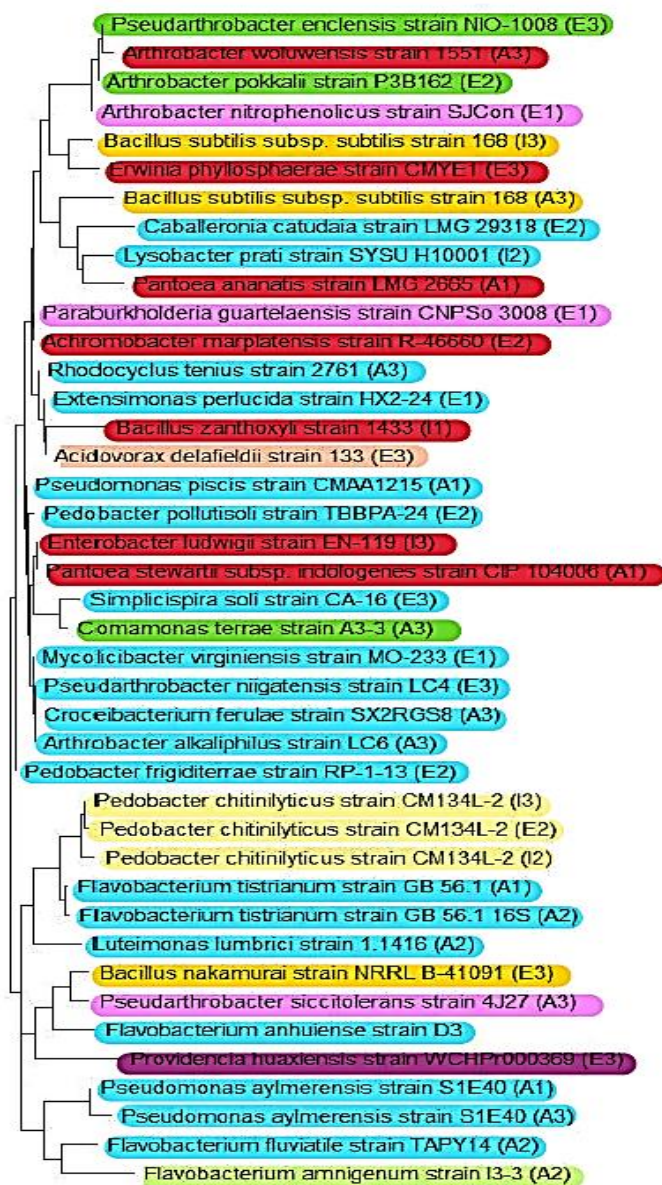
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671 Supplementary Figure 1: Phylogenetic tree of bacteria isolated from Jameson Park (I1),
 672 Kaydale (I2) and Rensburg (I3) soils before and after planting *Eragrostis curvula* Ermelo (E1:
 673 Jameson Park, E2: Kaydale and E3: Rensburg soils) and Agpal cultivar (A1: Jameson Park,
 674 A2: Kaydale and A3: Rensburg soils). The colours denote different functional traits where
 675 green: IAA production, red: phosphorus solubilisation, yellow: phosphorus solubilisation and
 676 nitrogen fixation, pink: nitrogen fixation, peach: siderophore production, light yellow: chitin
 677 degradation, purple: potassium solubilisation, light green: nitrogen cycling, and blue: bacteria
 678 isolated whose plant related functional traits are not reported in literature. *Flavobacterium*
 679 *anhuiense* strain D3 was isolated in Kaydale and Rensburg soils before and after planting both
 680 cultivars.

681 Supplementary Figure 2



682
683 Supplementary Figure 2: *Eragrostis curvula* Agpal (A) and Ermelo (B) cultivars with deep
684 roots penetrating soils at different depths