Scientific African 29 (2025) e02879

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Encephalartos lanatus-associated bacteria and extracellular enzyme activities improve soil nutrition in nutrient-deficient grassland ecosystems

Memory Sigasa [a,1], Anathi Magadlela [b,c], María A. Pérez-Fernández [d], Wilfred Otang-Mbeng [e], Terence N. Suinyuy [c,e,*]

[a] *School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Mbombela 1200, South Africa*
[b] *Centre for Global Change, Faculty of Natural and Applied Sciences, Sol Plaatje University, Private Bag X5008, Kimberley 8300, South Africa*
[c] *School of Life Sciences, College of Agriculture, Engineering and Sciences, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa*
[d] *Department of Physical, Chemical and Natural Systems, Universidad Pablo de Olavide, Carretera de Utrera Km 1, 41013 Seville, Spain*
[e] *School of Biology and Environmental Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Mbombela 1200, South Africa*

ARTICLE INFO

Editor: DR B Gyampoh

Keywords:
African cycads
Coralloid roots
Cycad-microbe-symbiosis
Nitrogen-fixation
Soil nutrient cycling

ABSTRACT

Encephalartos lanatus, commonly called 'Olifants River Cycad', is slow-growing and thrives in nutrient-deficient and acidic grassland soils. Like other *Encephalartos* spp., *E. lanatus* possess coralloid roots that host nutrient cycling microbes that may enhance soil and plant health and landscape productivity. However, identification of these microbes and their role in soil nutrient improvement have been documented for forest and savanna woodland *Encephalartos* spp., with limited knowledge on grassland species. The knowledge gap challenges the development of a holistic conservation strategy for grassland cycads. This study identified *E. lanatus* coralloid roots, rhizosphere, and bulk soils bacterial communities, and assessed the nutrient status and enzyme activities of both soils. Bulk and rhizosphere soils, and *E. lanatus* coralloid roots were sampled from a > 300 *E. lanatus* plant population growing in a rocky grassland at Botshabelo in Middleburg, Mpumalanga for nutrient analysis, enzyme activity assays, and bacterial identification. Nutrient concentration was higher in rhizosphere (20.54±3.40) than bulk (17.47±3.24) soils but not significantly different ($p = 0.55$). Concentration of magnesium and manganese were significantly higher ($p < 0.05$) in rhizosphere than bulk soils. Nitrogen, phosphorus, potassium and calcium were higher in rhizosphere than bulk soils but did not differ significantly ($p > 0.05$). Acid phosphatase and nitrate reductase enzyme activities were significantly ($p < 0.05$) higher in rhizosphere than bulk soils. Nutrient cycling bacteria of the genera *Bacillus, Burkholderia, Enterobacter, Paraburkholderia, Pseudomonas* and *Rhizobium* characterised coralloid roots, rhizosphere and bulk soils bacterial communities. The high nitrogen, phosphorus and potassium concentrations in rhizosphere as compared to bulk soils suggest that nutrient cycling bacteria in the coralloid roots, rhizosphere and bulk soils, and increased enzyme activities may be contributing to improving soil and *E. lanatus* health and productivity in nutrient-poor grassland

* Corresponding author.
E-mail address: Terence.Suinyuy@ump.ac.za (T.N. Suinyuy).
[1] Current Address: Agricultural Research Council, Institute for Tropical and Subtropical Crops, Private Bag X11208, Mbombela, 1200, South Africa.

https://doi.org/10.1016/j.sciaf.2025.e02879
Received 1 March 2025; Received in revised form 3 August 2025; Accepted 5 August 2025
Available online 6 August 2025

ecosystem. This underscores the role of *E. lanatus* in soil nutrient improvement and the need to develop holistic conservation approaches for grassland cycads.

Introduction

Cycads are the only gymnosperms that can establish a symbiotic relationship with nitrogen-fixing bacteria [1], and are among the most endangered plant groups globally, with over 70 % of cycad species listed as threatened in the 1997 IUCN Red List of Threatened Plants. According to Raimondo et al. [2], all species in the *Encephalartos* genus are included in the Red List of South African Plants, underscoring their conservation priority. This long-standing threat status, dating back decades, highlights the persistent and ongoing pressures on cycad populations. The grassland cycad *Encephalartos lanatus*, which is endemic to South Africa, has been reclassified as "Vulnerable" under the IUCN Red List criteria B1ab(v)+2ab(v) [3]. The extinction of species like *E. lanatus* would not only represent a loss of evolutionary heritage but would also disrupt essential ecosystem services such as carbon sequestration [4], nutrient cycling, and soil enrichment [5], which underpins the health and productivity of different ecosystems. Conserving *E. lanatus* is therefore critical, not only for preserving biodiversity but also for sustaining the ecological integrity of the threatened habitats it occupies.

Cycads such as *Cycas micronesica* have been reported to enhance soil fertility [6], highlighting the ecological significance of cycad species within their native environments. Studies by Ndlovu et al. [7] and Motsomane et al. [8] have further demonstrated that bacteria associated with the rhizosphere of *Encephalartos* species and their extracellular enzymes play a significant role in nutrient cycling. These findings provide a more comprehensive understanding of cycad–bacteria associations and their functional contributions to ecosystem nutrient dynamics. Extracellular enzymes secreted by soil microorganisms are critical in degrading complex organic matter into simpler, bioavailable forms that support microbial and plant nutrient uptake [9]. These enzymes mediate essential biogeochemical processes involved in the cycling, mineralisation, and regulation of key nutrients such as carbon (C), nitrogen (N), and phosphorus (P). Enzymes such as β-d-glucosidase facilitate C cycling by breaking down cellulose into glucose [10], while β-(D)-glucosaminidase and asparaginase catalyse the hydrolysis of chito-oligosaccharides and the conversion of asparagine into ammonia (NH_3) and aspartic acid (C_4H_7NO), respectively, processes through which N is made available in the soil [11].

Cycad-associated microbial communities include both cyanobacteria and a variety of bacterial taxa [12–14] that contribute to plant health and productivity. Several studies have characterised microbes associated with the coralloid roots of cycads belonging to the genera *Ceratozamia, Dioon, Lepidozamia, Macrozamia,* and *Cycas* [5,12–14]. However, these in-depth analyses display a geographical bias, as the studied species are distributed in the Americas and Australasia. In contrast, African cycads, particularly those within the *Encephalartos* genus, remain significantly understudied. Furthermore, studies on the contributions of cycads to soil fertility have predominantly focused on *Cycas* species [6], leaving a significant knowledge gap concerning *Encephalartos* species and how their associated bacterial communities regulate nutrient cycling and support ecosystem functioning, particularly in the nutrient-poor habitats where they occur in nature.

The findings of this study will bridge the existing knowledge gap by advancing our understanding of biodiversity and ecosystem service enhancement. This aligns with key conservation frameworks, including the Mpumalanga Biodiversity Sector Plan (MBSP) of 2015 [15], South Africa's National Biodiversity Strategy and Action Plan (NBSAP) 2015–2025, and the Convention on Biological Diversity (CBD) Strategic Plan for Biodiversity 2011–2020 and 2030–2050 [16], particularly Targets 14 and 15 of the Aichi Biodiversity Targets [17]. Additionally, the study will support conservation strategies for South African *Encephalartos* and other African cycads at large, particularly *Encephalartos* an African endemic genus and the most threatened cycad group comprising 65 species [18], of which 37 (over 50 %) are found exclusively in South Africa. Thus, this study aims to identify the bacterial communities associated with the coralloid roots of *E. lanatus* and those present in soils beneath and beyond its canopy, and to evaluate the species' contribution to soil nutrient dynamics in a nutrient-deficient grassland ecosystem. We hypothesize that the rhizosphere soils of *E. lanatus,* which are expected to host a more diverse bacterial community, have higher enzymatic activities involved in nitrogen and carbon cycling compared to bulk soils, thereby contributing to higher nutrient concentrations.

Materials and methods

Sites for collecting soil samples

The study was conducted in the Botshabelo Nature Reserve in Botshabelo Cultural Village, Middelburg District in Mpumalanga Province (coordinates are omitted due to cycads conservation concerns). The reserve lies within the mesic highveld grassland bioregion, which experiences rainfall in summer. Botshabelo Cultural Village is situated in the northern region of Middelburg with an annual rainfall of over 700 mm. The average annual summer and winter temperatures are 27.2 °C and 15.5 °C, respectively. The landscape is rocky and grassy and is occupied by baboons that feed on *E. lanatus* cones leaving droppings and urine in the soil.

Soil sampling

Sampling was conducted as per Marler and Calonje [6] and Marler and Krishnapillai [19]. Rhizosphere soils were defined as soils collected directly beneath the canopy of *E. lanatus*, while bulk soils were collected approximately five meters away from the plant canopy. Rhizosphere and bulk soils were collected at four main cardinal points around 10 plants, resulting in 10 replicates for the

treatment (rhizosphere soils) and control (bulk soils). Rhizosphere soils were collected approximately 10 – 20 cm away from the stem and at points within the leaves' canopy dripline at 0 - 10 cm and 10 - 20 cm depths, where the coralloid roots commonly occur. Bulk soils were collected five meters from the selected plants at the same depths. The collected soil samples were stored in sterile Ziploc bags and transported to the School of Life Sciences laboratory, University of KwaZulu-Natal for further analysis.

Physicochemical analysis

Twenty subsamples (500 g each) from rhizosphere and bulk soils were air-dried, pass through and 2 mm sieve, and submitted to the Department of Agriculture and Rural Development Analytical Services, Cedara, South Africa, for soil physicochemical property analyses. Chemical properties of the rhizosphere and bulk soils were determined following the procedures outlined by Manson et al. [20]. Total N concentration was quantified using Automated Dumas dry combustion and a LECO CNS 2000 (Leco Corporation, USA). The P and potassium (K) concentrations were calculated using the atomic absorption method, where 2.5 ml of the soil solution was mixed with 2.5 ml of ambic-2 solution at pH 8. Subsequently, the mixture was stirred using a multiple stirrer set at 400 rpm for ten minutes, and Whatman no 1 paper was used for filtering. The calcium (Ca) and magnesium (Mg) concentrations were measured using a similar procedure; however, 25 ml of 1 M KCl solution was used as a substitute for the ambic-2 solution. Soil pH was measured by mixing 10 mL of soil with 25 mL of 1 M KCl solution and stirring at 400 rpm for 5 min using a multi-stirrer. A gel-filled combination electrode was used to determine the pH of the suspension during agitation.

Collection of coralloid roots

After soil collection, coralloid roots were collected from the selected plants. The coralloid roots were washed in sterile distilled water, disinfected with 70 % (v/v) ethanol for 30 s, and soaked in 3.5 % (v/v) sodium hypochlorite solution for 3 min. Sterilised coralloid roots were submerged in 15 % glycerol and stored in the fridge until further analysis.

Extraction, sequencing, and amplification of bacteria from E. lanatus rhizosphere, bulk soils, and coralloid roots

Soil serial dilutions were conducted to extract bacteria from fresh soil subsamples (rhizosphere and bulk soils), and coralloid roots were submerged in 15 % glycerol and squashed with sterile tips to extract the bacteria. One hundred microlitres of serial dilution were inoculated in sterile petri dishes containing Tricalcium phosphate (P-solubilising bacteria), Simmons citrate (N-cycling), and Jensen (N-fixing) media. Each selective medium dish had three replicates. Ten microlitres of the coralloid root suspension were inoculated into sterile petri dishes containing yeast mannitol agar and replicated three times. The petri dishes were incubated at a temperature of 27 °C for 3 – 12 days. Pure bacterial colonies were obtained through subculturing.

After bacterial extraction, a Polymerase chain reaction (PCR) was used to amplify a small segment of pure bacterial colonies utilising the 16S rRNA gene primers: 63F (5′ CAGGCCTAACACATGCAAGTC 3′) and 1387R (5′-GGGCGGTGTGTACAAGGC 3′) as per protocols by [21]. EmaraldAmp GT Master Mix was used for PCR amplification under the following conditions: initial denaturation at 94 °C for five minutes, followed by thirty cycles of denaturation at 94 °C for 30 s, 30 s of annealing at 55 °C, two minutes of extension at 72 °C and the final elongation was performed at 72 °C for 10 min. Amplification products were viewed on 1 % (w/v) agarose gel electrophoresis conducted at 100 V for 60 min utilizing a Tris-acetate-EDTA (TAE) buffer under ultraviolet light. The amplified products were sent to Inqaba Biotechnical Industries (Pty) Ltd Pretoria, South Africa, for sequencing. The obtained DNA sequences were subjected to Nucleotide BLAST (NCBI) to find related bacteria in the GenBank database (www.ncbi.nih.gov).

Enzyme activities

Alkaline phosphatase, acid phosphatase, β-glucosidase, and N-acetylglucosaminidase activities were measured using the fluorescence-based technique outlined by Marler and Calonje [6]. Ten grams of soil and 100 ml of autoclaved water were placed inside jars wrapped with foil, homogenised in a shaker at a constant speed for 2 h, and kept overnight at 4 °C. Before being placed in the substrates, the supernatants were moved onto a black 96-well microplate. The sample run was a mixture made up of 200 µl soil aliquot and a 50 µl substrate that was close to the reference standards which consisted of 200 µl soil aliquot + 50 µl standard, a quench standard that had 200 µl soil aliquot + 50 µl standard, a sample control that had a mixture of 200 µl soil aliquot and 50 µl Buffer, a negative control which was a mixture of 200 µl buffer plus 50 µl standard and blanks consisting of only 250 µl buffer. After two hours of incubation at room temperature, 0.5 M NaOH was used to stop the reaction. The fluorescent absorbance was then quantified at 450 nm on a Glomax Multi Plus microplate reader. Before measuring acid phosphatase enzyme activity, the buffer and standard were adjusted to pH of 5.

The nitrate reductase activity was determined using a modified approach developed by Bruckner et al. [22]. An Erlenmeyer flask coated with foil was filled with 1 ml of 25 mM KNO_3, 4 ml of 0.9 mM 2,4-dinitrophenol, 5 ml of autoclaved water, and 5 g of soil were added to the solution. The flask was swirled and placed in an incubator for 24 h at 30 °C. After incubation, 10 ml of 4 M KCl was added to the mixture and filtered with Whatman number 1 filter paper into a new wrapped flask. To initiate the enzymatic reaction, 2 ml of the infiltrate was added to NH_4Cl buffer that had a pH of 8.5 and 0.8 ml of colour reagent containing 1 % of Sulfanilamide, 1 N HCl, and 0.02 % N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The solution was then incubated for 30 min at 30 °C. To measure the absorbance at 520 nm, an Agilent Cary 60 UV–Vis spectrophotometer was used.

Statistical analysis

The data on soil properties and enzyme activities in the rhizosphere and bulk were subjected to an Independent sample *t*-test to test for significant differences through Statistix 10 software, and the Shapiro-Wilk test was used to determine the normality of the residual distribution. Non-normal data were Log10 $(X + 1)$ and arcsine $\sqrt{(X \div 100)}$ for % variables transformed to improve normality. The homogeneity of variance assumption was tested using the F-test. Principal component analysis (PCA) was used to analyse the relationship between soil nutrients and the corresponding enzyme activities in both the rhizosphere and bulk soils of *E. lanatus*. The principal component analysis (PCA) was carried out utilizing RStudio software (version 3.6.0) with the assistance of the gg plot statistics package and the pr comp function.

Results

Soil physicochemical properties

The nutrient concentrations of the rhizosphere and bulk soils are presented in Table 1. The macro- and micro-nutrients in both soils occurred in varied concentrations, but the overall nutrient concentration in the rhizosphere soil (20.54±3.40; Mean ± *S*.E) was higher than in the bulk soil (17.47±3.24) and were not significantly different ($p > 0.05$). The magnesium (Mg) and manganese (Mn) concentrations were significantly higher in the rhizosphere than in bulk soils ($p < 0.05$) (Table 1).

There was a higher concentration of N in the rhizosphere compared to the bulk soils. However, the differences were not statistically significant ($t\,(18) = -0.92$, $p = 0.37$). Similarly, the P concentrations were higher in the rhizosphere than in the bulk soils, but the differences did not differ significantly ($t\,(18) = -1.53$, $p = 0.14$). Again, there was higher concentrations of K in the rhizosphere than the bulk soils, but no significant difference was found ($t\,(18) = -0.48$, $p = 0.64$). The concentration of Ca was higher in the rhizosphere compared to bulk soils, but the differences did not differ significantly ($t\,(18) = -0.41$, $p = 0.69$). There were no significant differences in copper (Cu) concentrations in the rhizosphere soils and bulk soils ($t\,(18) = -0.67$, $p = 0.51$). The zinc (Zn) concentrations [$t\,(18) = 0.89$, $p = 0.39$], pH [$t\,(18) = 1.45$, $p = 0.16$], and clay content [$t\,(18) = 0.89$, $p = 0.39$] of bulk soils were higher but not significantly different from those of rhizosphere soils. The total cation exchange capacity [$t\,(18) = -0.98$, $p = 0.34$], acid saturation [$t\,(18) = -0.01$, $p = 0.99$], total cation concentration [$t\,(18) = -1.77$, $p = 0.10$], and organic carbon content [$t\,(18) = -0.71$, $p = 0.48$] of rhizosphere soils were higher but did not significantly different from those bulk soils.

Identification of E. lanatus microbes from coralloid roots, rhizosphere, and bulk soils

The molecular identification of bacteria isolated from the coralloid roots is presented in Table 2. Five bacterial strains belonging to the families Pseudomonadaceae, Thermomonosporaceae, Burkholderiaceae, and Bacillaceae were isolated from the coralloid roots of *E. lanatus*.

Rhizosphere soils had 16 bacterial strains belonging to the families Burkholderiaceae (seven isolates), Rhizobiaceae (four isolates), Sphingobacteriaceae, Oxalobacteriaceae, and Enterobacteriaceae, with two isolates each (Table 3). The isolates in Rhizobiaceae had N_2-fixing traits, Oxalobacteriaceae isolates had P-solubilising traits, and Burkholderiaceae and Enterobacteriaceae isolates consisted of a diversity of N_2-fixing and P-solubilising traits (Table 3). A total of 14 bacterial strains isolated from bulk soils belonged to the Burkholderiaceae (six isolates), Rhizobiaceae (three isolates), Bacillaceae (two isolates), and Paenibacillaceae and Pseudomonadaceae (one isolate each) families (Table 4). The Rhizobiaceae family had N_2-fixing traits, while those in the family Bacillaceae,

Table 1
Soil nutrient concentration and relative acidity of *Encephalartos lanatus* rhizosphere and bulk soils in Botshabelo Cultural Village in Middleburg, Mpumalanga, South Africa.

Soil characteristics		Rhizosphere soils ($n = 10$)	Bulk soils ($n = 10$)
Macro-nutrients ($mg.kg^{-1}$)	*Primary nutrients*		
	Nitrogen	156.68±27.80[a]	124.88±20.58 [a]
	Phosphorus	13.81±2.94 [a]	8.92±2.29 [a]
	Potassium	1.13±0.01 [a]	1.12± 0.01[a]
	Secondary nutrients		
	Calcium	0.66±0.14 [a]	0.51±0.11 [a]
	Magnesium	1.29±0.12[a]	1.08±0.02[b]
Micro-nutrients ($mg.kg^{-1}$)	Manganese	16.96±5.19[a]	10.58±1.94[b]
	Copper	1.15±0.23 [a]	0.89±0.19 [a]
	Zinc	0.86±0.13 [a]	0.89±0.18 [a]
Soil relative acidity	pH (KCL)	3.84±0.03 [a]	3.92±0.05 [a]
	Acid saturation (%)	65.10±4.68 [a]	65.00±6.86 [a]
	Total cation exchange (cmol/kg)	3.09±0.31 [a]	2.42±0.22 [a]
	Exchangeable acidity (cmol/kg)	1.98±0.24 [a]	1.62±0.27 [a]
Other properties	Clay (%)	23.50±1.53 [a]	25.50±1.65 [a]
	Organic Carbon (%)	3.52±0.33 [a]	3.21±0.28 [a]

Values represent mean ± SE, $n = 10$, different letters indicate significant differences in soil physicochemical properties (Two sample *t*-test, $P \leq 0.05$).

Table 2
Molecular identification of the isolated bacteria in *Encephalartos lanatus* coralloid roots.

Family	Scientific name	Accession number	Similarity (%)	Function(s)
Pseudomonadaceae	*Pseudomonas putida*	OK037572.1	99.21	P-solubilizing
Thermomonosporaceae	*Actinoallomurus* sp.	KX533973.1	99.15	
Burkholderiaceae	*Burkholderia* sp.	MK559018.1	99.82	N-fixing
	Paraburkholderia sp.	MG182888.1	99.40	P-solubilizing
Bacillaceae	*Bacillus licheniformis*	MK418575.1	84.08	P-solubilizing N-fixing

Table 3
Molecular identification of the isolated bacteria in *Encephalartos lanatus* rhizosphere soils.

Family	Scientific name	Accession number	Similarity (%)	Function(s)
Rhizobiaceae	*Rhizobium miluonense*	MH236279.1	99.90	N-fixing
	Rhizobium tropici	MT539147.1	99.12	N-fixing
	Rhizobium miluonense	MT409530.1	97.43	N-fixing
	Agrobacterium rhizogenes	MN712240.1	96.28	Induce hairy root system
Sphingobacteriaceae	*Mucilaginibacter gossypiicola*	NR_116,406.1	94.48	
Oxalobacteriaceae	*Massilia phosphatilytica*	MK519185.1	98.61	P-solubilizing
	Massilia sp.	MH031738.1	96.46	P-solubilizing
Enterobacteriaceae	*Pantoea agglomerans*	MN758864.1	96.79	P-solubilizing N-fixing
	Enterobacter cloacae	KY660471.1	94.88	N-fixing P-solubilizing
Burkholderiaceae	*Burkholderia cenocepacia*	OR098458.1	97.80	P-solubilizing
	Caballeronia concitans	NR_145,603.1	97.51	N-fixing
	Paraburkholderia sp.	MZ031499.1	95.25	N-fixing P-solubilizing
	Burkholderia cepacia	MN691351.1	97.92	N-fixing P-solubilizing
	Paraburkholderia sabiae	MK139731.1	98.12	N-fixing
	Paraburkholderia fungorum	CP099647.1	99.34	N-fixing P-solubilizing
	Paraburkholderia lacunae	NR_165,707.1	99.40	N-fixing

Paenibacillaceae, and Pseudomonodaceae had P-solubilising traits. Based on Simpson's Diversity Index, it was observed that rhizosphere soils displayed greater ($D = 0.98$) species diversity in comparison to bulk soils ($D = 0.37$).

Soil extracellular enzyme activities

The extracellular enzyme activities of the rhizosphere and bulk soils are represented in Table 5. Acid phosphatase activity was significantly higher ($p < 0.05$) in the rhizosphere compared to bulk soils. Similarly, nitrate reductase exhibited higher enzymatic activity in the rhizosphere compared to the bulk soils ($p < 0.05$). In contrast, β-glucosidase activity was significantly higher ($p < 0.05$)

Table 4
Molecular identification of the isolated bacteria in *Encephalartos lanatus* bulk soils.

Family	Scientific name	Accession number	Similarity (%)	Function(s)
Rhizobiaceae	*Rhizobium tropici*	MT539147.1	99.65	N-fixing
	Rhizobium nepotum	MT533807.1	99.14	N-fixing
	Rhizobium sp.	MT269297.1	98.95	N-fixing
Acidobacteriaceae	*Terriglobus* sp.	KX555420.1	85.61	
Bacillaceae	*Bacillus licheniformis*	ON954648.1	99.67	P-solubilizing N-fixing
	Bacillus velezensis	MH040972.1	98.15	P-solubilizing N-fixing
Paenibacillaceae	*Paenibacillus* sp.	KY229692.1	94.09	P-solubilizing N-fixing
Pseudomonadaceae	*Pseudomonas koreensis*	MT409540.1	96.81	P-solubilizing
Burkholderiaceae	*Paraburkholderia* sp.	MT269305.1	99.66	P-solubilizing
	Paraburkholderia sp.	OM144561.1	97.67	P-solubilizing
	Paraburkholderia bengalensis	MT912698.2	97.51	N-fixing
	Trinickia sp.	LC661717.1	99.83	P-solubilizing
	Paraburkholderia atlantica	MK690530.1	96.31	N-fixing
	Burkholderia sp.	MK704288.1	97.39	N-fixing

Table 5
Soil enzyme activities of *Encephalartos lanatus* rhizosphere and bulk soils.

Enzyme Activity	Rhizosphere	Bulk soils
Acid phosphatase (nmolh^{-1} g^{-1})	0.49±0.03^{a}	0.40±0.04 ^{b}
Alkaline phosphatase (nmolh^{-1} g^{-1})	0.52±0.03 ^{a}	0.46±0.04 ^{a}
Nitrate reductase (μmolh^{-1} g^{-1})	3.61±0.08 ^{a}	3.32±0.20 ^{b}
N-acetyl-β-d-glucosaminidase (nmolh^{-1} g^{-1})	0.44±0.03 ^{a}	0.43±0.03 ^{a}
β-glucosidase (nmolh^{-1} g^{-1})	0.49±0.03 ^{a}	0.51±0.03 ^{b}

Values represent Mean ± SE, $n = 10$, different letters indicate significant differences in soil enzyme activities (Two sample *t*-test, $P \leq 0.05$).

in the bulk soils than in the rhizosphere. There were no significant differences observed in alkaline phosphatase [t (98) = −0.09, p = 0.92] and β-(D)-glucosaminidase [t (98) = −0. 05, p = 0.96] activities in rhizosphere and bulk soils.

Relationships between soil characteristics and enzyme activities of the rhizosphere and bulk soils

Relationships between soil characteristics and enzyme activities of the rhizosphere and bulk soils are presented in Fig. 1. Both PCA1 and PCA2 accounted for 45.2 % and 17.6 % of the overall variation respectively. The clusters revealed that the soil characteristics and enzymes were comparable in both the rhizosphere and the bulk soils. A negative correlation was observed between the soil P concentrations and the activity of acid and alkaline phosphatases. The enzymes nitrate reductase and β-d-glucosaminidase revealed a negative relationship with soil N concentration. The nitrate reductase was positively correlated with pH. Acid phosphatase was positively correlated with β-glucosidase. The N and P concentrations were positively correlated with organic carbon and total cation exchange.

Discussion

The results of this study provide compelling evidence that the rhizosphere soils of *E. lanatus* support a more diverse and functionally active bacterial community compared to bulk soils, which aligns with the hypothesis that higher bacterial diversity induces greater enzymatic activity related to C, P, and N cycling. This bacterial-driven nutrient cycling is particularly critical for *E. lanatus*, a cycad

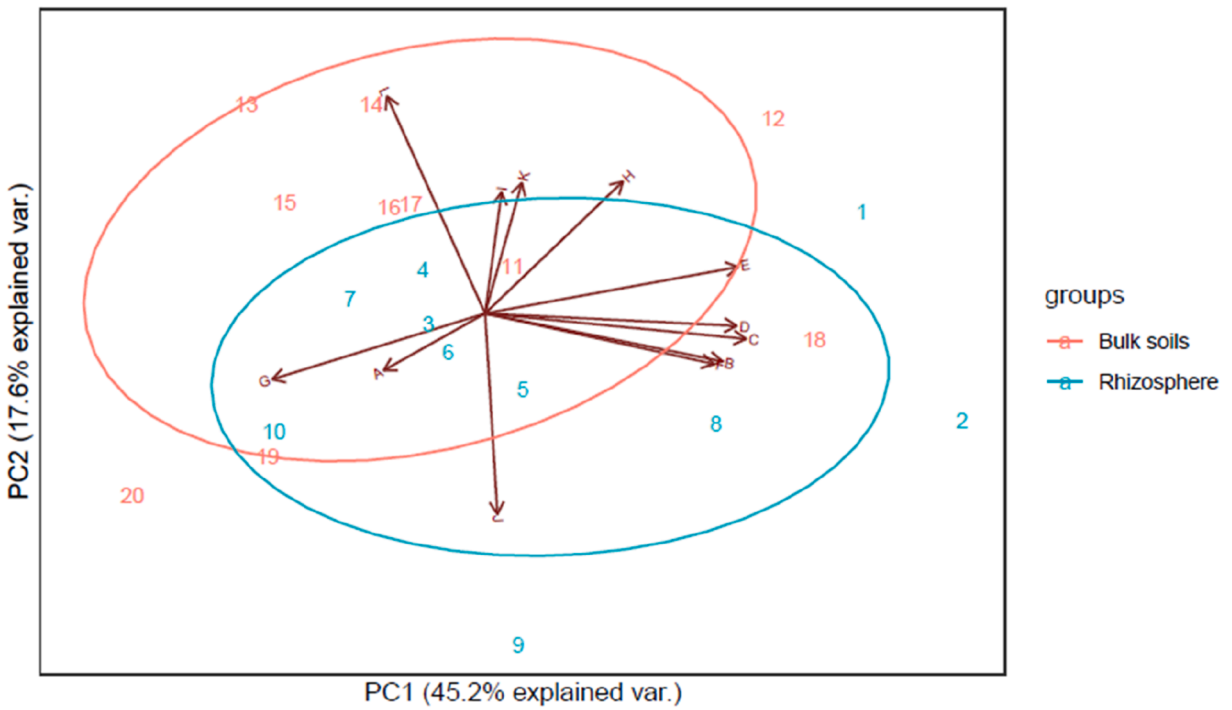

Fig. 1. Relationship between the soil traits and extracellular enzyme activities of *Encephalartos lanatus* rhizosphere and bulk soils. The following is a representation of soil attributes: *A* = nitrate reductase, *B* = nitrogen concentration, *C* = phosphorus concentration, *D* = organic carbon, *E* = exchangeable acidity, *F* = total cation exchange, *G* = pH, *H* = acid saturation, *I* = acid phosphatase, *J* = alkaline phosphatase, *K* = β-Glucosidase, *L* = *N*-acetyl-β-d-Glucosaminidase. Principal component analysis (PCA).

species that thrives in arid, nutrient-poor environments. The ability of *E. lanatus* to persist in such harsh conditions is likely facilitated by the synergistic relationships between its coralloid roots and the rhizosphere microbial communities, which enhance nutrient availability and support plant growth. The higher nutrient concentrations in rhizosphere soils compared to bulk soils therefore confirm that *E. lanatus* improves soil nutrient status where it occurs, a phenomenon that has been observed in *E. natalensis* [7], *E. villosus* [8], and *Cycas micronesica* [6,23].

Although the differences in most soil chemical properties were not statistically significant, the trends observed are consistent with previous studies demonstrating that rhizosphere soils are enriched in soil nutrients due to root-microbe interactions, as seen in other cycads [5]. This enrichment is significant in arid and nutrient-poor soils, where *E. lanatus* must rely on efficient nutrient acquisition strategies to survive. The higher organic C content, N, P, and K in the rhizosphere soils further supports the idea that bacterial activity contributes to nutrient accumulation and availability, which maintains soil fertility and promotes plant growth and development in harsh environments [23,24]. Among all the minerals, Mg and Mn concentrations in rhizosphere soils were significantly higher than in bulk soils (Table 1). Higher levels of Mg and Mn concentration recorded in *E. natalensis,* and *E. villosus* rhizosphere soils did not differ significantly from those of bulk soils [7,8]. High levels of Mg and Mn in the soil in this study can be attributed to plant root exudates and mineral weathering processes in the soil [25]. They are critical nutrients that support key physiological and biochemical processes in plants, particularly photosynthesis and enzyme function [26]. Soil microbes play a key role in soil nutrient cycling and mineralization processes, which enhances soil nutrient inputs, supports soil structure, and plant growth in the ecosystem. The higher microbial diversity in the rhizosphere, as indicated by the Simpson's Diversity Index ($D = 0.98$ for rhizosphere vs. $D = 0.37$ for bulk), suggests a more complex and functionally diverse microbial community. This diversity likely contributes to the observed differences in enzymatic activities, as microbial communities with higher diversity are often associated with greater functional redundancy and efficiency in nutrient cycling [27]. In the context of *E. lanatus*, this functional diversity is particularly advantageous in arid environments, where fluctuating conditions require resilient and adaptable microbial communities to maintain nutrient cycling processes, particularly N and P [7].

The most abundant group of microorganisms found in soils is bacteria which in this study is dominated by the genera *Bacillus, Burkholderia, Enterobacter, Paraburkholderia, Paenibacillus, Pseudomonas* and *Rhizobium* (Tables 2–4). The bacteria are involved in P-solubilisation and N-fixation, therefore making P and N available for plant growth. It has further been highlighted that soil nutrient availability may influence the plant-microbe symbiosis interactions. Hence, the bacterial community analysis in this study revealed a high abundance of P-solubilizing bacteria in the *E. lanatus* coralloid roots (Table 2), and a high diversity of N_2-fixing and P-solubilising bacteria in the rhizosphere and bulk soils. Specifically, the rhizosphere hosted 37.5 % of strictly N_2-fixing bacteria, 19 % of strictly P-solubilising bacteria, and 31 % of both N_2-fixing and P-solubilising bacteria (Table 3). The bulk soil hosted ~43 % of strictly N_2-fixing bacteria, 29 % strictly P-solubilising and 21 % of both N_2-fixing and P-solubilising (Table 4). Generally, precipitation and adsorption of P in acidic soil leads to low availability of P to plants. High abundance and diversity of P-solubilising bacteria in *E. lanatus* coralloid roots, rhizosphere and bulk soil may have been driven by limited P concentration in the soil (Table 1). The identification of *Bacillus, Burkholderia, Enterobacter, Paraburkholderia, Paenibacillus, Pseudomonas* and *Rhizobium* species in the coralloid roots, rhizosphere and bulk soils (Tables 2–4) known for their roles in P solubilisation, highlights the importance of these microbes in overcoming N and P limitations in *E. lanatus* nutrient-poor soils [20,22,28]. Bacteria of the genera *Achromobacter, Bacillus, Enterobacter* can solubilise Mn through the process of bioreduction [29] while *Caballeronia* can aid in weathering and mineralization of Mg [30]. The significantly higher concentrations of Mg and Mn in the rhizosphere may be due to the presence of specific microbes in the *E. lanatus* rhizosphere that mineralize and cycle these two key plant nutrients.

The presence of N-cycling bacteria, such as *Paraburkholderia* and *Burkholderia* species, further underscores the importance of microbial activity in N transformation processes, which are essential for sustaining plant growth in N-poor soils [27]. These microbes enhance nutrient cycling, growth and development of plants in nutrient-poor soils, and the functioning of the soil ecosystem [31]. The ability of *E. lanatus* to thrive in arid nutrient-poor soils can thus be attributed, at least in part, to the robust microbial communities in its rhizosphere. These microbes enhance nutrient availability through processes such as N fixation, P solubilization, and organic matter decomposition, which are critical for plant survival in resource-limited environments. Furthermore, the higher microbial diversity in the rhizosphere likely provides functional stability, enabling *E. lanatus* to withstand environmental stresses and maintain nutrient cycling even under adverse conditions.

Soil extracellular enzymes secreted by soil microorganisms are key bioindicators of soil microflora metabolism that can be used to assess the soil nutrient and productivity status; as they contribute to conservation, mineralisation and recycling of vital nutrients such as N, P, and C in soil, thus making nutrients available for uptake by plants [32]. Alkaline phosphatase, acid phosphatase, β-glucosidase, and N-acetylglucosaminidase are therefore crucial enzymes in studies of *E. lanatus* due to their roles in nutrient cycling and soil health. Studies show that the alkaline and acid phosphatases mineralize and recycle P in the soil, while *β*-glucosidase and N-acetylglucosaminidase are involved in organic matter decomposition, recycling C and N, which are vital for soil fertility and plant growth and nutrition [32]. Monitoring of these enzymes helps assess the ecological health and nutrient dynamics in *Encephalartos* plants. The increased activities of acid phosphatase, alkaline phosphatase, nitrate reductase, and β-(D)-glucosaminidase in rhizosphere soils suggest that microbial communities in these soils play a crucial role in nutrient mobilization and organic matter decomposition [33].

The high acid phosphatase and nitrate reductase activities in the rhizosphere soils further emphasize the role of microbial activity in facilitating P and N mineralisation [7]. The positive correlation between acid phosphatase and β-glucosidase suggests a synergistic relationship between phosphorus and carbon cycling, which could be influenced by root exudates stimulating microbial activity [34, 35]. This finding is consistent with the study of Ndabankulu et al. [27] in which rhizosphere soils exhibited enhanced enzymatic activities due to root exudates and stimulated microbial communities involved in nutrient cycling. A study by Magadlela et al. [32] showed increased acid, and alkaline phosphatases activities in P-limited and acidic soils. The increased acid phosphatase activity in the

E. lanatus rhizosphere soil may therefore indicate a greater demand for P in the P-limited and acidic soil. In contrast, the higher activity of β-glucosidase in bulk soils may indicate a microbial strategy favoring cellulose degradation in nutrient-limited environments [36]. These results are supported by previous studies which highlighted the rhizosphere as a hotspot for biochemical processes that support plant nutrition, especially in nutrient-deficient soils [22]. The elevated phosphatase activities observed suggest that the rhizosphere plays a pivotal role in enhancing P availability, which is critical for plant growth and development in ecosystems where P is a limiting factor, as it has been previously seen in other ecosystems [37].

N-acetyl-β-d-glucosaminidase (NAGase) is an important enzyme involved in the degradation of chitin, contributing to the cycling of nitrogen (N) and carbon (C) [7,38] in soils. The study revealed that NAGase levels were higher in the rhizosphere than in bulk soils, although this difference was not statistically significant (once again, most likely due to the disruptions induced in soil by grassland fires). This trend suggests that the rhizosphere may enhance NAGase activity due to root exudates and the associated microbial communities, which are known to thrive in the rhizosphere and contribute to organic matter decomposition and nutrient cycling [36]. Despite the lack of statistical significance, the observed increase in NAGase activity in the rhizosphere is in support of previous study of Ndanbakulu et al. [27] which also highlighted the rhizosphere as a hotspot for enzymatic activity.

The PCA results, which accounted for 62.8 % of the variation in soil characteristics and enzyme activities, reinforce the notion that enzymatic processes in both rhizosphere and bulk soils are shaped by soil's physicochemical properties. The negative correlation between P concentration and both acid and alkaline phosphatase activity suggests a feedback mechanism whereby microbial phosphatase production decreases in response to sufficient P availability [27,35,39]. Similarly, the inverse relationship between nitrate reductase and N concentration implies that microbial nitrate reductase activity is upregulated under N-limiting conditions to facilitate N assimilation [37].

Furthermore, the observed positive correlation between nitrate reductase activity and soil pH highlights the influence of soil acidity on N cycling processes [40]. Given that pH modulates microbial community composition and enzyme stability, it is likely that nitrate reductase-producing microbes thrive better under less acidic conditions [35]. The strong positive correlation between N, P, organic C, and total cation exchange capacity suggests that these factors collectively shape soil microbial communities and enzymatic activity, thereby influencing nutrient bioavailability and ecosystem functioning [35].

Conclusion

The findings of this study demonstrate that the rhizosphere of *E. lanatus* harbors a more diverse and functionally active microbial community compared to bulk soils. This microbial diversity drives enhanced enzymatic activities related to C and N cycling, supporting plant growth and nutrient acquisition in arid and nutrient-poor environments. The symbiotic relationships between *E. lanatus* and its associated microbes highlight the importance of microbial communities in the adaptation and survival of this species in harsh conditions. Future studies should explore the specific microbial taxa responsible for these enzymatic activities and their functional redundancy under varying environmental conditions, as well as the mechanisms by which these microbial communities influence nutrient dynamics and their potential applications in the conservation and restoration of *E. lanatus* habitats, particularly in the face of climate change and habitat degradation.

Author contributions

M.S. and T.N.S collected samples from the field. M.S. and M.P.F. analyzed the data and wrote the paper. T.N.S. and A.M. funded the study. T.N.S, A.M. and W.O.M. supervised the study; T.N.S, A.M. and M.P.F. proofread the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

The study was financed by the Vice Chancellor's Scholarship of the University of Mpumalanga to M.S., The National Research Foundation (Grant UID 129403) to T.N.S., and (Grant 138091) to A.M.

Declaration of competing interest

The authors declare that they do not have any competing interest.

Data availability statement

All the data is presented in the manuscript in figure and tables.

Acknowledgements

We acknowledge the assistance received from the University of KwaZulu-Natal for allowing us to carry out the experiments. We also thank the University of Mpumalanga for helping us with data collection. Furthermore, we appreciate the Mpumalanga Tourism and Parks Agency for granting a permit to conduct research on *Encephalartos lanatus*.

References

[1] J.L. Costa, P. Lindblad, Cyanobacteria in symbiosis with cycads, in: A.N. Rai, B. Bergman, U. Rasmussen (Eds.), Cyanobacteria in Symbiosis, Springer, Dordrecht, 2002, https://doi.org/10.1007/0-306-48005-0_11.
[2] D. Raimondo, L. von Staden, W. Foden, J. Victor, N. Helme, R.C. Turner, D.A. Kamundi, P.A. Manyama, Red list of South African plants, Strelitzia 25 (2009).
[3] J.D. Bösenberg, Encephalartos lanatus, IUCN. Red. List. Threat. Species (2022) e.T41933A51048427. Available from: https://www.iucnredlist.org/.
[4] Y. Ma, H. Jiang, B. Wang, G. Zhou, S. Yu, S. Peng, Y. Hao, X. Wei, J. Liu, Z. Yu, Carbon storage of cycad and other gymnosperm ecosystems in China: implications to evolutionary trends, Pol. J. Ecol 57 (2009) 635–646.
[5] K. Gutiérrez-García, E.D. Bustos-Díaz, J.A. Corona-Gómez, H.E. Ramos-Aboites, N. Sélem-Mojica, P. Cruz-Morales, M.A. Pérez-Farrera, F. Barona-Gómez, A. Cibrián-Jaramillo, Cycad coralloid roots contain bacterial communities including cyanobacteria and *caulobacter spp*. That encode niche-specific biosynthetic gene clusters, Genome. Biol. Evol 11 (2018) 319–334, https://doi.org/10.1093/gbe/evy266.
[6] T.E. Marler, M. Calonje, Two cycad species affect the carbon, nitrogen, and phosphorus content of soils, Horticulturae 6 (2020) 24, https://doi.org/10.3390/horticulturae6020024.
[7] S. Ndlovu, T.N. Suinyuy, M.A. Pérez-Fernández, A. Magadlela, *Encephalartos natalensis,* their nutrient-cycling microbes and enzymes: a story of successful trade-offs, Plants 12 (2023) 1034, https://doi.org/10.3390/plants12051034.
[8] N. Motsomane, T.N. Suinyuy, M.A. Pérez-Fernández, A. Magadlela, Exploring the influence of ecological niches and hologenome dynamics on the growth of *encephalartos villosus* in scarp forests, Soil. Syst 8 (2024) 21, https://doi.org/10.3390/soilsystems8010021.
[9] M. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, J.E. Nava-Saucedo, Polymer biodegradation: mechanisms and estimation techniques – a review, Chemosphere 73 (2008) 429–442, https://doi.org/10.1016/j.chemosphere.2008.06.064.
[10] C. Santi, D. Bogusz, C. Franche, Biological nitrogen fixation in non-legume plants, Ann. Bot 111 (2013) 743–767, https://doi.org/10.1093/aob/mct048.
[11] B.L. Turner, R. Baxter, B.A. Whitton, Seasonal phosphatase activity in three characteristic soils of the English uplands polluted by long-term atmospheric nitrogen deposition, Env. Pollut 120 (2002) 313–317, https://doi.org/10.1016/S0269-7491(02)00147-1.
[12] M.M. Gehringer, J.J. Pengelly, W.S. Cuddy, C. Fieker, P.I. Forster, B.A. Neilan, Host selection of symbiotic cyanobacteria in 31 species of the Australian cycad genus: *macrozamia* (Zamiaceae), Mol. Plant. Microbe. Interact 23 (2010) 811–822, https://doi.org/10.1094/MPMI-23-6-0811.
[13] J.L. Costa, P. Paulsrud, P. Lindblad, Cyanobiont diversity within coralloid roots of selected cycad species, FEMS. Microbiol. Ecol 28 (1999) 85–91, https://doi.org/10.1016/S0168-6496(98)00095-6.
[14] P.D.J. Suárez-Moo, A.P. Vovides, M.P. Griffith, F. Barona-Gómez, A. Cibrián-Jaramillo, Unlocking a high bacterial diversity in the coralloid root microbiome from the cycad genus *Dioon*, PLoS. One 14 (2019) e0211271, https://doi.org/10.1371/journal.pone.0211271.
[15] M.C. Lötter, Technical Report for the Mpumalanga Biodiversity Sector Plan – MBSP, Mpumalanga Tourism & Parks Agency, Mbombela (Nelspruit), 2015.
[16] C. Maney, D. Guaras, J. Harrison, A. Guizar-Coutiño, M.B.J. Harfoot, S.L.L. Hill, N.D. Burgess, W. Sutherland, National commitments to Aichi Targets and their implications for monitoring the Kunming-Montreal Global Biodiversity Framework, npj. Biodivers 3 (2024), https://doi.org/10.1038/s44185-024-00039-5.
[17] A. Marques, H.M. Pereira, C. Krug, P.W. Leadley, P. Visconti, S.R. Januchowski-Hartley, R.M. Krug, R. Alkemade, C. Bellard, W.W.L. Cheung, V. Christensen, H. D. Cooper, T. Hirsch, R. Hoft, J. van Kolck, T. Newbold, K. Noonan-Mooney, E.C. Regan, C. Rondinini, U.R. Sumaila, L.S.L. The, M. Walpole, A framework to identify enabling and urgent actions for the 2020 Aichi Targets, Basic. Appl. Ecol 15 (2014) 633–638, https://doi.org/10.1016/j.baae.2014.09.004.
[18] L.T. Mankga, K. Yessoufou, Factors driving the global decline of cycad diversity, AoB. Plants 9 (2017), https://doi.org/10.1093/aobpla/plx022 plx022.
[19] T.E. Marler, M.V. Krishnapillai, *Cycas micronesica* trees alter local soil traits, Forests 9 (2018) 565, https://doi.org/10.3390/f9090565.
[20] A.D. Manson, S.H. Bainbridge, G.R. Thibaud, Methods Used For the Analysis of Soils and Plant Material By Analytical Services At Cedara, KwaZulu-Natal Department of Agriculture and Rural Development, 2020.
[21] A. Magadlela, C. Beukes, F. Venter, E. Steenkamp, A. Valentine, Does P deficiency affect nodule bacterial composition and N source utilization in a legume from nutrient-poor Mediterranean-type ecosystems? Soil. Biol. Biochem 104 (2017) 164–174, https://doi.org/10.1016/j.soilbio.2016.10.021.
[22] A. Bruckner, J. Wright, C. Kampichler, R. Bauer, E. Kandeler, A method of preparing mesocosms for assessing complex biotic processes in soils, Biol. Fertil. Soils 19 (1995) 257–262, https://doi.org/10.1007/BF00336169.
[23] E. Rafique, M.Z. Mumtaz, I. Ullah, A. Rehman, K.A. Qureshi, Kamran M, M.U. Rehman, M. Jaremko, M.A. Alenezi, Potential of mineral-solubilizing bacteria for physiology and growth promotion of Chenopodium quinoa Willd, Front. Plant. Sci 13 (2022) 1004833, https://doi.org/10.3389/fpls.2022.1004833.
[24] B. Khoshru, D. Mitra, A.F. Nosratabad, A. Reyhanitabar, L. Mandal, B. Farda, R. Djebaili, M. Pellegrini, B.E. Guerra-Sierra, A. Senapati, P. Panneerselvam, Enhancing manganese availability for plants through microbial potential: a sustainable approach for improving soil health and food security, Bacteria 2 (2023) 129–141, https://doi.org/10.3390/bacteria2030010.
[25] M. Polgári, I. Gyollai, Geochemical constraints on the element enrichments of microbially mediated manganese and iron ores – an overview, Ore. Geol. Rev 126 (2021) 104203, https://doi.org/10.1016/j.oregeorev.2021.104203.
[26] S. Farzadfar, F. Zarinkamar, M. Hojati, Magnesium and manganese affect photosynthesis, essential oil composition and phenolic compounds of *tanacetum parthenium*, Plant. Physiol. Biochem 112 (2017) 207–217, https://doi.org/10.1016/j.plaphy.2017.01.002.
[27] K. Ndabankulu, S.O. Egbewale, Z. Tsvuura, A. Magadlela, Soil microbes and associated extracellular enzymes largely impact nutrient bioavailability in acidic and nutrient poor grassland ecosystem soils, Sci. Rep 12 (2022) 12601, https://doi.org/10.1038/s41598-022-16949-y.
[28] N. Motsomane, T.N. Suinyuy, M.A. Pérez-Fernández, A. Magadlela, How the right evolved partners in cycads and legumes drive enhanced growth in a harsh environment, Symbiosis 90 (2023) 345–353, https://doi.org/10.1007/s13199-023-00940-w.
[29] E.G. Baglin, E.G. Noble, D.L. Lamsphire, J.A. Eisele, Solubilization of manganese from ores by heterotrophic micro-organisms, Hydrometallurgy 29 (1992) 131–144, https://doi.org/10.1016/0304-386x(92)90009-o.
[30] C. Blanco Nouche, C. Cochet, M.P. Turpault, S. Uroz, Feedback effect of the size of mineral particles on the molecular mechanisms employed by *Caballeronia mineralivorans* PML1(12) to weather minerals, npj. Mater. Degrad 8 (2024) 129, https://doi.org/10.1038/s41529-024-00544-9.
[31] K. Kiprotich, E. Muema, C. Wekesa, T. Ndombi, J. Muoma, D. Omayio, D. Ochieno, H. Motsi, S. Mncedi, J. Tarus, Unveiling the roles, mechanisms and prospects of soil microbial communities in sustainable agriculture, Discov. Soil 2 (2025) 10, https://doi.org/10.1007/s44378-025-00037-4.
[32] A. Magadlela, Z. Lembede, S.O. Egbewale, A.O. Olaniran, The metabolic potential of soil microorganisms and enzymes in phosphorus-deficient KwaZulu-Natal grassland ecosystem soils, Appl. Soil. Ecol 181 (2023) 104647, https://doi.org/10.1016/j.apsoil.2022.104647.
[33] R.G. Burns, J.L. DeForest, J. Marxsen, R.L. Sinsabaugh, M.E. Stromberger, M.D. Wallenstein, M.N. Weintraub, A. Zoppini, Soil enzymes in a changing environment: current knowledge and future directions, Soil. Biol. Biochem 58 (2013) (2013) 216–234, https://doi.org/10.1016/j.soilbio.2012.11.009.
[34] X. Hu, J. Liu, A. Liang, H. Gu, Z. Liu, J. Jin, G. Wang, Soil metagenomics reveals reduced tillage improves soil functional profiles of carbon, nitrogen, and phosphorus cycling in bulk and rhizosphere soils, Agric. Ecosyst. Env. 379 (2025) 109371, https://doi.org/10.1016/j.agee.2024.109371.
[35] N.S. Zungu, S.O. Egbewale, A.O. Olaniran, M. Pérez-Fernández, A. Magadlela, Soil nutrition, microbial composition and associated soil enzyme activities in KwaZulu-Natal grasslands and savannah ecosystems soils, Appl. Soil. Ecol 155 (2020) 103663, https://doi.org/10.1016/j.apsoil.2020.103663.
[36] S. Allison, M. Wallenstein, M. Bradford, Soil-carbon response to warming dependent on microbial physiology, Nat. Geosci 3 (2010) 336–340, https://doi.org/10.1038/ngeo846.
[37] S. Ullah, C. Ai, S. Huang, J. Zhang, L. Jia, J. Ma, W. Zhou, P. He, The responses of extracellular enzyme activities and microbial community composition under nitrogen addition in an upland soil, PLOS. One 14 (2019) e0223026, https://doi.org/10.1371/journal.pone.0223026.
[38] V. Acosta-Martínez, L. Pérez-Guzmán, J.M. Johnson, Simultaneous determination of β-glucosidase, β-glucosaminidase, acid phosphomonoesterase, and arylsulfatase activities in a soil sample for a biogeochemical cycling index, Appl. Soil. Ecol 142 (2019) 72–80, https://doi.org/10.1016/j.apsoil.2019.05.001.
[39] A. Magadlela, N. Makhaye, M. Pérez-Fernández, Symbionts in *Mucuna pruriens* stimulate plant performance through nitrogen fixation and improved phosphorus acquisition, J. Plant. Ecol 14 (2020) 310–322, https://doi.org/10.1093/jpe/rtaa098.
[40] M. Krywult, D. Bielec, Method of measurement of nitrate reductase activity in field conditions, J. Ecol. Eng 14 (2013) 7–11, https://doi.org/10.12912/23920629/373.