Encephalartos villosus associated bacterial communities and extracellular enzymes improve soil nutrition in rhizosphere soils in forest ecosystem soils

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Research Article

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Abstract

Purpose Cycads are the only known gymnosperms associated with nitrogen-fixing bacteria housed in coralloid roots. Plant-associated bacteria, soil bacteria, and extracellular enzymes play a significant role in nutrient cycling. This study isolated and identified culturable bacteria in Encephalartos villosus coralloid roots, rhizosphere, and non-rhizosphere soils and investigated the role of soil bacteria and associated enzyme activities on soil nutrition in forest ecosystem soils in Rhebu and Oceanview, Eastern Cape, South Africa. Methods Encephalartos villosus coralloid roots were collected from mature cycad individuals for bacterial extraction and identification. Soil samples from E. villosus rhizosphere and non-rhizosphere soils were collected for bacterial identification, extracellular enzyme activity analysis, and soil characteristics (nutrient concentrations, pH, total cation, and exchange acidity). Results The bacteria isolated from the coralloid roots of E. villosus growing in Rhebu and Oceanview belonged to the Bacillus, Enterobacter, Peribacillus, Lysinibacillus, Stenotrophomonas, Rhizobium, and Paenibacillus genera. The Pseudomonas, Paraburkholderia, Burkholderia, Variovorax, Caballeronia, Stenotrophomonas, Novosphingobium, Caulobacter, Olivibacter, Cupriavidus, Arthrobacter, Gottfrieder, Dyella, Lysobacter, Xanthomonas, Neobacillus, Bradyrhizobium, Rhizobium, Enisfer, Chitinophaga, Paenarthrobacter, and Paenibacillus genera were isolated from rhizosphere and non-rhizosphere soils in Rhebu and Ocean View farm. There were no significant differences in the concentrations of soil macronutrients (N, P, K), alkaline and acid phosphatase, glucosaminidase, and nitrate reductase activity of E. villosus rhizosphere and non-rhizosphere soils in both localities, this may be attributed to dung and urine deposited by grazing cattle. Conclusion Our results show that soil bacterial communities with nutrient cycling and fixing functions may be linked to nutrient bioavailability contributing to nutrient enrichments in E. villosus rhizosphere soils.

Introduction

The *Encephalartos* genus is an African endemic and the largest genera in the Cycadales order (Viljoen and van Staden, 2006; Condamine et al., 2015) with 66 species out of which 38 species are indigenous to South Africa, making South Africa a hotspot for cycad diversity (Cousins et al., 2013; Williamson et al., 2016; Cousins and Witkowski, 2017). *Encephalartos villosus* is a forest understory plant, and the most abundant and widely distributed species belonging to the *Encephalartos* genus (Donaldson, 1997), and thus more prone to be harvested for traditional medicine (Yessoufou et al., 2014; Cousins et al., 2012). Using cycad species for traditional medicine has led to unsustainable harvesting and overcollection, threatening cycad diversity (Raimondo et al., 2009; Cousins et al., 2012; Williamson et al., 2016) and the associated ecosystem. Cycads are the only known gymnosperms to partake in a symbiotic association with nitrogen (N) fixing bacteria (Zheng et al., 2002; Chang et al., 2019). This association is believed to be one of the adaptations of cycads to nutrient-stressed ecosystems (Fisher and Vovides, 2004; Pecundo et al., 2021). The *Nostoc, Rhizobium, Bradyrhizobium, Azorhizobium, Burkholderia, Sytonema,* and *Richelia* genera are reported to be associated with cycad species (Barea et al., 2005; Gutièrrez-García et al*.,* 2019).

Plant symbionts are sourced from the soil and play a role in soil nutrient inputs; thus, the bioavailability of nutrients in the soil is dependent on soil bacteria (Cenciani et al*.,* 2008; Meena and Rao, 2021) that solubilise nutrients, making them available for plant assimilation (Latha et al*.,* 2011). Soil bacteria exude enzymes, such as phosphatase, urease, and β-glucosidase, that convert solid soil organic matter into dissolved compounds (Bandick and Dick, 1999; Barea et al., 2005; Merino et al., 2016; Adetunji et al., 2017). While studies such as those by Marler and Calonje (2020) report that cycads improve soil N, C, and P content, the role of soil bacterial communities and their associated enzyme activities is rarely studied. Thus, identifying the bacteria in coralloid roots and soils and determining the role of the associated enzyme activities will help in understanding cycad-microbe interactions, soil nutrient inputs by cycads and finding effective conservation strategies of threatened cycads. Conducting a study on *E. villosus* is essential because the species is widely distributed, of least concern, but co-occur and share similar life history to that of threatened cycads that cannot be studied due to their conservation status (Raimondo and Donaldson, 2003).

Hence, this study isolated and identified the culturable bacteria in *E. villosus* coralloid roots, rhizosphere, and non-rhizosphere soils. Also, this study examined the potential role of *E. villosus* on soil nutrient inputs in nutrient-deficient forest ecosystems. The objectives of this study included 1) determining the soil characteristics (pH, exchange acidity, total cation, and nutrient concentrations) in *E. villosus* rhizosphere and non-rhizosphere soils, 2) Identifying the N-fixing, P-solubilizing, and N-cycling bacteria found in *E. villosus* coralloid roots, rhizosphere, and non-rhizosphere soils, and 3) assayed the soil nutrient (P and N) cycling enzyme activities in *E. villosus* rhizosphere and non-rhizosphere soils. We hypothesized that the bacteria composition in coralloid roots, rhizosphere, and non-rhizosphere soils of *E. villosus* and associated enzyme activities indicate its contribution to the bioavailability of soil nutrition for *E. villosus* rhizosphere and surrounding plants in nutrient stressed forest ecosystems.

Materials And Methods

2.1. Soil sampling sites

Soil samples were collected from two scarp forests in Eastern Cape (South Africa), at Ocean View Farm in East London and Rhebu village in Port St Johns where *E. villosus* occurs (co-ordinates not included due to cycad conservation concerns). These two scarp forests like others occur in nutrient-poor, leached and shallow soils (Mucina et al., 2006). The forest floor is bare, with *E. villosus* as the dominant understory plant, and tall trees such as *Ptaeroxylon obliquum*, *Hyperacanthus amoenus*, *Protorhus longifolia*, and *Vepris undulata* adorning the forests (Mucina et al., 2006). In addition to muthi harvesting and collection of biofuels, the scarp forests in the Ocean View and Rhebu village are also disturbed by cattle that graze within the forests. In the process, the cattle deposit dung and urine which enriches the forest soils.

Encephalartos villosus rhizosphere soils were collected at the four cardinal points (North, East, West and South) around the plants at 0-10 and 10-20 cm depths. To avoid damaging the underground stem, rhizosphere soil was collected 50 cm away from the plant and at the leaf canopy drip line. Similarly, non-rhizosphere soils (similar depths) were collected from non-target sites defined by a radius of five meters from the base of each target plant. The direction of the non-rhizosphere samples was randomly selected using cardinal points of North, East, South, and West of the target plant. From each *E. villosus* target plant, the rhizosphere and non-rhizosphere soil samples collected at different depths and cardinal points were bulked to form composite samples used for bacterial extraction and identification, soil characteristic analysis and soil enzyme activity assays.

2.2. Soil characteristics analysis

Thirty 50 g soil samples (16 from Ocean View and 14 from Rhebu) collected from *E. villosus* rhizosphere and non-rhizosphere soils were sent to the KwaZulu Natal Department of Agriculture and Rural Developments Analytical services for soil nutrient concentrations, total cation concentrations, exchange acidity, and pH analysis. The soil characteristic analysis was performed per protocols explained by Manson and Roberts (2000). Briefly, the total soil N concentration was measured using the Automated Dumas dry combustion method with a LECO CNS 2000 (Leco Corporation, USA). The atomic absorption method was used to measure soil P and potassium concentrations, where 2.5 ml soil solution was mixed with 25 ml ambic-2 solution at a pH of 8. Thereafter, the mixture was stirred at 400 rpm for 10 minutes using a multiple stirrer and filtered with Whatman no.1 paper. The same methods as above was used to determine the soil calcium and magnesium concentration however, 25 ml 1 M KCl solution was used instead of ambic-2 solution. Soil pH was determined by mixing 10 ml soil solution was mixed with 25 ml 1 M KCl solution and stirred at 400 rpm for 5 minutes using a multiple stirrer. The pH of the suspension was measured using a gel-filled combination glass electrode while stirring.

2.3. Coralloid root surface sterilisation

Coralloid roots were harvested from eight and seven randomly selected mature *E. villosus* individuals in Rhebu and Ocean View, respectively. The coralloid roots were surface sterilized with 70% (v/v) ethanol for 30 seconds and soaked in 3.5% (v/v) sodium hypochlorite solution for 3 minutes. Thereafter, they were rinsed ten times with distilled water and stored at 4˚C in sterile Petri plates (90mm).

2.4. Bacterial extraction and identification from coralloid roots and soils

Soil samples collected from the rhizosphere and non-rhizosphere soils in Rhebu and Ocean View were subjected to serial dilutions, and the harvested coralloid roots were immersed in 100 µl of 15% glycerol in sterile Eppendorf tubes and crushed using sterile pipette tips for bacterial extraction. Ten µL of the root suspension and 50µL 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) to ensure that only bacteria with P-solubilizing, N-cycling, and N-fixing traits grew on the plates. 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' (21 bases)) and 1387R (5' GGGCGGTGTGTACAAGGC 3' (18 bases)) from Inqaba Biotechnical Industries (Pty) Ltd (South Africa) (Magadlela et al., 2023). The PCR amplification was performed using an EmaraldAmp 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, 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 Basic Local Aligned Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov).

2.5. Enzyme activities

N-acetylglucosaminidase, acid phosphatase, and alkaline phosphatase activities were assayed using the fluorescence-based method described by Jackson et al. (2013) and expressed as nmol $h^{-1}g^{-1}$. Briefly, soil samples (10g soil/100 ml autoclaved dH_2O) were homogenised at medium speed in a shaker for two hours and stored overnight at 4˚C. The supernatants were transferred into black 96-well microplates before adding their respective substrates. The sample run consisted of 200µl soil aliquot and 50µl substrate, alongside reference standards (200µl bicarbonate buffer + 50µl standard), quench standard (200µl soil aliquot + 50µl standard), sample control (200µl soil aliquot + 50µl buffer), negative controls (200µl buffer + 50µl substrate), and blanks (250µl buffer). The 96-well plate was incubated at 25˚C for 2 hours. Thereafter, the reaction was stopped by adding 5µl 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 Kandeler (1995). A volumetric flask wrapped in foil was filled with 1 ml of 25 mM KNO_3, 4 ml of 0.9 mM 2,4-dinitrophenol, and 5 ml of milliQ dH_2O. Thereafter, 5g 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, 10ml 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 2ml of the filtrate to 1.2ml 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 (NO_2^-) liberated into the medium was extrapolated from a prepared standard curve with KNO_3. The nitrate reductase activity was expressed as 0.1 µmol h^{-1} g^{-1}. There were no significant differences in the N-acetylglucosaminidase, acid phosphatase, alkaline phosphatase, and nitrate reductase activities of rhizosphere and non-rhizosphere soils in Rhebu and Oceanview (Figure S1).

2.6. Data analysis

The statistical software/program R (version 3.6.2) was used to test for differences in the micronutrients, pH, exchange acidity, soil enzyme activity, and total cation in soil samples collected in *E. villosus* rhizosphere and non-rhizosphere soils using independent samples T-test. The assumptions for normality and homogeneity of the variances were tested using the one-sample Kolmogorov-Smirnov normality and Levene's test, respectively ($p>0.05$). The Wilcoxon test, a non-parametric alternative, was used in cases where the assumptions were not met. A simple linear correlation analysis was done to determine if there is a relationship between soil enzyme activities and soil nutrition. A probability of $p\leq0.05$ was considered significant. Relations between soil nutrients and enzyme activities were determined using principal component analysis. The principal component analysis was performed using the gg plot statistical package and pr comp function on R (version 3.6.2).

Results

3.1. Soil characteristics

Overall, Ocean View and Rhebu rhizosphere soils had higher concentrations of macronutrients, intermediate nutrients, and micronutrients than the non-rhizosphere soils (Table 1). The micronutrients Magnesium (Mg) and zinc (Zn) concentrations in rhizosphere soils were significantly higher than in the non-rhizosphere soils in Ocean View (Table 1). The exchange acidity and total cation concentrations in Rhebu rhizosphere soils was higher than in the non-rhizosphere soils (Table 1). While there was no difference in exchange acidity of Ocean View rhizosphere and non-rhizosphere soils, the total cation concentration in non-rhizosphere soils was higher than in the rhizosphere soils (Table 1). The soil samples collected from rhizosphere and non-rhizosphere soils in Rhebu and Ocean View were relatively acidic (Table 1). In Rhebu and Ocean View, rhizosphere soils had a higher pH of 4.28 and 5.43 compared to pH of 4.14 and 4.97 for the non-rhizosphere soils, respectively. The organic N to carbon ratio (N:C) in rhizosphere soils were higher than in non-rhizosphere soils in Ocean View and Rhebu (Table 1).

3.2. Bacterial identification

3.2.1. Identification of bacterial isolates in the coralloid roots of *E. villosus* growing in Rhebu and Ocean View

The bacterial isolates in the coralloid roots of *E. villosus* growing in Rhebu had N-cycling, N-fixing, and P-solubilizing traits and belonged to the *Lysinibacillus, Peribacillus, Rhizobium,* and *Stenotrophomonas* genera (Table 2). The bacteria isolated in the coralloid roots of *E. villosus* growing in Ocean View had N-fixing and P-solubilizing traits and belonged to the *Lysinibacillus, Bacillus, Enterobacter,* and *Paenibacillus* genera (Table 2).

3.2.2. Identification of bacterial isolates in *E. villosus* rhizosphere and non-rhizosphere soils in Rhebu and Ocean View

The bacteria isolated from rhizosphere and non-rhizosphere soils in Rhebu and Ocean View had N-cycling, N-fixing, and P-solubilizing traits (Table 3). The rhizosphere soils in Rhebu had P-solubilizing bacteria belonging to the *Pseudomonas, Caballeronia, Paraburkholderia, Novosphingobium* and *Burkholderia,* N cycling bacteria belonging to the *Variovorax, Caulobacter, Olivibacter, Acinetobacter, Pseudomonas* and *Paraburkholderia* genera, and N-fixing bacteria belonging to the *Cupriavidus, Burkholderia, Novosphingobium, Pseudomonas*, and *Paraburkholderia* genera (Table 3). Non-rhizosphere soils in Rhebu had P-solubilizing bacteria belonging to the *Paraburkholderia, Pseudomonas,* and *Variovorax* genera (Table 3), N-fixing bacteria belonging to the *Paraburkholderia, Variovorax, Burkholderia, Stenotrophomonas,* and *Paraburkholderia* genera, and N-cycling bacteria belonging to the *Dyella, Stenotrophomonas, Gottfrieder, Arthrobacter, Caulobacter,* and *Burkholderia* genera (Table 3). Ocean View rhizosphere soils had P-solubilizing bacteria belonging to the *Lysobacter, Xanthomonas, Paraburkholderia, Variovorax and Hymenobacter* genera, N-fixing bacteria belonging to the *Paenibacillus, Bradyrhizobium, Variovorax, Pseudomonas, Paraburkholderia* and *Stenotrophomonas* genera, and N-cycling bacteria belonging to the *Paraburkholderia, Neobacillus, Cupriavidus, Chitinophaga, Dyella, Pseudomonas,* and *Paenarthrobacter* genera (Table 4). The non-rhizosphere soils in Ocean View had P-solubilizing bacteria belonging to the *Paraburkholderia* and *Variovorax* genera, N-fixing bacteria belonging to the *Enisfer, Burkholderia, Cupriavidus, Stenotrophomonas, Paraburkholderia* and *Variovorax* genera, and N-cycling bacteria belonging to the *Rhizobium, Caulobacter, Paraburkholderia* and *Pseudomonas* genera (Table 4).

3.3. Correlations between soil nutrients and enzyme activities

The first PCA explained 44.4% of the total variation and the second PCA explained 27.4% of the total variation (Figure 1). A separation between Ocean View rhizosphere and non-rhizosphere clusters showed distinct variations (Figure. 1). Variations in Ocean View rhizosphere soils were associated with alkaline phosphatase activity (represented as D) (Figure 1). There was an overlap of rhizosphere and non-rhizosphere soils in Rhebu, indicating an interaction between soil nutrients and enzyme activities (Figure 1). The PCA plot showed relationships between soil nutrients and enzyme activities; groups closer to each other represented high correlation (Figure 1). Acid phosphatase activity (represented as C) was highly correlated with soil P (represented as E) and soil N (represented as A) was correlated with N-acetylglucosaminidase activity (represented as B) (Figure 1).

Discussion

In the present study, *E. villosus* coralloid roots, rhizosphere, and non-rhizosphere soils were studied to determine the role of cycad-microbe symbiosis, soil bacterial communities, and associated enzyme activities on soil nutrient inputs. Microbes play a significant role in nutrient cycling by mediating carbon (C), nitrogen (N), and phosphorus (P) cycles (Zhao et al., 2014). This characteristic enables microbes to promote plant growth and development. Plant growth-promoting (PGP) bacteria can be free-living or in a symbiotic association with a host (Glick, 2012). Nitrogen cycling, nitrogen-fixing, and phosphorus solubilizing bacteria were isolated from the coralloid roots, non-rhizosphere soils, and rhizosphere of *E. villosus* growing in Rhebu and Oceanview (Table 1). The *Lysinibacillus xylanilyticus* and *Enterobacter cloacae* species isolated from *E. villosus* coralloid roots play a significant role in Indole-3-acetic acid (IAA) production (Verma et al., 2016), P-solubilisation (De Mandal et al., 2018), N-fixation, and the secretion of acetoin, a phytohormone that helps protect plants from phytopathogens (Khalifa et al., 2016). The *Lysinibacillus xylanilyticus* species has been reported to associate with *Encephalartos natalensis* (Ndlovu et al., 2023), which suggest that this species may play a role in the growth and development of cycad species in nutrient deficient and acidic environments. According to Ano and Ubochi (2007), the most abundant bacteria in soils belong to the *Paenibacillus, Lysinibacillus, Pseudomonas, Paraburkholderia, Bradyrhizobium,* and *Rhizobium* genera. These genera have plant growth promoting traits and play a role in N-cycling, N-fixing, and P-solubilization (Sitaram, 2015; Jaiswal et al., 2021; Favero et al., 2022; Bellès-Sancho et al., 2023; Pantoja-Guerra et al., 2023). Thus, the presence of bacteria belonging to the *Pseudomonas, Paraburkholderia,* and *Bradyrhizobium* genera in *E. villosus* rhizosphere and non-rhizosphere soils may be attributed to the abundance of these genera on soils and their plant growth promoting traits. The bacterial species isolated in rhizosphere and non-rhizosphere soils promote plant growth through P-solubilisation (Verma et al., 2016), N-fixation (Kong et al., 2017), siderophore (Haas and Dèfago, 2005), and IAA production (Gupta et al., 2015). The richness of plant growth-promoting bacteria in *E. villosus* coralloid roots, rhizosphere and non-rhizosphere soils can be interpreted as one of the reasons why the species is widely distributed and thrive in nutrient-deficient soils and indicates that

microbiota confer the nutritional advantages to cope with impoverished soils as the bacteria play a crucial role in nutrient cycling. They therefore increase the bioavailability of soil nutrition for *E. villosus* rhizosphere and surrounding plants in nutrient stressed ecosystems.

According to Merino et al. (2016) and Adetunji et al. (2017), free-living bacteria indirectly promote plant growth by secreting extracellular enzymes that solubilise solid organic matter, thus making nutrients available for plant uptake. Wouter and Buijsman (1980) studied the secretion of alkaline phosphatases and reported that *Bacillus licheniformis* secreted 30-40% of alkaline phosphatase in a medium containing low phosphate concentrations. Also, *Pseudomonas putida* is reported to improve soil urease and phosphatase activity in soils with low fertiliser input. According to Bansal et al. (2014), an increase in soil urease activity improves soil fertility, thus elucidating the role of soil microbes and their associated enzymes on soil nutrient inputs. *Variovorax paradoxus,* a bacterial species isolated from non-rhizosphere soils in Rhebu and Ocean View, reportedly produced 94.0 U/mL alkaline phosphatase in a liquid medium ten days after inoculation (Magadlela et al., 2023). The role of *Variovorax paradoxus* in P-solubilization through the production of alkaline phosphatase, as reported by Magadlela et al. (2023), suggests that the bacteria isolated from the rhizosphere and non-rhizosphere soils in Rhebu and Ocean View may play a role in soil nutrient inputs through extracellular enzyme activities. According to Caldwell (2005), soil nutrient concentrations are influenced by microbes and their associated enzyme activities.

There were no significant differences in the N-acetylglucosaminidase, acid phosphatase, alkaline phosphatase, and nitrate reductase activities in the rhizosphere and non-rhizosphere soils. This may have led to the lack of significant differences in the primary nutrient concentrations in rhizosphere and non-rhizosphere soils, which may be attributed to cattle that graze in the scarp forest of Rhebu and Ocean View. The cattle deposit dung and urine during grazing which may affect the soil microbial activity. Previous studies showed that application of cattle manure in soil increase the nitrate reductase activity (Purbajanti et al., 2019), alkaline phosphatase activity (Gautam et al., 2020), and N-acetylglucosaminidase activity (Zhao et al., 2022). Acid phosphatase activity is always higher in low pH soils (Reardon et al., 2022). Ndlovu et al. (2023) reported the lack of significant differences in the N-acetylglucosaminidase, acid phosphatase, alkaline phosphatase, and nitrate reductase activities of *E. natalensis* rhizosphere and surrounding soils in localities that experience cattle grazing, and these findings concur with the current study. The PCA plot (Figure 1) showed correlations between phosphorus (acid phosphatase) and N-cycling (N-acetylglucosaminidase) and soil P and N, justifying the contributions of extracellular enzyme activities to soil nutrient inputs. Therefore, high soil P and N concentrations indicate high P and N cycling enzyme activities. Similar findings were recorded by Ndlovu et al. (2023), where positive correlations between alkaline and acid phosphatases and extractable P in *E. natalensis* rhizosphere and non-rhizosphere soils. While Ndlovu et al. (2023) reported correlations between soil P and Alkaline phosphatase, in this study correlations were only recorded between acid phosphatase and soil P, this may be attributed to the acidic nature of the soils.

Sekaran et al. (2018) reported that N-rich soils had a higher enzyme activity, further supporting the correlation between N-cycling enzymes and soil N. Positive correlations between N-acetylglucosaminidase activity and soil N were recorded by Ndlovu et al. (2023), these findings concur with the findings of this study. N-acetylglucosaminidase promotes N and C cycling through the hydrolysis of chitin (Cenini et al., 2016). Ekenler and Tabatabai (2002) and Cenini et al. (2016) reported correlations between soil N and N-acetylglucosaminidase, further supporting the findings of this study. This study shows correlations between extracellular enzyme activities and soil nutrition, which is supported by Cenini et al. (2016), Ekenler and Tabatabai (2002), Sekaran et al. (2018) and Margalef et al. (2017), who reported positive correlations between P-Olsen and acid phosphatase. According to Allison *et al.* (2007), phosphatase enzyme activities are negatively correlated with soil P, our findings deviate from Allison et al. (2007) as positive correlations were recorded in Rhebu and Ocean View. Even though our findings deviate from Allison et al. (2007), the resource allocation model for extracellular enzyme activities states that soil microbes exude extracellular enzymes to mineralise and cycle deficient soil nutrients (Sinsabaugh and Moorhead, 1994). Thus, the positive correlations between the macronutrients and assayed enzyme activities may have been caused by the macronutrient deficiency in Rhebu and Ocean View soils.

Generally, cycads possess coralloid roots, which host N-fixing bacteria that function in converting atmospheric N to plant-usable N. It is therefore expected that nutrient concentration in soils of *E. villosus* rhizosphere in Rhebu and Ocean View will be higher than in non-rhizospheric soils. The results in this study is consistent with the hypothesis N-fixing bacteria in the coralloid roots of cycads aid in improving soil nutrient status. The rhizosphere soils of *E.villosus* contain higher nutrient concentrations than the non-rhizospheric soils. *Cycas micronesica*, and *Zamia integrifolia* have been shown to improve the soil nutrient status of the cycad rhizosphere (Marler and Krihnapillai, 2018; Marler and Calonje, 2020). In these studies, cycads alter the soil's chemical composition along the leaf's drip line canopy through the plant leaf litter (Marler and Krishnapillai, 2018). Marler and Calonje (2020) reported high concentrations of N and P in *Cycas micronesica* and *Zamia integrifolia* rhizosphere, showing cycads' role in ecosystem soils. Although the contributions of cycads to soil nutrition have been reported, the mechanism for these nutrient contributions is not discussed. The coralloid roots of *E. villosus* were predominated by N-fixing bacteria, suggesting that the high N content in the cycad rhizosphere (Table 1) is accounted for by the N-fixing bacteria which triggers biological N fixation (BNF) for plant uptake and survival. Also, arbuscular mycorrhizal fungi (AMF) is present in

cycad roots and it allow cycads to increase P uptake in soils which are low in P, and water availability for the plants (Muthukumar and Udaiyan, 2002). This way, plants are self-sufficient in terms of the deficient essential elements for plant growth (N and P). The immediate consequence is an increase in N-cycling bacteria that mobilize the nutrient. This explains the increased proportion of N-cycling bacteria in both rhizosphere and non-rhizosphere soils. In addition to that, bacteria such as *Bacillus, Pseudomonas, Paenibacillus,* and *Lysinibacillus* genera are known as plant growth-promoting rhizobacteria and enhance plant performance. Therefore, the current study shows that the contributions of *E. villosus* to soil nutrition are primarily through plant-associated microbes, soil microbes, and their associated enzyme activities. Marler and Calonje (2020) reported higher nutrient concentrations in rhizosphere soils than in non-rhizosphere soils, deviating from the findings of this study. This may be due to the presence of cattle in the study sites, differences in microbial diversity and composition, microbe-associated enzyme activities, and soil geochemical properties. Also, *E. villosus* is a forest understory plant, nutrient concentrations in the rhizosphere and non-rhizosphere soils may have been influenced by plant litter and deadwood. Deadwood has been reported to contribute 1-18% of N, P, K, and Ca inputs in forest litter (Laiho and Prescott, 2004).

Conclusion

This study showed that cycad-microbe symbiosis, identified soil microbes, and associated enzymatic activities may influence soil nutrient inputs in the rhizosphere and non-rhizosphere soils. Phosphorus solubilizing, N-cycling, and N-fixing bacteria enable cycads to improve soil health and fertility by enhancing nutrient cycling. These microbes also produce enzymes such as phosphatase and glucosaminidase that enhance nutrient bioavailability. The present study concluded that soil microbes and their associated enzyme activities influence soil nutrition and enable cycads to support the growth and development of plants in the ecosystem. Furthermore, this study highlights the role of cycads in maintaining ecosystem health through nutrient cycling. Therefore, cycads conservation should be prioritized globally due to their significant ecosystem services in nutrient-poor ecosystems.

Declarations

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Conflicts of interests

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

Availability of data and material

The data and material can be made available upon request.

References

1. Adetunji AT, Lewu FB, Mulidzi R, Ncube B (2017) The biological activities of β-glucosidase, phosphatase, and urease as soil quality indicators: A review. J Soil Sci Plant Nutr 17: 794–807.
2. Allison VJ, Condron LM, Peltzer DA, Richardson SJ, Turner BL (2007) Changes in enzyme activities and soil microbial composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biol Biochem 39: 1770–1781.
3. Ano AO, Ubochi CI (2007) Neutralization of soil acidity by animal manure: Mechanism of reaction. Afr J Biotechnol 2007: 364–368.
4. Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31: 1471–1479.
5. Bansal OP, Singh G, Katiyar P (2014) Effect of untreated sewage effluent irrigation on heavy metal content, microbial population and enzymatic activities of soils in Aligarh. J Environ Biol 35**:** 641–647.
6. Barea J, Pozo MJ, Azcòn-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56: 1761–1778.

7. Bellès-Sancho P, Beukes C, James EK, Pessi G (2023) Nitrogen fixing symbiotic *Paraburkholderia* species: Current knowledge and future perspectives. Nitrogen 4: 132-158.
8. Caldwell BA (2005) Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49: 637–644
9. Cenciani K, dos Santos Freitas S, Critter SAM, Airoldi C (2008) Microbial enzymatic activity and thermal effect in tropical soil treated with organic material. Soil Sci Plant Nutr 65: 1–7.
10. Cenini VL, Fornara DA, McMullan G, Ternan N, Carolan R, Crawley MJ, Clément JC, Lavorel S (2016) Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. Soil Biol Biochem 96: 198–206.
11. Chang ACG, Chen T, Li N, Duan J (2019) Perspectives on endosymbiosis in coralloid roots: Association of cycads and cyanobacteria. Front Microbiol 10: 1888.
12. Condamine FL, Nagalingum NS, Marshall CR, Morlon, H (2015) Origin and diversification of living cycads: A cautionary tale on the impact of the branching process prior in Bayesian molecular dating. BMC Evol Biol 15: 1–5.
13. Cousins SR, Williamson VL, Witkowski ETF (2012) Uncovering the cycad taxa (*Encephalartos* species) traded for traditional medicine in Johannesburg and Durban, South Africa. S Afr J Bot 78: 129–138.
14. Cousins SR, Williamson VL, Witkowski ETF (2013) Sifting through cycads: A guide to identifying the stem fragments of six South African medicinal *Encephalartos* species. S Afr J Bot 84: 115–123.
15. Cousins SR, Witkowski ETF (2017) African cycad ecology, ethnobotany, and conservation: A synthesis. Bot Rev 83: 152–194.
16. De Mandal S, Singh SS, Kumar NS (2018) Analysing plant growth promoting *Bacillus* sp. and related genera in Mizoram, Indo-Burma biodiversity hotspot. Biocatal Agric Biotechnol 15: 370–376.
17. Donaldson JS (1997) Is there a floral parasite mutualism in cycad pollination? The pollination biology of *Encephalartos villosus* (Zamiaceae). Am J Bot 84: 1398–1406.
18. Ekenler M, Tabatabai M (2002) Glucosaminidase activity of soils: Effect of cropping systems and its relationship to nitrogen mineralization. Biol Fertil Soils 36: 367–376.
19. Favero VO, Hilário de Calvalho R, Leite ABC, dos Santos DMT, Moura de Freltas K, Boddey RM, Xavier GR, Rumjanek NG, Urquiaga S (2022) *Bradyrhizobium* strains from Brazilian tropical soils promote increases in nodulation, growth, and nitrogen fixation in mung bean. Appl Soil Ecol 175: 104461.
20. Fisher JB, Vovides AP (2004) Mycorrhizae are present in cycad roots. Bot Rev 70: 16–23.
21. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012: 963401.
22. Gautam A, Udayakumar S, Jose G, Kovács P, Gonzalez, JLD, Sandeep K (2020) Responses of soil microbial community structure and enzymatic activities to long-term application of mineral fertilizer and beef manure. Environmental and Sustainability Indicators 8: 100073.
23. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. JMBT 7: 96–102.
24. Gutiérrez-García K, Bustos-Díaz ED, Corona-Gómez JA, Ramos-Aboites HE, Sélem-Mojica N, Cruz-Morales P, Pérez-Farrera MA, Barona-Gómez F, Cibrián-Jaramillo A (2019) Cycad coralloid roots contain bacterial communities including *Cyanobacteria* and *Caulobacter* spp. that encode niche-specific biosynthetic gene clusters. *GBE* 11: 319–334.
25. Haas D, Dèfago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3: 307-319.
26. Jackson CR, Tyler HL, Millar JJ (2013) Determination of microbial extracellular enzyme activity in waters, soils, and sediments using high throughput microplate assays. JoVE. 80: e5039.
27. Jaiswal SK, Mohammed M, Ibny FYI, Dakora FD (2021) Rhizobia as a source of plant growth promoting molecules: Potential applications and possible operational mechanisms. Front Sustain Food Syst 4: 1-14.
28. Kandeler E (1995) Potential Nitrification. In Schinner F et al. (eds.) Methods in Soil Biology. Spinger-Verlag Berlin Heidelberg, Berlin. pp. 146–149.
29. Khalifa AYZ, Alsyeeh A, Almalki MA, Saleh FA (2016) Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J Biol Sci 23: 79–86.
30. Kong Z, Deng Z, Glick BR, Wei G, Chou M (2017) A nodule endophytic plant growth promoting *Pseudomonas* and its effects on growth, nodulation, and metal uptake in *Medicago lupulina* under copper stress. Ann Microbiol 67: 49–58.
31. Laiho R, Prescott CE (2004) Decay and nutrient dynamics of coarse woody debris in northern coniferous forest: a synthesis. Can J For Res 34: 763–777.

32. Latha P, Anand T, Prakasam V, Jonathan EI, Paramathma M, Samiyappan R (2011) Combining *Pseudomonas, Bacillus* and *Trichoderma* stains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. Appl Soil Ecol 49: 215–223.
33. Magadlela A, Lembede Z, Egbewale SO, Olaniran AO (2023) The metabolic potential of soil microorganisms and enzymes in phosphorus deficient KwaZulu Natal grassland ecosystem soil. Appl Soil Ecol 181: 1–11.
34. Manson AD, Roberts VG (2000) Analytical methods used by the soil fertility and analytical services section. KZN Agri-report no. N/A/2001/04, Pietermaritzburg, South Africa.
35. Margalef O, Sardans J, Fernández-Martínez M, Molowny-Horas R, Janssens I A, Ciais P, Goll D, Richter A, Obersteiner O, Asensio D, Peñeulas J. 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7: 1–13.
36. Marler TE, Calonje M (2020) Two cycad species affect the Carbon, Nitrogen, and Phosphorus content of soils. Horticulturae 6: 24.
37. Marler TE, Krishnapillai MV (2018) *Cycas micronesica* trees alter local soil traits. Forests 9: 565.
38. Meena A, Rao KS (2021) Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semi-arid region, India Ecol Process 10: 1–10.
39. Merino C, Godoy R, Matus F (2016) Soil enzymes and biological activity at different levels of organic matter stability. J Soil Sci Plant Nutr 16: 14–30.
40. Mucina L, Scott-Shaw CR, Rutherford MC, Camp KGT, Matthews WS, Powrie LW, Hoare DB (2006) Indian Ocean coastal belt. In: Mucina L, Rutherford M C, (eds.). *The vegetation of South Africa, Swaziland and Lesotho*. Strelitzia 19, Pretoria: South African National Biodiversity Institute.
41. Muthukumar T, Udaiyan K (2002) Arbuscular mycorrhizas in cycads of Southern India. Mycorrhiza 12: 213–217.
42. Ndlovu S, Suinyuy TN, Pérez-Fernández MA, Magadlela A (2023) *Encephalartos natalensis*, Their Nutrient-Cycling Microbes and Enzymes: A Story of Successful Trade-Offs. Plants 12: 1034.
43. Pantoja-Guerra M, Burkette-Cadena M, Dunlap CA, Ramirez CA (2023) *Lysinibacillus* spp.: an IAA-producing endospore forming bacteria that promotes plant growth. Antonie van Leeuwenhoek: 1-15.
44. Pecundo MH, Chang ACG, Chen T, Cruz TEED, Ren H, Li N (2021) Full length 16S rRNA and ITS gene sequencing revealed rich microbial flora in roots of *Cycas* spp. In China. Evol Bioinform 17: 1–16.
45. Purbajanti E D, Slamet W, Fuskhah E, and Rosyida R. 2019. Effects of organic and inorganic fertilizers on growth, activity of nitrate reductase and chlorophyll contents of peanuts (*Arachis hypogaea* L.). IOP Conference Series: Earth and Environmental Science 250: 012048.
46. Raimondo D C, Donaldson J S. 2003. Responses of cycads with different life histories to the impacts of plant collecting: Simulation models to determine important life history stages and population recovering times. Biol. Conserv. 11: 345–358.
47. Raimondo D, von Staden L V, Foden W, Victor J E, Helmen N A, Turner R C, Kamundi, D A, Manyama P A. 2009. Red list of South African plants. Strelitzer 25, South African National Biodiversity Institute, Pretoria.
48. Reardon CL, Klein AM, Melle CJ, Hagerty CH, Klarer ER, Machado S, Paulitz T, Pritchett LD, Schlatter D, Smith SF, Wuest SB (2022) Enzyme activities distinguish long-term fertilizer effects under different soil storage methods. Appl Soil Ecol 177: 104518.
49. Sekaran U, McCoy C, Kumar S, Subramanian S (2018) Soil microbial community structure and enzymatic activity responses to nitrogen management and landscape positions in switchgrass (*Panicum virgatum* L.). Glob Change Biol 2019: 836–851.
50. Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biol Biochem 26: 1305–1311.
51. Sitaram R (2015) *Pseudomonas* spp. as models for plant-microbe interactions. Front Plant Sci 6: 787.
52. Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego. pp. 44–60.
53. Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK, Suman A (2016) Molecular diversity and multifarious plant growth promoting attributes in Bacilli associated wheat *Triticum aestivum* L. rhizosphere from six diverse agro-ecological zones in India. J Basic Microbiol 56: 44–58.
54. Viljoen CD, van Staden J (2006) The genetic relationship between *Encephalartos natalensis* and *Encephalartos woodii* determined using RAPD fingerprinting. S Afr J Bot 72: 642–645.
55. Williamson J, Maurin SNS, van der Bank H, Pfab M, Rilusa M, Kabongo RM, van der Bank M (2016) Exposing the illegal trade in cycad species (Cycadophyta: *Encephalartos*) at two traditional medicine markets in South Africa using DNA barcoding. Genome 59: 771–781.

56. Wouter JTM, Buijsman PJ (1980) Secretion of alkaline phosphatase by *Bacillus licheniformis* 749/C during growth in batch and chemostat cultures. FEMS Microbiol 7**:** 91–95.
57. Yessoufou K, Bamigboye SO, Daru BH, van der Bank M (2014) Evidence of constant diversification punctuated by a mass extinction in the African cycads. Ecol Evol 4: 50-58.
58. Zhao C, Hu J, Li Q, Fang Y, Liu D, Liu Z, Zhong R (2022) Transfer of nitrogen and phosphorus from cattle manure to soil and oats under simulative cattle manure deposition. Front Microbiol 13: 916610.
59. Zheng W, Song T, Bao X, Bergman B, Rasmussen U (2002) High cyanobacterial diversity in coralloid roots of cycads revealed by PCR fingerprinting. FEMS Microbiol Ecol 40: 201–222.

Tables

Table 1: Soil nutrient concentrations (mg/kg), relative acidity, and organic carbon to nitrogen ratio of *E. villosus* rhizosphere and non-rhizosphere soils in Rhebu and Oceanview, Eastern Cape.

	Study site	Ocean View		Rhebu	
	Parameter	Rhizosphere (n = 7)	Non-rhizosphere soils (n = 7)	Rhizosphere (n = 8)	Non-rhizosphere soils (n = 8)
Macronutrients	P	6.1±2.9[a]	3.86±1.9[a]	22.95±5.9 [a]	13.23±3.7 [a]
	K	139.5±33.6[a]	81.9±12.5 [a]	330.0±100.9 [a]	179.1±54.9 [a]
	N	3093.7±741.9 [a]	2638.0±438.2 [a]	5189.3±297.9 [a]	3473.3±1396.3 [a]
Intermediate nutrients	Ca	1555.3±274.8 [a]	1379.2±153.5 [a]	1534.9±974.2 [a]	1229.4±1087.2 [a]
	Mg	454.7±46.1[a]	382.12±35.3[b]	838.5±549.3 [a]	697.1±548.2 [a]
Micronutrients	Mn	55.3±21.1 [a]	44.8±15.9 [a]	89.7±58.0 [a]	48.1±20.2 [a]
	Zn	2.2±0.1[c]	1.3±0.4[d]	4.1±1.6 [a]	2.8±1.2 [a]
	Cu	1.1±0.2 [a]	0.6±0.1 [a]	0.9±0.1 [a]	0.6±0.2 [a]
Relative Acidity	Exchange acidity (cmol/L)	0.05±0.01 [a]	0.05±0.01 [a]	1.46±1.42 [a]	1.37±1.19 [a]
	Total cations (cmol/L)	11.53±1.65 [a]	11.65±1.62 [a]	15.26±8.13 [a]	15.10±9.48 [a]
	pH	5.43±0.41[e]	4.97±0.57[f]	4.28±0.61 [a]	4.14±0.58 [a]
Organic nitrogen to carbon ratio	Organic carbon (%)	3.2	2.8	4.8	3.4
	Nitrogen (%)	0.30	0.29	0.47	0.33
	C: N ratio	11:1	10:1	10:1	10:1

[1] In each row, different letters denote significant differences in the nutrient concentrations of soil samples within study sites (independent samples t-test, $p \leq 0.05$, means±SE, n=30).

Table 2: Molecular identification of P-solubilizing, N-cycling, and N-fixing bacterial isolates in the coralloid roots of *E. villosus* growing in Rhebu and Oceanview

Rhebu				Oceanview			
Strain	Accession number	Similarity (%)	Function	Strain	Accession number	Similarity (%)	Function
Lysinibacillus xylanilyticus strain LonMTB_3	KX254351	98.27	P-solubilizing	*Lysinibacillus fusiformis* strain DSM	NR_042072	99.07	P-solubilizing, N-fixing
Lysinibacillus sp. strain 1	MN911365	99.67	P-solubilizing, N-fixing	*Bacillus* sp.	KF010353	99.92	P-solubilizing
Lysinibacillus parviboronicapiens strain FJAT-4580	KY038664	99.09	P-solubilizing	*Lysinibacillus xylanilyticus* strain YEBFR4	MT332716	96.32	P-solubilizing
Peribacillus frigoritolerans	MN710450	99.50	P-solubilizing	*Lysinibacillus sp.* strain SH-2	MF465572	91.00	P-solubilizing
Stenotrophomonas sp. strain VV6	MG725655	99.58	N-cycling	*Enterobacter cloacae* strain TMX6	MK116463	98.92	N-fixing
Lysinibacillus fusiformis strain SYSU K30005	NR_175531	99.9	P-solubilizing, N-fixing	*Paenibacillus polymyxa* strain J	KT783525	99.15	N-fixing
Rhizobium huautlense strain KUDC1811	KC355318.1	98.41	N-fixing				

Table 3: Molecular identification of P-solubilizing, N-cycling, and N-fixing bacterial isolates in *E. villosus* rhizosphere and non-rhizosphere soils in Rhebu

Rhizosphere				Non-rhizosphere soils			
Strain	Accession number	Similarity (%)	Function	Strain	Accession number	Similarity (%)	Function
Pseudomonas koreensis strain Ps 9-14	NR_025228	99.96	P-solubilizing, N-cycling	*Paraburkholderia tropica* strain LMG 22274	NR_118080	85.61	P-solubilizing
Caballeronia fortuita strain LMG 29320	NR_145600	98.48	P-solubilizing	*Pseudomonas taiwanensis* DSM 21245 strain BCRC 17751	NR_116172	99.92	P-solubilizing
Paraburkholderia steynii strain HC1.1ba	NR_164972	89.42	P-solubilizing, N-fixing	*Paraburkholderia steynii* strain HC1.1ba	NR_164972	99.48	P-solubilizing, N-cycling, N-fixing
Pseudomonas migulae strain NBRC 103157	NR_114223	99.05	P-solubilizing, N-fixing	*Variovorax paradoxus* NBRC 15149	NR_113736	99.73	P-solubilizing, N-fixing
Paraburkholderia terricola strain LMG 20594	NR_118079	85.60	P-solubilizing	*Dyella marensis* strain CS5-B2	NR_042691	99.86	N-cycling
Burkholderia ambifaria strain AMMD	NR_074687	89.71	P-solubilizing	*Stenotrophomonas sp.* strain L145	MT505153	84.82	N-cycling, N-fixing

Pseudomonas mosselii strain CFML 90-83	NR_024924	82.69	P-solubilizing, N-cycling, N-fixing	*Gottfrieder acidiceler* strain CBD	NR_043774	72.48	N-cycling
Novosphingobium barchaimii LL02	NR_118314	99.00	P-solubilizing	*Arthrobacter globiformis* strain JCM 1332	NR_112192	99.58	N-cycling
Pseudomonas fulva strain NRIC 0180	NR_104280	99.65	N-cycling	*Caulobacter rhizosphaerae* strain 7F14	NR_156992	99.24	N-cycling
Variovorax guangxiensis strain GXGD002	NR_134828	99.38	N-cycling	*Burkholderia ambifaria* strain AMMD	NR_074687	95.96	N-fixing
Caulobacter rhizosphaerae strain 7F14	NR_156992	91.77	N-cycling	*Paraburkholderia sabiae* STM678	NR_118081	98.03	N-fixing
Olivibacter domesticus strain DC-186	NR_042571	99.20	N-cycling	*Paraburkholderia sabiae* strain Br3407	NR_115261	97.88	N-fixing
Acinetobacter oleivorans strain DR1	NR_102814	98.65	N-cycling				
Variovorax boronicumulans NBRC 103145	NR_114214	90.41	N-cycling				
Paraburkholderia steynii strain HC1.1ba	NR_164972	97.62	N-cycling				
Cupriavidus oxalaticus NBRC 13593	NR_113619	96.92	N-fixing				
Burkholderia ambifaria strain AMMD	NR_074687	99.20	N-fixing				
Novosphingobium resinovorum strain NCIMB 8767	NR_044045	92.59	N-fixing				

Figures

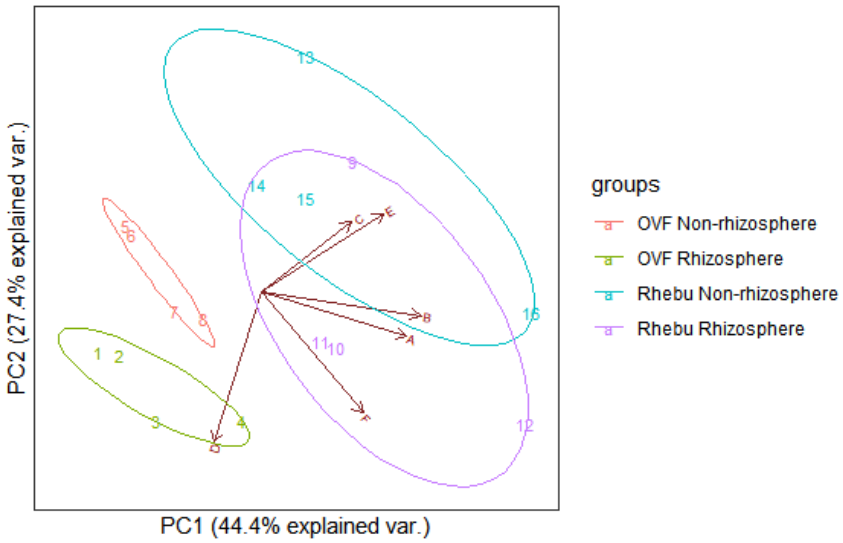

Figure 1

Correlations between soil nutrients and enzyme activities where A- Soil N; B- Glucosaminidase; C- Acid phosphatase; D- Alkaline phosphatase; E- Soil P, and F- Nitrate reductase. Principal component analysis (PCA).

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