



# Belowground traits of perennial grasses shape restoration strategies in tropical grasslands

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Received: 8 June 2025 / Accepted: 26 August 2025 / Published online: 25 September 2025  
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## Abstract

**Background and Aims** Multiple biotic and abiotic stressors increasingly threaten tropical grasslands, yet restoration efforts predominantly prioritise above-ground traits, neglecting critical belowground traits essential for ecosystem resilience and recovery. We proposed that these neglected traits shape ecosystem biodiversity, productivity, and stress tolerance. This study assessed biomass yield, root and rhizosphere traits, resource acquisition strategies, and soil microbial communities in selected perennial grasses used for forage and seed-based restoration. We also evaluated enzymatic activity and microbial functional potential related to carbon, nitrogen, and phosphorus cycling.

**Methods** In a long-term restoration project in a tropical semi-arid grassland, we measured plant biomass, root morphology, mycorrhization, extracellular enzyme activities, and rhizosphere microbial community composition and function.

**Results** *Eragrostis superba* had the highest shoot biomass, while *Cenchrus ciliaris* showed the highest root biomass and mycorrhization. The root trait–microbiome associations revealed distinct resource acquisition strategies: *Chloris roxburghiana* and *Enteropogon macrostachyus* displayed a ‘do-it-yourself’ strategy supported by copiotrophic rhizomicrobiome, while *C. ciliaris* displayed an ‘outsourcing’ strategy. Conversely, *E. superba* displayed high intraspecific root trait variation, exhibiting partially ‘do-it-yourself’ and ‘outsourcing’ traits. Grass species significantly influenced fungal, but not bacterial, community composition and favoured nutrient recycling over direct nutrient acquisition, with recycling strategies varying among species.

Responsible Editor: Michael Luke McCormack.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11104-025-07858-w>.

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**Conclusion** Root traits and belowground plant–microbe interactions shape ecosystem function in tropical grassland restoration. Selected species occupied distinct successional niches, from early colonisation (*C. ciliaris*) to late-stage productivity (*E. superba*). Integrating these traits into restoration frameworks is essential for enhancing ecosystem resilience, productivity, and successful long-term ecological restoration outcomes.

**Keywords** Plant-soil (below-ground) interactions · Sustainable management · Root and rhizosphere traits · Semi-arid savannahs · Degradation · Plant-climate interactions

### Abbreviations

|     |                                  |
|-----|----------------------------------|
| AMF | Arbuscular mycorrhizal Fungi     |
| MBC | Microbial biomass carbon         |
| MBN | Microbial biomass nitrogen       |
| PMB | Phosphorus-mineralising bacteria |
| PSB | Phosphorus-solubilising bacteria |
| RES | Root economic space              |

### Introduction

Tropical savannahs and grasslands cover approximately 20% of the global terrestrial surface, providing critical ecological and economic services (Boval et al. 2016; Lehmann & Parr 2016; Parr et al. 2014). However, these ecosystems are highly fragile and increasingly vulnerable to degradation due to biotic and abiotic stressors (Li et al. 2025b; Williams et al. 2022). Globally, nearly 50% of the grasslands have undergone some degree of degradation, with over 60% of them in poor condition, and less than 3% remaining intact (Niu et al. 2025; Wang et al. 2025a; Williams et al. 2022). Degraded grasslands are primarily found in arid and semi-arid drylands, where water scarcity and nutrient-poor soils limit their productivity (Li et al. 2025b). Multiple simultaneously occurring stressors, including land-use change, overgrazing, poorly managed fires, invasive species, and recurrent and prolonged droughts, are key drivers of grassland degradation (Li et al. 2025b; Lyons et al. 2023; Williams et al. 2022). Furthermore, poor grassland management practices further reduce species richness, diversity and productivity (Viruel et al. 2021; Wang et al. 2025a; Williams et al. 2022). Beyond

biodiversity loss, degradation reduces primary productivity and thus the C-sequestration potential of these ecosystems (Li et al. 2025b; Wang et al. 2025a).

Restoring degraded grasslands enhances ecosystem productivity and stability (Guo et al. 2021; Lyons et al. 2023; Mi et al. 2024). Due to slow natural regeneration potential, facilitated revegetation is often necessary for the degraded ecosystems (Atkinson & Bonser 2020; Stapleton et al. 2024). However, a combination of environmental constraints and climatic factors poses challenges, particularly in fragile, biotically and abiotically stressed drylands (Stapleton et al. 2024). Moreover, recently restored ecosystems have a high risk of reverting to a degraded state (Fogarty et al. 2025). Natural restoration methods are cost-effective but slow, whereas reconstructive restoration strategies, such as reseeded with indigenous perennial grasses, accelerate recovery (Atkinson & Bonser 2020; Mi et al. 2024; Slodowicz et al. 2023). Using indigenous grasses adapted to local environmental conditions enhances successful ecological restoration outcomes (Mganga et al. 2022). Characteristics and diversity of selected grasses may not only be decisive in achieving successful ecological restoration outcomes but also influence the productivity, functionality and resilience of restored ecosystems (Guo et al. 2021; Mganga et al. 2022; Weigelt et al. 2021).

Belowground characteristics of plants, such as root and rhizosphere traits, are crucial for plant resource acquisition and adaptation to stressors due to their plasticity in dynamic environments (Tao et al. 2024; Wild et al. 2024). Root architecture, morphology, physiology, and biotic interactions- including rooting depth, length, diameter, specific root length (SRL), respiration, exudation, and mycorrhization- affect resource uptake and ecosystem processes such as nutrient cycling (George et al. 2024; Laliberté 2017; McCormack et al. 2015). Roots with high tissue density (RTD) are typically long-lived, a ‘slow’ strategy, while those with high nitrogen (N) content and rapid turnover reflect a ‘fast’ strategy of the root economics spectrum (Matthus et al. 2025; Reich 2014). Absorptive roots (<2 mm) with larger diameters are also more likely to host mycorrhiza fungi, as they offer greater habitat volume for colonisation (Freschet et al. 2021). Arbuscular mycorrhizal fungi (AMF) enhance plant resource acquisition, an ‘outsourcing’ mechanism of the collaboration gradient of the root economic space (RES; Laliberté 2017; Li et al. 2025a; Wild et al. 2024). AMF

are dominant in our restored tropical grassland ecosystem under study (Bergmann et al. 2020; Weigelt et al. 2021). On the other hand, grass species with high specific root length (SRL) tend to follow a ‘do-it-yourself’ strategy, relying more on direct nutrient uptake than on symbiotic associations.

Resource acquisition strategies adopted by plants influence the rhizosphere and its microbial community composition and structure. ‘Fast’ strategists exhibit elevated exudation rates (Hou et al. 2025; Wen et al. 2022), which tend to support more dynamic and diverse microbial assemblages in the rhizosphere (Oram et al. 2025; Tomazelli et al. 2024; Wang et al. 2025b). Conversely, species allocating more C to mycorrhizal partnerships (i.e., an ‘outsourcing’ strategy) rely on symbionts to enhance nutrient uptake and improve stress mitigation, particularly under drought conditions (Begum et al. 2019; Kakouridis et al. 2022; Oram et al. 2025). Rhizosphere fungi (rhizofungi) and bacteria (rhizobacteria) communities play essential ecological functions, including organic matter (OM) decomposition, nutrient mining (mobilisation and transport), nutrient cycling and mediating plant-soil feedback mechanisms, thereby affecting ecosystem stress tolerance (Li et al. 2025a; Oram et al. 2025; Wang et al. 2025b).

Although rooting characteristics, rhizosphere interactions, and soil microbial communities sustain ecological functions and long-term ecosystem health, most grassland restoration programs prioritise above-ground traits, neglecting these critical belowground traits (Freschet et al. 2021; Guo et al. 2021; Tian et al. 2022; Tomazelli et al. 2024; Weigelt et al. 2021). We, therefore, hypothesised that these often underappreciated belowground traits significantly shape resource acquisition strategies and productivity of tropical grasslands in drylands. This study aimed to: 1. Analyse the biomass yield and root characteristics of selected perennial grasses to determine productivity and resource acquisition strategies. 2. Analyse fungal and bacterial communities’ composition and diversity to assess the influence of root traits and acquisition strategies. 3. Assess C, N and P degrading enzyme activities, microbial biomass, and potential microbial functions regulating C, N and P biogeochemical cycles within the rhizosphere of the selected grasses in these tropical grassland ecosystems. Understanding belowground interactions and mechanisms is crucial for maintaining biodiversity, enhancing productivity,

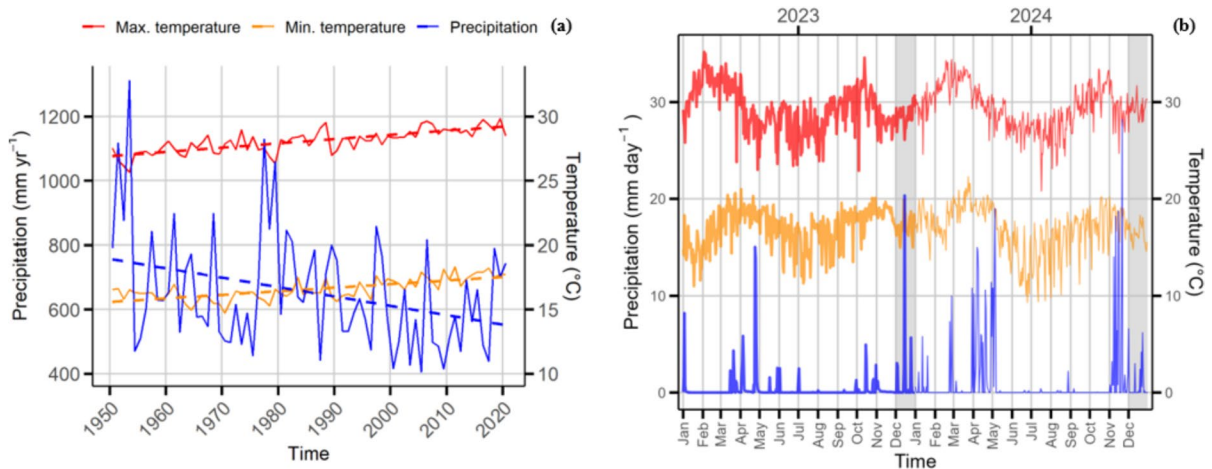
and improving restoration outcomes by enabling ecosystems to adapt to rapidly escalating and concurrent multiple stressors.

## Materials and methods

### Site description, experimental design and sampling

Soil and indigenous grasses were sampled in 2023 and 2024 from a field experiment linked to a long-term ecological restoration site in a semi-arid tropical grassland in Kenya (Mganga et al. 2021a, 2021b, 2022). The land is primarily used for crop and livestock production, with the local community being predominantly agropastoralists. The site is at South Eastern Kenya University (SEKU; Fig.S1). It Lies in semi-arid Kitui county, south-eastern Kenya, at an altitude of 1121 m. The bimodal rainfall ranges from 400–900 mm annually, with short rains between October and December and long rains between March and May. Over the last 70 years, study site precipitation decreased by 30.21 mm per decade, while Minimum and maximum temperatures rose by 0.28 °C and 0.33 °C per decade, respectively (1950 to 2020; Fig. 1a; World Bank 2024). We classified the soil as Haplic Acrisol with a sandy clay texture, based on the WRB soil classification guidelines (Table S1; IUSS Working Group WRB 2022). The soil has low organic matter (OM) and limited available nitrogen (N) and phosphorus (P), along with surface sealing properties, which reduce infiltration rates and increase vulnerability to erosion (Mganga et al. 2021b, 2022; Yageta et al. 2019).

The experiment was established during the short-rain season of 2017. The experimental design and initial site description are detailed in Mganga et al (2021b). Briefly, grass monocultures were established in 30 m<sup>2</sup> plots (10 m × 3 m), arranged in a randomised complete block design (RCBD), with a 2 m buffer between blocks. Locally collected seeds were hand-sown into shallow Micro-catchments spaced at 15 cm intervals, using the recommended seeding rate (5 kg ha<sup>-1</sup>). Sowing took place before the onset of the short rains, and no fertiliser was applied at planting. Weed control was conducted regularly to maintain pure stands of four tussock-forming grasses: *Cenchrus ciliaris* (African foxtail grass or Buffel grass), *Enteropogon macrostachyus* (Bush rye grass),



**Fig. 1** Climatic Data for Kitui County and SEKU Study Site. **(a)** Maximum temperature, Minimum temperature and annual precipitation trends for Kitui County from 1950 to 2020, displayed as a time series with trendlines showing long-term pat-

terns. **(b)** Daily precipitation and temperature data recorded at the SEKU study site during the sampling period in 2023 and 2024. Key: The grey bars indicate the sampling point

*Chloris roxburghiana* (Horsetail grass), and *Eragrostis superba* (Maasai love grass).

Aboveground biomass was harvested from a randomly selected 4 m<sup>2</sup> quadrat within each plot in December 2023 and 2024. Additionally, within this quadrat, a single representative tussock (~0.75 m<sup>2</sup>) was selected and manually excavated using hand tools to a depth of approximately 30–60 cm, depending on root penetration. The entire root system of the selected tussock was collected during the 2023 sampling campaign. Rhizosphere soil tightly adhering to the roots was collected by gently shaking and brushing. Soil samples were stored and transported at –20 °C for DNA analysis and at 4 °C for extracellular enzyme and microbial biomass carbon (MBC) assays. Shoot samples were oven-dried at 60 °C, while root samples were freeze-dried (at –56 °C and 1.00 mbar vacuum) for 72 h before dry weight measurement.

#### Root characterisation

##### Root functional traits

Root samples were transported in 70% ethanol from the field to the laboratory. They were thoroughly washed with tap water to remove soil and debris. The roots were gently separated and spread in a consistent position to minimise overlaps and clumping in DIN A3 (29.7×42.0 cm) water basins. Larger samples

were divided into smaller portions and scanned separately. The samples were placed in 0.9% sodium chloride (NaCl) at 4 °C to avoid desiccation during storage between scans. Scanning was performed with a DIN A3 scanner (Epson Expression 13000XL) fitted with a transparency unit at a resolution of 600 dpi (Image type: 16-bit Greyscale, Resolution: 600 dpi, scanning quality: high, Document type: colour positive film). The root scans were analysed with RhizoVision Explorer v2.0.3 (hereafter RhizoVision) to estimate root length (cm), diameter (mm), surface area (cm<sup>2</sup>), and volume (cm<sup>3</sup>). During analysis, we categorised roots based on their diameter: very fine (<0.5 mm), fine (0.5–2.0 mm), or coarse roots (>2 mm, hereafter transport roots) (Böhm 1979). We used absorptive roots, grouped as the total of very fine and fine roots, in the RES analysis. Specific root length (SRL) and root tissue density (RTD) were computed using Equations 1 and 2, respectively (Zhang et al. 2024).

$$\text{Specific root length (m g}^{-1}\text{)} = \frac{\text{Total root length (m)}}{\text{Root dry biomass (g)}} \quad (1)$$

$$\text{Root tissue density (g cm}^{-3}\text{)} = \frac{\text{Root dry biomass (g)}}{\text{Root volume (cm}^{-3}\text{)}} \quad (2)$$

### *Arbuscular mycorrhizal fungi (AMF) colonisation analysis*

Root tip samples for determining root colonisation were frozen at  $-20^{\circ}\text{C}$  during transport to the laboratory. They were stained using a modified ink and vinegar technique (Munene et al. 2025; Vierheilig et al. 1998). After washing with tap water to remove soil, the roots were cut into 10 mm segments, placed in biocassettes and remained in the biocassettes during the staining process. The biocassettes were heated in 10% potassium hydroxide (KOH) in a water bath at  $90^{\circ}\text{C}$  for 10 Min. After rinsing off the KOH, they were placed in 5% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 10

Min to reduce pigmentation. The roots were stained using 5% Pelikan blue ink-vinegar solution at  $90^{\circ}\text{C}$  for 3 min after rinsing  $\text{H}_2\text{O}_2$ . Excess ink was removed by destaining in acidified water for 20 Min. The stained roots were stored in glycerol at  $4^{\circ}\text{C}$  during Microscopy. An average of 55 stained root segments per grass species were observed at  $200\times$  total magnification using the Kern Digital Microscope Set OBN 135C825. Three images were captured at regular 3 mm intervals per segment. The images were fitted into grid squares, classified and counted using the Fungi Tagger master software, as described by Stock et al. (2021). The percentage of root mycorrhization was calculated using Equation 3.

$$\% \text{ root colonisation} = \frac{\text{Fungi colonised squares}}{(\text{Fungi colonised squares} + \text{root squares})} * 100 \quad (3)$$

where;

fungi colonised squares = total number of squares containing arbuscules, hyphae and vesicles;

root squares = Total number of squares lacking fungi structures.

### Rhizosphere traits

#### *Extracellular enzyme activity*

The kinetic activities of  $\beta$ -glucosidase (BG), leucine aminopeptidase (LAP), and acid phosphatase (AP) enzymes were measured to assess general Microbial activity, and N and P Mineralisation in soil. In the BG and AP standard plates, 4-methylumbelliferone (MUF) substrate with 0.1 M MES buffer was used, while amino-4-methyl-coumarin (AMC) substrate with 0.05 M TRIZMA buffer was used for LAP (Marx et al. 2001). 4-methylumbelliferone- $\beta$ -D-glucoside, 4-methylumbelliferone phosphate, and leucine 7-amino-4-methyl-coumarin hydrochloride were used as substrates for BG, AP, and LAP, respectively (Stock et al. 2019). Briefly, 50 ml of Milli-Q water was added to 0.5 g of fresh soil and sonicated at  $40 \text{ J s}^{-1}$  for 2 Min. 50  $\mu\text{l}$  of the soil suspension was pipetted into a 96-well Microplate with three replicates per sample. Afterwards, 50  $\mu\text{l}$  of MES or TRIZMA buffer and 100  $\mu\text{l}$  of the respective substrate (0, 10, 20, 30, 40, 50, 100, and 200  $\text{mol g}^{-1}$  for BG and LAP;

or 0, 10, 20, 30, 50, 100, 200, and 400  $\text{mol g}^{-1}$  for AP) added. For the standard plates, 50  $\mu\text{l}$  of composite soil suspension was pipetted. Then, 150, 140, 130, 100, 70, and 30  $\mu\text{l}$  of MES or TRIZMA buffer and 0, 10, 20, 50, 80, and 120  $\mu\text{l}$  of MUF or AMC standards were added respectively. The fluorescence was measured using the Victor<sup>3</sup> 1420–050 microplate reader (excitation: 355 nm, emission: 460 nm, slit width: 25 nm) at 0, 0.5, 1, and 2 h. The enzymatic activity, V, was calculated and fitted to the Michaelis–Menten equation. The  $V_{\max}$  and  $K_m$  were computed using equation 4.

$$V = \frac{V_{\max} * S}{K_m + S} \quad (4)$$

where;

S = substrate concentration added;

$V_{\max}$  = maximum enzymatic activity rate;

$K_m$  = substrate concentration at  $\frac{1}{2} V_{\max}$  = half-saturation constant.

#### *Microbial biomass carbon and nitrogen*

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform-fumigation extraction method (CFE) (Brookes et al. 1985; Joergensen et al., 2011). 7.5 g of fresh soil was fumigated with chloroform ( $\text{CHCl}_3$ ) for 24 h. 30 mL of 0.05 M potassium sulphate ( $\text{K}_2\text{SO}_4$ )

was added and shaken in a horizontal shaker (180 rpm) for one hour. Extractable C and N in fumigated and parallel unfumigated samples filtrate were measured using a C/TN analyser (CmultiN/C 2100; AnalytikJena). MBC and MBN were computed as the difference between fumigated and non-fumigated samples, applying a  $K_{ec}$  extraction factor of 0.45 and 0.54, respectively (Joergensen et al., 2011).

### DNA of microbial communities

Soil microbial DNA was extracted using the FastDNA Spin Kit (MP Biomedicals, United States) according to the manufacturer's protocol. DNA purification, library preparation, and sequencing were performed at the Quantitative Biology Center (QBiC), University of Tübingen. The V4 region of the 16S rRNA gene and the ITS region were sequenced to assess bacterial and Fungal communities, respectively. Primer pairs 515F (Parada): GTGCCAGCMGCCGCGGTAA and 806R (Apprill): GGACTACHVGGGTWTCTAAT were used for the 16S rRNA V4 region, while ITS1f: CTTGGTCATTTAGAGGAAGTAA and ITS2: GCTGCGTCTTCATCGATGC were used for the ITS region. Sequencing data were processed using the nf-core/ampliseq bioinformatics pipeline (version 2.12.0; Ewels et al. 2020; Straub et al. 2020).

Functional prediction for fungal and bacterial communities was conducted using FUNGuild v1.0 and Tax4Fun2, respectively (Nguyen et al. 2016; Wemheuer et al. 2020). The fungal community was analysed to predict its role in OM decomposition, while the bacterial community was assessed for its contribution to N cycling within the ecosystem. Fungal trophic levels were categorised into Pathotroph, Saprotroph, and Symbiotroph. OTUs assigned to multiple functional guilds were included in all relevant functional groups. Only OTUs with confidence levels classified as 'highly probable' or 'probable' were considered for analysis (Xu et al. 2021). Enzymes involved in the N-depolymerisation (Table S2; Landi et al. 2011; Sieradzki et al. 2023; Vranova et al. 2013) and N cycle (Table S3; Li et al. 2024; Sun et al. 2021), were selected based on annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Similarly, we selected P-solubilising (PSB) and P-mineralising (PMB) bacterial genes (Table S4; Pang et al. 2024; Pan & Cai 2023; Sun et al. 2022). The bacterial community was subsequently grouped

into N-depolymerisers, nitrifiers, denitrifiers, diazotrophs, PSB and PMB.

### Statistical analysis

Data compilation and arrangement were performed in Microsoft Excel, and statistical analyses were conducted using R statistical computing software (Version 4.4.2) (R Core Team 2024). The Shapiro–Wilk test and quantile–quantile (Q–Q) plots were used to assess data normality, while Levene's test was applied to evaluate homogeneity of variance. Data were log-transformed for positively skewed distributions or square root transformed for negatively skewed distributions before analysis. Differences among grass species were tested using one-way ANOVA, with means separated using Tukey's HSD test ( $P < 0.05$ ).

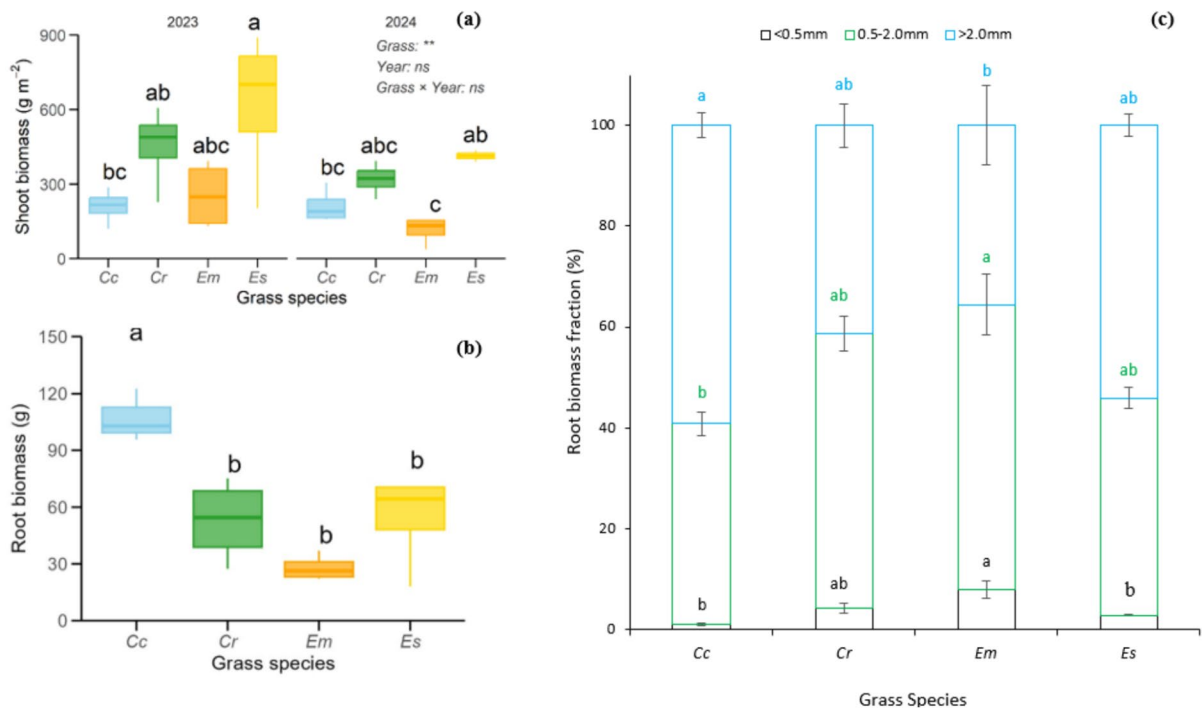
Alpha diversity indices, Chao 1 and Shannon ( $H'$ ), were used to assess richness and evenness of fungal and bacterial communities, respectively. Microbial community dissimilarity among grass species was quantified using Bray–Curtis distance matrices, with significance tested using Permutational Multivariate Analysis of Variance (PERMANOVA;  $P < 0.001$ ).

## Results

### Plant biomass

Shoot (Fig. 2a) and root biomass (Fig. 2b) varied significantly among grass species ( $P < 0.05$ ) but showed a similar pattern for aboveground biomass throughout the two sampling years. *E. superba* had the highest shoot biomass, two-fold, one-fold, and a half-fold greater than *C. ciliaris*, *E. macrostachyus* and *C. roxburghiana*, respectively. In contrast, *C. ciliaris* had the largest root biomass, which was one-fold greater than *C. roxburghiana* and *E. superba*, while *E. macrostachyus* showed the lowest root biomass, threefold lower than *C. ciliaris*. In consequence, root-to-shoot ratios differed significantly among species (Table S5;  $P < 0.05$ ). *C. ciliaris* had the highest ratio, 5–7 times greater than other grasses, while *E. macrostachyus* showed the lowest.

Biomass of very fine, fine and coarse roots also varied significantly among the grasses ( $P < 0.05$ ). *E. macrostachyus* had the highest proportion of very fine and fine roots (Fig. 2c), two- and six-fold greater than



**Fig. 2** Plant Biomass Data. **(a)** Shoot biomass and **(b)** root biomass are presented as box plots showing the median and interquartile range (IQR). **(c)** Root biomass fractions (very fine, fine, and coarse roots) based on root diameter, shown as

stacked bars. Data is presented as mean  $\pm$  standard error of the mean (SEM,  $n=4$ ), and letters indicate statistical significance at  $P<0.05$ . Key: Cc = *C. ciliaris*, Em = *E. macrostachyus*, Cr = *C. roxburghiana*, Es = *E. superba*

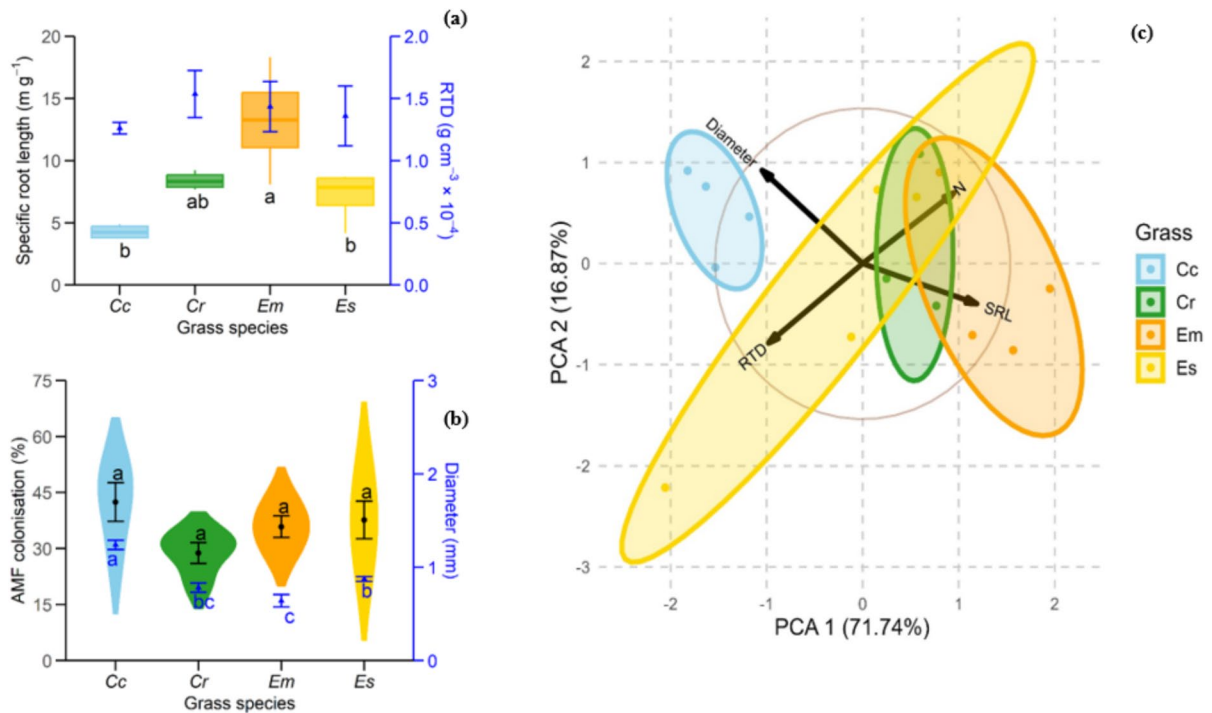
*E. superba* and *C. ciliaris*, respectively. Conversely, *C. ciliaris* had the highest proportion of coarse roots, 30% and 40% higher than *C. roxburghiana* and *E. macrostachyus*, respectively. Absorptive (<2 mm)-to-coarse (>2 mm) root biomass ratios differed significantly among grass species (Table S5;  $P<0.05$ ). *E. macrostachyus* allocated a greater proportion of biomass to absorptive roots, whereas *C. ciliaris* allocated significantly more to coarse roots. *E. superba* and *C. roxburghiana* exhibited moderate absorptive-to-coarse root allocation ratios among the studied grass species.

#### Root characterisation

Specific root length (SRL) differed significantly among the grasses ( $P<0.05$ ; Fig. 3a). *E. macrostachyus* had the greatest SRL, 37%, 46% and 68% higher than *C. roxburghiana*, *E. superba* and *C. ciliaris*, respectively. Root colonisation by AMF was not significantly different between the grasses, but *C. ciliaris* showed 16% and 32% greater AMF colonisation than

*E. macrostachyus* and *C. roxburghiana*, respectively (Fig. 3b). Root diameters varied significantly among grasses ( $P<0.05$ ), with *C. ciliaris* having the largest diameter, which was 30%, 37% and 48% larger than *E. superba*, *C. roxburghiana* and *E. macrostachyus*, respectively. Despite these significant differences in diameter, RTD did not vary significantly across species (Fig. 3a).

Moreover, the studied grasses occupied distinct positions within the RES (Fig. 3c). *E. macrostachyus* and *C. roxburghiana* were in the direction of the ‘fast and ‘do-it-yourself’ axis, characterised by high root N content and SRL, respectively. On the other hand, *C. ciliaris* aligned to the ‘outsourcing’ axis of collaboration gradient, characterised by its large absorptive root diameter. Additionally, its high RTD pointed towards the ‘slow’ axis of the conservation gradient. In contrast, *E. superba* displayed a high intraspecific variation of its root traits, without a clear strategy between the establishment of the symbioses and the ‘do-it-yourself’ strategy. Furthermore, a PCA of the transport root traits exhibited similar relationships observed



**Fig. 3** Root Characteristics. (a) Specific root length (SRL) of all roots presented as box plots, with root tissue density (RTD) displayed as blue triangles. (b) Arbuscular mycorrhizal fungi (AMF) colonisation of <2 mm roots displayed as violin plots, with the whole root system average root diameter shown as triangular blue symbols. (c) Root economic space of absorptive roots (<2 mm) presented via principal component analy-

sis (PCA). Note: Root diameter and SRL represent the collaboration gradient, while RTD and root nitrogen content (N) represent the conservation gradient. The PCA displays both intra- and interspecific trait variation. Means  $\pm$  SEM ( $n=4$ ) are shown, with statistical significance marked by letters at  $P<0.05$ . Key: *Cc*=*C. ciliaris*, *Em*=*E. macrostachyus*, *Cr*=*C. roxburghiana*, *Es*=*E. superba*

in the RES (Fig. S3). *C. ciliaris* loaded primarily on the diameter axis, *C. roxburghiana* on the RTD axis, and *E. macrostachyus* on the SRL-RTD axis. Consistent with its absorptive root traits, *E. superba* transport roots exhibited high intraspecific variation.

### Rhizosphere traits

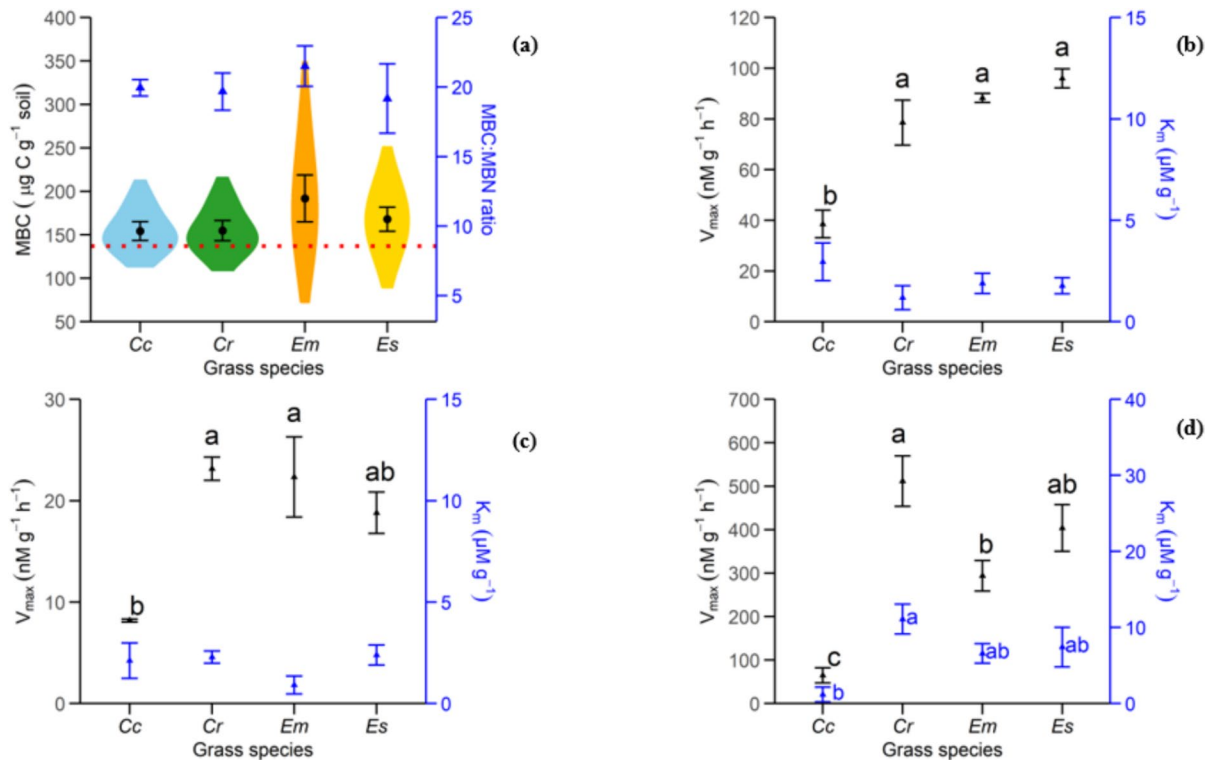
Our results showed similar MBC and MBC: MBN ratios among the grasses (Fig. 4a), with all MBC: MBN ratios being higher than double of the average stoichiometric ratio found in terrestrial ecosystems. Enzymatic activities varied significantly among the grasses for all enzymes analysed ( $P<0.05$ ; Fig. 4b, 4c, 4d).

The soil in the plots with *E. superba* had the highest  $\beta$ -glucosidase activity, 44% greater than *C. ciliaris*. *E. macrostachyus* had the highest leucine aminopeptidase activity, 28% and 69% greater than *E. superba* and *C. ciliaris*, respectively. *C. roxburghiana*

showed significantly higher acid phosphatase activity, two and eight times greater than *E. macrostachyus* and *C. ciliaris*, respectively. Among the grasses, *C. ciliaris* consistently exhibited the lowest overall enzymatic activities, despite maintaining microbial biomass levels comparable to the other species. The Km values for  $\beta$ -glucosidase and leucine aminopeptidase were comparable among the grasses. However, acid phosphatase Km values varied significantly, with *C. ciliaris* showing the lowest value, 25 times lower than *C. roxburghiana*.

### Microbial communities

The Chao1 alpha diversity index of fungal OTUs indicated significant differences in richness ( $P<0.05$ ; Fig. 5a). *C. roxburghiana* had the highest fungal OTU richness compared with *E. superba* and *E. macrostachyus*. However, the Shannon index of the



**Fig. 4** Rhizosphere Traits. (a) Microbial Biomass Carbon (MBC) presented as violin plots, with the MBC-Microbial Biomass Nitrogen (MBN) ratio shown by triangular blue symbols. Note: The red dotted line represents the average microbial C: N (60:7) stoichiometry in terrestrial ecosystems (Cleveland & Liptzin 2007). (b)  $\beta$ -Glucosidase, (c) Leucine aminopepti-

dase, and (d) Acid phosphatase extracellular enzyme activities (EEAs)— $V_{\text{max}}$  (black) and  $K_m$  (blue)—displayed as triangles representing means. Data is expressed as means  $\pm$  SEM ( $n=4$ ), with statistical significance indicated by letters at  $P<0.05$ . Key: Cc=*C. ciliaris*, Em=*E. macrostachyus*, Cr=*C. roxburghiana*, Es=*E. superba*

fungal OTUs was comparable across all grass species (Fig. 5a). Ascomycota and Basidiomycota were the dominant phyla, comprising on average 74% and 14% of the fungal community, respectively (Fig. S2a). Ascomycota abundance was highest in *E. superba*, 15% and 20% higher than in *C. roxburghiana* and *E. macrostachyus*, respectively. In contrast, *E. macrostachyus*, directly followed by *C. roxburghiana*, had the highest abundance of Basidiomycota, 25% and 49% higher than *C. roxburghiana* and *E. superba*, respectively. *E. macrostachyus* also showed the highest Glomeromycota abundance, while *C. roxburghiana* recorded the highest Chytridiomycota abundance.

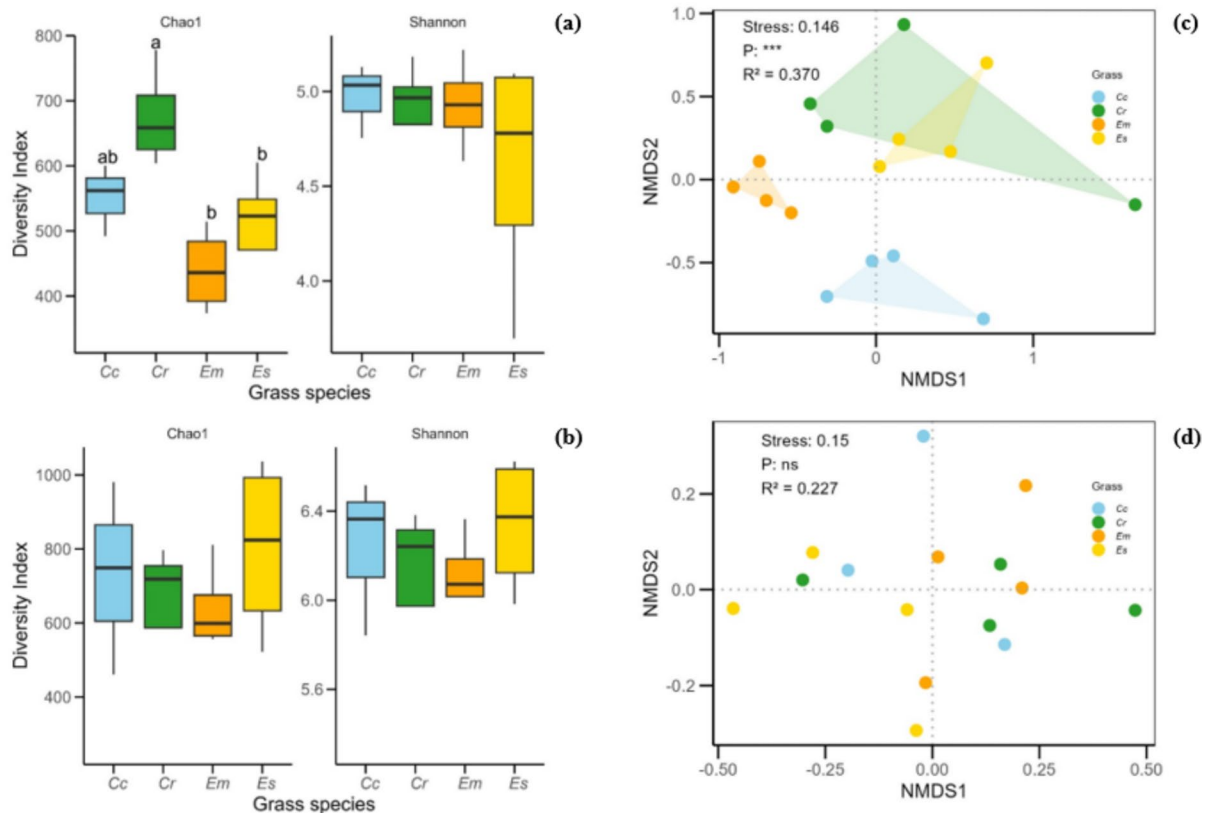
The bacterial OTUs, both the Chao 1 and Shannon alpha diversity indices, were comparable among the grasses (Fig. 5b). The dominant bacterial phyla across grass species were Actinobacteriota, Chloroflexi, Acidobacteriota, and Proteobacteria (Fig. S2b). *E. superba*

had the highest abundance of Actinobacteriota among the grass species, which was 21% higher than *E. macrostachyus*. Similarly, it had the highest Chloroflexi abundance, 32% and 40% greater than *C. ciliaris* and *E. macrostachyus*, respectively. In contrast, *E. macrostachyus* had the highest abundance of Acidobacteriota, 32% and 56% higher than *C. ciliaris* and *E. superba*, respectively. It also had the greatest abundance of Proteobacteria.

Grass species significantly influenced the fungal community ( $P<0.001$ ; Fig. 5c), with clear clustering observed along the NMDS axes. In contrast, no significant clustering was observed in the bacterial community based on grass species (Fig. 5d).

#### Rhizosphere microbial functions

Fungal community groups associated with OM utilisation differed significantly among grasses



**Fig. 5** Microbial Diversity. Fungal (a) and bacterial (b) communities Chao 1 and Shannon alpha diversity indices presented as box plots. Beta diversity analysis (Bray–Curtis distance) of fungal (c) and bacterial (d) communities displayed using non-metric multidimensional scaling (NMDS). Note: Stress values

indicate the goodness-of-fit of the NMDS ordination plots. \*\*\* indicates statistical significance based on PERMANOVA at  $P \leq 0.001$ . Key: *Cc*=*C. ciliaris*, *Em*=*E. macrostachyus*, *Cr*=*C. roxburghiana*, *Es*=*E. superba*

**Table 1** Relative abundance of microbial functional groups based on FUNGuild and Tax4Fun. Includes symbiotrophic and saprotrophic fungal communities, as well as bacterial groups

| Grass species           | Relative abundance (%) |             |                  |                |              |              |              |                |              |
|-------------------------|------------------------|-------------|------------------|----------------|--------------|--------------|--------------|----------------|--------------|
|                         | Symbiotroph            | Saprotroph  | N Depolymerisers | Ammonifiers    | Nitrifiers   | Denitrifiers | Diazotrophs  | PMB            | PSB          |
| <i>C. ciliaris</i>      | 22 ± 9.553ab           | 72 ± 4.998a | 1.41 ± 0.120     | 0.18 ± 0.024ab | 0.12 ± 0.046 | 0.19 ± 0.043 | 0.05 ± 0.019 | 0.15 ± 0.003a  | 0.25 ± 0.080 |
| <i>C. roxburghiana</i>  | 16 ± 1.627ab           | 52 ± 4.405b | 1.53 ± 0.010     | 0.14 ± 0.021b  | 0.09 ± 0.044 | 0.18 ± 0.038 | 0.06 ± 0.020 | 0.12 ± 0.028ab | 0.26 ± 0.044 |
| <i>E. macrostachyus</i> | 16 ± 1.505b            | 53 ± 1.951b | 1.46 ± 0.117     | 0.23 ± 0.020a  | 0.09 ± 0.042 | 0.18 ± 0.035 | 0.01 ± 0.016 | 0.12 ± 0.013ab | 0.25 ± 0.005 |
| <i>E. superba</i>       | 36 ± 4.301a            | 49 ± 5.040b | 1.49 ± 0.079     | 0.2 ± 0.022ab  | 0.10 ± 0.043 | 0.14 ± 0.037 | 0.04 ± 0.015 | 0.06 ± 0.008b  | 0.26 ± 0.058 |

Data is shown as means ± SEM (n=4). Means within a column followed by different letters are significantly different  $P < 0.05$

( $P < 0.05$ ; Table 1). *E. superba* showed the highest abundance of symbiotrophic Fungi, 40%, 55% and 57% higher than *C. ciliaris*, *C. roxburghiana*

involved in N and P cycling: N-depolymerizers, ammonifiers, nitrifiers, denitrifiers, diazotrophs, phosphate-mineralising bacteria (PMB), and phosphate-solubilising bacteria (PSB)

and *E. macrostachyus*, respectively. In contrast, *C. ciliaris* had a significantly greater proportion of saprotrophs, 28% and 32% higher than those in

the rhizosphere of *C. roxburghiana* and *E. superba*, respectively. Overall, saprotrophs were more abundant than symbiotrophs across the rhizosphere soil of the studied perennial grass species.

N-depolymerising bacteria (Table 1) and ammonifiers (Table 1), both jointly catalysing N recycling from OM, were the most abundant N-cycling groups in the rhizosphere of all grasses. The abundance of bacterial groups involved in the inorganic N cycle—nitrifiers (Table 1), denitrifiers (Table 1), and diazotrophs, fixing  $N_2$  from the atmosphere (Table 1)—were comparable among the grass species. Similarly, the relative abundance of PSB, mobilising inorganic P, (Table 1), was comparable among the grass species. However, the abundance of ammonifiers (Table 1) and of PMB (Table 1), both involved in recycling of N and P, respectively, varied significantly among grasses ( $P < 0.05$ ). *E. macrostachyus* had the highest and *C. roxburghiana* the lowest abundance of ammonifiers, while *C. ciliaris* had the highest and *E. superba* the lowest abundance of PMB.

## Discussion

### Biomass investment and resource acquisition strategies

Root and rhizosphere traits determine plant resource acquisition (Bergmann et al. 2020; Weigelt et al. 2021). Thicker absorptive roots provide a larger habitat for symbiosis (Bergmann et al. 2020; Laliberté 2017; Stock et al. 2021; Wang et al. 2025b), which aligns well with the observation that *C. ciliaris*'s large-diameter roots were strongly colonised by AMF. The combination of large root diameter, high mycorrhization, and low SRL indicates that *C. ciliaris* follows an 'outsourcing' resource acquisition strategy along the collaboration gradient (Matthus et al. 2025; Weigelt et al. 2021). Independently, its RTD reflects a slow-resource acquisition strategy along the conservation gradient, associated with long-lived, stress-tolerant roots.

In contrast, *E. macrostachyus* had a high SRL and a small absorptive root diameter, indicating a 'do-it-yourself' strategy along the collaboration gradient (Bergmann et al. 2020; Stock et al. 2021). Its high root N concentration, associated with rapid root biomass turnover, reflects a fast resource acquisition

strategy along the conservation gradient. Its thin and long roots maximise nutrient and water uptake, especially in resource-limited semi-arid tropical grassland ecosystems (Weigelt et al. 2021). Similarly, *C. roxburghiana* displayed root traits comparable to *E. macrostachyus*, suggesting that it too follows a predominantly 'do-it-yourself' and 'fast' strategy along the collaboration and conservation gradients, respectively. In contrast, *E. superba* exhibited a combination of high root N concentration, high RTD, high SRL and large absorptive root diameters with strong mycorrhization, without clear positioning along either the collaboration or conservation gradients of RES.

Species without distinct resource acquisition strategies, separation along the collaboration gradient; *E. superba* and partially *C. roxburghiana*, had the highest shoot biomass and low root-to-shoot ratios, indicating fast growth and a periodisation of aboveground productivity (Reich 2014; Weigelt et al. 2021). Consequently, they are often favoured by pastoralist communities (Mganga et al. 2015). However, the lower allocation of belowground C into the root system has significant ecological implications for their suitability in grassland restoration and forage production. Furthermore, the high aboveground productivity of *E. superba* and *C. roxburghiana* indicates that they follow a competitive life strategy. This suggests they are unlikely to cope well, e.g. under prolonged drought stress and grazing pressures. These aspects should be particularly considered when selecting grass species for restoration programs.

The high root biomass and root-to-shoot ratio of *C. ciliaris* highlight its greater ability to withstand abiotic stresses such as drought and to recover from severe grazing, as reported by Mganga et al. (2015, 2021b). This suggests that *C. ciliaris* is more stress-tolerant and disturbance-resistant, thus better suited for restoration programs, particularly given the increasing frequency of prolonged drought (Marshall et al. 2012). Moreover, its 'outsourcing' resource acquisition strategy, with persistent roots and symbiotic support, enhances its stress tolerance and resistance to disturbance. The high proportion of coarse roots not only facilitates anchorage but also enables *C. ciliaris* to access resources in soil layers that are mechanically compacted and not easily penetrated by finer roots, such as the Bt horizon in the Acrisol soils at the research site (George et al. 2024; IUSS Working Group WRB 2022; Wild et al. 2024). Its deeper

roots observed during sampling (~45 cm depth), demonstrate its ability to access water stored in the subsoil layer below the Bt horizon, thus highlighting its overall resilience to drought (Mganga et al. 2021c).

The similar trait relationships (RTD, N, D, and SRL) observed in absorptive and coarse roots of grasses likely reflect their comparatively small diameters, even in the > 2 mm fraction, relative to the much thicker woody roots of trees. Nevertheless, perennial grasses' roots also maintain the functional distinction (Glass et al. 2023), and thus absorptive roots align with resource acquisition strategies (McCormack et al. 2015; Sanaei et al. 2025; Wang et al. 2021), whereas coarse roots primarily support structure, transport, and storage (Sanaei et al. 2025; Wang et al. 2021; Zhang & Wang 2015). For instance, in absorptive roots, larger diameters enhance mycorrhization through extensive cortical tissue, while in coarse roots, diameter arises mainly from secondary development, where cortex is reduced and mycorrhization is limited (McCormack et al. 2015; Sanaei et al. 2025; Wang et al. 2016). Thus, diameter reflects symbiotic potential in absorptive roots, but transport and structural capacity in coarse roots (Matthus et al. 2025; Sanaei et al. 2025). This distinction may explain the high susceptibility of *E. macrostachyus* to uprooting by free-ranging grazing animals, particularly under moist soils during the rainy season (Mganga et al. 2015) despite its relatively high absorptive root SRL, a trait otherwise linked to mechanical strength (Mao et al. 2023). By contrast, high diameter and RTD in coarse roots likely enhance penetration into mechanically resistant soils, greater anchorage and stress tolerance in *C. roxburghiana* and *C. ciliaris*, as earlier discussed and reported by Mganga et al. (2015, 2021b).

#### Rhizosphere microbiome and its significance for resource acquisition strategies

Rhizosphere fungal communities were distinct among the studied grass species. *C. roxburghiana* and *E. macrostachyus* supported the highest and lowest fungal richness, respectively. Both species primarily follow a 'do-it-yourself' strategy, with the highest proportion of very-fine and fine roots among the studied grasses. While a high proportion of very-fine and fine roots of *C. roxburghiana* suggests a high rhizodeposition (Ling et al. 2022; Tomazelli et al. 2024)

supporting a rich fungal community, the overall low total root biomass of *E. macrostachyus* seems to dominantly shape its rhizosphere microbial community. Furthermore, *C. roxburghiana* showed a greater abundance of Chytridiomycetes, which degrade complex OM, compared to *E. macrostachyus* (Hanrahan-tan et al., 2022). Conversely, Basidiomycetes were most abundant in the rhizosphere of *E. macrostachyus*, which predominantly degrade lignin and recalcitrant litter. Their complex hyphal and mycelial structures optimise nutrient uptake and enhance soil aggregation, essential for infertile tropical grassland soils (Dang et al. 2018; Fall et al. 2022).

The high proportions of very fine and fine roots, along with associated rhizodeposition in *E. macrostachyus* and *C. roxburghiana*, likely contributed to the high abundance of copiotrophic Proteobacteria in their rhizospheres (Fu et al. 2023; Oram et al. 2025). Proteobacteria thrive in C-rich soils, rapidly utilising available resources for growth (Fu et al. 2023; Hua et al. 2024; Ling et al. 2022). Overall, although both grass species adopt a 'do-it-yourself' strategy, they shape their rhizosphere microbiomes in distinct ways, likely due to differences in the composition of their rhizodeposits (George et al. 2024; Ling et al. 2022; Wang et al. 2025b). They attract distinct microbes that support nutrient cycling and acquisition, thereby uniquely shaping their rhizosphere.

The symbiotic phylum Glomeromycota, comprising the AMF, is not only crucial for plant nutrient acquisition, particularly in P-limited tropical grasslands, but also AMF selectively recruit specific bacterial groups (Jin et al. 2024; Li et al. 2025a). This likely explains the high bacterial richness in the rhizospheres of *C. ciliaris* and *E. superba*, whose roots were also strongly colonised by AMF. Actinobacteriota, a group of gram-positive, aerobic saprophytes, which dominate nutrient-poor and drought environments (Li et al. 2025a; Oram et al. 2025), was found in high abundance in the rhizosphere of *E. superba* and *C. ciliaris*. These species exhibited a large proportion of coarse roots, thus likely low rhizodeposition rates (Ling et al. 2022), resulting in a rhizosphere microbial community that closely mirrors the bulk soil. Generally, plants with a large proportion of transport roots rely on high storage and/or on mycorrhization of their thicker absorptive roots (<2 mm) by AMF (Bergmann et al. 2020; McCormack

et al. 2015; Stock et al. 2021), both mechanisms lead to low rhizosphere activity in direct root proximity.

#### Rhizosphere microbiome functions regulating the savanna's C, N, and P cycles

Extracellular enzymes are secreted by both plants and microbes to overcome nutrient limitation by the release of OM-bound nutrients in soils (George et al. 2024; Guo et al. 2021; Mooshammer et al. 2014; Stock et al. 2019). The rhizosphere MBC content was comparable among the studied grasses despite significant variations in the maximal enzyme activities. This indicates that enzymatic activity was primarily mediated by plants or by microbes, irrespective of microbial abundance. The relatively low leucine aminopeptidase activity was likely due to the N-poor savannah litter combined with high N loss rates and frequent biomass removal that exacerbate N limitations, specifically of high-quality protein-based N, in savannas (Mooshammer et al. 2014; Veldhuis et al. 2016). The high C: N ratio of savannah litter goes along with the C: N stoichiometric imbalance of the microbial communities and explains the relatively higher  $\beta$ -Glucosidase activity degrading the litter carbohydrate backbone (Mooshammer et al. 2014; Stock et al. 2019). This N limitation is also reflected in the elevated MBC: MBN ratios observed across all grasses, suggesting strong microbial N demand relative to C (Cleveland & Liptzin 2007). Consequently, N limitation in the rhizosphere likely causes high N competition between the grasses and the microbes (Liu et al. 2016).

*C. ciliaris*'s larger proportion of coarse roots correlated with lower enzymatic activities across all the measured enzymes (Freschet et al. 2021; Stock et al. 2019), underpinning its symbiotic association with AMF for nutrient acquisition—an 'outsourcing' strategy. AMF likely played a critical role in P mobilisation for *C. ciliaris*. The low acid phosphatase activity measured in the rhizosphere soil of *C. ciliaris*'s may result from a large mycosphere network where the overall phosphatase activity is distributed across a much larger soil volume not covered by our sampling approach (Freschet et al. 2021; Mooshammer et al. 2014). In contrast, *C. roxburghiana*'s, following a predominantly 'do-it-yourself' acquisition strategy, maintains high acid phosphatase activities in direct root proximity. In consequence, 'do-it-yourself'

strategies require a different spatial pattern of nutrient exploitation than the 'outsourcing' strategy.

High AMF root colonisation and significantly high abundance of saprotrophs and PMB in *C. ciliaris*' rhizosphere indicate strong reliance on microbial partners for nutrient turnover. The high abundance of saprotrophs, N-depolymerisers, ammonifiers, and PMB genes among all grass species suggests that the ecosystem primarily recycles C, N and P (Abdallah et al. 2022; Vranova et al. 2013; Xu et al. 2025). In contrast, the relatively low abundance of diazotrophs, nitrifiers, and denitrifiers was relatively low among the studied grasses indicates that N gain and losses in the rhizosphere were not the dominant processes in the rhizosphere (Abdallah et al. 2022). This pattern points to a tightly closed N cycles in the system, characterised by intensive recycling and minimal losses (Abdallah et al. 2022; Pabst et al. 2016). The preference for N recycling over acquisition by diazotrophs may reflect the high energetic costs of  $N_2$  fixation, which would redirect substantial C amounts, leaving less available for stress tolerance mechanisms (Abdallah et al. 2022).

Similarly, the grasses showed comparable abundance of PSB genes, indicating that none of the grasses adopted a distinct strategy for weathering primary or secondary P from the soil minerals (Pang et al. 2024; Pan & Cai 2023; Sun et al. 2022). This highlights that, similar to N, the studied grasses primarily depend on P recycling rather than the acquisition of new inputs (Abdallah et al. 2022; Koester et al. 2021). The P recycling strategy is attributable to the low P content and high immobilisation typical of deeply weathered tropical soils (Maranguit et al. 2017; Pang et al. 2024). Overall, N and P recycling is the preferred strategy over acquisition mechanisms in this ecosystem, with distinct recycling strategies among the grasses.

Overall, *C. ciliaris*, with its deep rooting, high root biomass, strong mycorrhizal associations, high stress tolerance, and aggressive growth, is well-suited as an early-successional coloniser in restoration programs (Marshall et al. 2012; Mganga et al. 2019, 2021a). Conversely, *E. macrostachyus* and *C. roxburghiana* have relatively high allocation to absorptive roots, higher extracellular enzymatic activity, and robust vegetative growth, though with moderate stress tolerance (Mganga et al. 2015, 2019), and are thus suited to early- to mid-successional phases (Franklin et al.

2001; Hartnett et al. 2013). Despite the high intraspecific variation in root traits observed in the *E. superba* plants sampled in this study, the species' typical low root-to-shoot ratio, high aboveground productivity, partial shade tolerance, and superior foliage palatability (Mganga et al. 2019, 2021c), make it particularly suited to later stages of succession, where enhancing forage quality and supporting livestock are key restoration objectives (Avalos 2019; Franklin et al. 2001; Hilmers et al. 2018). Furthermore, while the RES framework has been more frequently applied in forest ecosystems (Matthus et al. 2025), our findings highlight its broader relevance for grasslands by capturing both inter- and intraspecific variation that underpins niche differentiation (Miao et al. 2025). The distinct strategies observed among the studied species, together with intraspecific variation in root traits linked to plant–soil interactions, suggest that trait variation within the RES is shaped not only by species identity but also by environmental processes that in turn drive ecosystem functioning (Matthus et al. 2025). Extending RES applications to grasslands across environmental gradients will therefore be essential for understanding root trait plasticity as a key dimension of grassland ecology, and for guiding species selection across successional stages in grassland restoration.

## Conclusions

The studied perennial grasses native to semi-arid African grasslands displayed distinct belowground biomass allocation patterns and resource acquisition strategies. *C. roxburghiana* and *E. macrostachyus* primarily relied on acquisitive root traits, adopting a 'do-it-yourself' strategy supported by copiotrophic rhizosphere microbiome, exploring and exploiting mainly the narrow root proximity for nutrients. In contrast, *C. ciliaris* displayed conservative root traits and largely depended on mycorrhizal fungi associations, adopting an 'outsourcing' strategy, exploiting larger soil volumes. *E. superba* adopted an intermediate resource acquisition strategy with the highest shoot biomass yields. Grass species significantly influenced rhizosphere fungal communities more than bacterial communities, highlighting plant-specific effects on microbial composition.

The observed variation in belowground traits among the studied tropical grasses highlights

differing capacities to withstand disturbance. Stress-tolerant species exhibited persistent root systems and strong symbiotic associations with AMF (namely *C. ciliaris*), while less tolerant species exhibited low belowground investment and high aboveground biomass production.

The tropical ecosystem was nutrient-poor, with N and P limiting plant and microbial productivity. Nutrient cycles were tightly closed, with both plants and microbes favouring nutrient recycling over acquisition from the atmosphere (N) or the geosphere (P). Overall, distinct resource acquisition strategies and their associated rhizosphere interactions offer valuable insights into maintaining ecosystem productivity and stability. The insights are essential in the selection of grassland species and species combinations for restoration efforts, particularly under increasing biotic and abiotic stress.

**Acknowledgements** This study was funded in the framework of the Hohenheim-Tübingen Africa projects. We sincerely acknowledge South Eastern Kenya University (SEKU), Kenya, for permitting the establishment and monitoring of the long-term restoration project. We also appreciate the Quantitative Biology Centre (QBiC), Tübingen, for supporting DNA amplicon sequencing and bioinformatics data processing. Additionally, we are grateful to the Katholische Akademische Ausländer-Dienst (KAAD) for providing financial support to MN during the study. Finally, we extend our appreciation to the Department of Agricultural Science and Technology at Kenyatta University, and the Geo-Biosphere Interactions group lab teams at the University of Tübingen, particularly Dr. Callum Banfield, Kallen Gacheri, Desara Roci, Aleksandra Okla, Jana Geiselhart, and the HIWI team, for their invaluable assistance during laboratory analyses.

**Author's contributions** KM, TA and MD conceived the ideas and designed the methodology; MN, SCS, RM and BM collected data; MN, SCS, IL, and MD analysed the data; MN, SCS, KM, and MD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This work was supported by the Hohenheim-Tübingen Africa Projects initiative.

**Data availability** All data and associated metadata generated or analysed during this study are available to researchers and the public upon reasonable request from the Geo-Biosphere Interactions research group at the University of Tübingen.

**Declarations**

**Competing interests** No potential conflict of interest was reported by the authors.

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