Received: 27 September 2022 | Revised: 6 January 2023 | Accepted: 17 January 2023

DOI: 10.1002/glr2.12040

Grassland Research

RESEARCH ARTICLE

Leaf gas exchange characteristics, biomass partitioning, and water use efficiencies of two C_4 African grasses under simulated drought

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Handling Editor: Lynn Sollenberger

Funding information
Academy of Sciences for the Developing World, Grant/Award Number: TWAS-DFG Cooperation Visits Program for scientist

Abstract

Background: Few studies have evaluated the effect of drought on the morpho-physiological characteristics of African C_4 grasses. We investigated how drought affects leaf gas exchange characteristics, biomass partitioning, and water use efficiencies of *Enteropogon macrostachyus* and *Cenchrus ciliaris*.

Methods: The grasses were grown in a controlled environment under optimum conditions, that is, 70% of the maximum water-holding capacity (WHC) for the first 40 days. Thereafter, half of the columns were maintained under optimum or drought conditions (30% of maximum WHC) for another 20 days.

Results: Under optimum conditions, *C. ciliaris* showed a significantly higher photosynthetic rate, stomatal conductance, and transpiration rate than *E. macrostachyus*. Drought decreased the photosynthetic rate, stomatal conductance and transpiration rate only in *C. ciliaris*. The net photosynthetic rate, stomatal conductance, and leaf transpiration of *E. macrostachyus* did not differ significantly under optimum and drought conditions. *E. macrostachyus* showed an increase in its water use efficiencies under drought to a greater extent than *C. ciliaris*.

Conclusions: Our results demonstrate that *C. ciliaris* is more sensitive to drought than *E. macrostachyus*. The decrease in the intercellular CO_2 concentration and the increase in stomatal limitation with drought in *C. ciliaris* and *E. macrostachyus* suggest that stomatal limitation plays the dominant role in photosynthesis of the studied African C_4 grasses.

KEYWORDS

biomass fractions, C_4 grasses, drought, photosynthesis, stomatal conductance, transpiration

INTRODUCTION

Desertification has been recognized as a serious threat to dryland environments (Verón et al., 2006). African drylands are particularly vulnerable because of recurrent droughts, infertile and fragile soils, and a depleted vegetation cover. This environmental challenge threatens millions of pastoralists who rely on dryland resources to support their livelihoods. Perennial C_4 grasses indigenous to Africa, notably *Cenchrus ciliaris* (African foxtail/Buffel grass) and *Enteropogon macrostachyus* (Wild rye grass), have been used to combat desertification in African drylands through seed-based restoration (Kimiti et al., 2017; Mganga, Musimba, & Nyariki, 2015; Mganga, Musimba, Nyariki, Nyangito, et al., 2015; Mureithi et al., 2016). Preference for these grasses is attributed to their evolved adaptive mechanisms for survival in arid environments characterized by droughts. Kenya drylands experience major droughts every decade and minor ones every 3–4 years. The current and projected trends in climate show increasing drought frequency and rainfall variability across African drylands, which intensifies land degradation and increases environmental sensitivity to drought (Hobbs et al., 2008; King et al., 2018).

The potential of perennial C_4 grasses to combat land degradation and enhance primary productivity in African drylands has been documented in previous studies (Kimiti et al., 2017; Mganga, Musimba, & Nyariki, 2015;

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Mganga, Musimba, Nyariki, Nyangito, et al., 2015; Mureithi et al., 2016). Generally, C_4 plants are expected to be better adapted to drought conditions than C_3 plants because they are recognized as species of warm and arid regions. C_4 plants are characterized by higher photosynthetic efficiency, CO_2 fixation rates, water use efficiency (WUE) and transpiration rate, which demonstrate their advantages compared to C_3 plants (Ghannoum, 2009; Ghannoum et al., 2001). Evidence demonstrates that photosynthesis in C_4 plants is highly sensitive to water stress characterized by a decrease in the photosynthetic rate and stomatal conductance with declining leaf water status (Ghannoum, 2009). However, leaf gas exchange characteristics, biomass accumulation and partitioning and water use efficiencies of perennial C_4 grasses native to African drylands under different soil moisture conditions remain largely understudied. The lack of such critical information poses a major challenge because water is a major factor limiting the productivity of C_4 grasses that dominate African rangelands and support a large pastoral industry (Ghannoum et al., 2002) and dryland ecological restoration programs.

The photosynthetic rate of plants is known to decrease with a reduction in the cellular water content and leaf water potential (Lawlor & Cornic, 2002). However, there is still a lack of consensus as to whether water stress mainly limits photosynthesis through stomatal closure or through metabolic impairment (Lawson et al., 2003). This is mainly because higher plants response to water deficiency often involves complex interactions of physical and metabolic processes in the entire plant system (Lawlor & Cornic, 2002). Nevertheless, the photosynthetic rate at the whole-plant level always decreases as a result of diversion of C allocation to nonphotosynthetic organs and defence molecules, with these changes in leaf biochemistry suppressing photosynthesis and limiting CO_2 diffusion through the leaf stomata (Chavez et al., 2002). Low CO_2 supply to the carboxylating enzymes because of reduced stomatal conductance decreases the photosynthetic rate (Da Silva & Arrabaca, 2004). Stomatal conductance depends on guard cell and leaf turgor, which are influenced by the balance between supply of water to the leaf from the soil and loss of water through transpiration (Tuzet et al., 2003). Under drought, the immediate response of terrestrial plants is stomatal closure, which not only diminishes water loss through transpiration but also reduces CO_2 uptake, consequently altering metabolic pathways such as photosynthesis. The adaptive capacity of plants to reduce the negative impacts of drought stress on photosynthesis, thereby resulting in a positive effect on WUE, will result in high yield potential (Basu et al., 2016).

Biomass yield and allocation to different plant organs depend on the species, ontogeny and environmental conditions. Higher plants have an incredible capacity to coordinate the growth of different organs, so that there is generally a tight balance between the biomass invested in shoot and root. For example, allocation to roots increases with decreasing nutrient or water availability, and allocation to shoots increases with decreasing irradiance (Poorter & Nagel, 2000). However, combining biomass allocated to leaf and stem fractions into a single (shoot) compartment, as commonly documented in previous studies, is disadvantageous because leaves and stems have different functions (Pearcy et al., 2005). Consequently, biomass partitioning as influenced by drought stress is presented as individual leaf, stem, and root fractions. We acknowledge that this approach of calculating fractions has been previously criticized because the biomass of the organ of interest is in both the numerator and the denominator (Müller et al., 2000). However, biomass fractions still retain key information and these three independent variables can be used to calculate all other biomass ratios (Poorter et al., 2012).

Biomass partitioning has a significant effect on whole-plant WUE. WUE has been used in grass growth models to predict productivity, pasture management, and land degradation in grasslands under current and future climates (Ghannoum et al., 2002). Therefore, it is critical to understand how environmental factors affect WUE of Africa's C_4 grasses. Previous studies have supported the basic assumption made by grass models that biomass accumulation in a large number of C_4 grasses can be predicted from water use, when they are grown under well-watered conditions and exposed to different irradiances and CO_2 concentrations (Ghannoum et al., 2001). Considering that the distribution of C_4 grasses is strongly associated with rainfall, we wanted to determine whether this relationship also holds under drought-stress conditions.

Here, we refer to drought as a period with declining soil moisture levels that are insufficient to meet plant demand (i.e., agricultural drought). Thus, we established *C. ciliaris* and *E. macrostachyus* under two contrasting soil moisture conditions, that is, optimum (70% of water-holding capacity, WHC) and simulated drought stress (30% of WHC), in a climate chamber. These species were chosen because they are native and adapted to African drylands. In nature, the *C. ciliaris* ecotype in this study is more adapted to deeper moister soils, while *E. macrostachyus* is adapted to drier environments characterized by more shallow soils occurring in rocky outcrops (Marshall et al., 2012; Mganga, Musimba, & Nyariki, 2015). Moreover, these grasses contribute significantly to seed-based ecological restoration in African arid and semi-arid lands and provide a source of forage for free-ranging livestock and wildlife. However, despite their socioecological significance and different ecophysiological adaptations to water stress conditions, limited studies, if any, have been conducted to investigate their response to drought in terms of leaf gas exchange, biomass fractions allocation, and WUE. Understanding the ecophysiological characteristics of these grasses will greatly improve restoration strategies and outcomes in severely denuded African drylands.

Thus, this study aimed to determine the effect of simulated drought on selected leaf gas exchange characteristics, biomass partitioning, and WUE of *C. ciliaris* and *E. macrostachyus*. Specifically, our objective was to address the following research question: How does the adaptation of *C. ciliaris* and *E. macrostachyus* to different soil moisture conditions contribute to the grasses showing contrasting behavior in terms of leaf gas exchange characteristics (e.g., photosynthetic rate, stomatal conductance, and transpiration rate), biomass fractions (leaf, stem, and root) and Whole-plant water

use efficiencies (WUE_t) under optimum and drought conditions? These physiological traits were selected because they have been measured to assess drought tolerance (Fracasso et al., 2016) and evasion (Guenni et al., 2002) mechanisms by plants. Subsequently, they will enable us to assess the contrasting responses of the grasses to drought and postulate their different survival strategies. We hypothesize that *E. macrostachyus*, which naturally occurs in drier environments, would be a more drought-adapted species than the *C. ciliaris* ecotype, which naturally occurs in deeper and moist soils.

MATERIALS AND METHODS

Soil

Topsoil (0–20 cm depth) was collected from a grassland site belonging to the agricultural teaching facility managed by the district of Upper Franconia at Bayreuth, Germany ("Landwirtschaftliche Lehranstalten," 49°55′46.2″ N, 11°33′2.376″ E). The soil was classified as loamy silt with 67% sand, 11% silt, and 22% clay and a pH of 5.9 ($CaCl_2$). After sampling, soil was air-dried and passed through a 5-mm sieve and stored in CO_2-permeable polypropylene bags before the start of the experiment.

Grass species, experimental design, and plant growth conditions

The general morphometric characteristics of the grasses selected for this study, that is, *C. ciliaris* and *E. macrostachyus*, are shown in Table 1.

Grass seeds for the experiment were obtained from the Arid and Range Lands Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO), Kenya. The experimental set-up included planting of five seeds as monocultures in 16 microcosm columns (⌀7 cm and 25 cm height) containing 1000 g of soil, for each species, totaling to 32 planted microcosms. All the grasses were established and maintained in one climate chamber throughout the experimental period with day and night temperatures of 29°C and 19°C, respectively, day and night relative humidity of 60% and 65%, respectively, and a 14 h photoperiod. During the first 40 days of plant growth (corresponding to the first 40 days after planting the seeds), optimum soil moisture conditions (70% of the maximum WHC) were maintained in all the microcosms. After the first 40 days of establishment, the 16 microcosms of each grass species were equally divided into two groups, and soil moisture in each group was adjusted to either (1) optimum conditions (70% of the maximum WHC) or (2) simulated drought conditions (30% of the WHC) for 20 days following a completely randomized block design. Previous studies that aimed to determine the effect of water deficit conditions on plant physiological traits similar to those measured in this study have simulated drought conditions for a period of 10–20 days (Carmo-Silva et al., 2008; Flexas et al., 2007; Galmés et al., 2007; Hu et al., 2010; Swarthout et al., 2009; Walter et al., 2011). This informed our selection of the duration of drought simulation (20 days) to enable us to observe the species-specific physiological responses. Moreover, the duration of water deficit conditions selected for this study is a typical representation of semi-arid drylands in Kenya characterized by high rainfall variability and where the grass species occur naturally.

TABLE 1 Morphometric traits of the C_4 perennial grasses native to African drylands.

Plant trait	Selected characteristics of perennial grasses native to African drylands	
	Cenchrus ciliaris	*Enteropogon macrostachyus*
Altitude distribution (m)	0–2000	300–1600
Leaf blade length (cm)	15–30	10–60
Leaf blade width (mm)	3–8	2–10
Stem width (mm)	1–2	1–2
Plant height (cm)	20–150	30–100
Rooting depth (cm)	Up to 240	Up to 220
Inflorescence type	Panicle	Spike
Inflorescence length (cm)	2–14	8–20
Optimal rainfall (mm)	300–750	550–800
Crude protein (%)	Up to 10	9–12

Source: Mganga et al. (2022).

Leaf-level gas exchange

The leaf net photosynthetic rate (Pn, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs, mmol m^{-2} s^{-1}), intercellular CO_2 concentration (C_i), and leaf transpiration rate (Tr, mmol m^{-2} s^{-1}) were measured on fully formed leaves using an open-flow infrared gas analyser equipped with a red-blue light source (LI-6400XT; Li-Cor). Measurements were conducted once at the end after drought simulation before plant biomass harvesting. Both grass species were at the elongation phase of development during measurements. Leaf cuvette conditions during measurement were maintained at a photosynthetic active radiation (PAR) of 475 μmol m^{-2} s^{-1}, a temperature of 29°C and relative humidity < 75%. The CO_2 concentration within the leaf cuvette (C_a) was 400 μmol mol^{-1}. The stomatal limitation value (L_s) was calculated as $1 - (C_i/C_a)$ (Song et al., 2020).

Whole-plant water loss and daily transpiration rates

Whole-plant transpiration was determined by continuously weighing the microcosms placed on metallic plates of an automated electronic balance connected to a computer. We added a thin layer of small white plastic pebbles on the soil surface to minimize evaporation. Water lost from the soil was added back to each individual microcosm to restore soil to 30% or 70% WHC. The transpiration rate (cm^3 day^{-1})

was determined by calculating the amount of water lost per day (24 h).

Plant biomass fractions and WUE

Whole-plant biomass was harvested at the end of the experiment (60 days) and separated into leaves, stems, and root biomass fractions. Roots were separated from soil by washing with running tap water through a 1-mm mesh size sieve to limit loss of fine roots. Harvested plant biomass was oven-dried for 48 h at 70°C and weighed. Dry matter yield reduction (DM_{red}) was calculated for each replicate separately as the difference between DM in control (optimum) and drought-treated grass species. WUE_t (i.e., for the entire growing period of 60 days) was calculated as the ratio of the total dry biomass produced over the total water transpired (Clifton-Brown & Lewandowski, 2000).

Data analysis

Statistics were performed using Software STATISTICA 10.0, StatSoft Inc. The experimental unit for the analysis was the microcosm in a completely randomized block design. Two-way analysis of variance was used to test for significant differences between grass species and treatments (optimum and drought conditions). Tukey's HSD post hoc test was used to separate significant differences between treatments at a $p < 0.05$ significance level. All the results shown represent arithmetic means of eight replicates for each measurement ($n = 8$).

RESULTS

Leaf-level gas exchange

Leaf-level gas exchange varied depending on the species and drought conditions. Under optimum conditions, *C. ciliaris* showed a significantly higher photosynthetic rate than *E. macrostachyus* (10.3 vs. 5.1 μmol CO_2 m^{-2} s^{-1}; $p = 0.0039$) (Figure 1). However, under drought, the photosynthetic rates of *C. ciliaris* (6.3 μmol CO_2 m^{-2} s^{-1}) and *E. macrostachyus* (5.5 μmol CO_2 m^{-2} s^{-1}) were comparable ($p = 0.2023$) (Table 2).

Similarly, under optimum conditions, *C. ciliaris* had more than double the stomatal conductance (0.09 mmol m^{-2} s^{-1}) ($p = 0.0361$) and leaf transpiration (1.2 mmol m^{-2} s^{-1}) ($p = 0.0456$) than *E. macrostachyus*, which had stomatal conductance of 0.04 mmol m^{-2} s^{-1} and transpiration rate of 0.5 mol m^{-2} s^{-1}. Stomatal conductance and transpiration rate of the two species under water-limited conditions were not significantly different (Table 2). The net photosynthetic rate, stomatal conductance, and leaf transpiration of *E. macrostachyus* did not differ significantly ($p = 0.1533$) under optimum and drought conditions (Table 2). The intercellular CO_2 concentration (C_i) and the ratio of intercellular and ambient CO_2 concentration (C_i/C_a) were significantly higher ($p = 0.0450$) under optimum than drought conditions for both species (Table 2). On the other hand, stomatal limitation (Ls) significantly increased ($p = 0.0047$) under drought conditions for both species (Table 2).

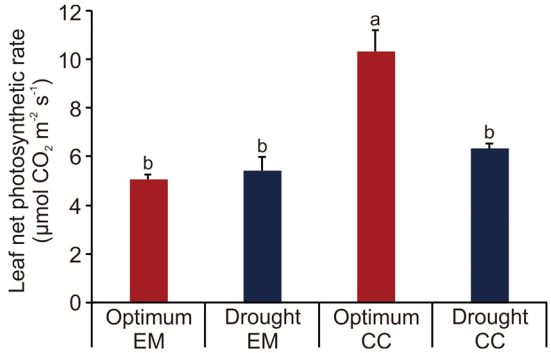


FIGURE 1 Leaf net photosynthetic rate (Pn, μmol CO_2 m^{-2} s^{-1}) of the two grasses under optimum and drought conditions. EM refers to *Enteropogon macrostachyus* and CC refers to *Cenchrus ciliaris*. The data shown are means ($n = 8$), and bars represent the standard error of means. Different letters in bar graphs denote significant differences ($p < 0.05$).

Whole-plant water loss, daily transpiration rates, and WUE_t

Cumulative whole-plant water loss during drought (the last 20 days of the experiment) was significantly higher ($p = 0.0013$) in plants under optimum than in drought conditions (Figure 2). Under optimum conditions, *E. macrostachyus* and *C. ciliaris* lost 292 and 302 cm^3 of water compared to 212 and 154 cm^3 under drought conditions, respectively. Cumulative amounts of water lost by the two species under drought stress were significantly different ($p = 0.0349$) (Figure 2). Hourly rates of whole-plant water loss were significantly different ($p = 0.0475$) and two to three times higher in plants under optimum conditions compared to drought conditions. These differences in loss of water were more distinct 6–7 days after drought simulation (Figure 3). WUE_t increased with drought in both species and was significantly higher ($p = 0.0086$) for *E. macrostachyus*, 13.5 g L^{-1}, compared to *C. ciliaris*, 12 g L^{-1} (Table 2).

Plant biomass and biomass fractions

Leaf, stem and root biomass of *E. macrostachyus* were not significantly different ($p = 0.0518$, 0.5789, and 0.0580, respectively) under optimum and drought conditions (Table 3).

However, *C. ciliaris* shoot and root biomass were significantly higher ($p = 0.0249$ and 0.0378, respectively) under optimum compared to simulated drought conditions (Table 3). The reduction in total and shoot biomass as a result of drought stress was much higher in *C. ciliaris*, with 1.23 and 0.97 g, compared to 0.43 and 0.25 g in *E. macrostachyus*, respectively. *E. macrostachyus* had higher leaf mass fractions compared to *C. ciliaris* under optimum and drought conditions, while *C. ciliaris* had higher stem mass fractions compared to *E. macrostachyus* under

TABLE 2 Leaf gas exchange characteristics and water use efficiencies of selected perennial C_4 grasses under optimum and drought conditions.

Grass species	Treatments	Gs (mmol m^{-2} s^{-1})	Tr (mol m^{-2} s^{-1})	WUE_t (g L^{-1})	C_i (μmol mol^{-1})	C_i/C_a	Stomatal limitation
EM	Optimum	0.04 ± 0.003^{b}	0.5 ± 0.01^{b}	9.0 ± 0.2^{c}	171 ± 15.8^{ab}	0.43 ± 0.04^{ab}	0.57 ± 0.04^{bc}
	Drought	0.03 ± 0.002^{b}	0.4 ± 0.02^{b}	13.5 ± 0.3^{a}	105 ± 17^{c}	0.26 ± 0.04^{c}	0.74 ± 0.04^{a}
CC	Optimum	0.09 ± 0.008^{a}	1.2 ± 0.11^{a}	9.6 ± 0.2^{c}	195 ± 5.9^{a}	0.49 ± 0.01^{a}	0.51 ± 0.01^{c}
	Drought	0.04 ± 0.008^{b}	0.6 ± 0.01^{b}	12.1 ± 0.5^{b}	115 ± 6.8^{bc}	0.29 ± 0.02^{bc}	0.71 ± 0.02^{ab}

Note: The data shown are means ± SE ($n = 8$). Different lowercase letters within the same column indicate significant differences ($p < 0.05$).

Abbreviations: C_a, CO_2 concentration within the leaf cuvette; CC, *Cenchrus ciliaris*; C_i, intercellular CO_2 concentration; EM, *Enteropogon macrostachyus*; Gs, stomatal conductance; Tr, leaf transpiration rate; WUE_t, total WUE.

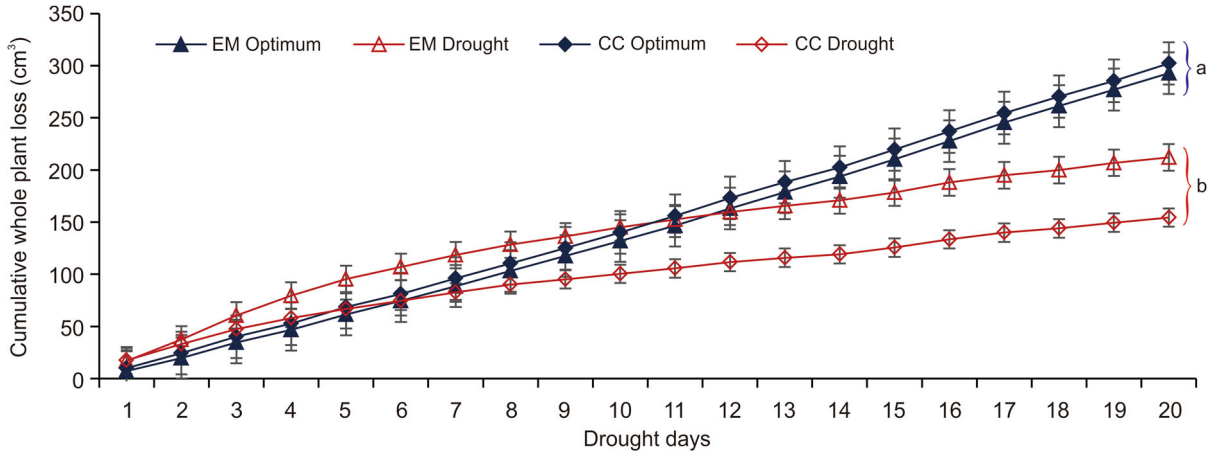


FIGURE 2 Cumulative whole-plant water loss during the last 20 days of the experiment. EM refers to *Enteropogon macrostachyus* and CC refers to *Cenchrus ciliaris*. The data shown are means ($n = 8$), and bars represent the standard error of means. Different letters in line graphs denote significant differences ($p < 0.05$).

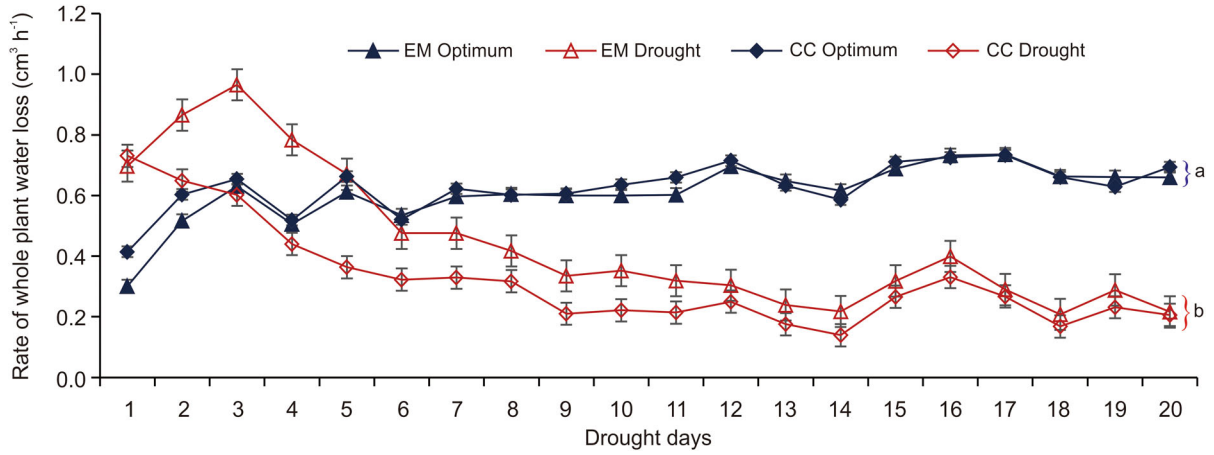


FIGURE 3 Rate of whole-plant water loss during the last 20 days of the experiment. EM refers to *Enteropogon macrostachyus* and CC refers to *Cenchrus ciliaris*. The data shown are means ($n = 8$) and bars represent the standard error of means. Different letters in line graphs denote significant differences ($p < 0.05$).

optimum conditions. Differences in root mass fractions were observed only under drought with a lower fraction of *E. macrostachyus* (Table 3).

DISCUSSION

C. ciliaris showed a significantly lower photosynthetic rate under drought than in optimum conditions. Also, stomatal conductance of *C. ciliaris* decreased by more than 50% under water-limited conditions. Our results suggest that drought-induced a decrease in the photosynthesis rate in *C. ciliaris* leaves. This suggests that drought-induced inhibition of photosynthesis in *C. ciliaris* might be of stomatal rather than metabolic origin. Previous studies have also shown that photosynthesis in water-stressed C_4 plant species was mainly limited by stomata with both rapidly and slowly imposed water deficits (Da Silva & Arrabaca, 2004; Taylor et al., 2011). The decrease in stomatal conductance in *C. ciliaris* might have led to reduced CO_2 availability to chloroplasts and, consequently, limited photosynthesis.

TABLE 3 Plant biomass characteristics of selected perennial C_4 grasses under optimum and drought conditions.

Grass species	Treatments	Leaf biomass (g)	Stem biomass (g)	Root biomass (g)	Shoot biomass (g)	Total biomass (g)	Shoot:root ratio	Leaf mass fraction	Stem mass fraction	Root mass fraction
EM	Optimum	2.4 ± 0.13^{a}	1.3 ± 0.06^{b}	1.3 ± 0.04^{a}	3.7 ± 0.1^{ab}	5.09 ± 0.2^{a}	2.8 ± 0.1^{ab}	0.48 ± 0.01^{a}	0.26 ± 0.01^{b}	0.27 ± 0.01^{ab}
	Drought	2.2 ± 0.03^{a}	1.4 ± 0.05^{b}	1.2 ± 0.01^{a}	3.5 ± 0.1^{b}	4.66 ± 0.05^{a}	3.1 ± 0.1^{a}	0.46 ± 0.01^{a}	0.29 ± 0.01^{ab}	0.25 ± 0.01^{b}
CC	Optimum	2.3 ± 0.15^{ab}	1.8 ± 0.09^{a}	1.5 ± 0.05^{b}	4.1 ± 0.2^{a}	5.59 ± 0.25^{a}	2.8 ± 0.2^{ab}	0.41 ± 0.01^{b}	0.32 ± 0.01^{a}	0.27 ± 0.01^{ab}
	Drought	1.8 ± 0.07^{b}	1.3 ± 0.10^{b}	1.2 ± 0.02^{a}	3.1 ± 0.1^{b}	4.36 ± 0.14^{b}	2.6 ± 0.1^{b}	0.42 ± 0.01^{b}	0.30 ± 0.01^{a}	0.28 ± 0.01^{a}

Note: The data shown are means ± SE ($n = 8$). Different lowercase letters within the same column indicate significant differences ($p < 0.05$).
Abbreviations: CC, *Cenchrus ciliaris*; EM, *Enteropogon macrostachyus*.

Plants show an increase in stomatal conductance and rates of photosynthesis under optimum soil moisture conditions (Li et al., 2017; Manzoni et al., 2011). However, *E. macrostachyus* showed low stomatal conductance even under optimum soil moisture conditions. Stomata conductance and photosynthetic rate in *E. macrostachyus* did not change significantly under optimum and water deficit conditions. A probable explanation is that a decrease in the soil moisture content did not increase the water cost of carbon (C) to the plant. Additionally, this suggests that *E. macrostachyus* relies mainly on functional traits and hydraulic architecture as its key "water-saving" strategies. Low stomatal conductance with increased soil water availability may decrease the rate of photosynthesis in *E. macrostachyus*. However, this would be beneficial in conserving soil moisture, especially in African drylands. Subsequently, rangelands dominated by *E. macrostachyus* will show prolonged soil moisture availability as water availability becomes limited. Our study suggests that *C. ciliaris* and *E. macrostachyus* show contrasting sensitivities of the marginal water cost of C uptake to declining soil water availability.

The direction of changes in the intercellular CO_2 concentration (C_i) and stomatal limitation (L_s) are good indicators of drought-stressed plants. Specifically, when C_i decreases and L_s increases, stomatal limitation (SL) plays a dominant role in photosynthesis, while when C_i increases and L_s decreases, nonstomatal limitation (NSL) plays a dominant role in photosynthesis (Song et al., 2020). In our study, C_i of both grasses decreased with drought, while the L_s increased with drought. This suggests that SL plays a dominant role in photosynthesis of *C. ciliaris* and *E. macrostachyus*. Decreased C_i due to reduced Gs has been considered proof of CO_2 limitation for C_4 photosynthesis shown by these species (Ghannoum, 2009).

Transpiration declined more significantly under drought in *C. ciliaris*, compared to *E. macrostachyus*, with a relatively constant leaf transpiration in optimum and drought conditions. Significantly higher leaf transpiration in *C. ciliaris* under optimum conditions can probably be interpreted as a "photosynthesis maximization" strategy. Minimizing loss of water during drought is an important adaptation strategy to water stress, especially for plants in drylands. Conversely, reduced transpiration of *C. ciliaris* under drought could be regarded as a "water-saving" strategy to minimize water stress. Simultaneous reduction in stomatal conductance suggests an adaptative efficient stomatal control of transpiration by *C. ciliaris*. *E. macrostachyus* showed continuous "water-saving" strategies under both optimum and drought stress conditions, while *C. ciliaris* showed "water-spending" strategies under optimum conditions and "water-saving" strategies under drought conditions. These results show that *C. ciliaris* is more sensitive and responsive to changes in soil moisture regimes than *E. macrostachyus*. Other perennial C_4 grasses have been shown to utilize these varied strategies and mechanisms to overcome water deficit conditions (Álvarez et al., 2011; Cardoso et al., 2015; Hessini et al., 2008).

E. macrostachyus and *C. ciliaris* maintained comparable higher rates of transpiration under optimum conditions, leading to significantly higher cumulative water loss compared to drought. Similarly, other forage crops (e.g., *Sorghum bicolor*) and forage grasses (e.g., *Dactylis glomerata, Fesctuca pratensis, Festulolium braunii*, and *Lolium multiflorum*) are known to have high stomatal conductance, small leaves with high boundary layer conductance, high stem, and root densities, factors that are important in maintaining high rates of transpiration when soil water supply is adequate (Fracasso et al., 2016; Staniak & Kocoń, 2015). Additionally, under drought, *C. ciliaris* showed significantly lower cumulative water loss than *E. macrostachyus*. Reduction in water loss under drought stress was more prominent in *C. ciliaris* than in *E. macrostachyus*. A probable explanation is that there was a gradual decrease of transpiration in *C. ciliaris* as drought advanced. This might explain the natural occurrence of *C. ciliaris* in deep soils that have more water available, compared to *E. macrostachyus*, which occurs naturally in rocky outcrop dryland environments (Marshall et al., 2012).

Our results indicate that both grasses have a combination of "evasion" and "tolerance" mechanisms to cope with drought. Comparable transpiration under optimum and drought conditions in *E. macrostachyus* suggests its "drought avoidance" and "conservative" water use even under optimum conditions. Conversely, the significant reduction in transpiration in *C. ciliaris* under limited water availability shows that it has a "drought evasion" mechanism and is more sensitive to drought. These results align well with other studies demonstrating that C_4 grasses, for example, *C. ciliaris, Hyparrhenia rufa* and *Trachypogon plumosus* have both

"evasion" and "tolerance" mechanisms to cope with water deficit conditions in dryland environments (Baruch & Fernández, 1993; Marshall et al., 2012).

WUE_t in both *E. macrostachyus* and *C. ciliaris* increased with drought. Previous studies have also demonstrated increased WUE in *C. ciliaris*, other C_4 grasses and plants under water deficit conditions (Boutraa et al., 2010; Ghannoum et al., 2002; Li et al., 2017). However, other studies have also shown that drought stress did not affect WUE_t of *S. bicolor* and other C_4 forage grasses (Bahrani et al., 2010; Clifton-Brown & Lewandowski, 2000). Under drought, *E. macrostachyus* had higher WUE_t compared to *C. ciliaris*. The greater ability of *E. macrostachyus* to improve WUE under drought compared to *C. ciliaris* is probably attributed to its superior stomatal adjustment. This demonstrates further that *E. macrostachyus* and *C. ciliaris* have slightly different water regulation mechanisms related to drought avoidance.

In drylands, where plants mainly rely on variable seasonal rainfall for growth, maximization of soil moisture use is a crucial component of drought resistance (avoidance), characterized by a lower WUE (Blum, 2005). The photosynthetic rate was comparable under optimum and drought conditions in *E. macrostachyus*, but significantly reduced under drought in *C. ciliaris*. These results indicate that *C. ciliaris* would probably have a higher WUE under optimum conditions, whereas *E. macrostachyus* would be more efficient and successful under more xeric conditions. Past studies have also shown that plant strategies to cope with drought are a mixture of drought avoidance mechanisms that vary with species and ecotype (Cardoso et al., 2015; Ghannoum et al., 2001, 2002; Staniak & Kocoń, 2015).

Leaf, stem, and root biomass of *C. ciliaris* were consistently higher in well-watered compared to drought-stressed plants. Previous studies have also shown a reduction in biomass allocation to different fractions of tropical forage grasses under water deficit conditions (Baruch, 1994; Ghannoum et al., 2002; Greco & Cavagnaro, 2002; van Staalduinen & Anten, 2005). Together with our results, these studies demonstrate some grass species will respond to water stress conditions by making adjustments that reduce water loss, increasing survival at the cost of reduced growth. Conversely, as observed in *E. macrostachyus*, other forage grasses, for example, climate-smart *Brachiaria* species (e.g., *B. decumbens, B. dictyoneura, B. humidicola*, and *B. mutica*), are less sensitive to a reduction in soil moisture (Bakker & Wilson, 2001; Dong et al., 2005; Guenni et al., 2002).

Greater reduction in the total plant biomass due to drought in *C. ciliaris* compared to *E. macrostachyus* suggests that *C. ciliaris* growth is more sensitive to changes in the soil moisture content. This suggests that the *C. ciliaris* ecotype is less drought-adapted and suffered greater productivity decline under drought, presumably due to photosynthetic rates declining by ~50%. In response to limited water conditions, *C. ciliaris* allocated a higher proportion of its plant biomass to root mass fractions. On the other hand, *E. macrostachyus* showed a relatively small decrease in DM yields, indicating resilience under drought. Our results indicate that grass species native to African dryland environments use different physiological and metabolic regulation strategies in response to water deficit conditions.

CONCLUSIONS

Leaf gas exchange characteristics, biomass partitioning in the leaf, stem and root fractions, and water use efficiencies varied between the two C_4 African rangeland grasses under water deficit conditions. Drought limited the photosynthetic rate mainly through stomatal closure. This suggests that stomatal limitation plays a dominant role in *C. ciliaris* and *E. macrostachyus* photosynthesis. Partitioning of biomass into leaves, stems and roots yielded more detailed information of the very different functions of the individual biomass fractions. *C. ciliaris* and *E. macrostachyus* showed different sensitivity and water regulation mechanisms associated with drought avoidance. *E. macrostachyus* showed an increase in its water use efficiencies under drought to a greater extent than *C. ciliaris*. Our results suggest that the *C. ciliaris* ecotype used in this study would grow well where water stress is not a dominating factor, while *E. macrostachyus* would better withstand water deficit conditions. These findings suggest that extreme climatic events, for example, drought, may lead to unequal adaptive responses to water deficit conditions among different C_4 grasses native to African drylands. Here, we investigated only two C_4 grasses established as monocultures during a short period under controlled environmental conditions. Thus, long-term field-based studies investigating multiple grasses established in their natural environment will broaden our understanding of African dryland grasses' leaf gas exchange characteristics, biomass partitioning, and WUE responses to varied soil moisture conditions. Consequently, this will improve management practices of the grasses under such prevailing conditions.

AUTHOR CONTRIBUTIONS

Kevin Z. Mganga: Conceptualization; formal analysis; funding acquisition; investigation; methodology; validation; writing—original draft; writing—review and editing. **Jana Kuhla**: Formal analysis; investigation; methodology; software; validation; writing—review and editing. **Andrea Carminati**: Conceptualization; formal analysis; funding acquisition; methodology; project administration; resources; supervision; writing—original draft; writing—review and editing. **Johanna Pausch**: Conceptualization; formal analysis; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing. **Mutez A. Ahmed**: Conceptualization; data curation; funding acquisition; investigation; methodology; resources; software; validation; writing—review and editing.

ACKNOWLEDGMENTS

Financial support for this research project was provided by TWAS-DFG's Cooperation Visits Program.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Mganga, K. Z., Kuhla, J., Carminati, A., Pausch, J., & Ahmed, M. A. (2023). Leaf gas exchange characteristics, biomass partitioning, and water use efficiencies of two C_4 African grasses under simulated drought. *Grassland Research*, *2*(1), 37–45. https://doi.org/10.1002/glr2.12040