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Effects of elevated carbon dioxide and climate change on biomass and nutritive value of Kyasuwa (*Cenchrus pedicellatus* Trin.)

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Summary

Atmospheric carbon dioxide enrichment enhances plant growth and development and may alter the nutritive value of grasses. The objective of this study was to evaluate growth, biomass partitioning and nutritive value of Kyasuwa under combinations of atmospheric CO₂ concentrations, watering and fertilization treatments. Plants were grown in two greenhouse chambers; with ambient (aCO₂; 400 ppm) and elevated CO₂ (eCO₂; 950 ppm), two watering and three fertilization regimes. Elevated CO₂ reduced stomatal conductance by 40%, root to shoot ratio by 8%, leaf to stem ratio (L:S) by 3%, protein content by 14% and Acid Detergent Lignin (ADL) by 23% with no significant changes in total biomass and C/N ratio however, slight increases in leaf area (2%) and Acid Detergent Fiber (ADF) by 4%. Higher fertilization resulted in increased biomass parameters only in well-watered plants while; a lower C/N ratio was recorded with higher fertilization. The L:S ratio was decreased with fertilization while ADL was increased at higher fertilization in well-watered plants. Interactive effects were recorded for ADF content and shoot height. Future eCO₂ will be unfavorable to Kyasuwa growth making them less competitive with a reduced nutritive value in drought prone and infertile soils.

Keywords: carbon dioxide, climate change, Kyasuwa, nutritive value, plant growth

Introduction

Cenchrus pedicellatus Trin. (formerly called *Pennisetum pedicellatum* Trin., “Kyasuwa”), belongs to the Poaceae family and is a C4 grass (SCHMELZER, 1996). It is native to West Africa and was introduced to India from where it has since spread to South East Asia and Northern Australia (SCHMELZER, 1996) where it is invasive and regarded as an environmental weed (QUDDUS et al., 2014). Kyasuwa is tolerant of disturbance with broad climatic amplitude, produces large quantities of seeds with an efficient dispersal mechanism (SCHMELZER, 1996). It occurs along road edges, disturbed and abandoned lands and thrives on soils of a wide pH scale in rainfall regimes ranging between 500-1500 mm with severe drought lasting 4-6 months (FAO, 2010; SCHMELZER, 1996). Kyasuwa provides succulent, palatable and nutritious forage over a long growing season including the dry periods of October-November and contributes to meeting Cattle fodder requirements in rural areas (SCHMELZER, 1996; FAO 2010). It is also used as a soil stabilizer, mulch and in soil erosion control (SCHMELZER, 1996; FAO, 2010).

Climate change is one of the most severe challenges of our time, with predicted increases in global mean temperature, length and severity of drought events and atmospheric CO₂ concentration, due to human activities (IPCC, 2014). Many plants respond to elevated atmospheric carbon dioxide (eCO₂) concentrations by increased growth, biomass

and productivity, with C3 plants generally benefitting more than C4 plants (SANTOS et al., 2014; AINSWORTH and ROGERS, 2007). Moreover, there are also reports of significant changes in the chemical composition of plants under eCO₂ (MYERS et al. 2014). Nonetheless, plant responses to eCO₂ are not easily predictable because they depend on multiple environmental factors which are not necessarily additive (ACKERLY et al., 1992). Water and nitrogen (N) availability are two of the most limiting plant resources and have been reported to interact with eCO₂ (ERBS et al., 2015). Several studies have shown that the photosynthetic capacity of plants grown at eCO₂ will be acclimated and even down-regulated due to feedback repression of accumulated carbohydrates (PAUL and DRISCOLL, 1997). This has especially been observed when the plant's C/N status is high due to deficient N supply, leading to a decrease in Rubisco activity and thus, lower photosynthetic rates (LEAKEY et al., 2012). Biomass enhancement of eCO₂ may therefore be dependent on sufficient N supply in some species (DONG et al., 2016). In contrast, some species may show enhanced C gain per leaf N due to a suppression of photorespiration under eCO₂ (LEAKEY et al., 2012).

There is a general lack of data on responses of tropical and subtropical plants to future climate changes and more research on plant responses of C3 than C4 species (LEAKEY, 2009). The purpose of this work was to determine the effects of future elevated atmospheric CO₂ concentration on biomass development which aids invasive potential and nutritive value of Kyasuwa grass. The objectives of the study were, (1) to assess the effects of eCO₂ on Kyasuwa growth, biomass allocation and nutritive value, (2) to evaluate how different water and fertilization regimes affect Kyasuwa growth, biomass allocation and nutritive value and, (3) to estimate interactive effects of the three resource factors on these traits of Kyasuwa. We hypothesize future increases in atmospheric CO₂ will increase Kyasuwa growth and biomass thereby enhancing competitiveness. We also hypothesize that nutritive value of Kyasuwa will increase with eCO₂ especially at high fertilization regimes.

Materials and methods

Plant preparation and growth conditions

Seeds for the study were harvested in January 2016 from 150 plants located in Tolon in the Guinea savanna zone of Ghana (09° 25'N, 00° 58'W). Seeds were transported within 3 weeks of picking to the greenhouse of Universität Hamburg, Germany (53° 30'N, 10° 12'E). Germination and pre-treatment growth were carried out as follows in the greenhouse. After a germination period of 3-6 days in germination trays, two plants were transplanted into a 3 L plastic pots of 15 cm height, filled with a mixture of 4:1:0.5 (v/v) sand (0.13-0.36 mm), clay and standard organic substrate (TKS1, Floragard Vertriebs-GmbH, Oldenburg). The bottom of the pots was secured with a weed mat to prevent loss of substrate. After pre-treatment growth for 20 days, the pots were randomly assigned to two growth chambers (each 28.5 m²) of different CO₂ concentrations in a green-

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house with controlled growth conditions (Day/night air temperature 25 °C/22 °C; 70%-80% relative air humidity; Length of photoperiod same as Hamburg area). We stimulated the atmospheric CO₂ concentration of 950 ppm based on the representative concentration pathway (RCP) 8.5 scenario by 2100 (IPCC, 2014). In the first growth chamber, the CO₂ concentration was the same as in ambient atmospheric air (aCO₂; 400 ppm) while in the other chamber, the CO₂ was elevated to 950 ppm (eCO₂).

Three fertilization treatments were applied using commercial liquid fertilizer with NPK values of 8-8-6 (WUXAL Super, Aglukon Spezialdünger GmbH, Düsseldorf, Germany). The treatments corresponded to the equivalent of 75 kg N ha⁻¹ (N-1), 100 kg N ha⁻¹ (N-2) and 125 kg N ha⁻¹ (N-3) and were applied at planting, 20 days after planting and 40 d after planting. The WUXAL super fertilizer has the full complement of macro and micro nutrients. During the experimental period of 68 days two watering regimes of continuous watering (wet), and a stimulated drought period of no watering (dry) for 2 weeks were implemented. The complete design of the study comprised of two levels of CO₂, two watering regimes, three fertilization regimes and six replicates, resulting in a total of 72 pots for the entire experiment. The pots were equally distributed among the two growth chambers which resulted in 36 pots in each chamber. To avoid edge and chamber effects, the positions of individual potted plants were rotated on the tables on a weekly basis but, it was practically impossible to switch CO₂ concentrations between the two chambers however, the growth conditions were similar. During the experiments, the CO₂ level, temperature and air humidity were monitored by the Computer Climate model CC 600 (RAM co. Measurement and Control, Germany) every 12 minutes. The light conditions in the two chambers were measured for a month with quantum sensors (LI-190R, LI-COR, Lincoln, NE, USA) connected to data loggers (CR 1000, Campbell Scientific, Logan, UT, USA). The grasses were exposed to the experimental treatments for a total of 68 days during the growth period (May-July 2016).

Plant growth and physiological measurements

To assess the effects of eCO₂, watering and fertilization regimes, the growth characteristics were recorded before harvesting the grass 80-90 days after sowing. Shoot height was measured from ground level to the base of the top-most, fully developed leaf or to the base of the panicle depending upon the stage of the particular plant. The stomatal conductance was measured on two leaves for three replicates within all treatments using a leaf porometer (SC-1, Decagon Devices, Pullman, WA, USA) on young but fully developed leaves. At harvest, the area of the individual grass leaves was determined using an area meter (LI 3100, LI-COR, Lincoln, NE, USA) while the fresh weight of leaves and stems were measured separately and the leaf to stem ratio (L:S) estimated from these measurements. Roots were thoroughly washed from the soil over a sieve with 1 mm mesh size. The biomass fractions (leaves, stems and roots) were oven dried for 48 hours at 65°C. Roots were denominated below-ground biomass (BGB). Above-ground biomass (AGB) was estimated by adding dry weights of leaves and stems, while total biomass (TB) was the sum of AGB and BGB. The root to shoot ratio (R:S) was calculated by dividing BGB by AGB.

Forage nutrient analysis

To evaluate the effects of eCO₂, watering and fertilization regimes on nutritive value of Kyasuwa, oven-dried leaves and stems were ground separately with a micro hammer mill (Culatti AG, Zürich, Switzerland) fitted with a 1 mm sieve. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured sequentially with the ANKOM filter bag method according to the manufacturer, using a fibre analyzer (ANKOM-200 Fiber Analyzer, ANKOM Technology,

Macedon, NY, USA). Ground leaf and stem material (500 mg) was placed in ANKOM F57 filter bags and sealed with heat. All 72 samples were first extracted with neutral detergent, and the residue was weighed to determine percentage of NDF. The NDF residue was then extracted with acid detergent solution, followed by extraction with 72% H₂SO₄ and ashing to determine the percentage of acid detergent lignin (ADL) (VAN SOEST, 1994; RYAN et al., 1990). The following nutrient parameters were estimated: % NDF, % ADF, and % ADL.

Total nitrogen and carbon concentration of leaves was measured in aliquots of oven dried samples by an elemental analyzer following pyrolysis (EURO-EA 3000, Euro Vector, Italy). Mass calibration was conducted by the use of the certified standard 2,5-bis (5-tert-butyl-2-benzoxazol-2-yl) thiophene (6.51% N; 72.52% C; HEKAtech, Germany). The percentage of proteins in leaves was measured according to BRADFORD (1976): Dried leaves of all treatments were finely ground using a Retsch mixer mill (MM-400, Fischer Scientific, Suwanee, USA) and thereafter digested in 0.1 M NaOH for 30 minutes (JONES et al., 1989). A volume of 100 µl aliquots of centrifuged supernatant were assayed with 50 µl Bio-Rad Bradford dye (Coomassie brilliant blue). Absorbance was measured at 595 nm after 15 minutes using a multi-mode microplate reader (Synergy HT, BioTek Instruments, Winooski, USA).

Data analysis

All statistical analyses were carried out using the software package Statistica 13 (Stat-Soft Inc., Tulsa, OK, USA). Growth, biomass and nutritive value parameters were analyzed by three-way analysis of variance (ANOVA) to test for significant main effects of the factors CO₂ concentration, fertilization levels and watering regime as well as all factor interactions, followed by the Tukey HSD post hoc test of significant differences. For analysis of nutritive value, data of stems and leaves were averaged after chemical assays. Prior to the 3-way ANOVA, all data were analyzed for homoscedasticity using Levene's test, and data were transformed appropriately where necessary. In addition, residual plots and normal probability plots were inspected to ensure that the assumptions of ANOVA were met.

Results

Environmental conditions during the study

Throughout the experiment, the monitored average daily temperature in both the ambient and elevated CO₂ chambers was 26 °C ± 1 °C. The relative humidity of the ambient CO₂ chamber was 79% ± 7% while that of the elevated CO₂ chamber was similarly 73% ± 8%. The light conditions of the two chambers were similar. Overall, the environmental conditions in two chambers were stable and similar throughout the duration of the study except the ambient CO₂ (400 ppm) and the elevated CO₂ (950 ppm) in the elevated chamber.

Effects of elevated CO₂ on Kyasuwa

Kyasuwa grass grew well in the two CO₂ chambers and showed no signs of nutrient deficiencies or pest attacks. Elevated CO₂ significantly reduced stomatal conductance ($p < 0.001$), leaf to stem ratio ($P = 0.04$) and root to shoot ratio ($P = 0.01$) by 40%, 3% and 5%, respectively (Tab. 1, Fig. 1). However, leaf area ($P = 0.014$) was significantly increased by 2% with increased carbon dioxide concentration (Fig. 1, Tab. 1). Above-ground and total biomass was not significantly affected by atmospheric CO₂ concentration (Tab. 1).

Growth under eCO₂ significantly increased structural carbohydrates (ADF) by 2% ($P < 0.001$). However, ADL and percentage protein were reduced by 23% ($P < 0.001$) and 14% ($P < 0.001$), respectively (Fig. 1, Tab. 1). The C/N ratio was not significantly affected by CO₂ concentration.

Tab. 1: F-values of three-way ANOVA of all measured parameters of Kyasuwa grass grown under elevated and ambient CO₂, three fertilization and two watering regimes. N=6

Parameters	Sources of Variation						
	CO ₂ (C)	Water (W)	Fertilization (N)	C*W	C*N	W*N	C*W*N
Growth & Biomass							
Height (cm)	10,63**	47,87***	0,77	0,58	0,07	1,42	4,15*
Leaf area (cm ²)	6,37**	0,03	0,44	0,00	0,18	0,64	0,33
Stomatal conduc-tance (mmol mol ⁻¹)	7,30**	0,73	0,13	2,54	0,23	0,73	1,03
Leaf to stem ratio	4,62*	2,49	7,04**	0,10	2,06	0,06	0,60
Root to shoot ratio	7,09**	2,41	0,46	0,09	0,17	0,23	0,09
Above ground biomass (g)	0,00	63,52***	9,67***	1,32	2,59	7,02***	2,56
Below ground biomass (g)	9,30**	36,69***	9,95***	3,11	2,12	4,90**	2,81
Total biomass(g)	0,71	54,04***	10,10***	1,26	2,26	5,68**	2,75
Nutritive value							
C/N ratio	0,59	53,98***	11,60***	1,64	0,39	5,88**	1,22
% Protein	30,38***	1,17	0,46	0,44	0,44	0,72	1,31
% Neutral detergent Fibre	23,45***	1,42	2,36	1,03	5,31**	2,34	0,55
% Acid detergent Fibre	111,34***	16,61***	1,79	0,00	1,44	0,82	0,23
% Acid detergent lignin	14,38***	2,06	0,78	3,87	1,64	3,23*	0,30

Statistically significant values in bold; * <0,05, **<0,01, ***<0,001 probability levels

Water and fertilization regime effects on Kyasuwa growth and nutritive value

The high-watering level significantly increased by 1% ($P < 0.001$) the ADF content (Fig. 1). Increased nutrient availability reduced the leaf to stem ratio by 6% between N-3 and N-1 ($P = 0.002$), and by 4% ($P = 0.04$) between N-2 to N-1 (Tab. 1, Fig. 1).

We found significant interactions between watering and fertilization regimes for AGB, BGB and, TB ($P = 0.02$; 0.01 ; and 0.005 ; respectively) (Fig. 2a). Increasing fertilization caused significant increases in these three biomass parameters but only with concurrently high water availability. At low water availability, no biomass responses to fertilization regime could be detected.

For nutritive components, we observed two way interactions of watering and fertilization regimes for the C/N ratio ($P = 0.005$) and ADL ($P = 0.046$) (Fig. 2b, c). The C/N ratio was lowered with higher levels of fertilization, but only in drought-exposed plants. The ADL concentration was higher at the highest than the two lower fertilization levels, but only in well-watered plants.

Interactive effects of elevated CO₂ with fertilization and/or watering regimes

There was a two-way interaction between carbon dioxide enrichment and fertilization regime for NDF ($P = 0.008$; Tab. 1; Fig. 3a). Here, eCO₂ had no effect on NDF at the low fertilization level, while it resulted in increased NDF at the higher nutrient availability N-3 (Fig. 3a). A significant three-way interaction ($P = 0.02$) of CO₂ concentration, water and fertilization regimes was observed for shoot height (Fig. 3b). Under aCO₂, the higher fertilization regimes (N-2 and N-3) increased shoot height in well-watered plants but decreased shoot height in drought-treated plants. However, under eCO₂, higher fertilization regimes (N-2) resulted in increased shoot height at lower water regimes, but well-watered plants had taller shoots than drought-treated plants though no fertilization effects were recorded.

Discussion

Growth and biomass

Most plants, both C3 and C4, respond to eCO₂ by reducing stomatal conductance (AINSWORTH and ROGERS, 2007; AINSWORTH and LONG, 2005). In the present study, Kyasuwa responded to eCO₂ by reducing its stomatal conductance (g_s), which usually translates into reduced water loss through lower transpiration rates and higher water use efficiency at the leaf level (EAMUS et al., 2008). A review of the effect of eCO₂ on both C3 and C4 vegetables pointed to down-regulating of stomatal conductance and lowering of transpiration, the combination of which leads to higher water use efficiency (BISBIS et al., 2018).

There are several studies showing that both tropical and temperate C4 grasses benefit from eCO₂ by down-regulating their stomatal conductance and thus, water loss, rather than increasing their above ground and or total biomass (XU et al., 2014; KAKANI and REDDY, 2007). Except shoot height, we did not find any interaction of watering regime and atmospheric CO₂ concentration on any other investigated parameters, so it is unlikely that eCO₂ had any effects on the integrated water-use efficiency of Kyasuwa, although g_s was lower under eCO₂, which at first sight might be indicative of an increased instantaneous water-use efficiency. In the case of shoot height there was a three-way interaction with differences recorded in the low water treatments of aCO₂ and eCO₂ in higher fertilization regimes. Since consequently the net CO₂ flux into the leaves might have been the same at both high and ambient CO₂ concentrations, irrespective of the water availability, the lower stomatal conductivity in eCO₂ was the result of an adjustment of the CO₂ influx to an apparently unchanged CO₂ demand reflecting a similar assimilation potential under both atmospheric CO₂ conditions (GHANNOUM et al., 2000). This suggestion is further supported by similar C/N ratios at aCO₂ and eCO₂.

The effects of eCO₂ on Kyasuwa growth and biomass seemed to be confined to changes in biomass allocation patterns in the present

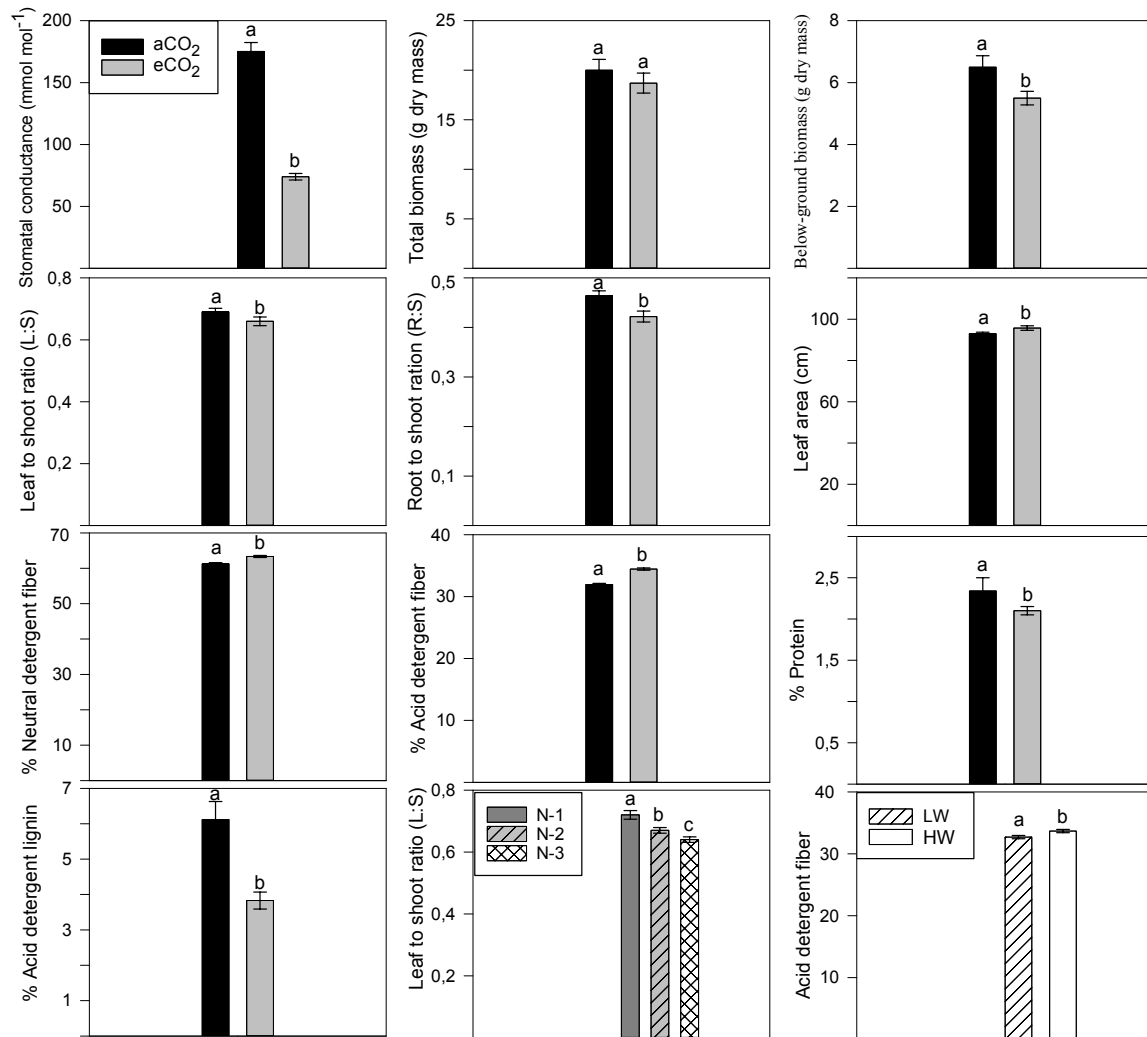


Fig. 1: Main effects of CO₂ concentrations, watering and fertilization regimes on growth, biomass parameters and nutritive values of Kyasuwa. Different lowercase letters represent significant differences among treatments. LSM \pm SE. eCO₂, elevated CO₂ concentration; aCO₂, ambient CO₂ concentration; LW, Low water treatments; HW, High water treatment; N-3, High fertilization regime; N-2, medium fertilization regime; N-1, low fertilization regime.

study. The decrease in the root to shoot ratio and the general reduction in below-ground biomass with eCO₂ has previously been observed (KAKANI and REDDY, 2007). The effect of eCO₂ on R:S is mechanistically not well understood, but it varies with plant types and resource supply (ROGERS et al., 1996). Other studies have both found an increase (ARNONE et al., 2000) and no change in R:S (KÖRNER et al., 1997). As a general principle of allocation, under changing resource availability (light, water, nutrients, CO₂), plants tend to enhance organs that can increase the capture of the resource that is becoming limiting, partly to the detriment of other organs. In our study, as in others, increased CO₂ supply caused lower stomatal conductivity leading to reduced water demand. Consistent with the above-mentioned principle, the allocation to root growth has been reduced, whilst a significant increase in leaf area as a response to eCO₂ has been observed which is also reported in other studies (ACKERLY et al., 1992). By lowering investment in below-ground biomass, the plant's rooted soil volume is reduced and, thus, not only less nutrients but also less soil water is available to the plant. Consequently under eCO₂ shorter dry spells could be withstood devoid of severe distress. However, Kyasuwa will be more susceptible to extended drought because less soil volume will be used for water uptake and thus less competitive in the drier savanna.

The observed decrease in leaf to stem ratio (L:S) signifies that eCO₂ resulted in higher allocation of biomass to stems, which has been previously reported in other C4 grasses (SANTOS et al., 2014). On one hand, if the lower L:S ratio is combined with the increased leaf area, plants grown at eCO₂ developed overall broader leaves and denser shoots than those grown at aCO₂, which is beneficial for future use of the species, as livestock prefer leaves with broader blades (BATISTOTI et al., 2012). On the other hand, a high leaf to stem ratio is generally preferred as an important factor in diet selection, quality and forage intake (SMART et al., 2004). However, L:S was significantly reduced with higher fertilization regimes because of higher accumulation of stems as reported by other studies (SALVADOR et al., 2016).

Water availability is one of the main biophysical limitations of grass growth in savannas (DEL GROSSO et al., 2008). Water in general is one of the most important limiting resources to plant growth, and in the present study, low soil water content decreased all the biomass parameters as well as grass shoot height. Water deficiency decreases transpiration rate via decreased stomatal conductance, which results in a decline of net photosynthetic rates (FLEXAS and MEDRANO, 2002). Although C4 species have inherently lower stomatal conductance than C3 and thus have higher water use efficiency, they can still

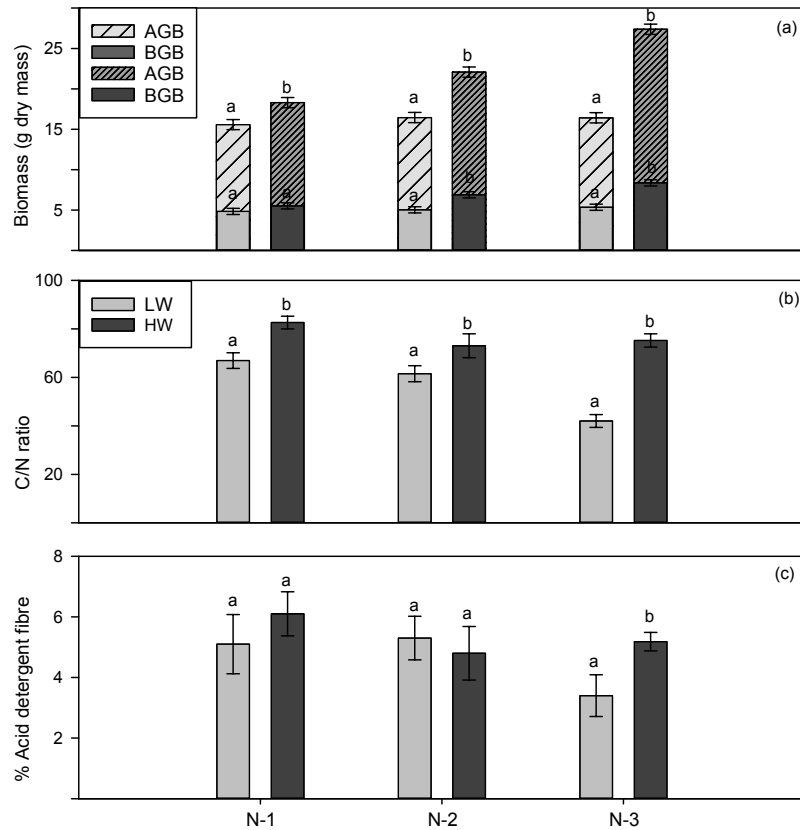


Fig. 2: Two-way interactions between watering and fertilization regimes on biomass of Kyasuwa (a). Interactive effects of fertilization and watering regimes on nutritive value parameters of Kyasuwa (b, c). Different lowercase letters represent significant differences among treatments. LSM \pm SE.

be threatened by drought. In the present study, it is more likely that the effects of water availability on growth were displayed through indirect effects for example lower mobility of nutrients in dry soil rather than a direct effect via stomatal conductance, which did not respond to watering regime. The water by fertilization regime interactions for Kyasuwa showed that water supply was the main limiting factor for biomass accumulation and height growth. Water availability is pivotal for increasing nutrient availability for Kyasuwa growth and biomass production, and elevated CO₂ did not help to improve the water use efficiency of this species. However, some plants do not reduce water loss through stomata closure but, react to water shortage by investing assimilates into protective substances (i.e. synthesis of LEA-proteins and other osmoprotectants, compatible solutes etc) and consequently they have a lower biomass under drought treatment (SCHULZE et al., 2005).

Kyasuwa tissue nutritive value

The significant reduction of the protein content under eCO₂ has been attributed more to a dilution-effect due to an overall increase in total non-structural carbohydrates, rather than an absolute decrease of the protein content in leaves (DUMONT et al., 2015). This is unlikely to be the case in the present study, since the C/N ratio was unaffected by CO₂ treatment. Other studies implicate the decrease in Rubisco concentration (AINSWORTH and LONG, 2005) due probably to carbohydrate-dependent decrease in expression of photosynthetic genes (MOORE et al., 1999) and decreased transpiration and stomatal conductance (DEL POZO et al., 2007). A meta-analysis on proteins in food crops also indicates a reduction of proteins at elevated CO₂ (TAUB et al., 2008). We suggest that a re-allocation of N to resources other than proteins occurred within Kyasuwa, for example to struc-

tural N-compounds or free amino acids or even to vacuolar nitrates, leaving a similar C/N ratio in the aCO₂ and eCO₂ treatment. The lack of effect of eCO₂ on the C/N ratio could be a result of C4 plants photosynthesis and biomass accumulation being less affected by eCO₂ (WANG et al., 2012). C4 plants are less affected by eCO₂ because of their C-accumulation strategy, which minimizes photorespiration through anatomical and biochemical specializations that concentrate CO₂ at the active site of Rubisco (SAGE, 2004) and are virtually CO₂ saturated already at aCO₂.

Low soil water potential generally impairs plant metabolism and there are suggestions that water availability plays a key role in nutrient limitations to grasses of semi-arid areas (LU et al., 2012). This is because of the vital role water plays in nutrient transport and availability for nutrient uptake in plant roots. The interactive effect of watering and fertilization regimes on C/N ratios showed higher fertilization regimes reducing C/N ratios when water availability was limiting. Several studies support the finding of an interaction between water availability and nitrogen nutrition for C/N ratio (LU et al., 2012). In nature, soil water availability influences soil N availability via many microbial aided pathways like litter decomposition (LIU et al., 2006) and N mineralization (WANG et al., 2006). In the present study, the high water availability probably had a direct effect on nutrient availability to plant roots by increasing their diffusion and mass flow in the soil. The lower C/N ratio may therefore be an expression for a relative decrease in C-assimilation rates under dry conditions when nutrient supply was high.

NDF concentration was increased at high fertilization regimes only under eCO₂, which is in concert with previous observations of soil N supply limiting the ability of plants to respond to eCO₂ (DONG et al., 2016). Plants with low N-demand, however, may respond to eCO₂ even in N-poor soils (NORBY et al., 1992). The present study

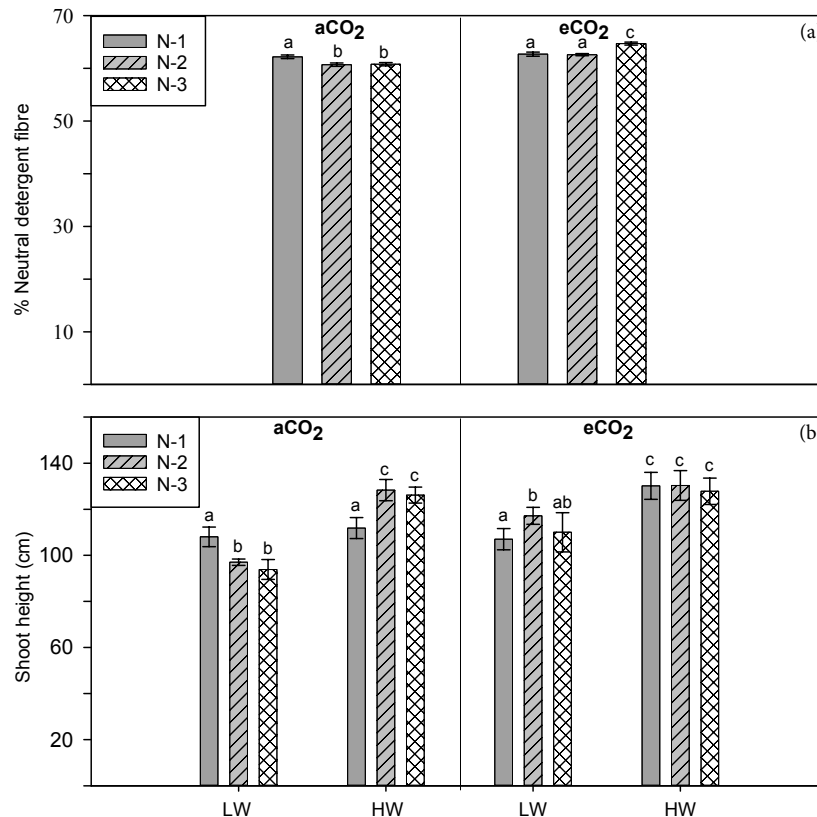


Fig. 3: Two-way interaction of CO₂ concentrations and fertilization regimes on neutral detergent fibre of Kyasuwa (a) and three-way interaction between CO₂ concentrations, watering and fertilization regimes on Kyasuwa shoot height (b). Different lowercase letters represent significant differences among treatments. LSM ± SE.

showed that the main effects of eCO₂ on NDF were significant but rather small, as reported by other studies (FRITSCHI et al., 1999; AKIN et al. 1995), and therefore future atmospheric CO₂ concentration can be expected to increase fiber content of Kyasuwa to a minimal extent, and only if a high fertilization load is provided.

ADF concentration was significantly reduced by drought, which may have been caused by delayed plant maturity due to stressful arid growth conditions (KÜCHENMEISTER et al., 2013). Plant fibre contents turn to increase with age since the stem to leaf ratio increases with age, and fiber content of stems is considerably higher than leaves (BRUINENBERG et al., 2002). The increases in structural carbohydrates with eCO₂ recorded in this study have previously been observed and seem to be species-dependent (DUMONT et al., 2015; MILCHUNAS et al., 2005; FRITSCHI et al., 1999; AKIN et al., 1995). In wheat for instance, AKIN et al. (1995) recorded increased contents in fibre fractions with eCO₂, while FRITSCHI et al. (1999) reported increases in structural carbohydrates of *Arachis glabrata* leaves. MILCHUNAS et al. (2005) likewise recorded increases in combined cellulose and hemicellulose in *Bouteloua gracilis* with eCO₂. However, a recent meta-analysis of forage quality of Mediterranean grasslands pinpointed no change in structural carbohydrates with eCO₂ (DUMONT et al., 2015).

ADL concentration was increased at the highest fertilization level, but only in well-watered plants, which could be the result of lower L:S ratio with higher fertilization regimes. Moreover, ADL was lowered by eCO₂, which means that the tissue quality as forage can be expected to be higher under future eCO₂ concentrations. The reducing effect of eCO₂ on lignin components of forages has been reported in previous studies (MILCHUNAS et al., 2005; AKIN et al., 1995) which may be caused by lignin being connected chemically to proteins and carbohydrates in the cell wall to form large macro-

molecules (MOORE and JUNG, 2001). A higher NDF concentration reduces animal intake while a higher ADF concentration decreases digestibility (SAHA et al., 2013). However, decreases in ADL increases digestibility because lignin limits digestion (MOORE and JUNG, 2001). We therefore propose that the overall quality of Kyasuwa as forage will be lower under eCO₂. Vegetables are reported to increase in sugars, vitamin C, phenols, flavonoids and antioxidant capacity as a result of eCO₂, however the macro and micronutrients are reduced (BISBIS et al., 2018).

Conclusion

Elevated CO₂ increased individual leaf area of Kyasuwa which would make the grass attractive as forage. However, eCO₂ resulted in a change in biomass allocation towards a lower R:S ratio, that ultimately may be harmful for the species especially under dry conditions and low nutrient availability making it less competitive. Moreover, eCO₂ will result in changes in the chemical composition of Kyasuwa with increases in structural carbohydrates (NDF and ADF) and reduction in ADL and protein which will reduce the nutritive value of Kyasuwa overall. Water and fertilization were the two most limiting resources for Kyasuwa compared to CO₂ and did not interact with CO₂ except in shoot height and NDF. As a compromise for future tissue quality, we suggest to avoid over-fertilization of Kyasuwa to avoid an undesirable increase in fibre content.

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