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Effects of clipping and irrigation on carbon storage in grasses: implications for CO₂ emission mitigation in rangelands

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ABSTRACT

Understanding how individual grasses respond to herbivory and rainfall has been hampered by the difficulty of quantifying above- and belowground carbon (C) storage in grasses. Particularly by restoring degraded rangelands through reseeding, their C storage potential can be greatly enhanced. The responses of reseeded grasses to the effects of herbivory and precipitation were assessed to evaluate the potential of individual grasses for C storage as a technique for climate change mitigation. Clipping experiments were conducted on mature grass tufts of two native grass species, *Chloris gayana* and *Cenchrus ciliaris*, in the semi-arid Borana rangelands, Ethiopia. Further, above- and belowground C storage of young grasses of the same species in pot and field plot trials was experimentally quantified under simulated grazing and variable rainfall. The results showed that aboveground C was significantly 4 times lower in the clipped compared to unclipped mature grasses. In contrast, 3 times higher C was found in young reseeded grasses that were clipped compared to unclipped ones. Clipping and irrigation in combination significantly influenced belowground C in young grasses, with reduced irrigation overriding clipping effects. The paper concludes that moderate grazing should be encouraged to enhance CO₂ uptake, consequently contributing to climate change mitigation in rangelands.

KEYWORDS

Borana; herbaceous layer restoration; herbivory; livestock management; rainfall variability

Introduction

Carbon storage and herbivory in rangelands

Carbondioxide (CO₂), the most abundant anthropogenic greenhouse gas, is a driving factor in climate change, which has increased by 40% from a pre-industrial value in the year 1750 of about 280 ppm to 391 ppm in 2011 [1,2]. Carbon storage through terrestrial sinks in woody and grassy vegetation is of high importance [101] and rangelands are playing a more important role than previously assumed [3,102]. Degraded semi-arid rangelands are increasingly reseeded with native grass species, which could substantially contribute to climate change mitigation [4]. In the Borana rangelands, southern Ethiopia, native grass species are reseeded to restore degraded rangeland areas, thus increasing forage production [5]. However, the ability of rangeland grasses to store a large amount of C is primarily controlled by two environmental stressors – herbivory and precipitation [103]. Herbivory removes aboveground and, consequently, changes belowground grass biomass and C storage in rangelands [6]. While heavy grazing strongly reduces C storage [104], moderate to light grazing can also increase root biomass [105] and, hence, lead to an overall higher plant C storage [7]. However, little is

known about responses of plants such as above- and belowground biomass allocation or individual C storage after herbivory [8], particularly in rangelands that face higher drought frequencies due to shifts in climatic conditions.

Reseeding activities as rangeland restoration

Plant roots are the major C source in the soil [9] and a reseeding of rangeland management to enhance forage production might enhance belowground C storages as a co-benefit in mitigation activities [106].

In the Borana rangelands, two perennial C4 grass species (*Cenchrus ciliaris* and *Chloris gayana*) are particularly important in the reseeding process because they are highly palatable native species [5]. Reseeding management with these species can also increase C storage, which is of paramount importance for mitigating greenhouse gas emissions in the face of climate change [107,108]. However, the response of these reseeded grass species to grazing frequency under variable rainfall remains unknown. Further, there are few studies dealing with individual responses of grass at different ages to grazing, which is important when devising appropriate grazing management for old and reseeded rangelands [109].

Stressors on rangelands through climate change

Climate change will result in multiple stresses for plants and animals in the coming decades. Both extreme precipitation and intensity of drought are likely to increase in the future due to climate change [2]. Drought suppresses plant productivity [10] and will diminish the recovery potential of overgrazed rangelands [11]. Large uncertainties exist regarding the C storage capacity of rangelands under different climatic regimes and management systems [12]. In addition, little is understood about the effects of combinations of climate- and herbivory-related stressors on the C storage of grasses [13].

This study focused on C allocation responses of grasses to climatic extremes and herbivore pressure. Treatments of rainfall variability and grazing pressure were combined to acquire knowledge on C storage of grasses, not only in reseeded young grasses but also in mature grass tufts under natural conditions. The C storage of the two native C4 grasses, *C. ciliaris* and *C. gayana*, was estimated under simulated grazing and rainfall regimes using clipping and irrigation experiments, respectively.

The objectives of this study were to quantify the effects of clipping on the C storage in mature and young grasses, and to assess the interactive effects of grazing and rainfall variability on the two native grass species in the Borana rangelands of southern Ethiopia. The following questions were addressed:

- How do clipping frequencies influence grass C storage in above- and belowground parts?
- What are the responses of grass C to increased or decreased irrigation?
- Are effects of clipping and irrigation interactive?
- What are the implications for C storage, in the face of climate change, of the study grass species in the Borana rangelands?

Materials and methods

Study site

Our experiments were conducted in the Borana rangelands, southern Ethiopia, at Yabello Pastoral and Dryland Agriculture Research Centre (N 04°52', E 038°08'; 1626 masl). The mean (\pm standard deviation, SD) annual rainfall of Yabello is 645 (\pm 232) mm, ranging from 327 to 1343 mm [NATIONAL METEOROLOGICAL AGENCY AND YABELLO WEATHER STATION, PERS. COMM.]. Rainfall is bimodal, with 52% of rain occurring during the main rainy season (from March to May) and 31% occurring during the short rainy season (from September to November). The mean annual temperature is 20 °C with average maximum and minimum temperatures of 26 °C and 14 °C, respectively [NATIONAL METEOROLOGICAL AGENCY AND YABELLO WEATHER STATION, PERS. COMM.].

Two separate experiments were conducted on two grass species, *Cenchrus ciliaris* and *Chloris gayana*, native to the Borana rangelands. The first experiment was run on already established mature tufts under natural rainfall conditions during the main rainy season. For both species, different clipping frequencies were used to simulate grazing pressure. Moving cages and cattle grazing were used to confirm whether clipping can sufficiently resemble herbivory impacts. In further experiments, clipping and irrigation were applied to young grasses in pots and field plots from November 2013 to February 2014 (i.e. during the dry season).

Clipping and grazing of mature grass tufts

In the first experiment, 58 tufts were selected of two mature grass species (*C. ciliaris* and *C. gayana*) of similar size from two sites. Sites were less than 500 m apart and, hence, their environmental conditions were assumed to be similar. Tuft selection considered independent, individual tufts within 5 m distance of each other, and of similar circumferences (63–79 cm and 27–36 cm circumference range for *C. ciliaris* and *C. gayana*, respectively). Grass dry biomass per area (g/cm^2) was calculated to standardize the data. Between April and June 2013, both species were subjected to a completely randomized block design with different clipping frequencies as treatments to resemble cattle herbivory. The growing season of both perennial grass species starts after the onset of rains in March and their maturity is reached at the end of the rainy season (i.e. late May to June). Twenty-eight exclosure cages (including the grazing treatment) were erected above each *C. ciliaris* tuft in seven replications within a cattle-grazed open rangeland site. Four treatments (defoliation levels) were used to simulate grazing pressure (weekly clipping = 'frequent', biweekly clipping = 'moderate' and no clipping = 'control', as well as a weekly grazing by cattle = 'grazing').

The 28 exclosure cages (1 m \times 1 m \times 1 m) were erected according to Veldhuis *et al.* [110] above the 28 experimental tufts and only the control treatment were not moved throughout the experiment period, while other treatments were moved based on the grazing and clipping frequencies. For instance, cages above weekly grazed and weekly clipped tufts were moved every week, while cages above moderately clipped tufts were moved every other week.

Moreover, at the end of the clipping/grazing experiment, the belowground biomass was measured for all tufts, which were hand-clipped at different frequencies and grazed by 4–6-year-old Boran cows (*Bos indicus*) on a weekly basis (= 'grazing'); the latter was used to record differences in response to grazing and clipping. Animals were taken directly to the experimental site before they accessed other feed so that they would graze on the dominant tufts (i.e., *C. ciliaris*), without making any strong selection [14]. It was observed that

cattle preferred *C. ciliaris*, which had been already recognized as palatable [15], while *C. gayana* was not present in cattle grazing sites. Hence, three clipping frequencies (frequent, moderate and control) were used on mature tufts, with 10 replications each. Grasses on this site had been hand-clipped once every year at the end of the growth period for hay making since 2008 and had been last clipped in 2012.

Clipping and irrigation of young grass seedlings

For the second experiment of pots and field plots, rainwater was collected in storage tanks during the rainy season and used for dry-season irrigation treatments, in addition to the clipping frequencies (frequent = weekly clipped, moderate = biweekly clipped, light = monthly clipped, control = unclipped). Grasses were sown in pots under controlled environmental conditions and in the field plots under natural conditions. Treatments were applied simultaneously in pots and plots during the dry season, with the assumption that C stored in grasses is primarily controlled by two environmental stressors – herbivory and precipitation [103].

Irrigation resembled the condition of a typically wet April, the main growing season of the two focus grass species [16]. The April average rainfall (158.8 mm) over 30 years (1984–2013) was divided by the average number of rainy days (~15 days over 30 years) to get the April rainfall amount per event and volume of water to be added as average rainfall (= ‘ambient’) treatment. Scenarios were then derived of a 5% rainfall increase (= ‘higher’) and a 30% rainfall decrease (= ‘lower’) from the ambient treatment. The irrigation amount was calculated as volume = water depth (daily April rainfall) × surface area (of field plot or pot). The irrigation treatments were hand-watered every other day throughout the experimental period.

In southern Ethiopia, overall annual rainfall is predicted to be lower with future climate change, albeit with more extremes and potentially higher rainfall amounts at one time [17]. Rainfall amount in the Borana rangelands strongly varies [18] and, hence, this variation was replicated in the irrigation treatments.

Grass seedlings of both species were raised on a seedbed in a lath house and transplanted individually into pots and field plots. One seedling of the same size per pot and per field plot was transplanted, with a total of 72 and 84 individual seedlings, respectively, per species. Seeds used for pot and field plot experiments were collected in the previous years from the same site (i.e. within a radius of $\frac{1}{2}$ km around the study site of mature tufts). Hence, it was assumed that the seeds used for pot and field plot experiments were genetically similar to the mature tufts used during the rainy season field experiment.

In the field plots, holes of 5 cm diameter were drilled, using a motorized soil auger, to a depth of

10 cm for seedling transplanting, without disturbing neighboring soil structure. In the pots and field plots, both grass species were subjected to a factorial experiment with randomized complete block design of the four clipping frequencies (frequent, moderate, light and control) and the three irrigation amounts (higher, ambient and lower). All experimental procedures were the same for field plot and pot experiments, except the number of blocks, which were seven and six, respectively. The soils for the pot experiment were collected from the same site where the field plot experiments were conducted, mixed and sieved to remove roots and other debris and to increase homogeneity. Hence, it was assumed all belowground plant materials harvested at the end of the experiments were from the present growth only.

Above- and belowground biomass collections

Before commencing the treatments in March, all mature grass tufts were cut down to a recommended height of 10 cm [19,20] to bring all grasses to similar starting conditions for the experiment. This height was kept throughout the experiments for each clipping treatment. At the end of the experiments, the 10-cm aboveground part was clipped to ground level and added to the aboveground biomass. The clippings were oven dried at 60 °C for 48 hours before weighing [21]. At the end of the experiments, weights were summed per clipping to obtain the total weight of individual grass tufts.

All root systems were excavated at the end of the experiments to assess belowground biomass [22]. The 58 mature grass tufts were dug out from a 100-cm-wide and 100-cm-deep hole, and all 168 root systems of the young grasses from the field plot experiment were dug out from a 50-cm-wide and 50-cm-deep hole. From the pot experiment, 144 root systems were isolated. All root systems were collected, with soil, soaked in water, washed and strained through a 1-mm sieve to remove fine roots from soils, and finally oven dried at 60 °C for 48 hours [21].

The C content of above- and belowground biomass of the study grass species was calculated based on the comprehensive standard that the C content of most plant tissue is in the range of 45–50% [23]. Therefore, a coefficient of 0.475 was used on average for calculating C storage in above- and belowground biomass.

Statistical analysis

The above- and belowground C of mature tufts was analyzed by one-way analysis of variance (ANOVA) with completely randomized block design, while data from field plots and pots were analyzed for significant interactions by two-way ANOVA with completely randomized block design at a significance level of $\alpha =$

0.05. Fisher's least significant difference (LSD) test was used for significant main effects (clipping and irrigation) and Tukey's Honest Significant Difference (HSD) test was used for significant interactions as a post hoc test with General Linear Model (GLM) procedure in SAS [24]. Analyses were done separately for each grass species and for above- and belowground parts in each experiment. Data were transformed with non-normal residual distribution after Shapiro–Wilk's test using square root, log and inverse transformation.

Results

Responses of mature grass tufts to clipping

Weekly grazing, frequent and moderate clipping significantly improved the belowground carbon (bgC) of *Cenchrus ciliaris* ($F_{(2,12)} = 4.61$; $P = 0.0146$) compared to no clipping (Figure 1(a)). The difference between grazing and clipping was not significant ($P = 0.3461$), nor was the difference in aboveground carbon (agC) in *C. ciliaris* ($F_{(2,12)} = 1.12$; $P = 0.3572$). The agC of mature *C. gayana* was about 4 times lower in clipped compared to unclipped tufts ($F_{(2,18)} = 17.05$; $P < 0.0001$; Figure 1(b)). There was no significant difference among the treatments for *C. gayana* in bgC ($F_{(2,18)} = 0.33$; $P = 0.7254$; Figure 1(b)).

Responses of young grasses to clipping

In the field plot experiment, moderate and light clipping significantly improved the agC in young *C. ciliaris* ($F_{(3,72)} = 5.41$; $P = 0.0021$; Figure 2(a)) and *C. gayana*

($F_{(3,72)} = 14.03$; $P < 0.0001$; Figure 3(b)) grasses compared to frequent and no clipping. The clipping effects did not show significant differences for bgC of *C. ciliaris* ($F_{(3,72)} = 0.76$; $P = 0.5229$; Figure 2(a)). In the pot experiment, unlike the field plot experiment, *C. ciliaris* bgC was slightly but significantly lower in the control than in the clipping treatments ($F_{(3,61)} = 3.01$; $P = 0.0367$; Figure 2(b)). In the field plot experiment, bgC of *C. gayana* was significantly higher ($F_{(3,72)} = 3.42$; $P = 0.0218$; Figure 3(b)) under moderate and light clipping than under the two extreme treatments, frequent and no clipping. Up to 3 times higher agC was found in clipped compared to unclipped young *C. gayana* ($F_{(3,72)} = 14.03$; $P < 0.0001$; Figure 3(b)). The effect of clipping was not significant for the agC of *C. gayana* ($F_{(3,61)} = 0.17$; $P = 0.915$; data not shown) in the pot experiment.

Combined effects of clipping and irrigation on young grasses

There were significant interactions between clipping and irrigation for bgC only in *C. gayana* ($F_{(6,55)} = 2.68$; $P = 0.0235$; Figure 3(a)). At lower clipping frequencies the bgC was significantly higher under increased irrigation, but did not differ when irrigation was low (Figure 3(a)). No significant interactions were observed in both field plots and pot experiments for agC of *C. gayana* ($F_{(6,55)} = 0.82$; $P = 0.5573$ and $F_{(6,55)} = 2.18$, $P = 0.0584$, respectively) and *C. ciliaris* ($F_{(6,55)} = 1.18$, $P = 0.3261$ and $F_{(6,55)} = 0.50$; $P = 0.8037$, respectively) and bgC of *C. ciliaris* ($F_{(6,66)} = 0.77$, $P = 0.5994$ and $F_{(6,55)} = 1.19$; $P = 0.3234$).

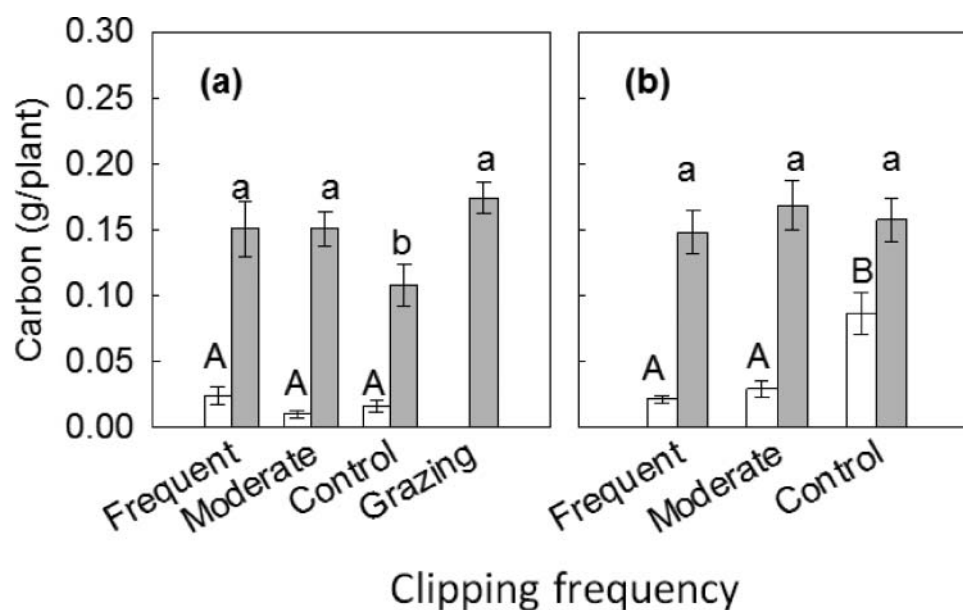


Figure 1. (a) Mean (\pm standard error) of aboveground (white bars) and belowground (gray bars) carbon of *Cenchrus ciliaris* across clipping frequencies (frequent, moderate, light, grazing, and control). (b) Mean (\pm standard error) of aboveground and belowground carbon of *Chloris gayana* tufts across clipping frequencies (frequent, moderate and control). Different letters denote significant differences across clipping frequencies by Fisher's least significant difference test.

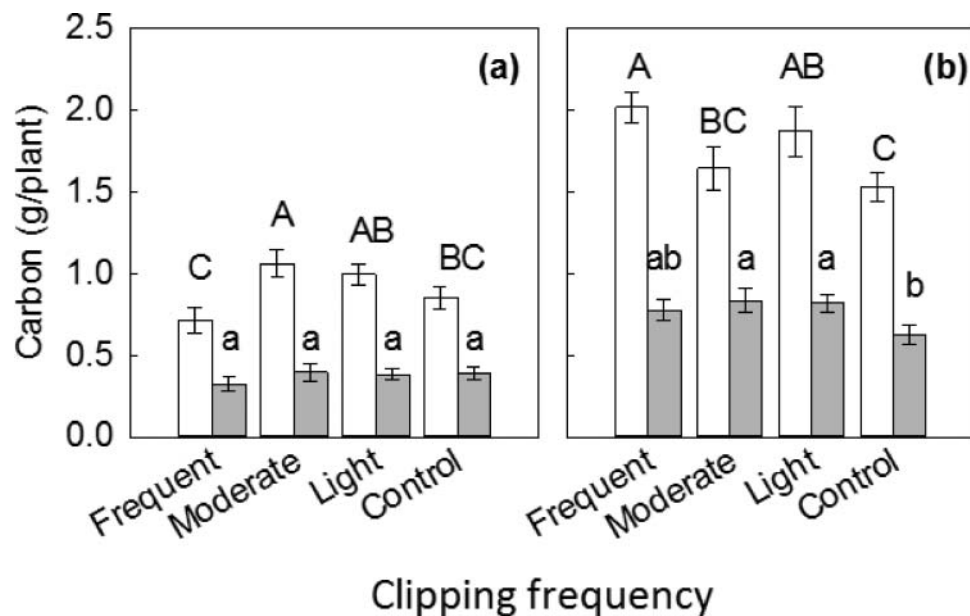


Figure 2. (a) Mean (\pm standard error) aboveground (white bars) and belowground (gray bars) carbon of *Cenchrus ciliaris* across clipping frequencies (frequent, moderate, light and control) in the field plots experiment. (b) Mean (\pm standard error) aboveground and belowground carbon of *Chloris ciliaris* across clipping frequencies (frequent, moderate, light, and control) in the pot experiment. Different letters denote significant differences across clipping frequencies by Fisher's least significant difference test.

In the pot experiments, decreased irrigation significantly reduced agC and bgC in *C. ciliaris* ($F_{(2,61)} = 8.49$, $P = 0.0006$ and $F_{(2,61)} = 9.55$, $P = 0.0002$, respectively; Figure 4(a)) as well as agC in *C. gayana* ($F_{(2,61)} = 15.03$, $P < 0.0001$; Figure 4(b)).

In contrast, in the field plot experiment, there were no significant differences between irrigation treatments in agC and bgC for both grass species (data not shown).

Discussion

Effects of clipping on carbon storage in mature grass tufts

The significantly higher agC in unclipped versus clipped *C. gayana* (Figure 1(b)) is similar to results found by Asgharnejad *et al.* [25] and indicates that grazing might play a significant role in reducing carbon (C) storage of mature grasses. The observed low agC

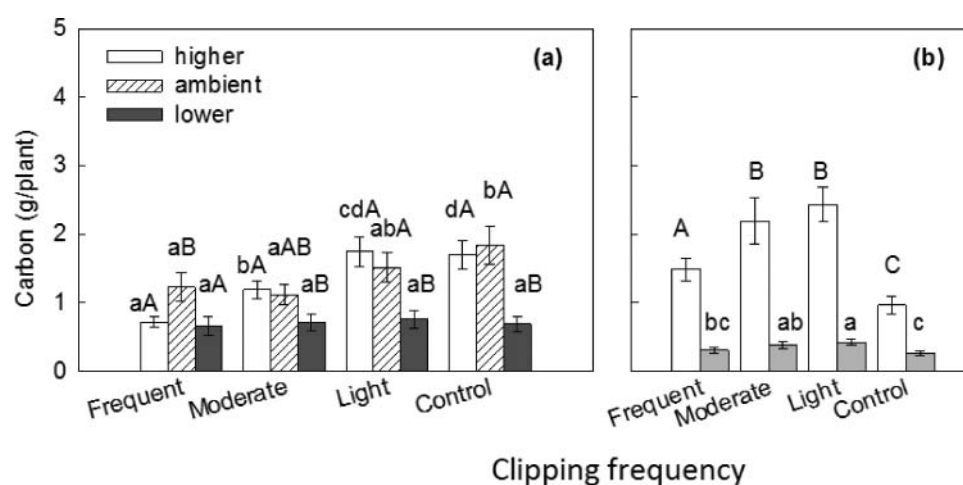


Figure 3. (a) Interactive effect of irrigation (higher, ambient and lower) and clipping (frequent, moderate, light and control) on belowground carbon storage of *Chloris gayana* in the pot experiment. Bar graphs with whiskers represent mean (\pm standard error) of belowground carbon across clipping frequencies and irrigation amounts in the pot experiment. Means in the same irrigation amount followed by the same lowercase letters, and means in the same clipping frequency followed by the same uppercase letters, are not significantly different by Tukey's HSD test; (b) Mean (\pm standard error) aboveground (white bars) and belowground (gray bars) carbon of *Chloris gayana* across clipping frequencies (frequent, moderate, light and control) in the field plot experiment.

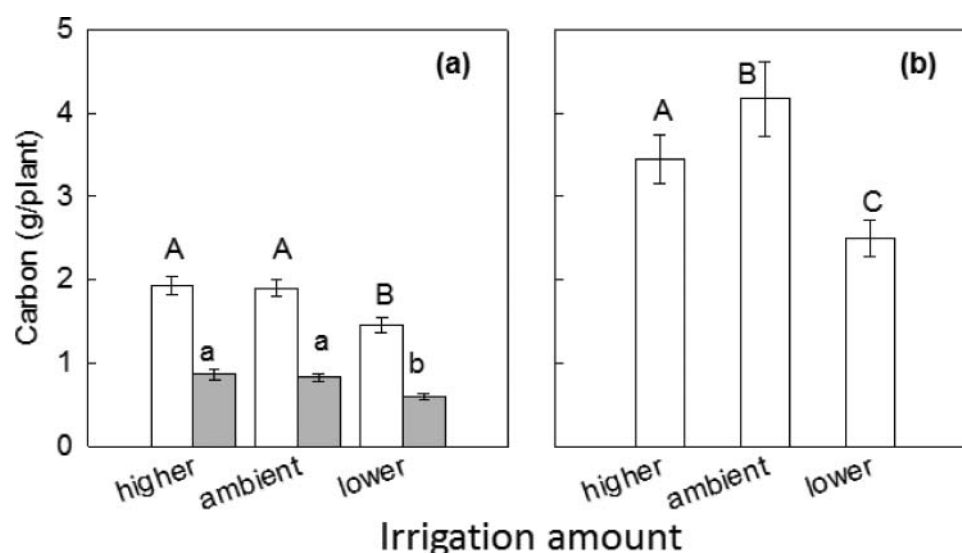


Figure 4. (a) Mean (\pm standard error) aboveground (white bars) and belowground (gray bars) carbon of *Cenchrus ciliaris* across irrigation (higher, ambient, lower), in the pot experiment. (b) Mean (\pm standard error) aboveground carbon of *Chloris gayana* as affected by irrigation (higher, ambient, lower) in the pot experiment; the belowground part has been omitted because it is presented in Figure 3(a). Different letters denote significant difference across treatments by Fisher's least significant difference test.

was caused by the removal of photosynthetic tissues, which was also reported by others [111]. On the other hand, the enhanced bgC under clipping and weekly grazing in *C. ciliaris* (Figure 1(a)) might be attributed to reallocation of resources away from the site of herbivory damage, as a tolerance strategy [26,27,112]. This was in accordance with grass responses in other field and greenhouse experiments [28] as well as under natural cattle grazing [113]. There is a general tendency of reduced agC in tropical grasses and higher agC in temperate grasses under grazing [6]. It was found that weekly grazing and clipping had the same effect, suggesting that clipping can be used as a proxy for grazing and its impact on grass C [29]. Other experiments that involved clipping and grazing observed different responses of grasses to the two treatments, mainly due to expected additional effects via nutrient cycling from urine and feces [30]. In contrast, the current experiment was based on short-term experimental responses and did not take into account long-term nutrient cycling processes. Further, the cattle were guided to only graze on experimental tufts and to move on, without the opportunity to recycle the nutrients from their urine or feces.

Effects of clipping on carbon storage in young grasses

A higher agC in lightly and moderately clipped freshly grown *C. gayana* and *C. ciliaris* compared to non-clipped grasses showed that light and moderate clipping can enhance C storage of grasses, in a young pasture. This finding concurs with results from semi-arid rangelands and a greenhouse experiment where significantly higher agC was found in defoliated

compared to non-defoliated *Festuca rubra* grasses [28]. The study further demonstrated that freshly reseeded grasses enhance their C more strongly under defoliation compared to already established grasses. This might be attributed to young foliage usually exhibiting greater photosynthetic capability than older tissues [31]. Further, regular grazing offtake is required for grasses, which co-evolved with large herbivores, to achieve a high individual-level C storage. Therefore, the findings contribute great knowledge to the current management of young grasses after reseeding [5,32] and highlight how to enhance their C storage [114].

Responses of carbon storage to changes in irrigation amount

Lower irrigation values reduced agC and bgC of both grass species in the pot experiment, highlighting that rainfall plays an overriding role in determining C storage of grasses in semi-arid environments. Irrigation treatment was not significant for both grass species in the field plot experiments, which might be attributed to an unavoidable horizontal and vertical seepage of water out of the field plots, diluting the treatment effects. Lower rainfall, as predicted in drought scenarios for eastern Africa under climate change [33], led to lower agC and bgC in the study grasses. This result implies that under drought conditions grazing is less important for C storage of grasses, as was also found by Habtemicael *et al.* [34] and Martin *et al.* [35]. Drought has been known to strongly constrain livestock production in the Borana rangelands [36]. Reducing cattle numbers before drought strikes as suggested by Tuffa and Treydte [51] would help grasses to easily recover in the following rainy season

and would further prevent losing animals due to starvation.

The significant interactions between clipping and irrigation for bgC in *C. gayana* suggest that lower grazing pressure can enhance bgC under average or above-average rainfall, but not under low rainfall conditions, similar to findings of other researchers [37]. Drought-induced morphological, physiological and biochemical changes in plants have been shown due to reduced CO₂ assimilation rates under low stomatal conductance [115]. Further, the high bgC in unclipped *C. gayana* at ambient irrigation indicates that under controlled grazing a high rainfall will greatly enhance the C storage of grasses. This might be an opportunity for some east African regions where higher rainfall is predicted under future climate change scenarios [38].

It is concluded that future grazing management should consider resting of grazing areas to enhance bgC storage of *C. gayana* reseeded areas. Belowground C plays a more relevant role than agC in mitigating climate change, since more than 90% of organic C in rangelands is comprised by roots [113]. Generally, organic carbon found in the soil mainly consists of plant debris [116] and plant roots [113]. Hence, a change of degraded lands to perennial rangeland through restoration activities, such as reseeding and continuous management afterward, can greatly contribute to C storage [108] as rangelands are important C sinks [117]. The management strategies for rangelands should focus on reseeding degraded areas with perennial grasses [39] which are suitable for dry environments [40,41]. Recent studies addressed the carbon sequestration potential of Borana rangelands [42,118]. Knowledge of the major drivers of individual-level grass response, as gained through this study, is important for understanding community-level response and up-scaling these processes on a landscape level [43,44,119]. The two study grass species have global distribution and, hence, their management will have widespread implications [45,120,121]. Generally, degraded areas bear a large potential for becoming a carbon sink as they are globally common [46], and their monitoring and sustainable management are essential.

Conclusion

We observed contrasting results in C storage between mature and young grasses, likely because young foliage exhibits greater photosynthesis and, thus, accumulates more C than older tissues. Hence, rangeland management should incorporate the age of the most dominant perennial grasses for better C storage in restored rangelands. Further, belowground C allocation differed across species; clipping/grazing enhanced belowground C in mature *C. ciliaris* while in mature *C. gayana* there was no observed difference. This

difference in response to clipping between mature *C. ciliaris* and *C. gayana* might be attributable to their difference in growth habit (rhizomatous and stoloniferous, respectively). Generally, light to moderate grazing can stimulate both above- and belowground C in young grasses, irrespective of the reseeding species. Therefore, our findings have important implications for the management of rangelands, particularly in areas where the two grass species tested are recommended for restoration of degraded areas. Further, our study showed that the high C storage of grasses was overridden by frequent grazing when rainfall was high. Hence, we recommend a resting period for years with increased rainfall. In contrast, reduced rainfall can also strongly reduce C storage in grasses, highlighting that under climate change an even more conservative management regime of low cattle grazing should be preferred. Therefore, long-term practiced enclosures and reseeded areas should be lightly and moderately clipped or grazed to enhance the overall C storage. Such grazing management will improve rangelands as CO₂ sinks and mitigate climate change. We conclude that appropriate grazing management is needed to foster the capacity of grasses to store more C after reseeding degraded rangelands.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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