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Population Dynamics in Mixed Canopies Composed of Kikuyu-Grass and Tall Fescue

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Abstract: The current work was based on the central hypothesis that grazing management strategies modulate tillering dynamics of mixed canopies composed of kikuyu-grass (C_4 perennial grass) and tall fescue (C_3 perennial grass). Among the five grazing management conditions evaluated, three (7, 12, and 17 cm) represented the heights up to which the grasses were kept by mimicking a continuous stocking method throughout the experimental period (from May 2016 to October 2017), and the other two consisted of the heights 12 and 17 cm with a single grazing to 7 cm in mid-autumn. Nitrogen fertilization was applied only during winter–spring. The results showed that under severe grazing (7 cm), kikuyu-grass predominated in the area and that tall fescue population predominated in the moderately lenient (12 cm) and lenient (17 cm) grazed pastures, regardless of an occasional autumn grazing to 7 cm. After two years of evaluation, kikuyu-grass tillers were reduced to 6% of the total population in those pastures managed at the heights of 12 and 17 cm. Grazing management strategies modulate species proportions in a mixed canopy composed of kikuyu and tall fescue. Moreover, our data also suggest that it seems unlikely that there is a stable association of tall fescue and kikuyu-grass when no nitrogen fertilization is applied during summer.

Keywords: canopy height; functionality; stress-tolerant; tiller demography

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1. Introduction

Areas of terrestrial globe with Cfa and/or Cfb climates (characterized by cool winters, well distributed rainfall and warm or mild summer, respectively) present favourable conditions for pasture-based animal production systems practically during all the year. The absence of a defined dry season and the annual thermal amplitude allow the use of warm-season forage species during spring and summer, and cool-season species during autumn and winter. Accordingly, a possible model for livestock production in pasture in those conditions would be the one comprised of warm and cool-season perennial grasses grown in a mixed canopy, which can thereby extend the period of pasture use, providing greater resistance and resilience to environmental stresses, and greater sustainability to the production system [1].

One of the greatest challenges to the success of binary systems constituting of mixed C_4 and C_3 perennial grasses is the choice of the cool-season species, since the chosen species has to tolerate a high degree of thermal stress and competition, especially during the summer period. In this sense, *Lolium arundinaceum* (Schreb) Darbysh (tall fescue) can be considered one of the few perennial grasses able to tolerate such stressful environments [2]. The literature has already produced information regarding the management of herbage and animal production in systems formed by mixed pastures of *Cynodon dactylon* (L) Pers. (warm-season perennial grass) and tall fescue in Cfa climate [3,4]. In this type of scenario, where the mean air temperature in the hottest month exceeds 23 °C, the persistence of tall fescue is basically ensured by its symbiotic association with wild endophytic fungi, or via inoculation with a fungal species, such as *Epichloë coenophiala*, since the fungus provides greater tolerance to biotic [5] and abiotic stresses [6,7].

The negative side of the symbiotic association between plant and fungus is the production of some metabolites by the endophyte, such as the ergot alkaloid (ergovaline) which can lead to reduction in herbage intake and animal performance [8]. Therefore, one of the alternatives to overcome this issue would be the use of tall fescue cultivars free of endophytes but, in this scenario, tall fescue can become more vulnerable to stressful conditions, and, thus, it is necessary to find out how grazing management strategies could modify interspecific competition to verify its persistence and longevity in mixed canopies. The results observed in unharvested [5] conditions suggest that lenient grazing could increase tall fescue persistence over time in binary mixtures.

Studying tillering dynamics in pure stands of tall fescue in a Cfb climate [9] found that tiller cohorts that appeared during autumn/winter were crucial to ensure tall fescue persistence until the same period of the next year. On the other hand, *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu-grass; a warm-season perennial grass) does not present a specific cohort that plays a fundamental role to guarantee its persistence [10], illustrating the relevance of a continuous replacement of tillers to extend its longevity over time. Kikuyu-grass plants are severely injured by frosts during autumn/winter but, normally experience regrowth in the spring [11], and therefore, this species seems to be a viable candidate to compose a stable mixture with tall fescue. However, it could be interesting to adopt strategies that reduce interspecific competition from mid-autumn to late winter and possibly stimulate and/or intensify the growth of tall fescue in this period. Thus, the removal of aerial biomass by hard grazing would allow greater light penetration at the base of the canopy, and possibly stimulate tillering [12–14] of tall fescue plants.

Given the above, this work was based on the hypothesis that grazing management strategies modulate the tillering dynamics of mixed canopies composed of kikuyu-grass (C_4 perennial grass) and tall fescue (C_3 perennial grass), endophyte-free. The objective of the current study was to evaluate changes in tillering dynamics as affected by grazing management strategies in mixed pastures composed of kikuyu-grass and tall fescue.

2. Materials and Methods

The present study was carried out in an experimental area of 2100 m^2 in Lages, SC, Brazil (27°48′ S, 50°19′ W and 900 m a.s.l.). According to the Köppen–Geiger classification, the climate of the region is Cfb type (temperate) with mild summer, cool winter, and a well-distributed rainfall throughout the year [15]. The climatic conditions during the evaluation period are presented in Table 1.

Table 1. Monthly average climatic variables during the study period (2015–2016 and 2016–2017), relative to the mean long-term values of rainfall and temperature in Lages, Santa Catarina, Brazil.

Climatic Variables	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
2015–2016												
Rainfall (mm)	208.1	107.4	236.4	186.7	136.8	120.6	24.9	131.5	139.0	107.9	224.8	73.8
Sun hours (h)	79.2	179.9	152.1	180.2	131.1	95.3	158.8	189.0	153.4	188.4	129.7	203.0
Average temperature (°C)	20.2	20.8	21.2	18.2	18.6	12.2	9.1	11.2	12.5	13.4	15.3	17.4
Frost (number of days)	0	0	0	0	2	4	5	2	0	0	0	0
2016–2017												
Rainfall (mm)	115.1	107.4	90.1	158.6	355.6	247.2	14.2	117.4	51.2	130.0	115.1	-
Sun hours (h)	188.8	175.5	169.8	128.5	108.3	147.2	239.1	171.5	178.7	168.6	188.8	-
Average temperature (°C)	21.1	21.6	19.0	15.6	14.7	12.4	12.2	13.1	17.2	15.9	21.1	-
Frost (number of days)	0	0	0	1	0	3	4	0	0	0	0	-
Long-term ↓												
Rainfall (mm)	145.7	171.1	156.6	122.3	107.3	112	114.6	132.2	123.9	163.7	180.8	138.2
Average temperature (°C)	19.3	20.4	20.3	19.1	16.3	13.2	11.4	11	12.3	13.6	15.7	17.4

↓ Long-term average values (1948–2015). Source: National Institute of Meteorology (INMET; http://www.inmet.gov.br/projetos/rede/pesquisa/).

The soil in the experimental area was classified as a typical inceptisol soil, and the 0–20 cm layer had the following physicochemical characteristics: mean soil pH (0.01 mol/L $CaCl_2$) = 5.7; organic matter = 4.2%; 35.8 mg P/dm^3 (Mehlich-I extraction method); 142 mg K/dm^3; 22 mmol$_c$ Ca/dm^3; 15 mmol$_c$ Mg/dm^3; 62 mmol$_c$ H + Al/dm^3; cation exchange capacity = 205 mmol$_c$/dm^3; and base saturation = 64%. The proportion of clay was 530 g/kg.

Tall fescue 'Rizomat' was no-till planted in July 2015 in a kikuyu-grass pasture established in the 1990s. Prior to tall-fescue seeding, kikuyu-grass was mowed to 10 cm height, and the tall fescue was seeded with 50 kg/ha at approximately 2 cm depth. The pastures were fertilized with 90 kg N/ha and 167 kg P_2O_5/ha 40 days after sowing. During the experimental period, pastures received 250 kg N/ha/year in four applications; two applications each of 70 kg/ha (one in May and another in September), and two each of 55 kg/ha (one in July and another in August) in both evaluation years. The option to apply all nitrogen in only four N splits in winter/spring was to give a strong stimulus to tall fescue tillering, which is almost completely concentrated during late autumn and winter [9]. On the other hand, no nitrogen was applied from late spring to autumn, and this was done to try to minimize a possible advantage to kikuyu-grass over tall fescue plants during the summer, a season where the competition between the plants, normally, would be greater.

The treatments comprised five management conditions allocated in experimental units in a randomized complete block design with three replicates. Three of them corresponded to conditions where pastures were kept at constant height (7 cm; 12 cm; and 17 cm treatments) throughout the entire experimental period. The other two treatments represented the management heights of the pastures combined to a single autumn grazing to 7 cm (12/7 cm and 17/7 cm treatments). After autumn grazing, the canopies were allowed to return to their original heights, which were maintained until the autumn of the second evaluation year, when the same procedure was repeated. The idea here was to remove large quantities of photosynthetically inactive kikuyu-grass tissues in autumn, such as leaves injured by frost, and allow more light to enter the base of the canopy, stimulating the production of new tall fescue tillers in winter/spring.

The data collection period extended from May 2016 (when the 12/7 and 17/7 cm treatments were grazed to a height of 7 cm) to October 2017. The pastures were managed in their respective treatment conditions since October 2015, defining a pre-experimental adaptation period of eight months. The canopy height in each treatment was measured from 30 readings using a ruler at random points within each experimental unit (140 m^2). The canopy height of the pastures was measured weekly throughout the experimental period, and height was maintained by dairy cattle grazing (±450 kg body weight) using the mob-stocking technique [16]. The animals were introduced to the experimental units before the canopy heights were 15% above their target height and removed before the grasses were 15% below their target height. The mean heights of each treatment were grouped into monthly values (Figure 1). The frequency with which the animals were used to maintain the canopy heights varied between 4 and 7 days, depending on the period of the year.

All evaluations were performed monthly throughout the experimental period as follows: (1) tiller population dynamics; evaluated in two 20 cm-diameter polyvinyl chloride (PVC) rings per paddock every 30 days throughout the experimental period. In the first evaluation, carried out on December 10, 2015, all live tillers of kikuyu-grass and tall fescue were marked with plastic rings of different colours. In the following evaluations, the new tillers that emerged during the time elapsed between the current and previous evaluations of both species were marked with plastic rings of different colours. The number of surviving tillers of each cohort was also counted. Thus, a total of 24 evaluations were carried out during the experimental period; (2) absolute number of emerged tillers of kikuyu-grass and tall fescue was obtained by monthly counting of new tillers that emerged inside of PVC rings and the number extrapolated by tiller/m^2; and (3) tiller population density (TPD); obtained from samples collected in two frames of 0.24 m^2, allocated in each experimental unit. The entire forage mass contained inside each frame was cut close to the ground and packed in plastic bags. In the lab, samples were manually separated, and the number of tillers of kikuyu-grass and tall fescue in the samples was counted. From the TPDs of kikuyu-grass and tall fescue, the total TPD of the pastures was calculated. It should be mentioned that through evaluations performed on plant tissues (leaf and stem) according to the methodological procedure described by [17], it was found that tall fescue cultivar 'Rizomat' used in the experiment was free of endophytic fungi.

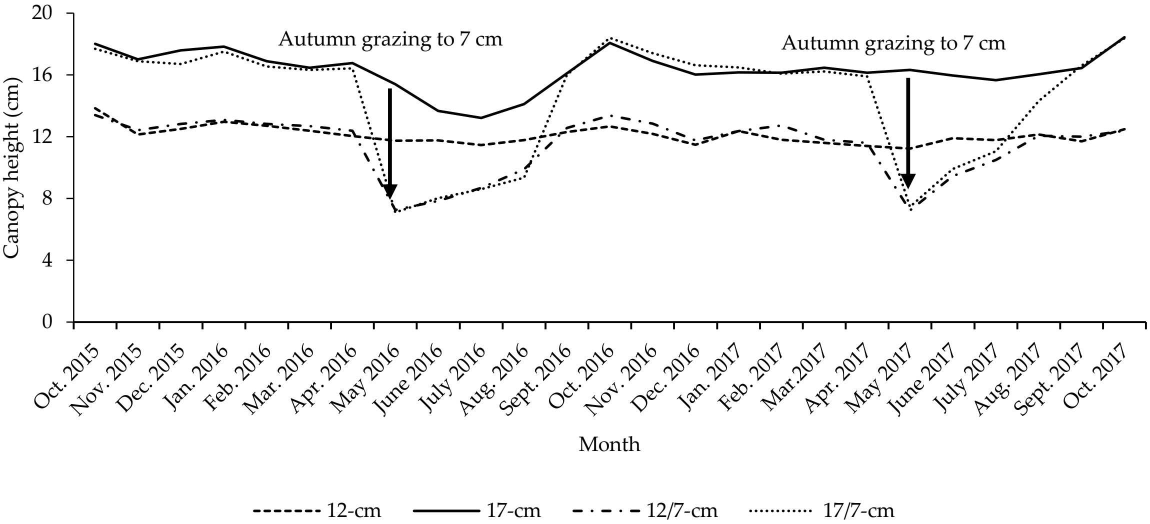

Figure 1. Canopy height of pastures composed of kikuyu-grass and tall fescue during the years 2015, 2016, and 2017. Treatments 12 cm and 17 cm represent canopy heights maintained uninterrupted throughout the experiment; treatments 12/7 cm and 17/7 cm represent the canopy heights with autumn grazing to 7 cm height in May.

For statistical analysis, the data were grouped into four seasons: summer (represented by January, February, and March), autumn (April and May), winter (June, July, August, and September), and spring (October, November, and December). It should be noted that the year of 2016 was composed of two seasons, once the experimental period began in the May of that same year. Subsequently, the data were submitted to analysis of variance using the MIXED procedure (mixed models) of the statistical package SAS® (Statistical Analysis System; SAS Institute Inc., Cary, NC, USA), version 9.2. The Akaike Information Criterion (AIC) [18] was used to select the covariance matrix that best fitted the data sets. The effects of canopy height, the presence or not of a single autumn grazing to 7 cm, and season were considered as fixed effects. Data collected in each month were considered as repeated measurements over time. The means of each variable were estimated by the LSMEANS and were compared by Tukey's test, using a 5% significance level.

3. Results

The absolute number of emerged tillers of kikuyu-grass was affected by treatment (p = 0.002 in 2016 and $p < 0.0001$ in 2017) and season of the year (p = 0.0022 in 2016 and $p < 0.0001$ in 2017). In 2016, pastures kept at a height of 7 cm presented, on average, 70% more tillers than the other treatments. Moreover, the values recorded in winter were 2.2 times lower than those observed in spring. In 2017, the highest values were recorded in the pastures kept at 7 cm, and the number of tillers recorded in autumn was, on average, 47% and 69% higher than those observed in the winter and spring, respectively (Table 2). In both evaluation years, the tall fescue absolute number of emerged tillers varied according to the season of year ($p < 0.0001$), and the highest values were recorded during winter (Table 3).

Table 2. Absolute number of emerged tillers of kikuyu-grass (tillers/m^2) in mixed canopies of kikuyu-grass and tall fescue submitted to grazing, in different seasons.

Season	**2016**						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Winter	1041	226	213	325	511	471 B	113
Spring	2373	1191	244	741	660	1042 A	
Mean	1708 a	730 b	228 c	532 b	585 b		
SEM *	179						
Season	**2017**						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Summer	1681	1020	293	690	553	848 AB	92
Autumn	2061	1058	424	1034	485	1012 A	
Winter	1074	558	127	493	435	538 BC	
Spring	788	546	48	122	87	318 C	
Mean	1401 a	796 b	223 c	585 bc	390 bc		
SEM *	103						

* Standard error of the mean. Values followed by the same capital letter in the column and lowercase letters in the row do not differ ($P < 0.05$).

Table 3. Absolute number of emerged tillers of tall fescue (tillers/m^2) in mixed canopies of kikuyu-grass and tall fescue submitted to grazing, in different seasons.

Season	**2016**						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Winter	1315	1798	1796	1870	1943	1744 A	83
Spring	489	315	209	240	180	287 B	
Mean	902 a	1057 a	1002 a	1055 a	1061 a		
SEM *	132						
Season	**2017**						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Summer	28	49	65	64	69	55 C	44
Autumn	79	32	148	69	122	90 BC	
Winter	1449	1266	1744	1472	1444	1475 A	
Spring	323	261	111	225	286	242 B	
Mean	470 a	402 a	517 a	458 a	480 a		
SEM *	49						

* Standard error of the mean. Values followed by the same capital letter in the column and lowercase letter in the row do not differ ($p < 0.05$).

Total tiller population density (TPD) was affected by an interaction between the treatment and season of the year ($p < 0.0001$) in both evaluation years. In the spring of 2016, the highest value was recorded in the pastures kept at 7 cm high. In 2017, the highest TPDs were observed during the summer and autumn seasons in the pastures kept lower (7 cm). However, in the winter and spring seasons, the TPDs were practically similar in all management conditions, with the exception of the canopies managed with 17 cm, which during the winter presented 34% less tillers, compared to the pastures kept with 7 cm (Table 4).

Table 4. Tiller population density (tillers/m^2) in mixed canopies of kikuyu-grass and tall fescue submitted to grazing in different season.

Season	2016						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Winter	2713 Ba	3152 Aa	2624 Aa	3670 Aa	2764 Aa	2984	109
Spring	5233 Aa	3157 Abc	2612 Ac	3925 Ab	3388 Abc	3663	
Mean	3973	3154	2618	3797	3076		
SEM *	174						
Season	**2017**						
	Canopy Height (cm)						
	7	**12**	**17**	**12/7**	**17/7**	**Mean**	**SEM ***
	Tillers/m^2						
Summer	8474 Aa	2972 Abc	2271 Ac	3586 ABb	2583 Abc	2924	101
Autumn	5746 Ba	3299 Abc	2129 Ac	3948 ABb	2341 Ac	3492	
Winter	3744 Ca	2911 Aab	2463 Ab	2927 Bab	2577 Aab	2924	
Spring	4055 Ca	3728 Aa	3298 Aa	4346 Aa	3416 Aa	3769	
Mean	5505	3227	2540	3701	2729		
SEM *	112						

* Standard error of the mean. Values followed by the same capital letter in the column and lowercase letters in the row do not differ ($p < 0.05$).

In order to have a broader view of the tiller population dynamics of kikuyu-grass and tall fescue, we generated figures with demographic patterns to illustrate the monthly variation of the cohorts of each species within each treatment during the experimental period (Figure 2a–d; Figure 3a,b; Figure 4a–d). Subsequently, the populations of kikuyu-grass and tall fescue were combined to generate new graphs that illustrated the total tiller population of the pastures as being the sum of the populations of the two grass species (Figure 2e,f; Figure 3c; Figure 4e,f). In general, kikuyu-grass decreased from the beginning to the end of the experimental period. In addition, the population reduction of kikuyu-grass was intensified as the height of pasture management increased, making the tiller populations reach values close to zero in treatments 17 and 17/7 cm at the end of experimental period.

On the other hand, tall fescue presented an inverse pattern of response to those observed for kikuyu-grass plants, with their population increasing from the beginning to the end of the experimental period, regardless of the treatment condition. In addition, the tall fescue population overlapped the kikuyu-grass population as the canopy height increased. It was also observed that the pastures managed at the heights of 12 and 12/7 cm, as well as those managed at the heights of 17 and 17/7 cm, presented similar results.

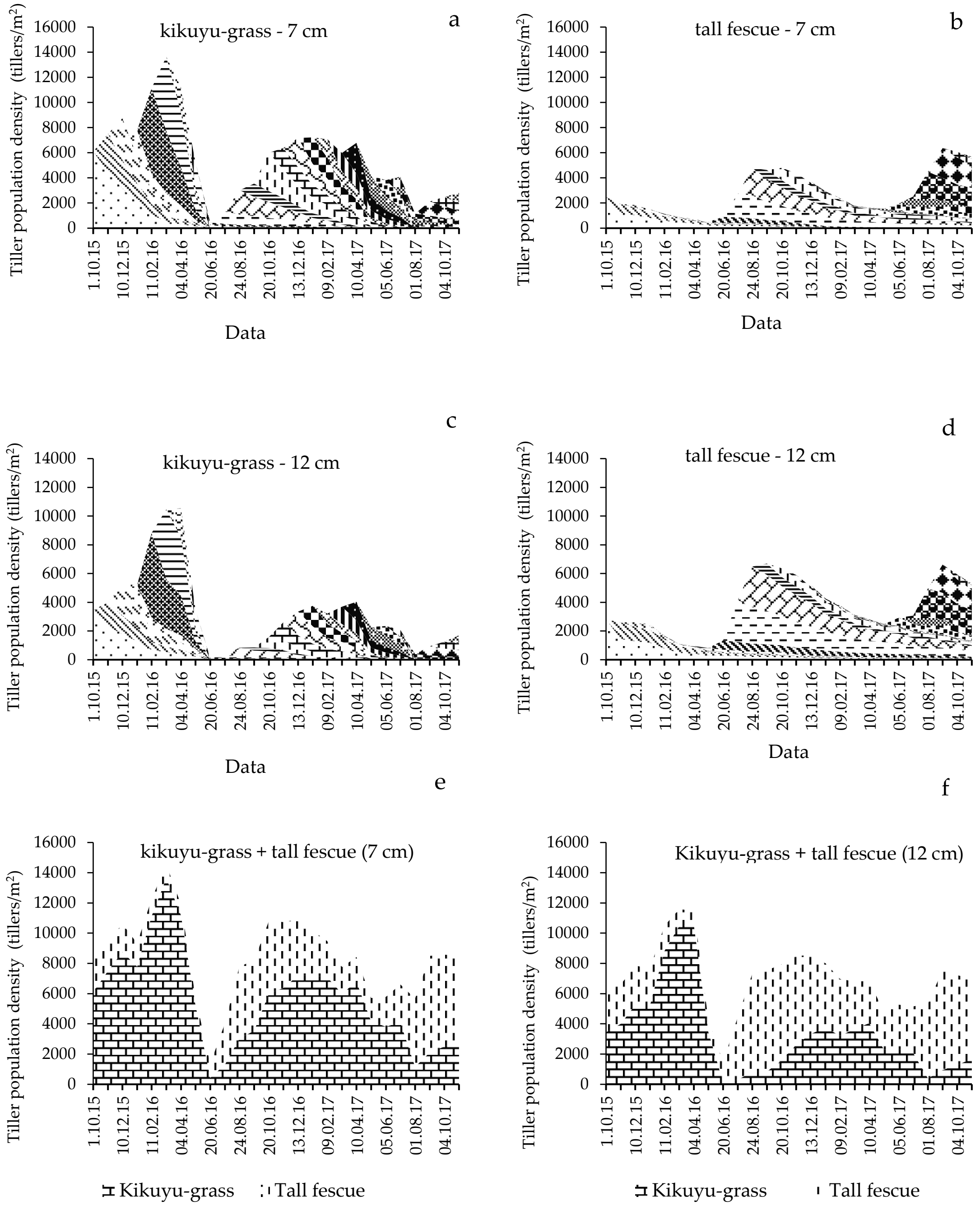

Figure 2. Cohort survival diagrams for tillers of kikuyu-grass (**a**,**c**) and tall fescue (**b**,**d**) cultivated in mixed canopies maintained at 7 and 12 cm and their overlapping population demography (**e**,**f**).

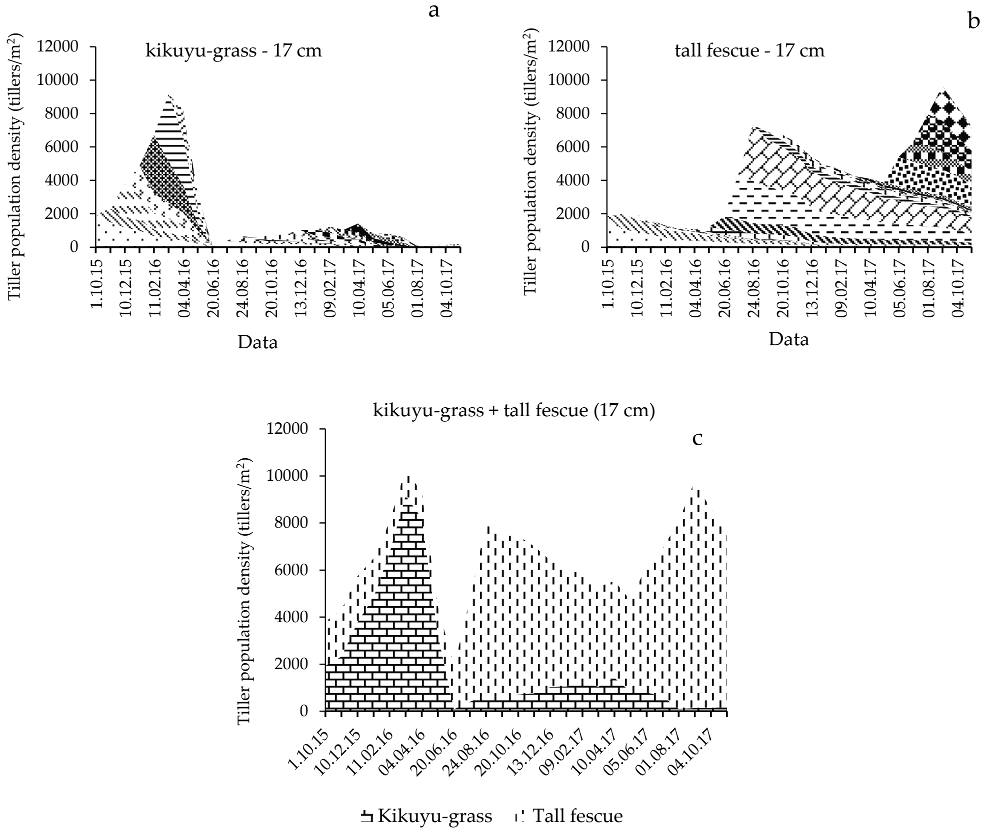

Figure 3. Cohort survival diagrams for tillers of kikuyu-grass (**a**) and tall fescue (**b**) cultivated in mixed canopies maintained at 17 cm and their overlapping population demography (**c**).

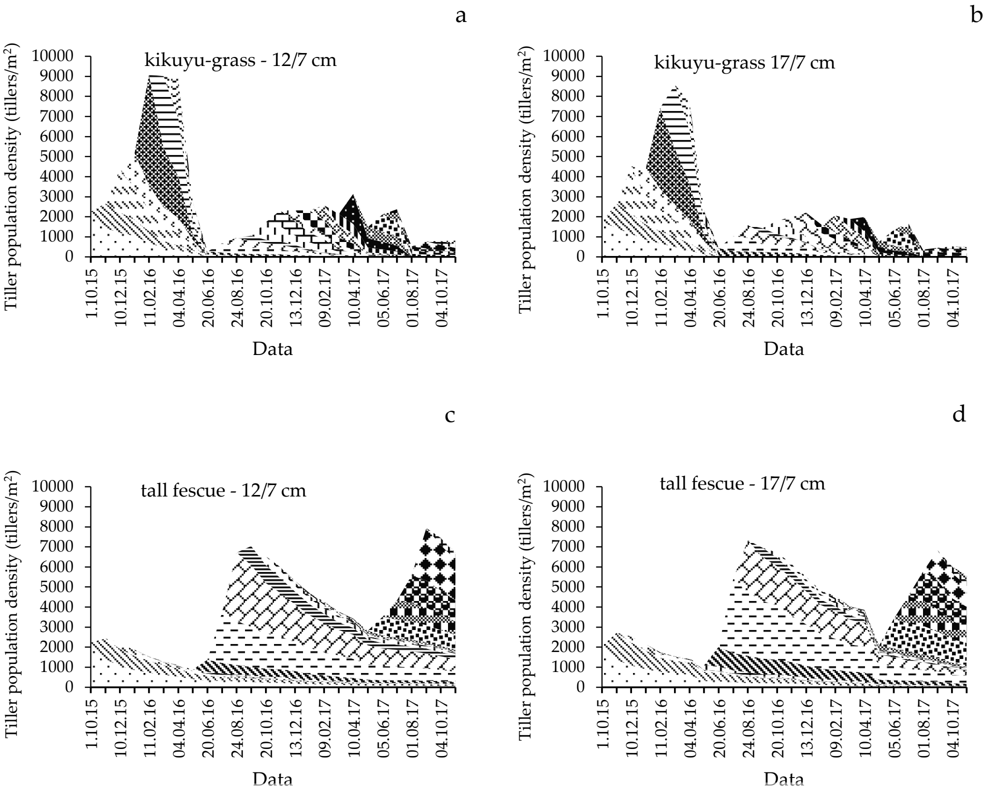

Figure 4. *Cont.*

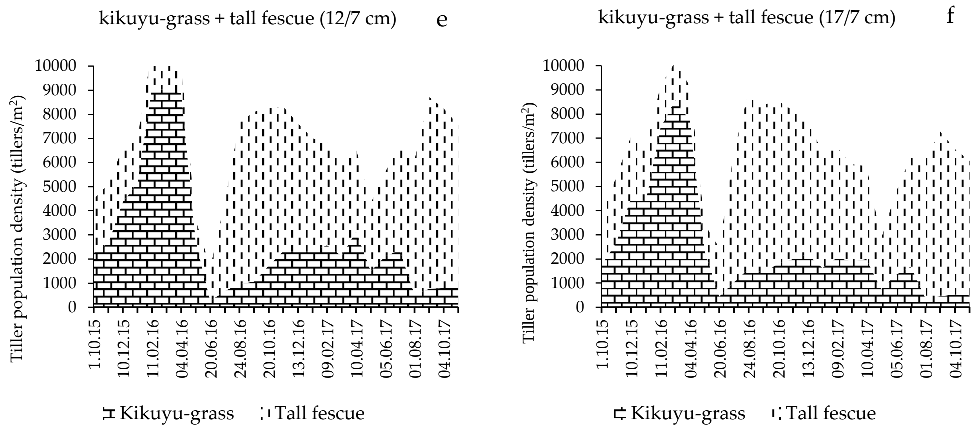

Figure 4. Cohort survival diagrams for tillers of kikuyu-grass (**a**,**b**) and tall fescue (**c**,**d**) cultivated in mixed canopies maintained at 12 and 17 cm and submitted to a single grazing to 7 cm in mid-autumn and their overlapping population demography (**e**,**f**).

4. Discussion

The canopy heights were kept close to the intended targets during the entire experimental period. The hypothesis that a single autumn grazing to 7 cm would change tillering dynamics was rejected, once the total (and tall fescue) tiller populations were similar between canopies subjected or not to an autumn grazing to 7 cm (Table 4). The isolated effect of grazing management based on canopies' heights generated unique effects on the tillering dynamics for each species. The number of tillers of tall fescue appearing in each cohort, and its longevity, were practically insensitive to such management variations, except in those maintained at 7 cm. The tall fescue can be classified as a stress-tolerant plant, characterized by a low persistence in environments with a high degree of disturbance [19], which can help to explain the low number of tall fescue tillers appearing in canopies managed at the height of 7 cm.

On the other hand, kikuyu-grass tillering decreased as the canopy height increased. Kikuyu-grass persistence was dependent on the frequent recruitment of tillers, since it was characterized by short-lived tillers (3–4 months, on average), even under more lenient management conditions. This fact, associated with the occurrence of frost during the autumn of 2016 and greater shade due to an increase in the tall fescue population in the more lenient management conditions during winter, likely prevented the restoration of kikuyu-grass during the summer of 2017. Moreover, probably, the amount of carbohydrates stored in the roots and stems [20] of kikuyu-grass was not sufficient to provide an adequate regrowth in summer to withstand the highly stressful/disturbing cool season (autumn and winter), which led to an almost null population of this plant at the end of the experimental period. In addition, the absence of nitrogen fertilization in the summer might have directly affected the loss of persistence of kikuyu-grass growing under more lenient grazing managements (17 and 17/7 cm), considering that plants functionally defined as exploitative, such as kikuyu-grass, tend to deplete water and carbohydrate reserves when continuously submitted to low-fertility environments [21].

It should be highlighted that, regardless of grazing management strategies adopted, tall fescue presented a pattern of tillering concentrated almost exclusively during the winter. Tiller cohorts of tall fescue appearing in July and August (winter in the Southern Hemisphere) were essential to increase its population, ensuring tall fescue persistence until the winter of the second year, and to maintain the population during the hottest period of the year (summer). This pattern of response of tall fescue tillering had already been observed by other authors [9,22], and in our study, even the competition with kikuyu-grass, or the greater light penetration into the canopy caused by autumn grazing, was not enough to change it. This result suggests that the control of tall fescue tillering could be a result of a more complex interaction between temperature, photoperiod [23], and hormonal effects [24].

On the other hand, as expected, kikuyu-grass tiller population renewal occurred during the spring, starting mostly in October, and did not present any remarkable tiller cohort like tall fescue. This pattern of response is relatively common in tropical grasses and can be observed in several others plants, like those belonging to genus *Cynodon* [25], *Urochloa* [26], and *Digitaria* [27], except in some more conservative C_4 plants like *Andropogon* species [28].

5. Conclusion and Implications

As shown throughout the manuscript, grazing management strategies can modulate species proportions in mixed pastures composed of kikuyu-grass and tall fescue. Under severe grazing (7 cm), kikuyu-grass predominated in the area, and tall fescue population predominated in the moderately lenient (12 cm) and lenient (17 cm) grazed pastures, regardless of an occasional autumn grazing to 7 cm. After two years of evaluation, kikuyu-grass tillers were reduced to 6% of the total population in those pastures managed at the heights of 12 and 17 cm. A straight consequence of our findings is that it seems unlikely that there is a stable mixture of kikuyu-grass and tall fescue when the pastures are subjected to hard grazing, or when no nitrogen fertilization is applied during summer. In this sense, to ensure the mid- and long-term persistence of these grasses growing together, it would be interesting to adopt management strategies, such as summer N application, that could increase the kikuyu-grass population during its predominant growing season. Moreover, an experimental protocol could be designed to test whether a strategic defoliation performed in late spring to promote a specific disturbance in tall fescue could stimulate kikuyu-grass tillering during summer.

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