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Yield and Stability of Three Tropical Forage Grasses Cultivated as Pure or Mixed Stands

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ABSTRACT

Multispecific pastures are considered more sustainable to animal production than monocultures. However, studies on mixtures of tropical grasses are underexplored. We aimed to assess whether a mixture of three contrasting growth strategies of perennial tropical forage grasses could outperform their respective monocultures in terms of forage yield and stability. The four treatments included three perennial tropical forage grasses: Andropogon gambagrass (AG; *Andropogon gayanus* cv. Planaltina), Massai guineagrass [MG; *Megathyrsus maximus* cv. Massai] and Piatã palisadegrass [PP; *Urochloa brizantha* cv. BRS Piatã], cultivated as monocultures and as a mixture of the three species. Treatments were randomly assigned to sixteen 180 m^2 plots and managed intermittently under manual harvest at pre- and post-harvest heights of 35 and 17.5 cm, respectively, for 2 years. The botanical composition of the mixture remained relatively stable, with a predominance of MG (68.9%), followed by PP (20.1%) and AG (6.8%). Annual forage yield varied between years ($p = 0.0017$) with a difference of 3974 kg DM ha^{-1} $year^{-1}$, but not according to treatments ($p > 0.05$) with an average of 23,012 kg DM ha^{-1} $year^{-1}$. No effect was observed for the intra-annual forage yield variance among treatments ($p > 0.05$). The association among PP, MG and AG in a mixture allows species to coexist with a relatively stable botanical composition, as well as forage yield and stability, similar to their monocultures.

1 | Introduction

Grasslands are complex and dynamic ecosystems where the dominant vegetation consists of native and/or exotic herbaceous species (Hadley 1993). In addition to providing forage resources for grazing ruminants, which produce high-quality protein sources, these ecosystems also offer essential environmental services, contributing to the sustainable development of humanity (Bengtsson et al. 2019). When managed exclusively to maximise animal productivity, typical systems rely on high-yield monoculture grasses coupled with high fertiliser inputs and pesticide spraying (Finn et al. 2013; Lüscher et al. 2014). However, these management practices can degrade biodiversity and jeopardise the range of services delivered by these ecosystems (Finn et al. 2013).

Multispecific swards have been investigated worldwide as a more sustainable alternative to monocultures (Wang et al. 2021). Most successful mixtures are usually composed of species that complement themselves by exploring ecological niches differently (Fridley 2001), with contrasting growth strategies (i.e., conservative or competitive in the use of resources). More competitive species thrive in fertile environments due to their efficiency and speed in capturing resources and tissue renewal, in addition to a greater number of axillary buds that favour tillering (Davies 1974). In contrast, more conservative species succeed in poorer environments and are known to have lower specific leaf area, longer leaf lifespans and slower tissue renewal rates (Cruz et al. 2002).

Several ecological and agronomic benefits have been reported for multispecific swards, including greater resilience to extreme weather events (Hofer et al. 2016), efficient use of growing resources (Spehn et al. 2000; Storkey et al. 2015), soil organic carbon storage (Bai and Cotrufo 2022) and greater forage yield and stability (Duchini et al. 2019; Nölke et al. 2022). These benefits are likely due to differences among species in stress and disturbance tolerance, growth resource requirements throughout the year and canopy architectural complementarity (Medeiros-Neto et al. 2023). In addition, from an animal point of view, more diverse pastures have been reported to increase herbage intake rates (Otaviano et al. 2024) and animal performance (Maxwell et al. 2023).

Despite the numerous benefits reported in the literature regarding mixed swards over monocultures, tropical pasture-based systems predominantly rely on monocultures of palisadegrasses due to their adaptability to a range of edaphic and climatic conditions. Other forage grasses, however, such as gambagrasses and guineagrasses, are also well adapted to tropical conditions and have contrasting strategies of resource use and might be good candidates for composing stable and productive mixtures with *Brachiaria*. We hypothesised that a mixture of palisadegrass, gambagrass and guineagrass can coexist under frequent and non-severe defoliation management, as well as yield more forage with lower intra-annual variation relative to their respective monocultures. Therefore, this study aimed to assess whether this mixture of three perennial tropical forage grasses could outperform their respective monocultures in terms of forage yield and stability.

2 | Materials and Methods

2.1 | Site Description

The study was carried out uninterruptedly from February 2020 to March 2022 at the Luiz de Queiroz College of Agriculture, University of São Paulo, in Piracicaba, SP, Brazil (22°42′35″ S, 47°38′24″ W and 546 m a.s.l.). According to the Köppen classification, the local climate is humid subtropical with hot summers and dry winters (Cwa; Alvares et al. 2013). The rainy season is historically observed from October to March, and the dry season from April to September. The historical accumulated rainfall during both rainy and dry seasons is 992 and 282 mm, respectively (1917–2023 average). A meteorological station located 1.9 km from the study site was used as a reference for climate and weather data during the experimental period (Figure 1).

The soil is classified as Eutroferric Red Nitossol (Santos et al. 2018) with the following chemical and physical characteristics at

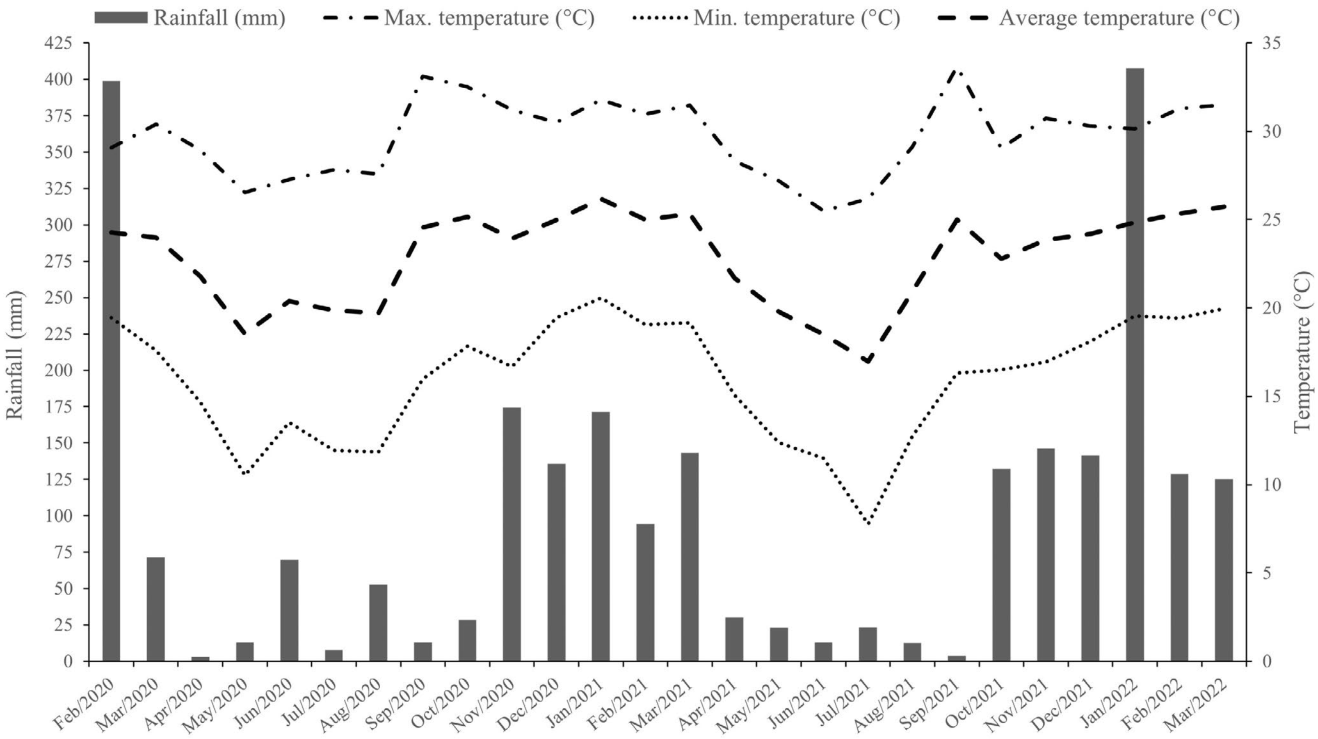

FIGURE 1 | Monthly maximum, average and minimum air temperatures (°C) and rainfall (mm) from February 2020 to March 2022, Piracicaba, SP, Brazil.

the 0 to 20 cm depth based on spring 2019 soil sampling: pH $CaCl_2 = 4.50$; organic matter = 33.8 g dm^{-3}; $p = 49.5$ mg dm^{-3}; K = 3.45 mmol$_c$ dm^{-3}; Ca = 30.3 mmol$_c$ dm^{-3}; Mg = 12.5 mmol$_c$ dm^{-3}; Al = 1.50 mmol$_c$ dm^{-3}; H + Al = 72.5 mmol$_c$ dm^{-3}; S = 25.8 mg dm^{-3}; Cu = 4.51 mg dm^{-3}; Fe = 176 mg dm^{-3}; Zn = 5.54 mg^{-3}; Mn = 35.2 mg dm^{-3}; B = 0.26 mg dm^{-3}; sum of bases = 46.0 mmol$_c$ dm^{-3}; cation exchange capacity = 119 mmol$_c$ dm^{-3}; base saturation = 39.0%; aluminium saturation = 3.50%; sand = 358 g kg^{-1}; clay = 446 g kg^{-1}; and silt = 196 g kg^{-1}. Dolomitic limestone was used to increase the soil base saturation to 70% (Cantarella et al. 2022) in late winter 2019, before the establishment of the experimental plots in January 2020.

2.2 | Treatments and Experimental Design

The four treatments were three perennial tropical forage grasses: Andropogon gambagrass (AG; *Andropogon gayanus* Kunth cv. Planaltina), Massai guineagrass [MG; *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Massai] and Piatã palisadegrass [PP; *Urochloa brizantha* (Hochst. ex A. Rich.) R. Webster cv. BRS Piatã], cultivated as monocultures and as a mixture comprised of the three species. Treatments were allocated to experimental units according to a randomised complete block design, with four replications, totalling 16 paddocks of 180 m^2 each (12 × 15 m). The soil slope was considered as a blocking criterion.

The forage species to compose the mixed stand were selected based on their contrasting strategies of resource use (i.e., capture and conservation), as well as plant architecture and growth habits (Loreau and Hector 2001; Pontes et al. 2012), similar to the protocol reported by Duchini et al. (2018, 2019). The most conservative species was AG, a tall, tufted, tussock-forming grass characterised by its low specific leaf area and high leaf longevity (Sousa et al. 2010), as well as field tolerance to spittlebug attacks (Ferrufino and Lapointe 1989). Conversely, PP was the most competitive species in capturing resources, known for its high rates of tillering, leaf appearance and high specific leaf area (Silveira et al. 2010). As an intermediate species, MG has a semi-upright growth habit, intermediate specific leaf area and slower renewal of leaves and tillers than PP (Martuscello et al. 2015).

2.3 | Establishment and Management

Experimental plots were sown manually in January 2020 at a rate of 300 pure viable seeds m^{-2}, with this amount being equally split among all three species in the mixed stand plots. The plots were immediately compacted after sowing, using a compactor roller weighing approximately 100 kg. Nitrogen was applied 21 days after sowing at a rate of 40 kg ha^{-1} as ammonium nitrate. At the end of February, a standardisation clipping was made at 17.5 cm of stubble height when canopies reached a height of 35 cm.

All treatments had the same defoliation management. The pre-harvest height followed the concept of critical leaf area index (LAI) when canopies reached 95% of light interception during regrowth on rotational stocking (Da Silva et al. 2015). According to this criterion, the pre-grazing heights for PP, AG and MG were 35 (Crestani et al. 2017), 50 (Sousa et al. 2010) and 55 cm (Barbosa et al. 2010), respectively. However, studies have reported the possibility of reducing up to 40% of the corresponding canopy height at 95% light interception without decreasing herbage accumulation (Sbrissia et al. 2018). In this more flexible scenario, AG and MG could be harvested when their canopies reached pre-harvest heights of up to 30 and 33 cm, respectively. Therefore, a pre-harvest height of 35 cm was considered to be compatible with all three forage grass species. The post-harvest height was equivalent to 50% of the pre-harvest height, 17.5 cm. It allows for non-severe and frequent defoliations by ensuring high residual LAI (Giacomini et al. 2009) at lower levels of stress (e.g., light competition) and disturbance (e.g., grazing severity).

A total of 200 kg N ha^{-1} was applied throughout the warm and rainy period (October to March) in each year using ammonium nitrate (32% N), always at post-harvest. A daily rate of 1.7 kg N ha^{-1} per rest day (Congio et al. 2019) was used to calculate the amount of N to be applied in each application, ensuring equal amounts of the nutrient to be applied in all paddocks at the end of each season.

2.4 | Samplings

2.4.1 | Canopy Height and Light Interception

Canopy height was measured concomitantly with canopy light interception measurements. Both pre- and post-harvest heights were measured using a sward stick, from ground level to the top leaf horizon by taking 40 readings per paddock (Carnevalli et al. 2021). Canopy light interception was monitored either at dawn or dusk using a LAI-2000 canopy analyser (LI-COR, Lincoln, NE, USA) by taking eight readings above the canopy and 40 at ground level per paddock (Da Silva et al. 2021).

2.4.2 | Herbage Mass, Yield and Botanical Composition

Pre- and post-harvest herbage mass assessments were performed once every season. Two herbage samples per paddock were clipped at ground level using 0.25 m^2 rectangular frames randomly placed in representative sites (i.e., visual assessment of canopy height and herbage mass) at the time of sampling. After harvesting, a subsample was taken to the laboratory. Firstly, the number of tillers was counted, and botanical and morphological components were determined by manual separation. For monocultures, plant-part components were separated into leaves (green leaf blade), stems (stem + sheath), seedheads, weeds and dead material. For mixtures, morphological components were individually separated by grass species, except dead material, owing to the lack of distinction among species. All samples were then dried at 65°C in a forced-air oven until a constant weight was reached, and the data were used to calculate pre- and post-harvest herbage mass.

Additional samples were taken at each regrowth cycle by clipping the herbage at the post-harvest height of 17.5 cm, when

TABLE 1 | Pre- and post-harvest canopy heights (±SD) and total number of regrowth cycles (±SD) for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as monocultures or as a mixture (MIX) from April 2020 to March 2022, Piracicaba, SP, Brazil.

Season	PP	MG	AG	MIX
Pre-harvest canopy height (cm)				
Late Spring	35.2 ± 0.1	35.3 ± 0.2	35.4 ± 0.1	35.3 ± 0.3
Summer	35.2 ± 0.2	35.1 ± 0.1	35.3 ± 0.2	34.7 ± 0.5
Aut./Win./Early Spring	30.8 ± 0.5	34.9 ± 0.2	34.2 ± 0.8	34.5 ± 0.6
Post-harvest canopy height (cm)				
Late Spring	17.3 ± 0.3	17.1 ± 0.3	17.6 ± 0.2	17.2 ± 0.1
Summer	17.5 ± 0.1	17.2 ± 0.1	17.5 ± 0.1	17.3 ± 0.1
Aut./Win./Early Spring	17.3 ± 0.2	17.3 ± 0.1	17.5 ± 0.1	17.3 ± 0.2
Total number of regrowth cycles				
	14 ± 0.8	20 ± 1.5	19 ± 1.3	20 ± 1.5

Note: Late spring: average referring to November 2020, December 2020, November 2021 and December 2021; summer: average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: average referring to April to October 2020 and April to October 2021.

TABLE 2 | Pre- and post-harvest herbage mass for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as monocultures or as a mixture (MIX) from April 2020 to March 2022, Piracicaba, SP, Brazil.

Season	PP	MG	AG	MIX	Mean	SEM
Pre-harvest herbage mass (kg DM ha^{-1})						
Late Spring	7790^{Ba}	6680^{Bbc}	6450^{Ac}	7390^{Bab}	7080^{B}	182
Summer	8650^{Aa}	7660^{Ab}	6590^{Ac}	7330^{Bbc}	7560^{A}	182
Aut/Win/Early Spring	8970^{Aa}	7470^{Ab}	6470^{Ac}	8580^{Aa}	7870^{A}	182
Mean	8470^{a}	7270^{b}	6500^{c}	7770^{b}		
SEM	234	234	234	234		
*Pre-harvest herbage mass – Mixture (kg DM ha^{-1})**						
Late Spring	1100^{Ab}	2670^{Ba}	290^{Ac}	—	1350^{ns}	107
Summer	1080^{Ab}	3660^{Aa}	90^{Ac}	—	1610^{ns}	107
Aut/Win/Early Spring	800^{Ab}	3860^{Aa}	170^{Ac}	—	1610^{ns}	107
Mean	990^{b}	3390^{a}	180^{c}			
SEM	62	62	62			
Post-harvest herbage mass (kg DM ha^{-1})						
Late Spring	5830^{Ba}	5430^{Aa}	5710^{Aa}	5590^{Aa}	5640^{AB}	169
Summer	5660^{Ba}	6060^{Aa}	4180^{Cb}	5950^{Aa}	5460^{B}	165
Aut/Win/Early Spring	6820^{Aa}	5560^{Abc}	4920^{Bc}	6070^{Ab}	5840^{A}	169
Mean	6230^{a}	5680^{b}	4940^{c}	5870^{ab}		
SEM	185	185	179	179		

Note: Means with a common uppercase letter in the columns or lowercase letters in the rows are not different ($p > 0.05$). SEM indicates the standard error of the mean. *Pre-harvest herbage mass in the mixture does not include dead material and weeds. Late spring: average referring to November 2020, December 2020, November 2021 and December 2021; summer: average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: average referring to April to October 2020 and April to October 2021.

canopies reached the 35 cm pre-harvest height. Immediately after collecting these samples, paddocks were entirely mowed at 17.5 cm using a backpack mower and the material removed from the area using a rake. Samples were processed as described above, and the following yields were calculated: total forage (all plant parts and botanical components), net forage (excluding

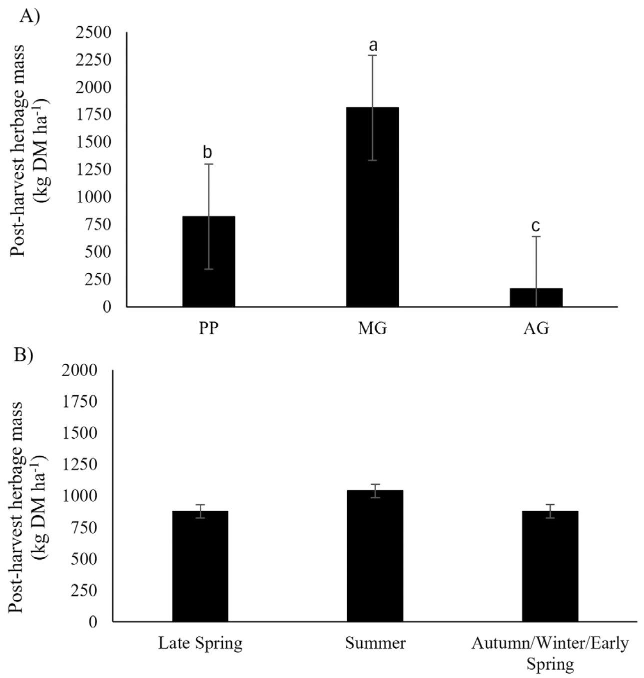

FIGURE 2 | Post-harvest herbage mass for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as a mixture from April 2020 to March 2022, Piracicaba, SP, Brazil: (A) treatment effect and (B) season of the year effect. Means with a common lowercase are not different ($p > 0.05$). The bars indicate standard errors of the mean. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

dead material and weeds), leaves, stems and forage from each species in the mixture (Duchini et al. 2019).

2.4.3 | Intra-Annual Forage Yield Variance

According to Pontes et al. (2012), the intra-annual stability of forage yield across seasons of the year can be assessed by comparing the productivity variance between mixed plots and the average of monocultures of their constituent species. The authors state that this analysis allows the identification of overyielding, meaning whether species in the mixture performed better than expected compared to monocultures. This response is essential to identify plant combinations that enhance biodiversity while maintaining or increasing forage yield relative to monocultures.

The intra-annual forage yield variance among seasons of the year was calculated differently for each treatment (all plants/species) and populations (each species separately in the mixture). For plants/species together, the intra-annual forage yield variance was calculated according to Pontes et al. (2012), considering the standard error of the mean (SEM) for different seasons. The coefficient of variation (CV) was used for the plant populations in the mixture, considering that the proportions of areas occupied by each species in the mixture relative to their monocultures were different (Duchini et al. 2019).

2.4.4 | Overyielding and Underyielding Calculations

The expected forage yield of each species in the mixture (E_i) was calculated according to Loreau (1998):

$$E_i = p_i M_i$$

where p_i is the proportion of species i in the mixture, and M_i is the observed forage yield for species i in monoculture. Overyielding and/or underyielding of each species population was computed by the proportional deviations from the expected yield (D_i) based on the forage yield of their monocultures (Loreau 1998):

$$D_i = \frac{O_i - E_i}{E_i}$$

where O_i is the observed population of species i in the association. Because the proportion of each species in the mixture throughout the experimental period differed from the sown proportion, the values for the proportions of species (p_i) were calculated based on the tiller population density for each species (Duchini et al. 2019). Similarly, D_i was calculated for stem and leaf yields, considering their respective values of O_i and M_i. Overyielding and/or underyielding for the plant community were quantified as the average weighted ($\overline{D}$) and total proportional deviations (D_t) from the D_i, as proposed by Loreau and Hector (2001):

$$\overline{D} = \sum_i p_i D_i$$

$$D_t = \frac{O_t - E_t}{E_t}$$

where

$$O_t = \sum_i O_i \quad \text{and} \quad E_t = \sum_i p_i M_i$$

2.5 | Statistical Analysis

Since treatments were managed by clippings based on pre- and post-harvest canopy heights, the timing of clippings varied among both treatments and replications throughout the experimental period. Therefore, all variables were pooled by season to allow for comparisons under the same weather conditions. The experimental period was then divided into the following seasons of the year: summer I (February and March 2020), autumn, winter and early spring I (April to October 2020), late spring I (November and December 2020), summer II (January to March 2021), autumn, winter and early spring II (April to October 2021), late spring II (November and December 2021) and summer III (January to March 2022). Summer I, however, was considered an adaptation period in which pastures were being established and data were presented separately (Table S1). Averages for each season of the year were subsequently calculated, and the data were pooled as follows: late spring; summer; and autumn, winter and early spring. Data were subjected to variance analysis using the MIXED procedure in the SAS statistical package (version 9.3; SAS Institute

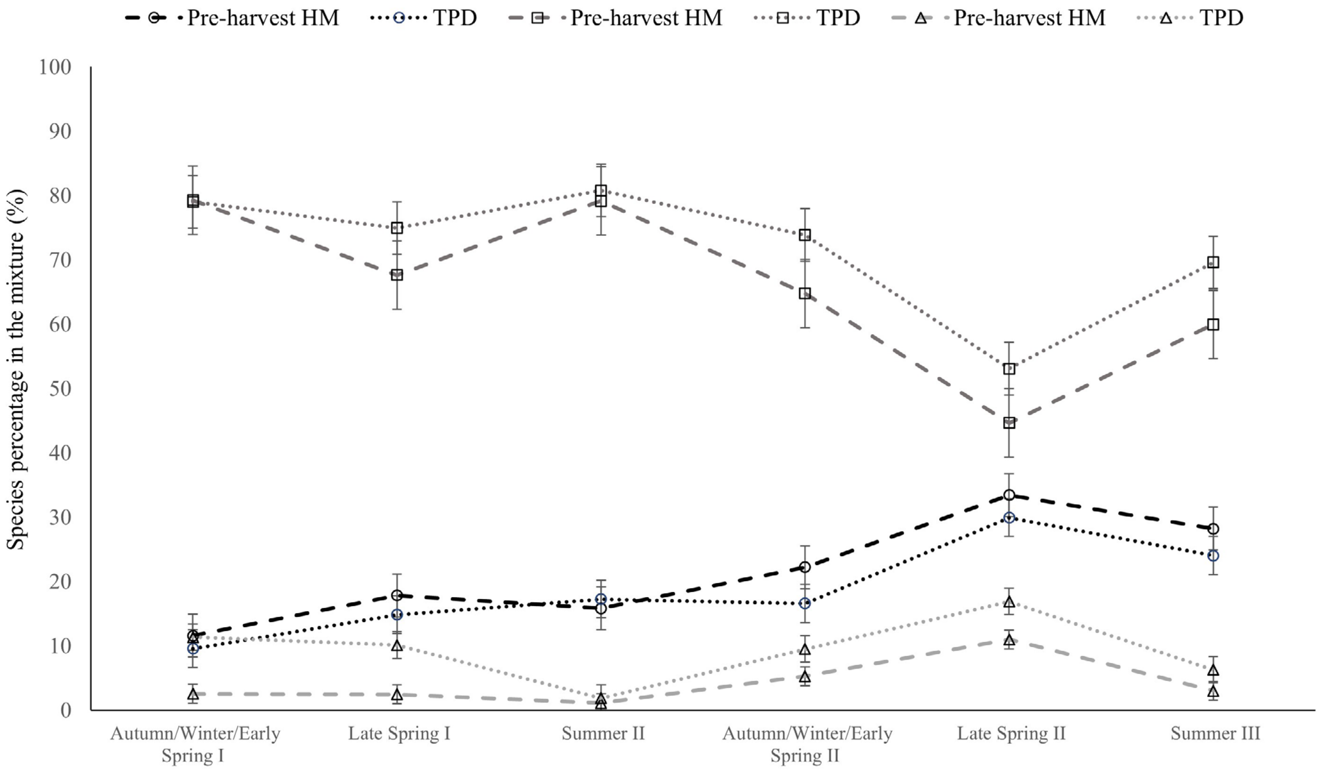

FIGURE 3 | Percentage of Piatã palisadegrass (PP) (circles), Massai guineagrass (MG) (squares) and Andropogon gambagrass (AG) (triangles) cultivated as a mixture from April 2020 to March 2022, Piracicaba, SP, Brazil. Dashed lines indicate values based on pre-harvest herbage mass (HM), and the dotted lines indicate values based on tiller population density (TPD). The bars indicate standard errors of the mean.

Inc., Cary, NC, USA). Different structures of the variance–covariance matrices were tested, and the Akaike information criterion (AIC) was adopted to select the best-fit matrix. The analysis was performed considering treatments, seasons of the year and their interactions as fixed effects and blocks as random effects (Littell et al. 2000). The seasons of the year were treated as repeated measurements. A descriptive analysis throughout all seasons of the year was performed for the botanical composition of the mixture. Treatment means were estimated using the LSMEANS statement and, when appropriate, comparisons were made using the probability of difference (PDIFF) with the Student's *t*-test at $p < 0.05$. Confidence interval (CI) of 95% was used to consider deviations from the expected yield (D_i), as well as the proportional deviations (D^- and D_t) for each species and their deviations in the yield of each morphological component (i.e., leaf and stem), as different from zero.

3 | Results

3.1 | Pre- and Post-Harvest Heights

Overall, both pre- and post-harvest heights (35 and 17.5 cm, respectively) were kept close to the planned values throughout the entire experimental period, except for the PP during the autumn/winter/early spring season in both years of the experiment, where a clipping was performed on all replications before the planned pre-harvest height was reached (Table 1).

3.2 | Canopy Light Interception

Canopy light interception at pre-harvest varied with treatment ($p = 0.0025$) and season of the year ($p = 0.0332$), with average values very close to 95%. Greater values were observed for PP (95.3%), followed by the mixture, AG and MG (average of 94.3%). At post-harvest, it changed according to treatment ($p = 0.0005$), season of the year ($p < 0.0001$) and treatment × season of the year interaction ($p = 0.0019$). Overall, greater values were recorded for PP and the mixture, as well as for all treatments during autumn/winter/early spring compared to other seasons of the year (Table S2).

3.3 | Pre- and Post-Harvest Herbage Mass

Both pre- and post-harvest herbage mass varied with treatment ($p < 0.0001$), season of the year ($p = 0.0001$ and $p = 0.0332$, respectively) and treatment × season of the year interaction ($p = 0.0174$ and $p < 0.0001$, respectively). At pre-harvest, greater values were recorded in summer and autumn/winter/early spring, and smaller values were recorded during late spring (Table 2). Piatã palisadegrass showed the greatest values, followed by the mixture and MG, and AG had the smallest values. Regarding the species in the mixture, pre-harvest herbage mass varied with grass species ($p < 0.0001$) and its interaction with the season of the year ($p = 0.0069$). Massai guineagrass consistently showed the greatest herbage mass values, whereas AG had the smallest. There was no difference among seasons

TABLE 3 | Total forage yield and net accumulation rate for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as monocultures or as a mixture (MIX) from April 2020 to March 2022, Piracicaba, SP, Brazil.

Season	PP	MG	AG	MIX	Mean	SEM
Total forage yield (kg DM ha^{-1}*)*						
Late Spring	5370^{Ca}	5020^{Ca}	5830^{Ba}	4860^{Ca}	5270^{C}	324.9
Summer	$10{,}410^{Ab}$	$11{,}990^{Aa}$	$10{,}660^{Ab}$	$11{,}640^{Aab}$	$11{,}180^{A}$	234.9
Aut/Win/Early Spring	6650^{Ba}	6510^{Ba}	5190^{Bb}	6730^{Ba}	6270^{B}	324.9
Mean	7480^{ns}	7840^{ns}	7230^{ns}	7740^{ns}		
SEM	393.59	393.59	393.59	393.59		
Net forage accumulation rate (kg DM ha^{-1} day^{-1}*)*						
Late Spring	88^{Ba}	82^{Ba}	96^{Ba}	80^{Ba}	86^{B}	3.2
Summer	116^{Abc}	133^{Aa}	111^{Ac}	129^{Aab}	122^{A}	2.8
Aut/Win/Early Spring	31^{Ca}	30^{Cab}	24^{Cb}	31^{Ca}	29^{C}	1.5
Mean	78^{ns}	82^{ns}	77^{ns}	80^{ns}		
SEM	3.8	3.8	3.9	3.8		
Net forage accumulation rate – mixture (kg DM ha^{-1} day^{-1}*)*						
Late Spring	21^{Bb}	45^{Ba}	7^{Ac}	—	25^{B}	1.5
Summer	32^{Ab}	79^{Aa}	7^{Ac}	—	39^{A}	2.0
Aut/Win/Early Spring	4^{Cb}	23^{Ca}	1^{Bc}	—	9^{C}	1.0
Mean	19^{b}	49^{a}	5^{c}			
SEM	1.3	1.3	1.3			

Note: Means with a common uppercase letter in the columns or lowercase in the rows are not different ($p > 0.05$). SEM indicates the standard error of the mean. Late spring: average referring to November 2020, December 2020, November 2021 and December 2021; summer: average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: average referring to April to October 2020 and April to October 2021.

of the year ($p = 0.2218$). At the post-harvest condition, smaller values were observed during the summer, particularly for AG. Massai guineagrass and the mixture showed relatively stable values across seasons of the year, with the mixture presenting the second-greatest value (Table 2). For the species in the mixture, variation occurred only with grass species ($p < 0.0001$), following the same response pattern described for pre-harvest (Figure 2).

3.4 | Botanical Composition of the Mixture

The proportion of species in the mixture showed a consistent pattern of variation, whether calculated using pre-harvest herbage mass or tiller population density data (Figure 3). Massai guineagrass had the largest percentage (average of 65.9% in pre-harvest herbage mass and 71.9% in tiller population density), whereas AG had the smallest percentage (average of 4.3% in pre-harvest herbage mass and 9.4% in tiller population density), and PP showed an intermediate proportion in the mixture, averaging 21.5% in pre-harvest herbage mass and 18.7% in tiller population density. Except for late spring II, when MG showed a slight decrease in its population, the contribution of each species in the mixture remained relatively stable throughout the entire experimental period.

3.5 | Forage Yield and Contribution of Each Species in the Mixture

The total forage yield (considering all plant and botanical components) varied with the season of the year ($p < 0.0001$) and treatment × season of the year interaction ($p = 0.0084$). Overall, greater values were recorded during summer and smaller values were recorded during late spring (Table 3). This pattern was the same for all treatments, except for AG, which showed similar yields in autumn/winter/early spring and late spring. The net herbage accumulation rate (excluding dead material and weeds) varied according to the season of the year ($p < 0.0001$) and treatment × season interaction ($p = 0.0005$). In general, greater values were observed during summer and smaller during autumn/winter/early spring. There was no difference among treatments, with net herbage accumulation rates averaging 79.3 kg DM ha^{-1} day^{-1}. During summer, MG showed the highest net accumulation rate, followed by the mixture, whereas AG had the lowest rates. Piatã palisadegrass had net herbage accumulation rates similar to those of the mixture and AG (Table 3). During autumn/winter/early spring, AG showed the lowest net accumulation rate compared to the other treatments (Table 3). For the species in the mixture, net herbage accumulation rate varied with treatment ($p < 0.0001$), season of the year ($p < 0.0001$) and treatment × season of the year interaction ($p < 0.0001$).

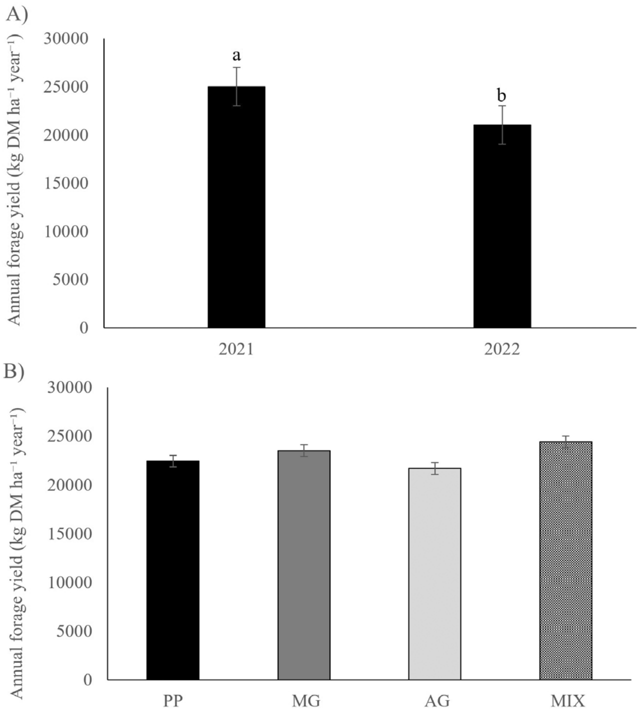

FIGURE 4 | Annual total forage yield for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as monocultures or as a mixture (MIX) from April 2020 to March 2022, Piracicaba, SP, Brazil: (A) year effect (April 2020 to March 2021 represents the year 2021 and April 2021 to March 2022 represents the year 2022) and (B) treatment effect. Means with a common lowercase are not different ($p > 0.05$). The bars indicate standard errors of the mean.

The same response pattern described for the monocultures was observed for the species in the mixture. However, due to their greater and smaller percentages in the mixture, MG and AG showed the highest and lowest net herbage accumulation rates, respectively (Table 3).

Total forage yield varied between experimental years ($p = 0.0007$). In 2021, the average yield was 3974 kg DM ha^{-1} greater than in 2022 (Figure 4A). No differences were found among treatments ($p = 0.2610$), and an average value of 23,012 kg DM ha^{-1} year^{-1} was recorded (Figure 4B).

3.6 | Intra-Annual Forage Yield Variance

No effect was observed ($p = 0.1357$) for the intra-annual forage yield variance among the species when cultivated in the mixture relative to their respective monocultures (Figure 5A). Still, no effect was observed ($p = 0.1232$) for the intra-annual forage yield variance among treatments (Figure 5B).

3.7 | Expected Forage Yield of the Mixture and Overyielding

Net forage yield above the expected values was not observed within the 95% CI among seasons of the year for the mixture; in other words, the values of weighted deviations ($\overline{D}$) and total deviations (D_t) were similar to the expected yields for the seasons of the year (Figure 6). However, considering each species individually in the mixture, a negative D_i was observed for MG during summer, indicating that D_i was different from zero within the 95% CI (Figure 7B). However, neither PP nor AG showed the same pattern in any season of the year (Figure 7A–C).

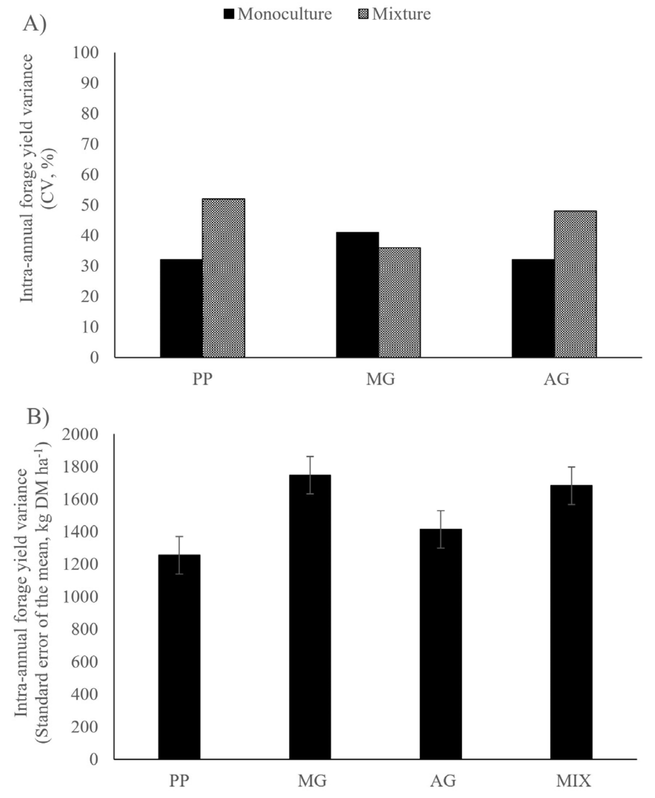

FIGURE 5 | Intra-annual forage yield variance (among seasons of each year) for Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG) cultivated as monocultures or as a mixture (MIX) from April 2020 to March 2022, Piracicaba, SP, Brazil: (A) calculated using coefficient of variation (CV) based on plant populations considering each species separately in the mixture and (B) calculated using standard error of the mean (SEM) considering all plants/species together. Means with a common lowercase are not different ($p > 0.05$). The bars indicate standard errors of the mean.

No significant D_i values were observed for leaf yield, whereas stem yield was greater than expected for MG, indicating that *Di* was different from zero within the 95% CI (Figure 8A). No significant D_i values were observed for the seasonal averages (Figure 8B).

Considering each species in the mixture, MG showed a 17% lower (95% CI) leaf yield than expected during the summer (Figure 9B). Both PP and AG yielded leaves similar to those expected throughout all seasons of the year (Figure 9A–C). Regarding stem yield, only AG showed an underyielding of 86% (95% CI; Figure 10C), whereas no significant D_i values were observed for both PP and MG across seasons of the year (Figure 10A,B).

4 | Discussion

Overall, both pre- and post-harvest canopy heights were kept very close to the targets throughout the experimental period,

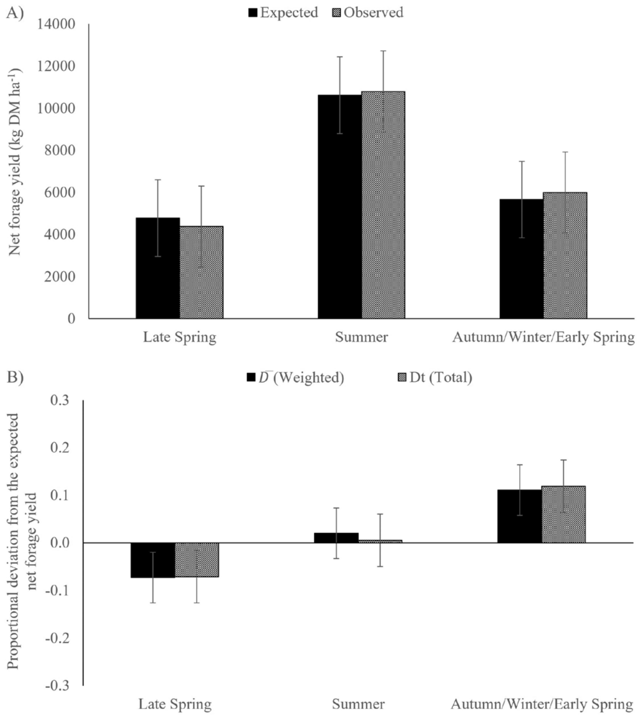

FIGURE 6 | Observed and expected net forage yield (calculated based on the proportion of each species as mixture and its net forage yield as monoculture) of a mixture cultivated by Piatã palisadegrass, Massai guineagrass and Andropogon gambagrass, throughout the seasons from April 2020 to March 2022, Piracicaba, SP, Brazil (A) and weighted (D^{-}) and total (D_t) proportional deviations from the expected net forage yield for the mixture (B). The bars indicate standard errors of the mean. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

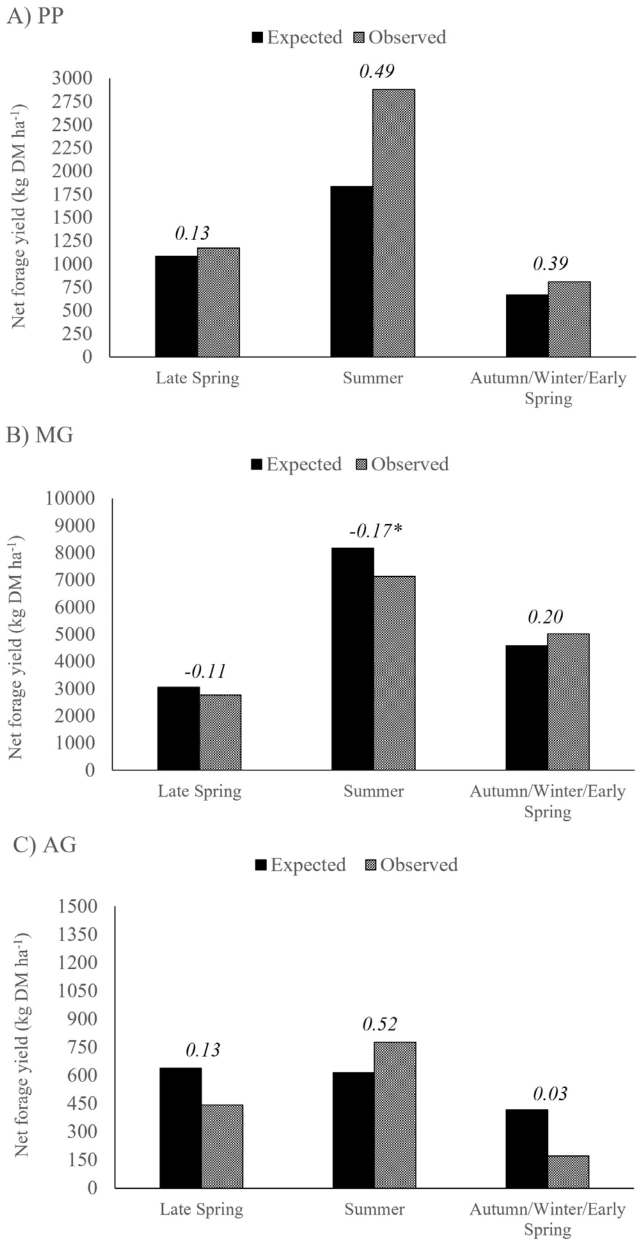

FIGURE 7 | Observed and expected net forage yield (calculated based on the proportion of each species as mixture and its net forage yield as monoculture) of a mixture cultivated by Piatã palisadegrass, PP (A), Massai guineagrass, MG (B) and Andropogon gambagrass, AG (C), throughout the seasons of the year from April 2020 to March 2022, Piracicaba, SP, Brazil. Values on top of the bars in italic indicate D_i, and asterisks indicate that D_i differs from zero (0.0) at a 95% confidence interval. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

ensuring effective control of experimental conditions (Table 1), and the pre-harvest canopy light interception values remained close to 95%, a condition known to prevent intense light competition and avoid excessive accumulation of stems and dead material in the herbage mass (Da Silva et al. 2009). Our results agree with those from Sbrissia et al. (2018), which suggest the possibility of reducing up to 40% of the canopy height corresponding to 95% light interception without decreasing herbage yield, as the plants adjust their LAI through tiller density/size compensation to intercept the same amount of light. Leaf angle is also a characteristic that changes according to the height at which the canopies are managed and is partially responsible for this reduction in the height at which both AG and MG intercept 95% of the light (Da Silva et al. 2015). In this more flexible scenario, AG and MG could optimally have their regrowth interrupted in this range of 35–50 and 35–55 cm, respectively. However, it is important to note that the bottom range of pre-grazing heights can be used when nutrients are not limited, given the high energy cost associated with tiller turnover, the key mechanism by which the canopy can adjust their LAI in order to intercept light (Martins et al. 2021).

Pasture herbage mass is dependent on several factors, including weather conditions, nutrient availability, management and the functional group to which the cultivated plants belong (Langer 1963; Cruz et al. 2002; Da Silva et al. 2019). In this experiment, the first three factors were identical for all treatments. Therefore, the differences observed in herbage mass and net accumulation rate were due to the morphological and functional characteristics of each species (Table 2), which also led to

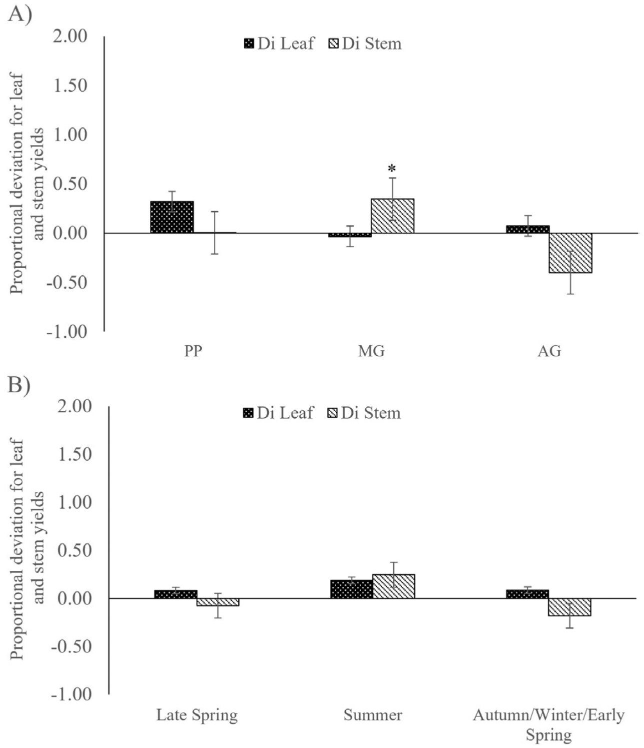

FIGURE 8 | Proportional deviations (D_i) for leaf and stem yields (observed and expected, calculated based on the proportion of each species as mixture and its yields as monoculture) of a mixture cultivated by Piatã palisadegrass (PP), Massai guineagrass (MG) and Andropogon gambagrass (AG), throughout the seasons of the year from April 2020 to March 2022, Piracicaba, SP, Brazil: (A) treatment effect and (B) season of the year effect. Asterisks indicate that D_i differs from zero (0.0) at 95% confidence interval. The bars indicate standard errors of the mean. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

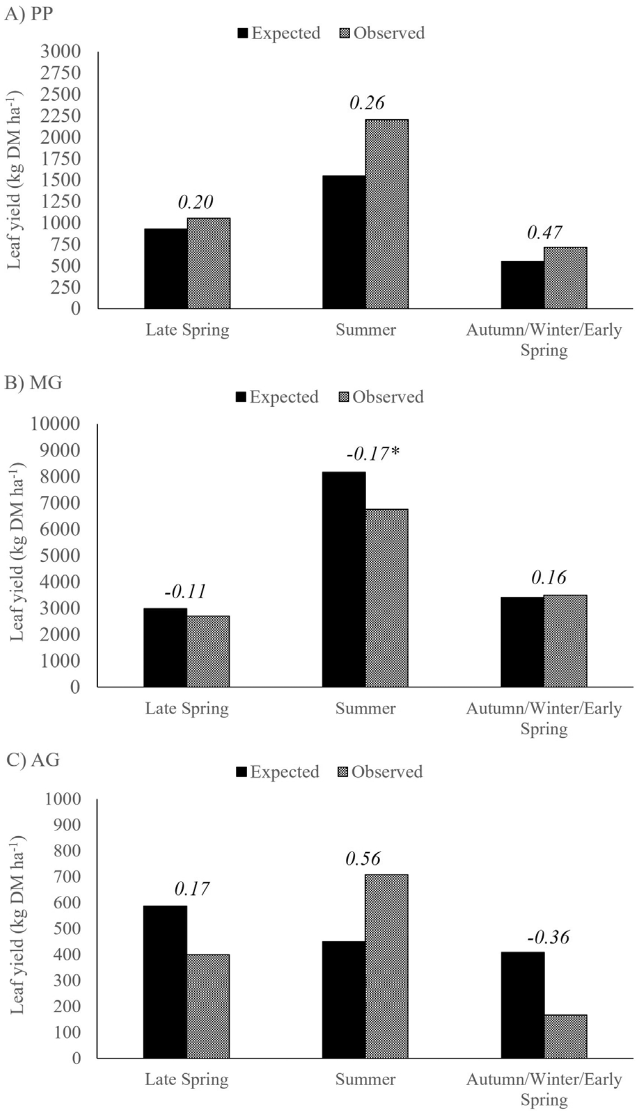

FIGURE 9 | Observed and expected leaf yield (calculated based on the proportion of each species as mixture and its leaf yield as monoculture) of a mixture cultivated by Piatã palisadegrass, PP (A), Massai guineagrass, MG (B) and Andropogon gambagrass, AG (C), throughout the seasons of the year from April 2020 to March 2022, Piracicaba, SP, Brazil. Values on top of the bars in italic indicate D_i, and asterisks indicate that D_i differs from zero (0.0) at 95% confidence interval. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

changes in the number of regrowth cycles (Table 1) but did not influence annual forage yield between monocultures and the mixture (Figure 4B).

In experiments conducted concomitantly, MG showed a greater leaf angle (more vertical), a greater leaf elongation rate, with a smaller LAI in the top 10 cm of the canopy (Silva 2023), and a longer root length (Gomes 2023), whereas PP had a greater LAI but a smaller leaf angle (more horizontal), a smaller leaf elongation rate (Silva 2023) and a greater root mass (Gomes 2023). AG had fewer leaves per tiller, lower yield performance despite having growth rates similar to those of MG (Silva 2023), and smaller root mass and length (Gomes 2023). Thus, the species exhibited contrasting traits that resulted in architectural complementarity in the vertical sward profile (Spehn et al. 2000). Similarly, Naeem et al. (1994) and Spehn et al. (2000) showed that greater plant diversity in an ecosystem allows for better three-dimensional distribution and canopy space occupation. However, this also resulted in less intense competition for light, with MG positioning its leaves more upright and allowing for greater penetration of light into the canopy (Silva 2023). Consequently, MG was prevalent in the botanical composition of the mixture, followed by PP and AG regardless of the calculation method used (Figure 3). Therefore, the dynamics among species in the mixture were most likely determined by competitive and coexistence interactions driven by their complementarity growth traits.

Complementarity in plant communities occurs when species coexist due to sufficiently contrasting characteristics and resource requirements (Mason et al. 2020; Wang et al. 2021),

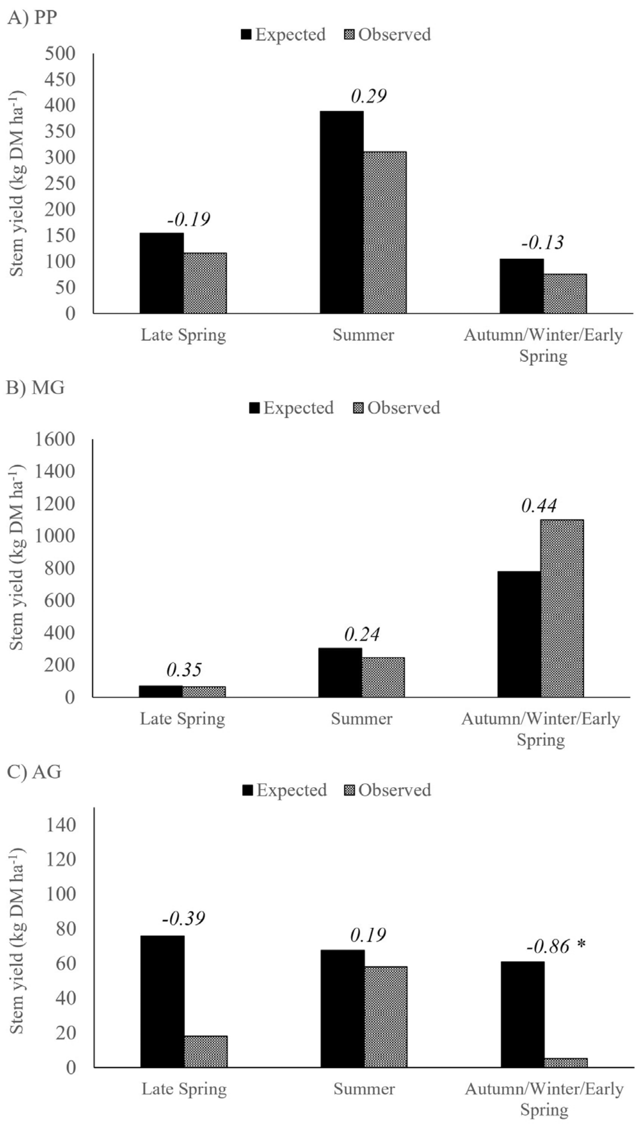

FIGURE 10 | Observed and expected stem yield (calculated based on the proportion of each species as mixture and its stem yield as monoculture) of a mixture cultivated by Piatã palisadegrass, PP (A), Massai guineagrass, MG (B) and Andropogon gambagrass, AG (C), throughout the seasons of the year from April 2020 to March 2022, Piracicaba, SP, Brazil. Values on top of the bars in italic indicate D_i, and asterisks indicate that D_i differs from zero (0.0) at a 95% confidence interval. Late spring: Average referring to November 2020, December 2020, November 2021 and December 2021; summer: Average referring to January to March 2021 and January to March 2022; autumn, winter and early spring: Average referring to April to October 2020 and April to October 2021.

indicating that there are benefits to species living together. However, the selection effect occurs when competitive interactions result in one or a few species dominating others in the mixture, with the functional responses of the mixture being strongly influenced by the functionality of the dominant species (Tilman 1999; Yang et al. 2021). According to Loreau and Hector (2001), dominance occurs due to the greater adaptability of some plants to their environment, allowing them to outperform others in the community. As a result, the response pattern of the plant community, as well as morphogenetic rates, is conditioned by the characteristics of the dominant species (Fridley 2001; Loreau and Hector 2001). This was exemplified in the present study by the prevalence of both MG and PP in the mixture as they shared similar levels of responses (e.g., herbage mass, forage yield, net herbage accumulation rate).

Although no reduction in intra-annual forage yield variance was observed in the mixture, species showed fluctuations in their proportions during the transition from the dry season to the rainy season, with increases in PP and AG, particularly in the second year of the experiment (Figure 3). According to Hector et al. (2010) and Tilman et al. (2006), biodiversity increases the temporal fluctuations of the population and primary production of each species in a mixture but decreases the variability at the community level. Furthermore, asynchrony in population fluctuation and overyielding of certain species contribute to increasing the stability of biodiverse ecosystems. This may indicate that a mixture encompassing greater proportions of PP and AG could have greater yield stability if these fluctuations alternate the dominant species throughout the year, as reported by Allan et al. (2011). The authors, assessing diversity gradients over 7 years, concluded that the long-term stability in biodiverse ecosystems is mainly due to the asynchrony in the population fluctuation of species present in these communities, as the dominant species differed over the years. Ultimately, greater yield stability determined by smaller intra-annual forage yield variance could favour longer and more stable periods of forage supply throughout the year in grazing systems and decrease reliance on external feeding.

According to Loreau and Hector (2001), the net effect of biodiversity on ecosystem functions (e.g., primary production) results from the balance among the selection effects of species in a mixture and the complementarity among them. Although no overyielding was observed in the present study, the forage yield in the mixture was as expected across all seasons of the year. This was likely due to the temporal complementarity of the species, especially by PP and AG, which together represented 35% of the net forage yield during late spring (Table 3). Regarding the species contribution in the mixture, although MG showed a slight leaf underyielding, this was not enough to reduce the net productivity of the mixture, representing an adaptive response of the species (Figure 9). In addition, AG showed stem underyielding during autumn/winter/early spring (Figure 10), which may be contextually beneficial because the nutritional value of stems is smaller than that of leaves (Griffin and Jung 1983). Moreover, greater proportions of stems in the upper canopy layers could negatively affect herbage intake by the grazing animals (Benvenutti et al. 2006) and, ultimately, their performance (Congio et al. 2018). Duchini et al. (2016) did not report greater stem yields in a mixture of black oats and annual ryegrass compared to their monocultures, but a transgressive overyielding was observed for leaf yield during the vegetative growth phase. Although no overyielding was observed among the species, the mixture was not underyielded and was as productive as its respective monocultures. Our results indicate that these three species coexisted during 2 years under a frequent and non-severe cutting regime. Future research should include herbivores as long-term defoliators as well as investigate potential ecosystem services provided by this mixture, especially under broader environmental conditions.

5 | Conclusions

The association among PP, MG and AG in a mixture cultivated in fertile soil under frequent and non-severe defoliation management allows for species coexistence with a relatively stable botanical composition, as well as forage yield and stability similar to their monocultures. While MG emerged as the dominant species, the temporal fluctuations in PP and AG proportions, especially during seasonal transitions, suggest potential for enhanced system resilience. These findings move a step forward in our understanding of tropical mixed-species pasture dynamics and indicate that such associations could be viable alternatives for intensive pasture-based systems.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.