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*Review*

# Mixing Tropical Perennial Forage Grasses in Pastures—An Opportunity for Sustainable Intensification

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**Abstract:** Botanical richness and diversity play crucial roles in regulating ecosystem functions and contribute to the sustainable intensification of perennial grasslands. This approach can be achieved through simultaneous or partial production of grasses in the same field, leading to enhanced productivity, reduced dependence on inorganic fertilizers and pesticides, and mitigating effects of edaphoclimatic variations. However, the existing literature predominantly focuses on associations between forage grasses and legumes or annual species. Furthermore, the subject should be explored under tropical conditions and environments, particularly considering the associations among well-managed perennial forage grasses. The interaction among perennial tropical forage grasses presents an alternative for exploration in the tropics, given the vast diversity of species and genotypes available. This review discusses the existing literature on multispecific pastures, elucidating the potential benefits for pastoral ecosystems. The association of perennial tropical forage grasses represents an emerging research area offering strategic opportunities for the sustainable intensification of animal production systems. Mixed pastures may be an economical and ecological alternative and enhance the production and sustainability of forage systems in the tropics. However, challenges persist in selecting plant species to achieve multifunctionality and understanding the underlying mechanisms shaping botanical diversity and productive performance within an association. This review emphasizes that understanding the morphological and agronomic characteristics of species and genotypes intended for cultivation in association is key to grasping the dynamics of competition for aboveground and belowground resources and creating combinations that deliver specific ecosystem services.

**Keywords:** functional diversity; grass intercropping; mixed pastures; sustainable pastures; tropical ecosystems



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## 1. Introduction

Biodiverse pastures have been shown to enhance the delivery of ecosystem services while mitigating the impact of anthropogenic and edaphoclimatic factors [1]. At field scale, achieving richness and diversity in pastures involves the simultaneous or partial production of at least two plant species or genotypes in the same pasture. These species can be spatially arranged in different ways, such as mixed rows, alternating rows, stripes, or random seedings [2]. Previous studies, encompassing meta-analyses [3,4] and biodiversity experiments such as the Cedar Creek Experiment [2] and Jena Experiment [5], have provided robust evidence for the positive correlation between species richness/diversity and ecosystem service provision. These studies highlight that biodiverse grasslands generate good results from complementarity, selection, or facilitation effects [6–10].

A major challenge in supporting the development of multispecific pastoral systems remains selecting species that will compose mixed pastures to achieve multifunctionality. This approach requires understanding the underlying mechanisms that shape the mixtures' botanical proportions and productive performance. Recent studies suggest that functional diversity is more important for ecosystem functioning than the number of taxonomic groups in the mixture [1,11]. Furthermore, greater dissimilarity in traits indicates reduced niche overlap, leading to more efficient resource capture in both space and time [12,13].

Although the benefits of using multispecific pastures were demonstrated in several studies with forage species from temperate climates [14], few studies have been conducted regarding mixing perennial tropical forage species. Mixing tropical grass species is an emerging research topic that presents an opportunity for sustainable intensification of pastures. In this context, this review aims to summarize existing information regarding multispecific pastures, elucidate the benefits offered to forage livestock systems, and offer possible applications under Brazilian conditions.

## 2. Functionality of Multispecific Pastures

Spatial and temporal resource partitioning are highlighted as primary drivers of success in multispecies pasture systems [15]. Spatial resource partitioning occurs when different forage species within mixed pastures can explore resources at different strata of the forage canopy or soil depth. This capability enables more efficient resource utilization [16]. For instance, mixing species with contrasting characteristics, such as growth habit (erect or prostrate), morphogenetic responses (leaf lifespan, leaf elongation, senescence rate, and stem elongation rate), and structural traits (leaf size and number of live leaves per tiller), may lead to a better distribution of leaf area and a more effective use of incident light from the canopy [17]. Belowground, spatial partitioning of resources may occur for the water and nutrient uptake from different soil depths due to variations in root length and/or diameter, resulting in a more even occupation of soil volume [18,19]. In this case, efficient belowground resource utilization can also positively impact aboveground sward canopy performance in terms of production. Temporal resource partitioning involves species within mixtures being dominant at different times based on genetics, phenological state, or resource availability. This mechanism results in reduced intra-annual variation in herbage accumulation, thus minimizing seasonality [20].

The balance of biodiverse ecosystems influences two central characteristics that determine ecological niche complementarity and multifunctionality: (i) richness of present species and (ii) homogeneity of distribution among individuals of those species [21]. Various factors, including environmental inputs and management practices, can influence the complementary interactions among species within a mixture. In this context, fertile environments subject to frequent and lenient defoliations tend to result in lower levels of disturbance (defoliation intensity) and stress (competition for resources), particularly concerning competition for light and nutrients. These conditions favor ideal conditions for species to coexist and express their traits according to their genetic programming throughout different seasons of the year [22–24]. Competition for resources can occur both aboveground and belowground [25] or solely aboveground for light [26,27]. Competition for light suggests that understory species experience reduced light availability as productivity increases, potentially leading to their exclusion by faster-growing or taller species that efficiently capture this resource [26].

The hypothesis provided to explain greater competition for light and less competition for soil nutrients in the same ecosystem is based on the idea that light is a resource that comes only from above and must be used immediately, offering a limited opportunity to differentiate species' space niches. Conversely, soil resources such as macronutrients, micronutrients, and water can be acquired by roots in various dimensions and time scales [28–30]. Enhanced resource availability in the soil can alleviate resource depletion and promote greater richness and diversity of species belowground than aboveground [29].

Functional associations of forage plant species with contrasting growth strategies managed and fertilized to minimize competition for resources pose a current challenge. Many studies addressing this topic focus on ecological aspects and overlook agronomic management. However, given the demand for increased food production, the intensification of pastoral production systems through nitrogen fertilization is crucial, particularly for species and genotypes of perennial tropical grasses, which typically respond to nitrogen fertilization. Some studies have highlighted that inorganic fertilizers in biodiverse ecosystems favor plant competition, especially for light [23,26]. Additionally, research indicates that resource-conservative forage species groups tend to dominate unfertilized grasslands, whereas exploitative species groups prevail in grasslands with nutrient-rich soil [31]. However, other studies suggest that herbivory may offset species losses and mitigate competition for light [22,23]. Thus, these findings underscore the importance of adopting appropriate defoliation frequency as a management strategy to avoid competition for light and to facilitate the intensification of multispecific pasture systems.

## 3. Plant Species Selection to Compose an Association

The selection of forage plant species or genotypes to establish an association must consider, among other factors, characteristics associated with complementarity and/or facilitation of access to resources [32]. Species may use the same or different resources at different times or spaces (complementarity) [33], both aboveground [34] (Figure 1A) and belowground [18] (Figure 1B). Similarly, some species may alleviate adverse conditions and increase the availability of resources for other groups of species, a species-inherent mechanism beneficial to the associated species [35] (Figure 1C). This mechanism can mitigate potential impacts of diseases and pests when at least one species in the association is tolerant or resistant (facilitation effect) [10,36,37] (Figure 1D). Therefore, species with contrasting growth strategies and different survival mechanisms and interactions with neighboring species may coexist and maintain stable populations when the management practices are adopted to control competition for resources. It is also important for each ecosystem to consider the objectives outlined for the mixtures, specifically the functional traits of the species used and possible ecosystem services that the mixed pasture may offer. According to Hanisch [38], in a systematic review of the literature based on results from pastures in temperate climate regions, 40 functional characteristics and 11 ecosystem services were identified in 108 studies. This suggests that the functional characteristics of species are related to services provided by ecosystems. However, it is necessary to highlight that not all ecosystem services can be improved simultaneously in large-scale operations outside research plots. Therefore, understanding the functional traits of the species and their ecosystem services is vital for selecting different species combinations that form associations tailored to specific objectives.

Another strategy used to select forage species for composing mixtures is the combination of plants with contrasting growth strategies, such as conservative or resource-gathering plant groups. The functional traits of individual species delineate their capacity to capture and utilize available resources, which are inherently tied to their ecological roles within the ecosystem. Consequently, these traits serve as pivotal indicators for categorizing species into functional groups, facilitating informed decisions on selecting species to combine in mixtures. In this context, Cruz [39] suggested a specific classification for pastoral ecosystems emphasizing the ability of plants to acquire or, conversely, conserve nutrients as well as adapt to different levels of defoliation (Table 1).

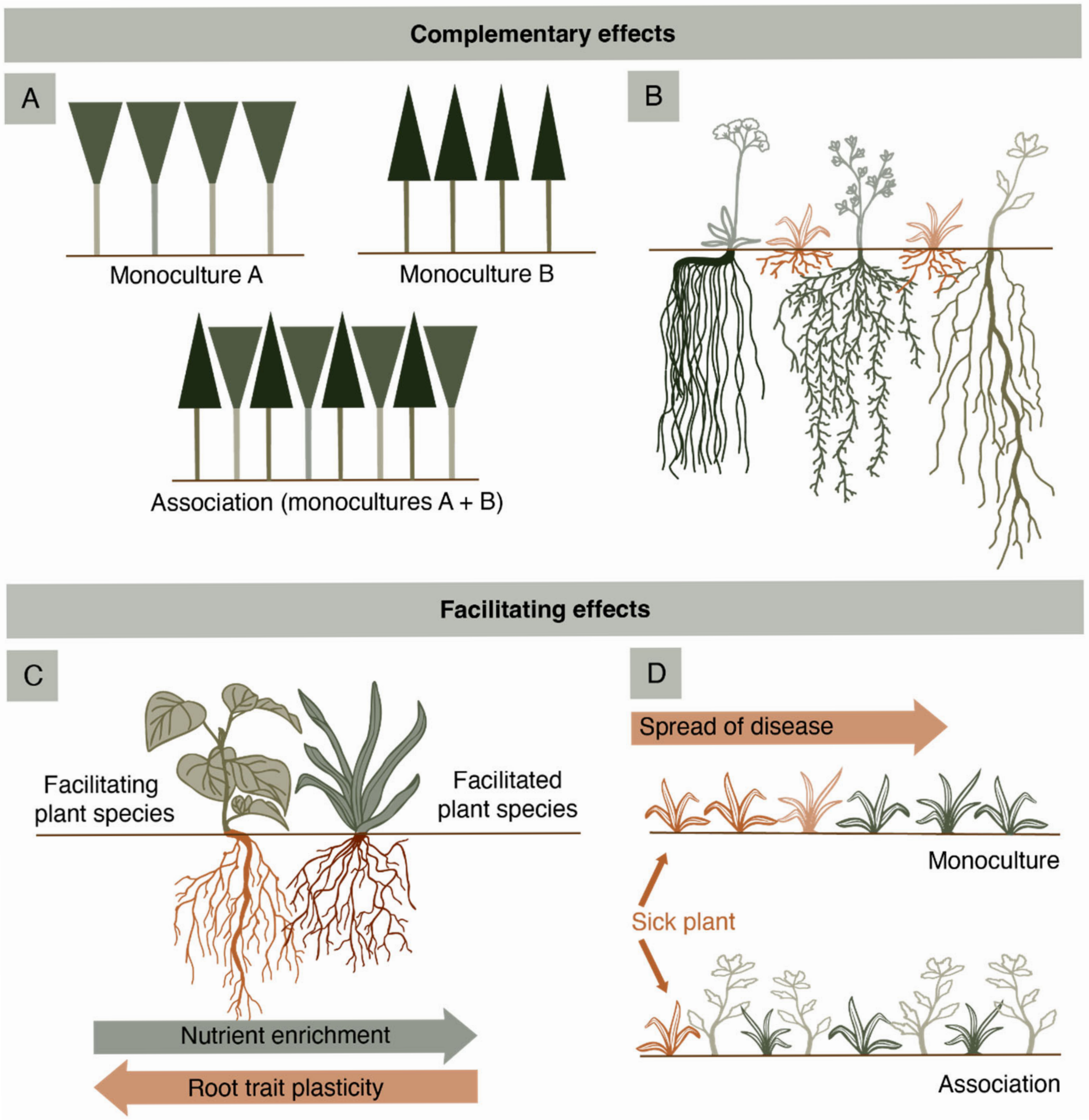


**Figure 1.** Potential effects of species association on canopy space filling in even-aged stands when plant crowns have complementary shapes (**A**), adapted [34]. Categories of root traits that can potentially impact ecosystem processes (**B**), adapted [18]. Belowground interspecific facilitation that requires matching of benefactors and beneficiaries (**C**), adapted [35]. Overview of disease transmission in monocultures and plant associations with resistant species (**D**), adapted [36].

Based on this classification, different species within the same family can be grouped into distinct functional groups according to their growth mechanisms in response to defoliation frequency and nutrient availability [39] These groupings are determined by their strategies for capturing and using resources. Agronomic characteristics and associated biological traits, summarized in Table 1, play a vital role in shaping plant growth strategies. Characteristics such as leaf lifespan and leaf appearance rate are key factors influencing herbage mass accumulation over time and grazing management. Species with more pronounced vertical development are less suited to frequent defoliation but exhibit better herbage mass accumulation. Certain studies, such as [40], have outlined that species selection holds greater significance than the complexity of the mixture aiming at greater forage mass and forage nutritive value. In this scenario, selecting species with complementary growth and resource use strategies is viable and can be an alternative to productive and stable stands [14,41]. In this context, some possible associations aimed at functional coexistence can be suggested, such as combinations of type A or B plants (resource capture) with type C or D plants (resource conservation).

**Table 1.** Plant characteristics grouped by growth strategy or functional group as a function of fertility and defoliation level, adapted.

| | Resource Capture (Fertile Environments) | Resource Conservation (Not Fertile Environments) |
| --- | --- | --- |
| Rapid tissue cycling; frequent and severe defoliation | **Type A**<br>- High growth rate;<br>- Peak of growth achieved quickly;<br>- High digestibility and nutrient content;<br>- Low efficiency in the use of soil minerals. | **Type C**<br>- Low growth rate;<br>- Peak growth achieved late;<br>- Low digestibility and nutrient content;<br>- High efficiency in the use of soil minerals. |
| Slow tissue cycling; infrequent and lenient defoliation | **Type B**<br>- High growth rate;<br>- Peak growth achieved late;<br>- Satisfactory digestibility and mineral content;<br>- Moderate efficiency in the use of soil minerals. | **Type D**<br>- Low growth rate;<br>- Peak growth achieved late;<br>- Very low digestibility and nutrient content;<br>- High efficiency in the use of soil minerals. |

A different theory postulated by [24] classified plants based on their different forms of growth and adaptation to a wide variety of environments as competitors (C), stress-tolerant (S), ruderals (R), and their combinations (Figure 2A). In that study, a general perspective was drawn on the range of strategies encompassed by certain life forms or taxonomic groups. However, perennial herbs and ferns (Figure 2D) were classified as encompassing three growth forms, indicating that the current classification by functional groups warrants further investigation. Additionally, in a global meta-analysis, ref. [42] analyzed several functional traits of plants and concluded that they are isolated poor predictors of long-term ecosystem functioning. They emphasized the need to incorporate data on additional factors, including interactive abiotic factors components (e.g., soil characteristics, topography, climate, disturbances, and their intricate interactions with plant attributes). This holistic approach is crucial for enhancing ecosystem linkages and accurately classifying plants into specific functional groups. Consequently, selecting plant species to form an association remains an unresolved task.

The increase in richness and diversity of plant species or genotypes in pastures has been associated with improved ecosystem services, such as greater forage production [43–45], productive stability [14,46], nutritional value [40,47], root mass and depth, improved quality of the soil [48–51], mitigation of environmental impacts [50,52,53], reduced weed pressure [54], and improved animal performance [41,55–57]. Despite its many benefits, research involving the association of forage species is primarily focused on pastures from temperate climate regions, especially associations between forage grasses and legumes or those including annual species. On the other hand, this type of study is rarely conducted under tropical conditions and environments, particularly when well-managed perennial grasses are involved (Figure 3).

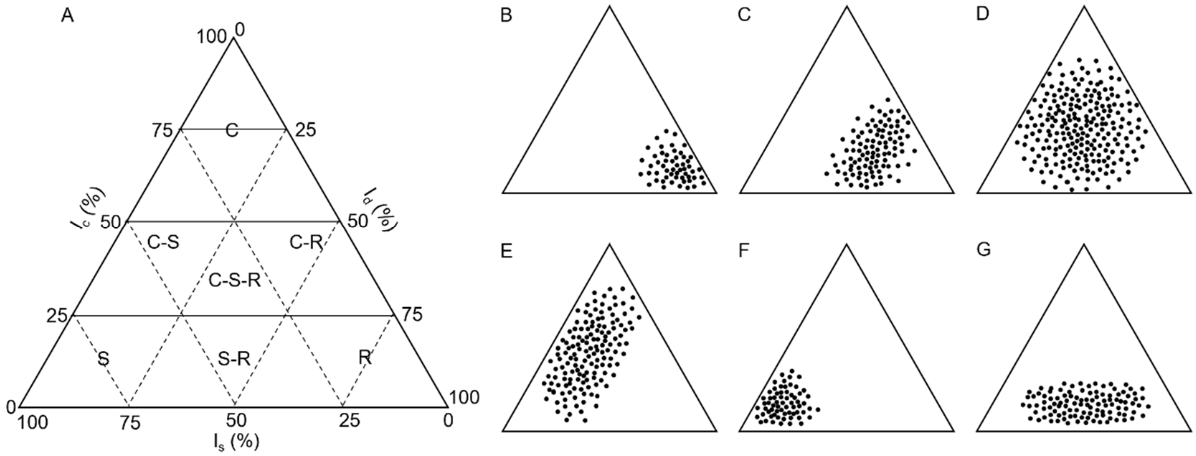


**Figure 2.** Classification of plants based on different forms of growth and adaptation to different environments such as competitor (C), stress-tolerant (S), ruderal (R), competitor-ruderal (C-R), competitor stress-tolerant (C-S), ruderal stress-tolerant (S-R), and C-S-R plants (**A**). Diagrams describing the range of strategies encompassed by annual herbs (**B**), biennial herbs (**C**), perennial herbs and ferns (**D**), trees and shrubs (**E**), lichens (**F**), and bryophytes (**G**), adapted [24].

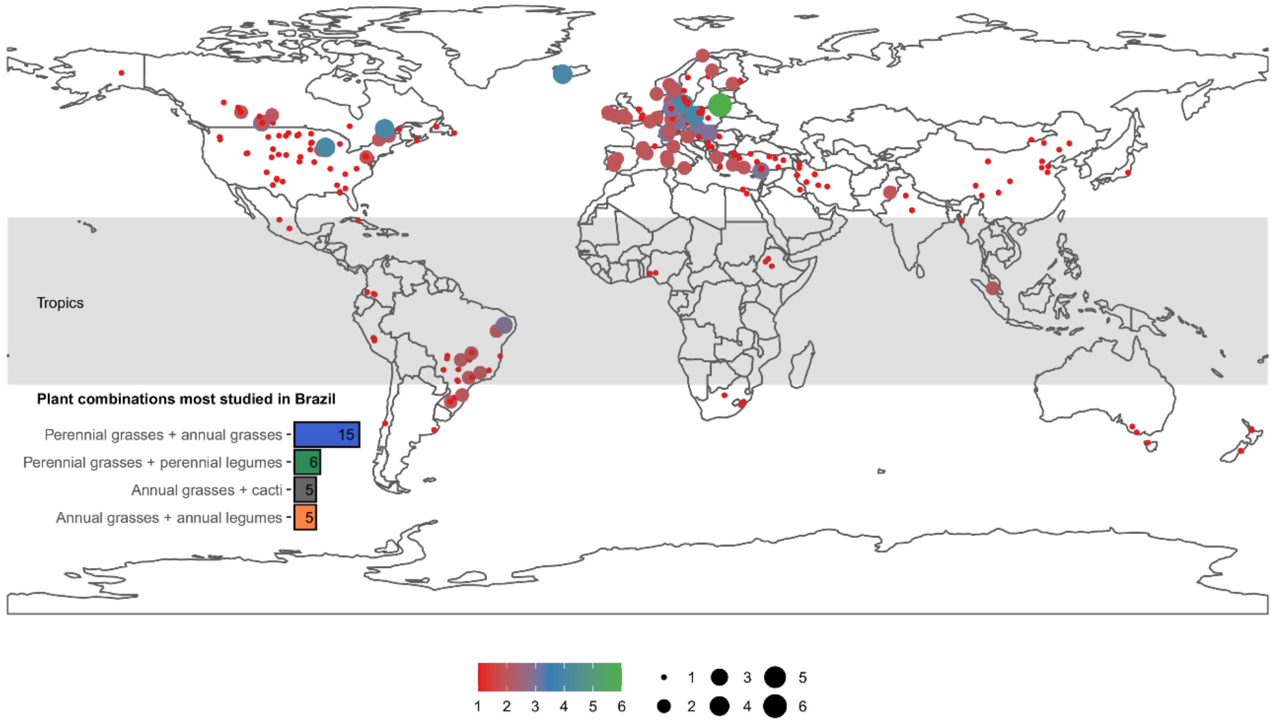


**Figure 3.** Global distribution of research involving associations of forage plants and types of plant combinations. A systematic search was conducted on a data set (ISI Web of Science Core Collection) (title session) to identify research articles published between 1 January 2000 and 17 September 2022. The search terms used were (“grassland*” or “pasture*” or “forage*”) and (“association*” or “mixture*” or “intercropping*”). The result was a total of 571 non-duplicated research articles. Titles and abstracts were screened to select articles that reported the association of forage plants in rangelands and/or grasslands, or those involving multiple associations with annual crops. Subsequently, the geographic coordinates reported in the selected articles were extracted to assemble the database. Single studies may have collected data from different locations. The total number of articles included in the study was 248.

In Brazil, these associations normally comprise perennial grasses + annual grasses (e.g., *Brachiaria* + *Zea mays*) and perennial grasses + perennial legumes (e.g., *Brachiaria* + *Arachis*) in the Cerrado and Atlantic Forest regions; annual grasses + cacti (e.g., *Sorghum bicolor* + *Opuntia*) in the Caatinga and part of the Northern Cerrado region; and annual grasses + annual legumes (e.g., *Avena sativa* + *Vicia faba*) in the Southern Atlantic Forest and Pampa regions.

## 4. Opportunity to Explore Grass Mixtures in Brazil

In Brazil, pastures cover approximately 45% of agricultural lands, totaling around 160 million hectares [58]. Given the edaphoclimatic and seasonal production characteristics and wide diversity of species and genotypes of forage grasses (Table 2) [59], the association of forage grasses in pastures would be relevant and strategic to expand the potential for use, productivity, and sustainability of pasture-based systems. In this context, the association of perennial forage grasses would have a high potential for use, favoring their dissemination in production areas, especially in those where pasture recovery is necessary.

**Table 2.** Species and genotypes of tropical perennial forage grasses.

| | |
|---|---|
| **Genus *Brachiaria*** | |
| *Brachiaria brizantha* cv. Marandu | *Brachiaria humidicola* cv. Llanero |
| *Brachiaria brizantha* cv. La Libertad | *Brachiaria humidicola* cv. Tupi |
| *Brachiaria brizantha* cv. Xaraés | *Brachiaria ruziziensis* cv. Kennedy |
| *Brachiaria brizantha* cv. BRS Piatã | Híbrido BRS RB331 Ipyporã |
| *Brachiaria brizantha* cv. BRS Paiaguás | Híbrido Mulato II |
| *Brachiaria brizantha* cv. MG 13 Braúna | Híbrido Mavuno |
| *Brachiaria decumbens* cv. Basilisk | Híbrido CIAT BR02/1752 Cayman |
| *Brachiaria decumbens* cv. Tully | Híbrido CIAT BR02/1752 Cobra |
| **Genus *Cynodon*** | |
| *Cynodon* spp. cv. Coastcross | *Cynodon* sp. cv. Florakirk |
| *Cynodon* spp. cv. Tifton 78 | *Cynodon nlemf.* Vand. cv. Tifton 68 |
| *Cynodon* spp. cv. Tifton 85 | *Cynodon nlemf.* V. var. *nlemf.* cv. Florico |
| *Cynodon* spp. var. Jiggs | *Cynodon nlemf.* V. var. *nlemf.* cv. Florona |
| **Genus *Panicum maximum*** | |
| *Panicum maximum* cv. Mombaça | *Panicum maximum* cv. Aruana |
| *Panicum maximum* cv. Tanzânia | *Panicum maximum* cv. Vencedor |
| *Panicum maximum* cv. Massai | *Panicum maximum* cv. Centenário |
| *Panicum maximum* cv. BRS Zuri | *Panicum maximum* cv. Centauro |
| *Panicum maximum* cv. BRS Tamani | *Panicum maximum* cv. Áries |
| *Panicum maximum* cv. BRS Quênia | *Panicum maximum* cv. Atlas |
| *Panicum maximum* cv. Sempre Verde | *Panicum maximum* cv. Tobiatã |
| **Genus *Paspalum*** | |
| *Paspalum atratum* | *Paspalum guenoarum* |
| *Paspalum notatum* | *Paspalum dilatatum* |
| *Paspalum regnellii* | *Paspalum plicatulum* |
| **Genus *Pennisetum purpureum*** | |
| *Pennisetum purpureum* cv. BRS Capiaçu | *Pennisetum purpureum* cv. Mineiro |
| *Pennisetum purpureum* cv. BRS Kurumi | *Pennisetum purpureum* cv. Cameroon |
| *Pennisetum purpureum* cv. BRS Canará | *Pennisetum purpureum* cv. Roxo Botucatu |
| *Pennisetum purpureum* cv. Pioneiro | *Pennisetum purpureum* cv. Paraíso |
| *Pennisetum purpureum* cv. Napier | |
| **Genus divers** | |
| *Andropogon gayanus* cv. Planaltina | *Hyparrhenia rufa* (Ness) Stapf |
| *Andropogon gayanus* cv. BRS Sarandi | *Digitaria decumbens* Stent. |
| *Chloris gayana* Kunth | *Setaria anceps* Stapf. ex. Massey |
| Melinis minutiflora, Beauv. | Pennisetum cland. Hochst. ex. Chiov |

Given the diverse edaphoclimatic conditions and seasonal production characteristics, as well as the wide variety of forage grass species and genotypes (Table 2) [59], incorporating combinations of these grasses in pastures presents an intriguing and strategic approach to expanding the utilization, productivity, and sustainability of pasture-based systems.

These associations are already used informally in pasture-based animal production systems in tropical biomes. However, many questions must be answered before their widespread adoption. A comprehensive understanding of resource utilization dynamics, agronomic performance, and interspecific competition among species comprising these mixtures is crucial. Such knowledge is crucial to understanding these ecosystems, as well as determining and defining their management requirements.

In Brazil, the most widely used species and genotypes of tropical perennial forage grasses, along with their morphological and agronomic traits, are described in Table 3 [59,60].

These morphological and agronomic traits of common species and genotypes in Brazil can help identify suitable mixture combinations, considering the ecosystem services these mixtures may provide [38]. For example, if the goal is to have denser canopies, mixtures can be chosen between plants with different growth habits and heights, making the canopies more filled along the vertical profile [61]. It is also possible to combine plants so that at least one species is tolerant of pests and diseases, promoting facilitation mechanisms [10,37,38]. Or, if the goal is to avoid simultaneous flowering of the pasture, mixtures of plants that flower at different times can be combined.

A major challenge with these mixtures is keep them perennial and prevent them from turning into monocultures through competition for resources. A study conducted by [61] evaluated three tropical forage grasses (*Andropogon gayanus* cv. Planaltina, *Panicum maximum* cv. Massai, and *Brachiaria brizantha* cv. BRS Piatã) grown both in monoculture and in association (with the three species in equal proportions based on viable seed numbers). All treatments were managed under the same defoliation regime with a pre-cut height of 35 cm and post-cut height of 17.5 cm. It was observed that the association had the same forage mass productivity as the most productive monoculture. However, the association was shaped by light competition and little nitrogen competition, with *Panicum maximum* cv. Massai showing a higher botanical proportion. The functional traits of the species in monoculture indicate that *Panicum maximum* cv. Massai has a greater leaf angle and low leaf area index in the top 10 cm, resulting in a higher light proportion in the vertical profile of the canopy and favoring rapid leaf elongation. This resulted in a higher population density of tillers, favoring the shading of the other two species in the association and causing competition for light. *Brachiaria brizantha* cv. BRS Piatã exhibited a lower rate of leaf elongation per tiller, which may have been caused by the combination of a large leaf area index and a smaller leaf angle, leading to self-shading of the leaves at the base of the pasture canopy. *Andropogon gayanus* cv. Planaltina showed the greatest final leaf length and the fewest leaves per tiller, which explains its lower proportion and productivity in both monoculture and association. The study concludes that leaf angle, leaf elongation rate per tiller, number of leaves per tiller, and leaf area index are functional traits that shape the dynamics of light competition, botanical proportion, and productive performance of grass species in association, and should be considered when selecting grass species for new mixtures. The same study highlighted that no nitrogen competition was observed between plants in the association.

**Table 3.** Common species and genotypes of tropical perennial forage grasses in Brazil and their morphological and agronomic traits.

| Forage Species | Growth | Plant Height Under Grazing (cm) | Leaf Architecture | Leaf Blade Width (mm) | Flowering | Fertility Requirement | Drought Tolerance | Flooding Tolerance | Resistance to Pasture Leafhoppers | Resistance to Foliar Diseases | Shade Tolerance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Andropogon gayanus* cv. Planaltina | Caespitose | 50 | Erect | 15 | March–April | Low–Medium | High | Low | Medium | Medium | Low |
| *Brachiaria brizantha* cv. BRS Paiaguás | Intermediary | 30 | Erect | 14 | December | Medium | Medium | Low | Very low | Medium | Medium |
| *Brachiaria brizantha* cv. BRS Piatã | Semi-erect | 35 | Arched | 15 | January–February | Medium | High | Low | High | Medium | Medium |
| *Brachiaria brizantha* cv. Marandu | Intermediary | 30 | Arched | 19 | March | Medium | Medium | Very low | High | Low | Medium |
| *Brachiaria brizantha* cv. Xaraés | Semi-erect | 30 | Arched | 24 | May–June | Medium | Medium | Low | Medium | Medium | Medium |
| *Brachiaria decumbens* cv. Basilisk | Semi-prostrate | 30 | Geniculate | 14 | December | Low–Medium | High | Low | Very low | High | Low |
| *Brachiaria* Híbrida BRS Ipyporã | Semi-prostrate | 35 | Erect | 18 | April | Medium | Medium | Very low | Very low | High | Medium |
| *Brachiaria humidicola* cv. Llanero | Semi-prostrate | 30 | Erect | 10 | January–February | Low–Medium | Medium | High | Medium | Medium | Low |
| *Brachiaria humidicola* cv. Tully | Prostrado | 30 | Erect | 7 | January | Low–Medium | Medium | High | Medium | Medium | Low |
| *Brachiaria ruziziensis* cv. Kennedy | Intermediary | 35 | Arched | 16 | March | Medium–High | High | Low | Very low | High | Medium |
| *Panicum maximum* cv. BRS Quênia | Caespitose | 70 | Arched | 31 | February–March | Medium–High | High–Medium | Low | High | Medium | Medium |
| *Panicum maximum* cv. BRS Tamani | Caespitose | 50 | Arched | 17 | March | Medium–High | High–Medium | Low | High | High | High |
| *Panicum maximum* cv. BRS Zuri | Caespitose | 70 | Arched | 47 | April | Medium–High | High–Medium | Medium–High | High | High | Medium |
| *Panicum maximum* cv. Massai | Caespitose | 55 | Erect | 18 | March-April | Medium | Medium | Medium–High | High | High | High |
| *Panicum maximum* cv. Mombaça | Caespitose | 90 | Geniculate | 36 | March–April | Medium–High | High–Medium | Medium–High | High | High | High |
| *Panicum maximum* cv. Tanzânia | Caespitose | 70 | Arched | 32 | March–April | Medium–High | High–Medium | Medium–High | High | Medium | High |

In studies conducted concurrently in the same experimental area mentioned above, it was observed that the association had complementarity effects and changes in resource use, contributing to a reduction in $N_2O$ emissions from the soil [62]. Additionally, the canopy structure of the mixture allowed for higher forage intake rates than monocultures during winter/early spring, indicating the possibility of combining tropical forage grass species without compromising the canopy structure and grazing animal responses, compared to pastures of a single grass species [63].

Perennial grasses have undergone a long evolution, allowing them to adapt to grazing and expand their storage capacity for organic reserves. This adaptation ensures resilience and perenniality, as well as the rapid regrowth and restoration of canopy leaf area after defoliation, a process that occurs more slowly in other forage species [30]. In addition, $C_4$ grasses use resources more efficiently than legumes which can lead to disproportionate interspecies competition or excessive grazing of legumes, reducing diversity and ecosystem success in some cases [43]. In this context, mixtures comprising only well-managed perennial grasses would be an adaptable alternative, with benefits that would allow sustainable production and ensure the provision of important ecosystem services. Furthermore, understanding how a grass-only association works may be important for future legume species to be inserted into the ecosystem to provide nitrogen for grasses.

## 5. Research Priorities

Future studies should focus on more diverse associations while minimizing resource competition. Initial efforts should prioritize fieldwork to produce several grass species or forage genotypes from tropical climates across different edaphoclimatic conditions, fertility, and management. In light of the data obtained, grouping the grass species into functional groups 6o distinguish resource conservers from competitors, becomes essential. Assessing how functional traits vary across different scenarios and identifying grass species that best adapt across different biomes and technological contexts is important, along with understanding the ecosystem services they can provide when grouped. This functional grouping can also be achieved by synthesizing existing research findings through meta-analysis, particularly focusing on traits that characterize conservative and competitive plant species. This approach will identify key functional traits for selecting grass species to compose complementary mixtures, while also informing adequate management strategies such as defoliation intensity and fertilization. Emphasis should be placed on strategies ensuring long-term pasture persistence and multifunctionality.

Additionally, evaluating established methods for these mixtures is crucial—for example, comparing methods such as broadcast seeding, mixed-row planting, and interspersed-row planting. Investigating seed proportions, germination rates, dormancy periods, developmental timelines, and fertilization levels of grass mixtures is essential to understanding the dynamics and complexity of tropical forage grass associations. These insights will enable the formulation of more functional multispecific pastoral ecosystems.

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## References


1. Li, W.; He, S.; Cheng, X.; Zhang, M. Functional diversity outperforms taxonomic diversity in revealing short-term trampling effects. *Sci. Rep.* **2021**, *11*, 18889. [CrossRef] [PubMed]
2. Tilman, D.; Reich, P.B.; Knops, J.; Wedin, D.; Mielke, T.; Lehman, C. Diversity and productivity in a long-term grassland experiment. *Science* **2001**, *294*, 843–845. [CrossRef] [PubMed]
3. Chen, C.; Chen, H.Y.H.; Chen, X.; Huang, Z. Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nat. Commun.* **2019**, *10*, 1332. [CrossRef] [PubMed]
4. Martin-Guay, M.O.; Paquette, A.; Dupras, J.; Rivest, D. The new Green Revolution: Sustainable intensification of agriculture by intercropping. *Sci. Total Environ.* **2018**, *615*, 767–772. [CrossRef]
5. Scherber, C.; Eisenhauer, N.; Weisser, W.W.; Schmid, B.; Voigt, W.; Fischer, M.; Schulze, E.D.; Roscher, C.; Weigelt, A.; Allan, E.; et al. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **2010**, *468*, 553–556. [CrossRef]
6. Callaway, R.M. Positive interactions among plants. *Bot. Rev.* **1995**, *61*, 306–349. [CrossRef]
7. Loreau, M. Separating Sampling and Other Effects in Biodiversity Experiments. *Oikos* **1998**, *82*, 600. [CrossRef]
8. Loreau, M.; Hector, A. Partitioning selection and complementarity in biodiversity experiments. *Nature* **2001**, *412*, 72–76. [CrossRef]
9. Wang, S.; Isbell, F.; Deng, W.; Hong, P.; Dee, L.E.; Thompson, P.; Loreau, M. How complementarity and selection affect the relationship between ecosystem functioning and stability. *Ecology* **2021**, *102*, e03347. [CrossRef]
10. Wright, A.J.; Wardle, D.A.; Callaway, R.; Gaxiola, A. The Overlooked Role of Facilitation in Biodiversity Experiments. *Trends Ecol. Evol.* **2017**, *32*, 383–390. [CrossRef]
11. Roscher, C.; Schumacher, J.; Gubsch, M.; Lipowsky, A.; Weigelt, A.; Buchmann, N.; Schmid, B.; Schulze, E.D. Using Plant Functional Traits to Explain Diversity–Productivity Relationships. *PLoS ONE* **2012**, *7*, e36760. [CrossRef] [PubMed]
12. Gross, N.; Bagousse-Pinguet, Y.L.; Liancourt, P.; Berdugo, M.; Gotelli, N.J.; Maestre, F.T. Functional trait diversity maximizes ecosystem multifunctionality. *Nat. Ecol. Evol.* **2017**, *1*, 132. [CrossRef] [PubMed]
13. Wagg, C.; Ebeling, A.; Roscher, C.; Ravenek, J.; Bachmann, D.; Eisenhauer, N.; Mommer, L.; Buchmann, N.; Hillebrand, H.; Schmid, B.; et al. Functional trait dissimilarity drives both species complementarity and competitive disparity. *Funct. Ecol.* **2017**, *31*, 2320–2329. [CrossRef]
14. Duchini, P.G.; Guzatti, G.C.; Echeverria, J.R.; Américo, L.F.; Sbrissia, A.F. Can a Mixture of Perennial Grasses with Contrasting Growth Strategies Compose Productive and Stable Swards? *Agron. J.* **2019**, *111*, 224–232. [CrossRef]
15. Hooper, D.U. The role of complementary and competition in ecosystem responses to variation in plant diversity. *Ecology* **1998**, *79*, 704–719. [CrossRef]
16. Barry, K.E.; van Ruijven, J.; Mommer, L.; Bai, Y.; Beierkuhnlein, C.; Buchmann, N.; de Kroon, H.; Ebeling, A.; Eisenhauer, N.; Guimarães-Steinicke, C.; et al. Limited evidence for spatial resource partitioning across temperate grassland biodiversity experiments. *Ecology* **2020**, *101*, e02905. [CrossRef]
17. Duchini, P.G.; Guzatti, G.C.; Ribeiro-Filho, H.M.N.; Sbrissia, A.F. Intercropping black oat (Avena strigosa) and annual ryegrass (Lolium multiflorum) can increase pasture leaf production compared with their monocultures. *Crop Pasture Sci.* **2016**, *67*, 574–581. [CrossRef]
18. Bardgett, R.D.; Mommer, L.; de Vries, F.T. Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* **2014**, *29*, 692–699. [CrossRef]
19. Mommer, L.; van Ruijven, J.; de Caluwe, H.; Smit-Tiekstra, A.E.; Wagemaker, C.A.M.; Joop Ouborg, N.; Bögemann, G.M.; van der Weerden, G.M.; Berendse, F.; de Kroon, H. Unveiling below-ground species abundance in a biodiversity experiment: A test of vertical niche differentiation among grassland species. *J. Ecol.* **2010**, *98*, 1117–1127. [CrossRef]
20. Hector, A.; Hautier, Y.; Saner, P.; Wacker, L.; Bagchi, R.; Joshi, J.; Scherer-Lorenzen, M.; Spehn, E.M.; Bazeley-White, E.; Weilenmann, M.; et al. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* **2010**, *91*, 2213–2220. [CrossRef]
21. Magurran, A.E. *Measuring Biological Diversity*, 1st ed.; Blackwell Publishing: Oxford, UK, 2004.
22. Borer, E.T.; Seabloom, E.W.; Gruner, D.S.; Harpole, W.S.; Hillebrand, H.; Lind, E.M.; Adler, P.B.; Alberti, J.; Anderson, T.M.; Bakker, J.D.; et al. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* **2014**, *508*, 517–520. [CrossRef] [PubMed]
23. Eskelinen, A.; Harpole, W.S.; Jessen, M.T.; Virtanen, R.; Hautier, Y. Light competition drives herbivore and nutrient effects on plant diversity. *Nature* **2022**, *611*, 301–305. [CrossRef] [PubMed]
24. Grime, J.P. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory on JSTOR. *Am. Nat.* **1977**, *111*, 1169–1194. [CrossRef]
25. Grime, J.P. Competitive Exclusion in Herbaceous Vegetation. *Nature* **1973**, *242*, 344–347. [CrossRef]

26. Hautier, Y.; Niklaus, P.A.; Hector, A. Competition for light causes plant biodiversity loss after eutrophication. *Science* **2009**, *324*, 636–638. [CrossRef] [PubMed]
27. Suding, K.N.; Collins, S.L.; Gough, L.; Clark, C.; Cleland, E.E.; Gross, K.L.; Milchunas, D.G.; Pennings, S. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4387–4392. [CrossRef]
28. Hiiesalu, I.; Öpik, M.; Metsis, M.; Lilje, L.; Davison, J.; Vasar, M.; Moora, M.; Zobel, M.; Wilson, S.D.; Pärtel, M. Plant species richness belowground: Higher richness and new patterns revealed by next-generation sequencing. *Mol. Ecol.* **2012**, *21*, 2004–2016. [CrossRef]
29. Pärtel, M.; Hiiesalu, I.; Öpik, M.; Wilson, S.D. Below-ground plant species richness: New insights from DNA-based methods. *Funct. Ecol.* **2012**, *26*, 775–782. [CrossRef]
30. Parton, W.J.; Stewart, J.W.B.; Cole, C.V. Dynamics of C, N, P and S in grassland soils: A model. *Biogeochemistry* **1988**, *5*, 109–131. [CrossRef]
31. Quétier, F.; Thébault, A.; Lavorel, S. Plant Traits in a State and Transition Framework as Markers of Ecosystem Response to Land-Use Change. *Ecol. Monogr.* **2007**, *77*, 33–52. [CrossRef]
32. Volaire, F.; Barkaoui, K.; Norton, M. Designing resilient and sustainable grasslands for a drier future: Adaptive strategies, functional traits and biotic interactions. *Eur. J. Agron.* **2014**, *52*, 81–89. [CrossRef]
33. Naeem, S.; Thompson, L.J.; Lawler, S.P.; Lawton, J.H.; Woodfin, R.M. Declining biodiversity can alter the performance of ecosystems. *Nature* **1994**, *368*, 734–737. [CrossRef]
34. Pretzsch, H. Canopy space filling and tree crown morphology in mixed-species stands compared with monocultures. *Ecol Manag.* **2014**, *327*, 251–264. [CrossRef]
35. Yu, R.P.; Lambers, H.; Callaway, R.M.; Wright, A.J.; Li, L. Belowground facilitation and trait matching: Two or three to tango? *Trends Plant Sci.* **2021**, *26*, 1227–1235. [CrossRef] [PubMed]
36. Ampt, E.A.; van Ruijven, J.; Zwart, M.P.; Raaijmakers, J.M.; Termorshuizen, A.J.; Mommer, L. Plant neighbours can make or break the disease transmission chain of a fungal root pathogen. *New Phytol.* **2022**, *233*, 1303–1316. [CrossRef]
37. Wright, A.J.; Mommer, L.; Barry, K.; van Ruijven, J. Stress gradients and biodiversity: Monoculture vulnerability drives stronger biodiversity effects during drought years. *Ecology* **2021**, *102*, e03193. [CrossRef]
38. Hanisch, M.; Schweiger, O.; Cord, A.F.; Volk, M.; Knapp, S. Plant functional traits shape multiple ecosystem services, their trade-offs and synergies in grasslands. *J. Appl. Ecol.* **2020**, *57*, 1535–1550. [CrossRef]
39. Cruz, P.; Duru, M.; Therond, O.; Theau, J.P.J.P.; Ducourtieux, C.; Jouany, C.; Khaled, R.A.H.; Ansquer, P. Une nouvelle approche pour caractériser les prairies naturelles et leur valeur d'usage—Association Francophone pour les Prairies et Fourrages. *Fourrages* **2002**, *172*, 335–354.
40. Deak, A.; Hall, M.H.; Sanderson, M.A. Grazing Schedule Effect on Forage Production and Nutritive Value of Diverse Forage Mixtures. *Agron. J.* **2009**, *101*, 408–414. [CrossRef]
41. Nieman, C.C.; Albrecht, K.A.; Schaefer, D.M. Temporal Composition of Alfalfa–Grass Pastures and Productivity Response of Holstein Steers. *Agron. J.* **2019**, *111*, 686–693. [CrossRef]
42. Plas, F.; Schröder-Georgi, T.; Weigelt, A.; Barry, K.; Meyer, S.; Alzate, A.; Barnard, R.L.; Buchmann, N.; de Kroon, H.; Ebeling, A.; et al. Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nat. Ecol. Evol.* **2020**, *4*, 1602–1611. [CrossRef] [PubMed]
43. Grace, C.; Boland, T.M.; Sheridan, H.; Lott, S.; Brennan, E.; Fritch, R.; Lynch, M.B. The effect of increasing pasture species on herbage production, chemical composition and utilization under intensive sheep grazing. *Grass Forage Sci.* **2018**, *73*, 852–864. [CrossRef]
44. Haughey, E.; Suter, M.; Hofer, D.; Hoekstra, N.J.; McElwain, J.C.; Lüscher, A.; Finn, J.A. Higher species richness enhances yield stability in intensively managed grasslands with experimental disturbance. *Sci. Rep.* **2018**, *8*, 15047. [CrossRef]
45. Sonkoly, J.; Kelemen, A.; Valkó, O.; Deák, B.; Kiss, R.; Tóth, K.; Miglécz, T.; Tóthmérész, B.; Török, P. Both mass ratio effects and community diversity drive biomass production in a grassland experiment. *Sci. Rep.* **2019**, *9*, 1848. [CrossRef] [PubMed]
46. Gross, K.; Cardinale, B.J.; Fox, J.W.; Gonzalez, A.; Loreau, M.; Wayne Polley, H.; Reich, P.B.; van Ruijven, J. Species richness and the temporal stability of biomass production: A new analysis of recent biodiversity experiments. *Am. Nat.* **2014**, *183*, 1–12. [CrossRef]
47. Nobilly, F.; Bryant, R.H.; Mckenzie, B.A.; Edwards, G.R. Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. *Proc. N. Z. Grassl. Assoc.* **2013**, *75*, 165–172. [CrossRef]
48. Bennett, J.A.; Koch, A.M.; Forsythe, J.; Johnson, N.C.; Tilman, D.; Klironomos, J. Resistance of soil biota and plant growth to disturbance increases with plant diversity. *Ecol. Lett.* **2020**, *23*, 119–128. [CrossRef] [PubMed]
49. Bresciano, D.; del Pino, A.; Borges, A.; Tejera, M.; Speranza, P.; Astigarraga, L.; Picasso, V. Perennial C4 grasses increase root biomass and carbon in sown temperate pastures. *N. Z. J. Agric. Res.* **2018**, *62*, 332–342. [CrossRef]
50. Cardinale, B.J. Biodiversity improves water quality through niche partitioning. *Nature* **2011**, *472*, 86–89. [CrossRef]

51. Yang, Y.; Tilman, D.; Furey, G.; Lehman, C. Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nat. Commun.* **2019**, *10*, 718. [CrossRef]
52. Abagandura, G.O.; Sekaran, U.; Singh, S.; Singh, J.; Ibrahim, M.A.; Subramanian, S.; Owens, V.N.; Kumar, S. Intercropping kura clover with prairie cordgrass mitigates soil greenhouse gas fluxes. *Sci. Rep.* **2020**, *10*, 7334. [CrossRef] [PubMed]
53. Lange, M.; Eisenhauer, N.; Sierra, C.A.; Bessler, H.; Engels, C.; Griffiths, R.I.; Mellado-Vázquez, P.G.; Malik, A.A.; Roy, J.; Scheu, S.; et al. Plant diversity increases soil microbial activity and soil carbon storage. *Nat. Commun.* **2015**, *6*, 6707. [CrossRef] [PubMed]
54. Finn, J.A.; Kirwan, L.; Connolly, J.; Sebastià, M.T.; Helgadottir, A.; Baadshaug, O.H.; Bélanger, G.; Black, A.; Brophy, C.; Collins, R.P.; et al. Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *J. Appl. Ecol.* **2013**, *50*, 365–375. [CrossRef]
55. Dillard, S.L.; Hancock, D.W.; Harmon, D.D.; Mullenix, M.K.; Beck, P.A.; Soder, K.J. Animal performance and environmental efficiency of cool- and warm-season annual grazing systems. *J. Anim. Sci.* **2018**, *96*, 3491–3502. [CrossRef]
56. Jonker, A.; Farrell, L.; Scobie, D.; Dynes, R.; Edwards, G.; Hague, H.; McAuliffe, R.; Taylor, A.; Knight, T.; Waghorn, G. Methane and carbon dioxide emissions from lactating dairy cows grazing mature ryegrass/white clover or a diverse pasture comprising ryegrass, legumes and herbs. *Anim. Prod. Sci.* **2018**, *59*, 1063–1069. [CrossRef]
57. Pembleton, K.G.; Hills, J.L.; Freeman, M.J.; McLaren, D.K.; French, M.; Rawnsley, R.P. More milk from forage: Milk production, blood metabolites, and forage intake of dairy cows grazing pasture mixtures and spatially adjacent monocultures. *J. Dairy Sci.* **2016**, *99*, 3512–3528. [CrossRef]
58. IBGE. URL 2017. Available online: https://censoagro2017.ibge.gov.br/templates/censo_agro/resultadosagro/index.html (accessed on 1 September 2023).
59. Fonseca, M.D.; Martuscello, J.N. *Plantas Forrageiras*, 2nd ed.; Editora UFV: Viçosa, Minas Gerais, Brazil, 2022.
60. Pasto Certo. **Pasto Certo: Soluções para Pastagens e Nutrição Animal. 2023. Available online: https://www.pastocerto.com/ (accessed on 1 September 2023).
61. Silva, A.M.S. Association of Tropical Forage Grasses in Pastures: Agronomic Aspects That Determine Plant Performance. Ph.D. Thesis, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, Brazil, 2023. [CrossRef]
62. Gomes, C.M. Association of Tropical Forage Grasses in Pastures: An Opportunity for Sustainable Intensification. Ph.D. Thesis, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, Brazil, 2023. [CrossRef]
63. Otaviano, E.K.S.; Mammana, A.F.; Gomes, C.M.; Silva, A.M.S.; Carvalho, L.F.G.; Sbrissia, A.F.; Barbosa, R.A.; Sollenberger, L.E.; Silva, S.C. Canopy structure and herbage intake rate of three tropical forage grasses cultivated as pure or mixed stands. *Crop Pasture Sci.* **2024**, *75*, CP24125. [CrossRef]