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DANIEL AUGUSTO BARRETA

HERBAGE PRODUCTION AND VEGETATION DYNAMICS OF MULTISPECIES PASTURES MANAGED AT TWO PRE-GRAZING CANOPY HEIGHTS AND THREE NITROGEN FERTILIZATION RATES

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Dedico este compilado de pensamentos à
minha família, pela inspiração de sempre!

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“*Verba volant, scripta manent*”
– Proverbio Latim

Por mais longe que um homem vá,
jamais fugirá de si.
- Jayme Caetano Braun

Les circuits de consécration sociale, sera
d'autant plus efficace plus la distance
sociale de l'objet consacrée / “Qual é o
elogio mais ineficaz do mundo? O próprio”
– Pierre Bourdieu / Clóvis de Barros Filho

RESUMO

BARRETA, Daniel Augusto. **Produção de forragem e dinâmica da vegetação em pastos multiespecíficos manejados em duas alturas de pré-pastejo e três doses de fertilização nitrogenada**. 2023. 119p. Tese (Doutorado em Ciência Animal – Área: Produção Animal). Universidade do Estado de Santa Catarina. Programa de Pós-Graduação em Ciência Animal. Lages, 2023.

A construção de ambientes pastoris com plantas funcionalmente distintas tem sido considerada como uma ferramenta importante para a intensificação sustentável dos sistemas produtivos. Entretanto, o desafio destes sistemas é a preservação da diversidade das espécies ao longo do tempo. O objetivo deste trabalho foi determinar estratégias de manejo que combinem diversidade de espécies e produção de forragem em uma pastagem multiespecífica de gramíneas e leguminosas e identificar se o manejo adotado durante a estação quente promove um efeito na dinâmica da vegetação na estação fria subsequente. Para isto, uma área experimental foi formada com as espécies *Arachis pintoi*, *Cenchrus clandestinus*, *Cynodon* spp., *Lotus corniculatus* L. e *Trifolium repens* L., sobressemeadas anualmente com *Lolium multiflorum* L. na estação fria. O delineamento utilizado foi de blocos completos casualizados, em arranjo fatorial 2x3 com três repetições por tratamento. Os tratamentos foram duas alturas em pré-pastejo do pasto, 17 e 23 cm, associadas a três doses de adubação nitrogenada, 50, 150 e 250 kg de N ha^{-1}. Este manejo foi adotado durante a estação quente de 2020, 2021 e 2022 (novembro a abril), enquanto na estação fria dos mesmos anos (maio a outubro), o manejo foi o mesmo para todos os tratamentos, com altura em pré-pastejo de 20 cm associados a uma única adubação com 50 kg de N ha^{-1}. O método de lotação em ambas as épocas foi intermitente com a meta de altura pós-pastejo correspondente a 60% da altura em pré-pastejo. Não houve efeito de altura de manejo sobre a produção de forragem na estação quente, enquanto as doses de 150 e 250 kg de N ha^{-1} aumentaram a produção de forragem, mas diminuíram a diversidade de espécies, principalmente de leguminosas, comparadas à dose de 50 kg de N ha^{-1}. As espécies *Cenchrus clandestinus* e *Cynodon* spp. compuseram majoritariamente o dossel durante a estação quente e coexistiram em proporções similares independentemente do manejo. Na estação fria, a produção de forragem foi semelhante independentemente da dose de N aplicada no verão, enquanto a diversidade de espécies foi menor quando os pastos foram manejados com alturas de 17 cm no verão, com significativa redução no percentual de *Lotus corniculatus* L. na mistura. Nossos achados sugerem que em uma pastagem formada por gramíneas e leguminosas, um suprimento de N no verão que mantenha as gramíneas em um status de nutrição nitrogenada satisfatório (INN 0,8-1,0) reduz a diversidade de espécies no dossel e não afeta a produção de forragem na estação fria seguinte. Além disso, manejar uma mistura em uma altura próximo ao limite superior de altura de manejo correspondente ao $\text{IAF}_{\text{crítico}}$ é uma alternativa para atenuar a perda de diversidade de espécies, sem comprometer a produção de forragem.

Palavras-chave: coexistência entre plantas, diversidade funcional, multifuncionalidade, pastos multiespecíficos, serviços ecossistêmicos.

ABSTRACT

BARRETA, Daniel Augusto. **Herbage production and vegetation dynamics of multispecies pastures managed at two pre-grazing canopy heights and three nitrogen fertilization rates**. 2023. 119p. Thesis (Doctorate in Animal Science - Area: Animal Production) - Santa Catarina State University. Post Graduate Program in Animal Science, Lages, 2023.

The establishment of pastoral environments with functionally distinct plants has been considered an important tool for the sustainable intensification of productive systems. However, the challenge of these systems is the preservation of species diversity over time. The objective of this work was to determine management strategies that combine species diversity and forage production in a multispecific pasture composed by grasses and legumes and to identify whether the management adopted during the warm season promotes an effect on vegetation dynamics in the pasture growth in subsequent cool season. For this, an experimental area was formed with the species *Arachis pintoi*, *Cenchrus clandestinus*, *Cynodon* spp., *Lotus corniculatus* L. and *Trifolium repens* L., and sown *Lolium multiflorum* L. in the cool season. The experiment design was a complete randomized block design, assigned in a 2x3 factorial arrangement with three replications per treatment. The treatments were two pre-grazing heights, 17 and 23 cm, associated with three rates of nitrogen fertilization, 50, 150 and 250 kg of N ha^{-1}. This management was adopted during the warm season of 2020, 2021 and 2022 (November to April), while in the cool season of the same years (May to October), the management was the same for all treatments, with pre-grazing height of 20 cm associated with a single fertilization with 50 kg of N ha^{-1}. The stocking method in both seasons was intermittent with the target post-grazing height corresponding to 60% of the pre-grazing height. There was no effect of management height on forage production in the warm season, while rates of 150 and 250 kg of N ha^{-1} increased forage production, but decreased species diversity, mainly of legumes, compared to the 50 kg of N ha^{-1} rates. The species *Cenchrus clandestinus* and *Cynodon* spp. corresponded to around 85% the canopy during the warm season and coexisted in similar proportions regardless of management. In the cool season, forage production was similar regardless of the N rate applied in summer, while species diversity was lower when pastures were managed with heights of 17 cm in summer, with a significant reduction in the percentage of *Lotus corniculatus* L. in the mixture. Our findings suggest that in mixed pastures composed by grasses and legumes, a supply of N in the summer that maintain the grasses in a nitrogen nutrition status from 0.8 to 1.0 reduces species diversity in the canopy and does not affect forage production in the following cool season. In addition, managing a mixture at a height close to the upper limit of management height corresponding to the critical LAI is an alternative to attenuate the loss of species diversity, without compromising forage production.

Keywords: ecosystem services, functional diversity, multifunctionality, multispecific pastures, plants coexistence

FIGURES LIST

TABLES LIST

SUMMARY

1 INTRODUCTION

Brazil has a natural agricultural vocation, and it faces the challenge of remaining a world leader in food production while keeping to its environmental commitment. In this scenario, pasture-based animal production systems are critical since grasslands cover more than two-thirds of the country's agricultural acreage and have the capacity to minimize the environmental problems associated with livestock production. The southern region of Brazil is predominantly influenced by a subtropical climate, a condition defined by a large intra-annual thermal amplitude and the absence of a dry season. These attributes allow the cultivation of warm- and cool-season forage species in the same area during different periods of the year and the establishment of more complex and diverse plant communities, including many species of grasses and legumes.

Regarding the study and establishment of more diverse pastures, research conducted particularly in Europe, New Zealand and USA (Jaramillo et al., 2021a) over the past two decades demonstrates that mixed grasslands with up to five forage species outperform monocultures. Additional gains promoted by the inclusion of more species are marginal (Storkey et al., 2015) for two main reasons: only a few of the most productive species tend to become dominant in the community (selection effect), and the likelihood of new species exploiting a niche decrease with increasing species richness, thereby reducing the positive effects of plant complementarity (Lüscher et al., 2022).

Therefore, it is necessary to establish criteria in the selection of plants to compose more complex communities according to the system objectives, as it is the functional diversity of the species, and not the species diversity *per se*, that provides opportunities for more efficient resource use in a temporally or spatially defined environment (Daz & Cabido, 2001; Gross et al., 2017). Despite the potential benefits of multispecies grasslands, sustaining the species diversity is difficult, hence preventing farmers from adopting more diverse pastures (Lüscher et al., 2014).

This study assessed the influence of two pre-grazing height management strategies and three N rates on the vegetation dynamics of grass-legume mixed pastures. The adoption of these management (applied during the warm season) was followed by the overseeding of an annual cool-season grass in the area to determine whether the warm season management carried over an effect into the subsequent cool-season forage growth. In addition, following the observation that two structurally similar grass species (*Cynodon* spp. and *Cenchrus clandestinus*) was coexisting in the area for three years in similar proportion, we concentrated a chapter on describing some functional attributes that could explain this coexistence under a variety of management practices.

2 LITERATURE REVIEW

2.1 GRASSLANDS THOUGHT BEYOND | SHIFTING PERSPECTIVES ON GRASSLANDS RESEARCH

More than 40% of the earth's ice-free land area is covered by grasslands (Hewins et al., 2018), and grazing livestock is the most common type of land use (Lambin & Meyfroidt, 2011). As a result, grasslands play a critical role in providing the world's food supply, particularly through the production of milk and meat, as ruminants can efficiently convert forage into foods high in both energy and protein (O’Mara, 2012). The value of grasslands extends far beyond the provision of forage for animal production systems (Huguenin-Elie et al., 2018; Sollenberger et al., 2019; Petermann & Buzhdygan, 2021; Pellaton et al., 2022). In recent times, grasslands have garnered extensive attention regarding the development of sustainable ecosystems and livelihoods. They provide socio-economic, cultural, and ecological benefits (Bardgett et al., 2021), referred to as Ecosystem Services (ES), which are “the benefits people obtain from ecosystems” (Millennium Ecosystem Assessment, 2005). The importance of this theme is exhibited further in current trends in grassland research, where the primary focus has shifted from traditional, production-oriented studies to more ecological and environmental-related topics (Zhao, 2022), in addition to the general increase in scientific literature describing the modulation of ES in grassland systems (Bengtsson et al., 2019; Lemaire, 2012; Sollenberger et al., 2019). The same tendency is reflected in conference themes, as the topic of the 29th general meeting of the European Grassland Federation was "Grasslands at the heart of circular and sustainable food systems" (Delaby et al., 2022).

Among the many ES that grasslands provide, some of the most notable are the housing of wildlife and pollinators, roles in nutrient cycling, improving water quality, soil carbon storage and erosion control, and the mitigation of livestock greenhouse gas emissions (Sollenberger et al., 2019; Richter et al., 2021). Therefore, understanding and discovering ways to better manage, exploit, and protect grasslands to combine farmers' needs with the general public's need for a more sustainable food production system is of utmost concern (Zhao, 2022).

Current strategies that can be utilized to improve grassland ES delivery include the increased use of legumes in grass swards (Jaramillo et al., 2021b), improved defoliation management (Congio et al., 2018; Pereira et al., 2018), increased species diversity in swards (Schils et al., 2022) which prioritize functional divergence (Gross et al., 2017; Hanisch et al., 2020), and moderate N fertilizer inputs (Huguenin-Elie et al., 2018). These options are best suited for planted grassland areas, where the apex system might possess a mixture of several grasses and legumes, defoliated in a way that promotes highly digestible feed without compromising each species' persistence and stability, with the lowest N possible to still produce high forage yields while maintaining a high diversity of pollinator species, etc. Each of these characteristics, however, would be difficult to achieve in one permanent grassland system. At the farm level, it is impractical to incorporate all these features (Jordon et al., 2022; Savage et al., 2021), but a growing body of literature has been written to address this conceptual framework.

2.2 LIMITATIONS TO THE ADOPTION OF MULTISPECIES GRASSLANDS

One well known strategy to improve the multifunctionality of grasslands is the inclusion of legumes in grass monoculture pastures (Lüscher et al., 2014). An increased proportion of leguminous plants has been suggested to promote carbon sequestration (Li et al., 2016), improve herbage production (Egan et al., 2018) and animal performance (Lagrange et al., 2020), reduce the use of mineral N (Enriquez-Hidalgo et al., 2018), and decrease GHG emissions (Eugène et al., 2021) among many other perceived benefits. Despite these advantages, producer adoption is limited (Sollenberger & Dubeux, 2022), and primarily attributed to legumes' lack of persistence and yield stability over many years (Ortega et al., 2018), which implies significant additional costs to re-establish the species within short periods of time.

The establishment and persistence of multi-specific grasslands is a challenge, because the same functional dissimilarity among plants that promotes ecosystem multifunctionality (Díaz et al., 2007), mainly through niche complementarity and positive interspecific interactions leading to diversity effects

(Nyfeler et al., 2009), reduces the flexibility of farm-scale management (Jordon et al., 2022). The problem is that the adopted practice can sort plant species according to their functional strategy axis and trait convergence trend (Fischer et al., 2019), resulting in invariably less multifunctionality (Gross et al., 2017). The effect of management practices in species richness has been studied more often in native grasslands (Streit et al., 2022; Zanella et al., 2021) than in sown grasslands (but see Savage et al., 2021). Regardless of the grassland type, fertilization and defoliation management, i.e., cutting frequency or stocking rate, are factors with the greatest influence on plant species richness (Huguenin-Elie et al., 2018). Systems under both high fertilization and high defoliation intensities elicit a more extreme environment where only the most adapted species can survive, while more lax management regimes permit species to be selected by other environmental filters (Fischer et al., 2019). Thus, the development of studies that consider all factors present in a system and test practical management strategies for farmers should be of top priority in research that intersects agriculture and ecology (Wood et al., 2015).

2.3 GRASSLAND MANAGEMENT PRACTICES

A theoretical threshold proposed that 95% interception of incident radiation (Light Interception – LI) corresponds to a species' critical Leaf Area Index (LAI) to determine ideal defoliation frequencies (Brougham, 1956). Thus, an intermittent stocking method (Allen et al., 2011), when the sward reaches values around 90-95% LI, is the optimal structure to be grazed (Congio et al., 2018; Zanine et al., 2011). This range coincides with maximum accumulation of leaf dry matter, high rates of herbage intake, and high nutritive value (da Silva et al., 2013). The breaching of this limit is implied in canopies with a lower proportion of leaves and green material and a higher proportion of stems, as the competition for light becomes more critical (da Silva et al., 2019a). This concept is applicable at the farm level because sward LI is highly correlated with canopy height (Hodgson & da Silva, 2002) and has been successfully demonstrated as a grazing management tool for a wide range of species (da Silva et al., 2015).

Sward height targets are also helpful in determining appropriate defoliation severities. The adoption of moderate grazing intensity, at 40-50% pre-grazing height reduction, can increase forage dry matter production, individual animal performance, and productivity per land area when compared with more severe defoliation (Costa et al., 2021). Defoliation height can also help to define the range of pre-grazing management heights where net production of green herbage is essentially constant (Bircham & Hodgson, 1983), since grasses can reach 90 to 95% LI under different pre-grazing sward conditions (herbage mass, LAI, or height) (Duchini et al., 2014; Sbrissia et al., 2018) through the existence of a compensatory mechanism that operates between tiller population density (n^o tiller m^2) and tiller mass (g $tiller^{-1}$) (Matthew, 1995). For example, no differences in forage accumulation rate were found for C4 kikuyu grass submitted to 15, 20, and 25 cm pre-grazing target heights despite different average LAI values of 2.6, 3.2, and 3.6, respectively (Sbrissia et al., 2018).

These two approaches, defoliation severity and management height, allow more flexibility to manage grasses and could serve as a starting point for identifying management targets for grass-legume mixtures. Since forage yield is an important aspect of livestock systems and many grasses can adjust their morphology without compromising forage production over a range of pre-grazing heights, we can further explore this variation to determine whether, near the lower or upper threshold, there is facilitation for the persistence of companion plants such as legumes, forbs, or grasses with differing traits. However, the choice of species cannot be arbitrary, as it is necessary to consider the previous similarities among species, such as height of management. Trying to rectify incompatibility among plants through grazing management is unfeasible, i.e. mixing tall, upright growing grass species with stoloniferous legumes will hinder legume persistence due to light restriction (Andrade et al., 2015; Annicchiarico et al., 2015).

Although legume breeding programs often select for competitive ability against associated grasses (Annicchiarico et al., 2015), it is widely known that grasses have a competitive advantage over legumes regarding nutrient utilization (Haynes, 1980), and it is difficult to provide N fertilizer to the legume-grass mixture without depleting legumes over time (Ortega et al., 2018). N fertilization increases both above and below ground competition, which can cause a legume to have limited energy production via photosynthesis due to above ground competition for light by companion species, mainly grasses. These conditions also reduced legume N_2-fixation, due to the greater availability of nutrients below the ground, so the legume no longer has its main asset (Haynes, 1980; Kristensen et al., 2022; Soussana & Tallec, 2010). Many of these effects were studied in white clover, a stoloniferous legume species, which revealed its remarkable plasticity of yield and morphology in response to differential defoliation management (Annicchiarico et al., 2015).

Numerous studies have been conducted to determine the range of N fertilizer rates that can improve mixed sward herbage yields while having little impact on legume persistence. In general, research has indicated that 100 to 200 kg N ha yr^{-1} fulfilling this criteria, but these studies are mainly restricted to temperate regions with mixtures containing *Lolium perenne* L. and no clear management height targets (Egan et al., 2018; Enriquez-Hidalgo et al., 2016; Kristensen et al., 2022; Suter et al., 2021). Louault et al. (2017) also evaluated the effects of fertilization and herbage utilization levels, however, only a high stocking density and half of the full stocking density were tested without clear management targets. The authors suggested that future studies should consider a combined modification of fertilization and grazing factors for refined management guidelines to foster plant diversity. Beyond this, additional perennial species should be considered, mainly because the white clover has some tradeoffs, such as high drought-sensitivity (Inostroza & Acuña, 2010).

The *Lotus* genus produces superior quality forages with high nutritional value. Additionally, these forages improve the utilization of proteins in the rumen, help to avoid ruminal bloat as they contain condensed tannins (Piluzza et al., 2014; Rose et al., 2021), and even have an intrinsic ability to tolerate extended periods of water stress (Inostroza et al., 2015). *Arachis* is another promising genus. Its benefits include: clonal propagation, which enables young stolons to survive even after the death of the main axes (Faverjon et al., 2017), the capacity to increase population density if there is a reduction in the size of individuals (phenotypic plasticity) (Black, 2009), and the presence of growth points close to the soil surface, thus when grazing, animals are unlikely to damage apical meristems (Boddey et al., 2020). On the other hand, these species possess a generally low tolerance to cooler temperatures and frosts (Tambara et al., 2017), which may limit their adoption in regions with more severe winters.

2.4 GRASS-LEGUME MIXTURES IN CFA AND CFB CLIMATE ZONES

In terms of climate classification, the model originally developed by Wladimir Köppen, referred to as the Köppen-Geiger classification, is still the most frequently used. An updated world map was proposed by Peel et al., (2007). Considering this approach, climate class C (temperate) is the fourth most common individual climate type. However, there are nine sub-classifications in temperate climates, so we restrict our approach to regions that are temperate (C), without a dry season (f), and with hot (a) and warm (b) summers, respectively classified as Cfa and Cfb (Alvares et al., 2013; Beck et al., 2018; Peel et al., 2007). These areas include New Zealand, France, Ireland, the United Kingdom, the Netherlands, a portion of the United States, Argentina, Uruguay, Brazil, and the east coast of Australia, all of which are important locations for grazing livestock production. In Brazil, Cfa and Cfb climate zones represent about 9% of the country and are located mainly in the south (Alvares et al., 2013). The intra-annual temperature variation in these zones allows for both C3 and C4 species to coexist (Miqueloto, et al., 2020; Sbrissia et al., 2017), thereby enabling the conceptualization of many possible plant species combinations.

In the southern US, one experiment assessed if N fertilization could be halved by introducing alfalfa (*Medicago sativa* L.) or rhizoma peanut (*Arachis glabrata* Benth.) into Tifton 85 bermudagrass monocultures, without compromising productivity. The grass monoculture received 90 kg N ha^{-1} per harvest, while the mixtures received half that amount, and all swards were clipped to 7,5 cm seven times a year. The proportion of legumes present in mixtures was often low, at less than 8% for rhizoma peanut and less than 1% for alfafa at the last cutting (Santos et al., 2021). In order to better maintain legumes in mixed swards, the authors proposed that future experiments examine multiple harvest frequencies and N fertilization rates.

Interesting results were found in southern Brazil by da Motta et al. (2020), who tested mixtures of white clover and birdsfoot trefoil with different interspecific

Paspalum hybrids compared with the hybrid monocultures under multiple N rates. Over a two-year period, mixtures with no added nitrogen performed similarly to grass swards fertilized with 60 and 120 kg N ha^{-1}, producing legume proportions greater than 20% in almost all genotypes. Incidentally, the legume species had to be replanted in the second year of the study because of a drought that occurred during the summer of year one, which decreased plant stands. Additionally, harvests were defoliated mechanically as opposed to by grazing, considering the height of the taller *Paspalum* ecotype. Some caution should be taken when adopting these particular mixtures, since the authors also suggest that further research would be needed for testing the impact of animal grazing. Nevertheless, this study is an example of one more promising step to improve the comprehension of grass-legume mixtures.

Switzerland has become a testament to the adoption of forage mixtures in recent times, as the grasslands in the country are almost exclusively comprised of grass-legume swards. The key components of their success were providing stakeholders with the clear purpose of the mixture (from intensive forage production to biodiversity improvement); and the development of experiments and on-farm trials to ensure robustness and feasibility, under the joint efforts of extension services, the seed industry, and researchers, taking place over several decades (Suter et al., 2019). The development of guidelines for subtropical applications of grassland mixtures is still limited. However, the recent exponential rise of pasture research in Brazil (Zhao, 2022), combined with the demand for more sustainable pastoral systems and improvements in plant breeding programs to compose mixtures in Cfa and Cfb climatic zones (da Motta et al., 2021; Quesenberry et al., 2022), holds promise for developing site-specific guidelines, rather than adopting inadvertent global practices (Gao & Carmel, 2020).

2.5 LEGACY EFFECT

In accordance with all the literature described previously, herbivore-plant interactions and abiotic factors, such as N fertilization, have remarkable effects on forage production, sward structure, and species composition. Furthermore, the outcomes of these elements extend far beyond the short term, and can promote a “legacy effect”, which is defined as any implications that persist even after the biotic and abiotic interactions cease (Wurst & Ohgushi, 2015). For instance, defoliation intensity can determine if more or less of the primary production will be consumed by grazing animals and returned as manure or decomposed as vegetative material (Díaz & Cabido, 2001), these changes in litter quality can impact litter decomposition, nutrient cycling, and potentially future crop performance (Wurst & Ohgushi, 2015). In the same way, species diversity, and particularly species identity, can alter soil microbial communities, which in turn affect the subsequent crop performance (Fox et al., 2020).

The legacy effect was identified in one study of the performance of planted Italian ryegrass following a two-year ‘grassland phase’. The grassland phase consisted of different species diversity (from monocultures to six-species mixtures, considering functional groups of grasses, legumes, and herbs), fertilizer levels, and drought conditions. After two years, grassland phase areas were treated with herbicide, and new species were sown in the following year. Grass monoculture under high N fertilization (300 kg ha yr^{-1}) did not promote a positive legacy effect beyond low N efficiency use, while plots with moderate N levels and high proportions of legumes (>30%) promoted a positive legacy effect in the production of future crops (Grange et al., 2022).

Given the characteristics of the Cfa and Cfb climatic zones, we can divide the year into at least two seasons: the warm and cool seasons, which the cool season occur between October and April in the Northern hemisphere and May and September in the Southern hemisphere. In these regions, the temperature variation and frost occurrences during the cool season limit the production of C4 species (Sbrissia et al., 2017), and freezing conditions would particularly mark changes in sward composition. To overcome declines in forage production, a common practice in southern Brazil is to overseed annual C3 grasses into perennial C4 pastures (Schmidt, 2022). The remarkable difference in swards in the same year raises the hypothesis that management practices in one intermittent season may impact the subsequent growing season, mainly considering biodiverse pastures and possible changes in botanical composition. In southern Brazil, studies have shown that N fertilization in cool season grasses can increase forage yields and may even improve the performance of succeeding crops, such as maize, due to N cycling and carryover effects (Sartor et al., 2018), but literature in grassland-grassland system intra-annual is incipient.

2.6 FUNCTIONAL TRAITS AND COEXISTENCE AMONG SPECIES

The recent trend in grassland science toward ecology- and environment-related research and ES has brought grassland scientists and ecologists closer together, in a sense. While ecologists are interested in underlying mechanisms, the first group is interested in how management practices affect community assemblages and the practical implications of this. However, these fields overlap in many areas, and literature that includes both angles has increased over the past few decades.

A central issue of community ecology is finding mechanisms that explain the composition and abundance of coexisting species within a community (Lhotsky et al., 2016). Some studies have suggested that in grasslands, processes such as habitat filtering and niche differentiation jointly determine species diversity in a community (Maire et al., 2012). The habitat filtering process is comparable to natural selection in those environmental factors, both biotic and abiotic, eliminate traits that are inappropriate for the environment (Keddy, 1992) and select organisms with similar traits to better withstand stress or local disturbances (Grime, 1973). The other process that can modulate species coexistence is niche differentiation, which occurs when species present some dissimilar traits that avoid competitive exclusion, because they differ in their resource-acquisition traits and promote the complementarity of resource use in space and/or time (Macarthur & Levins, 1967). Thus, in accordance with this theory, the total number of species in a community is proportional to the total range of the environment divided by the specificity of each species, since there is a limit to the degree of similarity in which the species can coexist (Pacala & Tilman, 1994).

An extensive body of literature suggests that functional diversity, rather than species diversity, enhances ecosystem functions (Díaz & Cabido, 2001; Tilman et al., 1997), because the complementarity of species and the consequent decrease in competition intensity, rather than facilitation, frequently explain the overyielding of mixtures to monocultures (Gross et al., 2007; Hooper et al., 2005; Wagg et al., 2017). The growth in trait-based research rather than species-only research has been dubbed "the biodiversity revolution" in ecology (Cernansky, 2017). Since then, studies aiming to enhance the multifunctionality of sown grasslands have tried to match complementary species to develop “ready-to-use” mixtures. For instance, Suter et al. (2021) selected four species based on the factorial combination of functional traits, temporal establishment (fast-establishing *vs*. temporally persistent), and N acquisition (non-fixing and N_2-fixing) and found that they outperformed monocultures in the delivery of ES. One of the main drivers of biomass production in multispecies communities appears to be large niche differentiation (Godoy et al., 2020).

The term “niche” is generally defined as a hyper-volume in the multidimensional space of ecological variables, within which a species can maintain a viable population (Hutchinson, 1957). Thus, in terms of niche complementarity, this can occur along many axes and become further modulated by environmental interactions. Using a multiple trait-based approach, Maire et al. (2012) evaluated the relative role of habitat filtering and niche differentiation in determinate species abundances. The authors identified four independent axes of functional trait specialization that together explained the majority of species abundance differences. The axes included traits of roots (i.e. specific root area; thin roots), leaves (leaf lifespan, leaf N content), and plant physiology (root uptake capacity for nitrate (NO_3) vs. ammonium (NH_4)). Some of the axes were specifically involved in some species responses; for instance, when the supply of N is the main environmental factor, the species preference for one kind of molecule (NO_3 or NH_4) may better explain the species abundance.

Considering the various specialized approaches possible, trying to explore many of them at once can prove difficult when coupled with practical management. Tall species, for example, can limit light allowance to shorter species. One of the most widely used classifications differentiates species based on their resource acquisition or conservation strategies (Cruz et al., 2010; Duru et al., 2008). This axe reflects a tradeoff between traits that confer an ability for high rates of resource acquisition and use in productive habitats and those responsible for retention of resource capital in less productive conditions (Grime et al., 1997). Therefore, markers such as specific leaf area (SLA) and leaf dry matter content (LDMC) can be utilized as indicators for this axe (Cornelissen et al., 2003; Wilson et al., 1999). While they do provide a general overview of plant function, these “classic” functional traits show some limitations under specific conditions, including drought and plant-herbivore interactions (Volaire et al., 2020), and a more complete approach can be obtained using additional traits (Díaz et al., 2016). Added traits would then usually be considered an independent axis of specialization (i.e., root traits) (Fort et al., 2017).

At the species level, functional traits determine how plants respond to their environment. These quantitative traits have an average and a specific level of variability, with the average representing the trait's central tendency and the variability representing the overall capacity of an individual to alter their morphology and physiology in response to environmental conditions (Pontes et al., 2015). Phenotypic plasticity varies among species: a more exploitative species (*Dactilys glomerata*) was more able to show it than a conservative species (*Festuca paniculata*) along a productivity gradient (Grassein et al., 2010), but opposite results also were found in response to grazing treatments (Streit et al., 2022). On the other hand, under similar conditions, i.e., high fertilization level, a specific trait can overlap between two species, while a different trait can have an opposite effect on the two species (de Bello et al., 2021; Price et al., 2014). This means that caution should be taken when using trait mean values from databases, as these resources would not incorporate site specific interactions between plants and the environment (Weisser et al., 2017).

To summarize, community assembly is mediated by multiple traits operating in a multidimensional space, which has boosted the development of mathematical models that consider environmental factors and intraspecific trait variation to predict the relative abundance of species, thereby building more resilient and functional grassland ecosystems (de Bello et al., 2021; Laughlin & Laughlin, 2013). Although testing these models in the field is challenging, it is extremely necessary to continue developing grassland models (Hetzer et al., 2021). Tools such as grazing intensity and frequency may alter the outcome of competition between plant species within the community in a number of ways, by favoring the expression of different sets of traits (Fischer et al., 2019; Louault et al., 2017; Streit et al., 2022), which are the underpinning mechanisms that drive species coexistence, and, consequently, grassland community assembly.

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3 HYPOTHESIS

3.1 GENERAL

Nitrogen fertilization and grazing height drive the vegetation dynamics of multispecies pastures.

3.2 SPECIFICS

a) Lower grazing height increases diversity but reduces forage yield in multispecies pastures.
b) The management applied in the warm season in a C_4 perennial-based pasture changes forage production and canopy diversity in subsequent cool-season pastures.
c) Plants belonging to the same functional spectra can coexist in contrasting management scenarios since they preserve dissimilarities in functional traits.

4 OBJECTIVE

4.1 GENERAL

Determine management strategies (grazing heights and nitrogen fertilization) for multi-species pastures that cope species diversity and forage production.

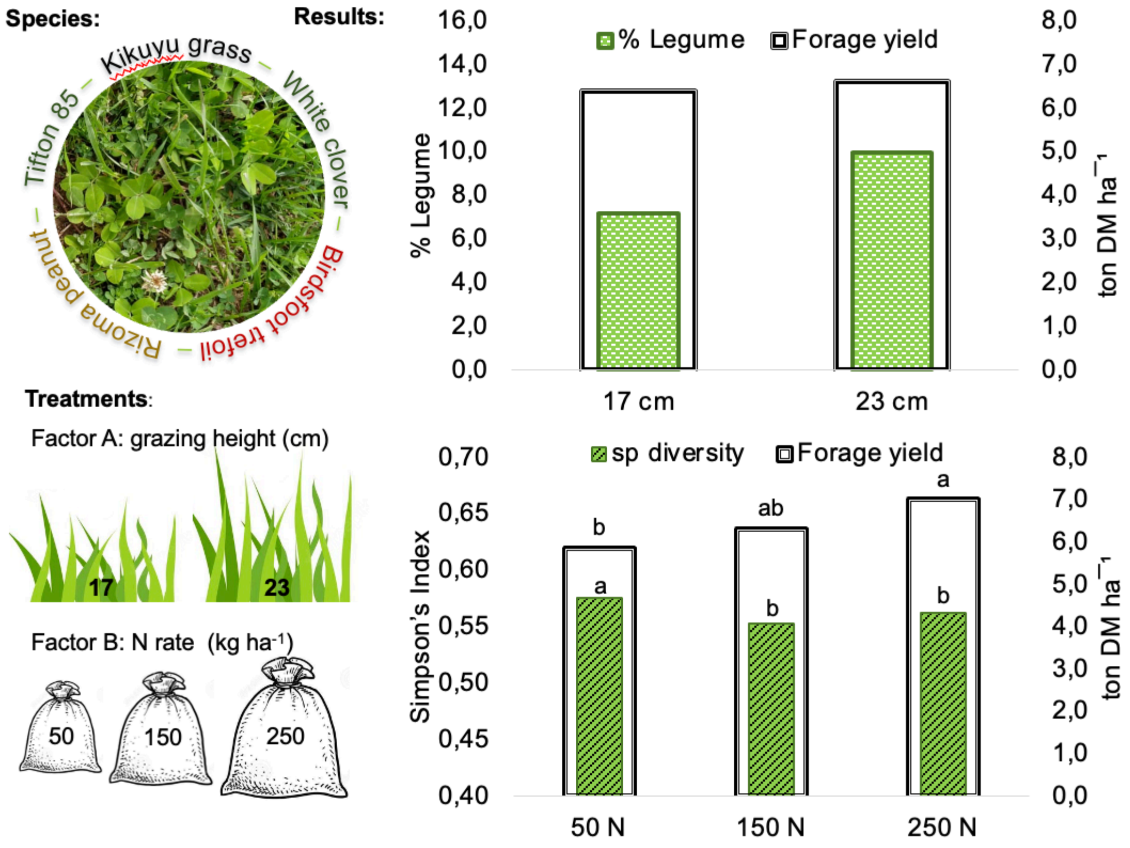
4.2 SPECIFICS

a) Identify whether two grazing heights and three nitrogen fertilization rates applied in the warm season affect species diversity in the following cool season.

b) Identify the underlying mechanisms that could explain the stable coexistence of kikuyu grass and Tifton 85 in a range of fertilization gradient.

5 GRAZING HEIGHT DOES NOT CHANGE FORAGE PRODUCTION AND FUNCTIONAL DIVERSITY IN A FIVE-SPECIES MIXTURE SWARD

5.1 GRAPHICAL ABSTRACT



5.2 ABSTRACT

The establishment of pastoral environments containing different plant functional groups is a strategy for sustainable intensification of animal production systems. However, maintaining species diversity is a major challenge in systems containing grasses and legumes. The objective of this study was to identify management strategies that maintain species diversity in a mixed pasture containing five species, including grasses and legumes. The species were Tifton 85 (*Cynodon* spp.), kikuyu grass (*Cenchrus clandestinus*), pintoi peanut, (*Arachis pintoi*) white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*). The experimental treatments were all combinations of two management heights (17 and 23 cm) associated with three nitrogen fertilizer rates (50, 150, and 250 kg N ha^{-1}), using a randomized complete block design with three replicates per treatment. The stocking method was intermittent with a 40% defoliation severity for all treatments. The experimental period lasted for three warm seasons (November – April) of 2020, 2021 and 2022. Forage accumulation, botanical composition, light interception, canopy leaf area index, specific leaf area and leaf dry matter content of all species, leaf N concentration, tiller population density, and average weight per tiller of the grasses were estimated. Forage accumulation was similar for both management heights, but the greater N rates increased forage accumulation in the mixture. The botanical composition of the canopy was mostly Tifton-85 and kikuyu grasses, which coexisted in similar proportions in all treatments. Species diversity and dissimilarity of community traits were less in the pastures managed with the greatest N rates, a condition in which the grasses in the mixture had an adequate nitrogen nutrition index (NNI>0.8). Management of the sward at heights close to 23 cm achieved greater species diversity. Managing a mixed grass-legume pasture at a pre-grazing height close to the critical leaf area index may be a strategy to mitigate loss of species diversity in the canopy, without compromising forage production.

Keywords: biodiversity, legumes, pasture management, multispecies pastures

5.3 INTRODUCTION

Grasslands are an extensive ecosystem throughout the world, occupying about a third of the earth's surface (Suttie et al., 2005). They are important environments that provide a wide variety of services, including supplying forage (which animals turn into important products such as meat and milk), protecting soil and aquifers, and preserving fauna and flora (Bengtsson et al., 2019). Despite their importance, many of the world's pastoral environments are in a state of degradation or are occupied by monoculture grasses in intensive systems requiring large inputs of nitrogen fertilizer (Lüscher et al., 2014). An alternative to both problems is to establish multispecies pastures that contain nitrogen-fixing legumes. Compared with monocultures, multispecies grasslands are reported to be more resource-use efficient (Storkey et al., 2015), produce at least the same amount of forage as monocultures (Finn et al., 2013), reduce occurrence of invasive plants (Connolly et al., 2017), increase resistance and resilience to extreme weather events (Hofer et al., 2016), improve animal performance (Grace et al., 2019), and reduce the intensity of greenhouse gas emissions (Ghani et al., 2022). Despite their ecosystem services, a major challenge of managing biodiverse swards is to define practices that support high levels of forage accumulation while maintaining species diversity.

Both grazing height and nitrogen fertilizer are management practices that alter species dynamics over time. High rates of nitrogen fertilization usually reduce species richness (Hautier et al., 2009), especially legumes (Soussana & Oliveira Machado, 1999). Negative effects of N on yield and persistence of legumes in mixtures have been observed in various environments and management conditions (Egan et al., 2018; Suter et al., 2021). This has been attributed to superior resource use by grasses, particularly grasses that better exploit resources (Cruz et al., 2010).

Grazing regulates light entering into the canopy, indirectly acting on the competition among plants (Grime, 1973). Managing pastures at taller heights usually favors the persistence of erect plants, while compromising the persistence of prostrate plants (Tamele et al., 2018). Thus, management can alter the functional characteristics of the community, either by changing the botanical composition of the sward (Fischer et al., 2019) and/or by intraspecific trait variation (Streit et al., 2022). These changes can alter trait dissimilarity and complementarity among species in the community (Wagg et al., 2017), which is more important than species diversity *per se* in promoting multifunctionality of pastoral environments (Díaz & Cabido, 2001; Gross et al., 2017).

In single-species (especially grasses) pastures, a range of management heights (or LAIs) have been relatively well established at which forage production is similar, both when pastures were managed under continuous (Bircham & Hodgson, 1983; Pinto et al., 2001; Sbrissia et al., 2020) and rotational stocking (Mocelin et al., 2022; Parsons et al., 1988; Sbrissia et al., 2018). Similar forage production in structurally different pastures can be explained by the principle of self-thinning (Matthew et al., 1995; Yoda et al., 1963), in which swards managed at taller heights have fewer tall tillers and vice versa. However, in the case of mixtures, this compensatory process between height and the number of tillers may not operate in the same way, since plants have different productive capacities (Payne et al., 2021) and, as mentioned, management can alter the botanical composition of the sward and modify the compensation mechanism between size and number of individuals.

Hence, we hypothesize that shorter management height increases species diversity but reduces forage production in a 5-species sward mixture. The objective of this study was to identify management strategies that maintain species diversity in a mixed grass-legume sward.

5.4 MATERIAL AND METHODS

This experiment was submitted and approved by the Ethics Committee on Animal Use of the Santa Catarina State University (CEUA/UDESC; 1093021219). The experiment was conducted at the Agroveterinary Sciences Center of Santa Catarina State University (Universidade do Estado de Santa Catarina - UDESC), Lages, Santa Catarina, Brazil (27º47′ S, 50º18′ W). The experimental area has a typical Inceptisol soil (*Cambissolo Húmico Alumino Típico modular*) (EMBRAPA, 2006). Soil fertility was corrected prior to planting the species according to the Manual for Fertilization and Liming for the states of Rio Grande do Sul and Santa Catarina, Brazil (Comissão de Química e Fertilidade do Solo - RS/SC, 2016). According to Koppen’s classification, the region has a humid subtropical climate

(Cfb) with severe winters (10–15 frosts per year), mild summers, and the absence of a defined dry season (Figure 1).

The warm-climate species – Tifton 85 (*Cynodon spp.*) and forage peanut (*Arachis pintoi* cv. Amarillo) were established in March 2018 over an already established pasture of kikuyu grass (*Cenchrus clandestinus*). The area was plowed and harrowed, and Tifton-85 was planted using 4 rooted cuttings per square meter and forage peanut seeded with 8 kg of seeds ha^{-1}. In May 2019, the area was overseeded with 12 kg ha^{-1} of birdsfoot trefoil, (*Lotus corniculatus* L. cv. São Gabriel), 4 kg ha^{-1} of white clover (*Trifolium repens L.* cv. Zapican), and 40 kg/ha of annual ryegrass (*Lolium multiflorum* L. cv. La Estanzuela 284). In December 2019, all paddocks were grazed to 10 cm to mark the beginning of the experimental period. The experiment was conducted during the warm season (November to April) for three consecutive years (2019/2020, 2020/2021, and 2021/2022).

5.4.1 Treatments

Treatments were pastures managed at two pre-grazing canopy heights (17 and 23 cm) that were associated with three nitrogen rates (50, 150, and 250 kg N ha^{-1}). The treatments were distributed in a randomized complete block design in a 2 × 3 factorial arrangement with three replications each. The experimental units were 315 m^2. The pastures were grazed using the intermittent stocking method (Allen et al., 2011). The defined post-grazing management targets were 60% of the initial height (Martins et al., 2021); therefore, the pastures in which grazing was initiated at heights of 17 and 23 cm were grazed to sward heights of 10.2 and 13.8 cm, respectively. In the first year (January 2020), N (urea 45%) was applied once at the treatment rates. In subsequent years, the treatment rates were subdivided into two equal applications, one in November and the other in January.

During the cold season (May to October) of 2020 and 2021, all the paddocks were managed identically to avoid confounding of winter management with the vegetation dynamics of the subsequent warm season. During this period, the area was reseeded annually with annual ryegrass (20 kg ha^{-1}) and fertilized with 50 kg N ha^{-1} (using 45% N urea) at the tillering stage. The pre-grazing canopy height in this period was 20 cm with an exit height of 12 cm. At the end of the cool season (November), all paddocks were grazed to 10 cm to mark the beginning of the new warm season evaluation period.

5.4.2 Measurements

The average canopy height was determined using a ruler at 30 points in the paddock. The light interception (LI) by the canopy was measured with LAI 2200 equipment (LI-COR Biosciences Lincoln, NE, USA) in pre- and post-grazing conditions during February in all years. Herbage mass was determined indirectly using a rising plate meter (Farmworks®; F200, New Zealand) calibrated for pre- and post-grazing conditions. Equations were generated using calibration data collected during the three experimental years: $y=147*x + 1474$ ($R^2=0.58$) (pre-grazing) and $y=188*x + 1038$ ($R^2=0.52$) (post-grazing), where y= herbage mass (kg of dry matter ha^{-1}) and x= average canopy height measured by rising plate meter. Forage accumulation was calculated by the difference between the pre-grazing herbage mass in cycle n and the post-grazing herbage mass in cycle n-1. Total forage production was determined as the sum of forage accumulation across all the inter-grazing intervals.

The botanical composition of the sward was determined in the middle (January) and the end (April) of the growing season. Three 0.5-m^2 quadrats (0.5 x 1.0 m) were sampled to ground level at representative points in the paddock, and a fraction (~20%) of each sample was manually separated into species and their botanical components (leaf and stem). The number of grass tillers (Tifton 85 and kikuyu grass) in the subsample was also counted. The detached leaves were then scanned using the LAI-3100C leaf area meter (LI-COR Biosciences Lincoln, NE, USA), and all components were placed in a forced-air circulation oven at 65 °C for 72 h before being weighed. From the data collected from the subsamples and the weight of the samples and botanical components, the botanical composition of the sward, the leaf area index (LAI), and the tiller population density (TPD) of each grass and canopy were determined. The species diversity of the community was calculated using Simpson's Index (Simpson, 1949).

Leaves separated for botanical composition were used to determine the leaf N concentration. Measuring the N concentration of leaves exposed to sunlight is a robust proxy for plant N status (Farruggia et al., 2004). Total N concentration was measured according to the Dumas combustion method 968.07 (Association of Official Analytical Chemists, 1990) using the Leco FP 528 instrument (LC, Leco Corporation, Saint Joseph, MI, USA). The leaf nitrogen concentration was used to calculate the nitrogen nutrition index (NNI) of the two main grasses present in the mixture (Tifton-85 and kikuyu grass) employing the formula proposed by Ziadi et al. (2009), in which $NNI = -0.035 + 0.028\ N_L$, when N_L corresponds to the leaf N concentration in dry matter (DM) (mg/g).

Supplemental leaf collections of the species were conducted in the second and third years to determine the specific leaf area (SLA) and leaf dry matter content (LDMC) of the species according to the recommendations of Cornelissen et al. (2003). For Tifton 85 and kikuyu grass, 35 fully expanded leaves were collected in March 2021. The legume leaves were collected in November 2021. From the botanical composition of the sward, SLA and LDMC values and categorical variable grass/legume, the Rao Index (Rao, 1982) was estimated. It is characterized as a metric of functional community diversity because this index is sensitive to changes in trait values and species abundance (de Bello et al., 2007).

The morphogenetic characteristics of kikuyu grass and Tifton 85 were evaluated using the marked tiller technique (Davies, 1993). The evaluations covered one grazing cycle, just in the third year (2022), starting in the post-grazing condition until the next grazing. Ten tillers of each species were selected along a transect at points that represented the condition of the pasture. The tillers were identified with metal wires and evaluated at seven-day intervals. Pseudostem length was measured from ground level to the last visible leaflet. For mature leaves (visible leaflet), the length was determined from the leaf tip to the leaflet, while for expanded leaves, the length was determined between the leaf tip and the leaflet of the last mature leaf (Duru & Ducrocq, 2000).

5.4.3 Statistical analysis

Botanical composition, LAI, and TDP data were pooled to represent the average for the evaluation period in each year. Data analysis was performed using R statistical software (R Core Team, 2021). Simpson's and Rao indexes were calculated using the Rao.diversity function from the SYNCSA package (Debastiani & Pillar, 2012). Analysis of variance was performed using mixed models from the lme4 package (Bates et al., 2015), considering block and year as random effects and management height, nitrogen rate, and their interaction as fixed effects. The best model was selected based on Akaike's information criterion. Means with significant differences ($P<0.05$) were compared by Tukey's Test at a 5% significance level.

5.5 RESULTS

The management height did not affect forage production, and no interaction was observed between management height and nitrogen fertilizer rate (Table 1). Fertilization affected forage production; at the highest rate of N (250 kg N ha^{-1}), the production was greater than at the lowest rate (50 kg N ha^{-1}), while at intermediate rates (150 kg N ha^{-1}), forage production was equivalent to the lowest and highest rates of N (Table 1).

The NNI indicated an effect of N rate, with the greatest values observed with the greatest N rates and the lowest with the lowest rate (0.97 and 0.92; 0.77 and 0.70 for kikuyu grass and Tifton 85 grass, respectively; Figure 2). The treatments did not alter the growth dynamics of the tillers, with average elongation of 0.87 and 0.83 cm tiller day^{-1} and senescence averages of 0.58 and 0.30 cm tiller day^{-1} for kikuyu grass and Tifton 85, respectively.

Pre- and post-grazing (herbage) forage mass, LI, and pre-grazing LAI differed between management heights, with the highest values observed in the 23 cm pasture (Figure 3). The pre-grazing LAI also varied as a function of the N rate, with values increasing as the N rate increased (Figure 3).

The TPD was greatest at the shortest grazing height for both species (Figure 4). Only the kikuyu grass increased TPD at the greater N rates compared with the lower rate (Figure 4). Tillers were tallest at the 23-cm management height for both species.

Grasses comprised more than 85% of the canopy biomass in all treatments. The proportion of kikuyu grass and Tifton 85 in the canopy was similar (~5% difference) except in treatments 17/250 and 23/50. Under these conditions, the difference in the proportion between the grasses was 13% (Table 2). The proportions of pintoi peanut and white clover were similar among the treatments. On the other hand, the proportion of birdsfoot trefoil increased by a factor of more than two at the height of 23 compared with 17 cm (Table 2).

Considering Simpson's Index, the lowest rate of N resulted in greater species diversity than the highest N rates. Management height also affected diversity (p=0.09), with greater diversity at 23 compared with a 17-cm height (Table 3). Rao's Index was similar between management heights and greatest at the lowest N rate, which is the same pattern observed for species diversity (Table 3).

5.6 DISCUSSION

5.6.1 Forage yield

Both management heights lead to similar forage production, which caused us to reject one of our hypotheses. Although pre-grazing LI and LAI differed between management heights (LI: 92.6 and 95.1%; LAI: 2.38 and 2.75, in pastures at 17 and 23 cm, respectively), some authors have shown that forage production can be similar when pastures are managed at a range of LIs or a range of heights (Mocelin et al., 2022; Sbrissia et al., 2018; Zanine et al., 2011). The cited authors worked with monocultures, and in the current study the presence of 15% legumes in a mixture with a predominance of grasses did not seem to change the flexibility in height targets at which forage production is similar with moderate grazing defoliations.

In pastures managed to shorter heights, plants compensate for a smaller tiller size and a lower leaf expansion rate with an increased tiller number and a reduced leaf senescence rate (Bircham & Hodgson, 1983, 1984; Matthew et al., 1995). However, in our experiment, the elongation and leaf senescence rates were similar in both management heights for both grasses evaluated, indicating that homeostasis in forage accumulation occurred exclusively as a function of the trade-off between tiller size and number (Figure 4), which is an ecological mechanism extensively studied in cultivated pastoral environments (Matthew et al., 1995; Sbrissia et al., 2003; Calsina et al., 2012; Duchini et al., 2014; Sbrissia et al., 2018).

Forage production was affected by N rate (Table 1). Lower forage production in swards fertilized with 50 vs. 250 kg of N ha^{-1} could be due to both predominant grasses in the mixture having NNI values below 0.8 in less-fertilized pastures, a level which is considered a threshold for loss of productive potential (Duru et al., 1997). The NNI at the remaining rates was between 0.8 and 1.0 for both grasses, within a theoretical range of satisfactory nutritional status (Duru et al., 1997; Farruggia et al., 2004).

As the leaf elongation and senescence rates did not differ among N rates and between species, the increase in forage production at the greater N rates was exclusively due to an increase in TPD, particularly for kikuyu grass (Figure 4). Although Tifton 85 and kikuyu grass have the same ecological spectrum of functionality (resource exploitative/acquisitive) (Cruz et al., 2002), their functional characteristics linked to nutrient uptake and use have important differences (Barreta et al., 2021). Thus, the increase in both forage production and LAI (Figure 3) in the pastures that received the greatest N rates is the result of more efficient use of this resource by the species that was most competitive for resources (kikuyu grass).

The satisfactory nutritional condition of both grasses starting at N rates of 150 kg ha^{-1} (NNI above 0.8) suggests that the observed forage production of 6500–7000 kg of DM ha^{-1} (average forage accumulation rate of 45 kg of DM ha day^{-1}) is close to the agronomic limit for these plants in the studied region. This indicates that other factors may have limited production, as C4 grasses have higher production potential than this range. Our hypothesis is that temperature and water shortage experienced during the experimental period may have contributed to limit production. In the three evaluation periods (six months each year), monthly precipitation was at least 50% less than the historical average in eight months, and the average temperature was below 20 °C for most of the growing season (Figure 1). Under water stress conditions, N supplementation is less efficient in promoting plant growth in general, but the limitation may be enhanced for kikuyu grass, because this plant is more sensitive to water restriction than other tropical grasses (Murtagh, 1988b, 1988a).

5.6.2 Botanical composition

The proportion of legumes was less than usually recommended for an association with grasses, which according to Lüscher et al. (2014) should be around 30%. However, compared with grass monocultures, mixed swards can deliver greater ecosystem functionality (including forage production, yield stability, and atmospheric N fixation) across a wide range of legume proportions. Benefits have been observed with proportions of 5–10% (Suter et al., 2021). However, high rates of applied N (450 kg ha year^{-1}) to the mixture can limit these benefits because N fertilization can modulate species richness, particularly with short defoliation intervals (Hautier et al., 2009; Huguenin-Elie et al., 2018; Suter et al., 2021).

Kikuyu and Tifton 85 maintained similar proportions in virtually all managements, indicating that these species are candidates for coexistence in multispecies swards. Nevertheless, there was a slight imbalance between them in the two most "dissimilar" managements. In the greatest N rate associated with the shortest height, kikuyu grass predominated. This species can exploit resources better than Tifton 85 (Barreta et al., 2021). However, under the lower N rate associated with taller height, Tifton 85 contributed more to canopy composition. Therefore, height management and nitrogen fertilization could be used to stabilize the population of one or the other of these species when grown in association.

For legumes, pintoi peanut made a negligible contribution to the herbage mass (Table 2). Similar results were found in a mixture of Tifton 85 and *Arachis glabrata* Benth, in which the legume accounted for less than 2% of the herbage mass (Santos et al., 2021). In another experiment that studied the overseeding of *Arachis pintoi* cv. Amarillo into *Cynodon dactylon* (L.) with different stubble heights, the participation of the legume was also low, less than 6% regardless of the management adopted (Sanchez et al., 2018). In general, pintoi peanut establishes slowly, which can compromise the contribution in herbage mass in areas with grasses (Homem et al., 2021).

White clover produces mainly in spring and early summer in subtropical climatic regions, and its persistence is almost exclusively through stolon maintenance. Thus, more frequent defoliation management applied in late summer/early fall decreased its proportion in a mixture with kikuyu grass in Australia (Fulkerson & Reeves, 1996). In a mixture of white clover and *Cynodon dactylon* (L.), annual nitrogen fertilization starting at 112 kg N ha^{-1} compromised the participation of this legume in the mixture (Payne et al., 2021).

The greater participation of birdsfoot trefoil in the tallest management height may be due to the lesser frequency of defoliation in this treatment (Table 3). The persistence of this species is guaranteed with longer intervals between cuttings that allow a greater accumulation of reserves in its roots and crown (Scheffer-Basso et al., 2011), especially when in mixture with competing grasses, including those of the genus *Cynodon* (Boyle et al., 2020).

5.6.3 Species and trait diversity

In general, managing pastures at taller heights mitigated the loss of canopy diversity without compromising forage production, also causing us to reject one of our hypotheses, while increasing the N rate resulted in greater forage production but lesser diversity (Table 3). The proportion of legumes was greater at the lowest N rate, a condition in which the grasses presented slight nutritional stress (NNI 0.7-0.8).

The lowest rate of N led to greater dissimilarity of traits in the community, which is considered an important indication of the support of various functions in the ecosystem (Gross et al., 2017). This broader approach that considers the various services that pastures can provide is important. Qualifying them for use based only on their forage production can be very reductionist and lead to systems that are misaligned with sustainable production goals (Schellberg & Pontes, 2012), goals which require maximizing food production while also providing efficient and conscientious use of resources (Suter et al., 2021).

5.7 CONCLUSIONS AND IMPLICATIONS

Managing a mixed sward at a pre-grazing height close to the critical LAI (around 23 cm for a mixture of predominantly Tifton-85 and kikuyu grass) is a strategy to maintain species diversity in the canopy without compromising forage production. Furthermore, our data indicate that functional diversity is increased when grasses have a suboptimal N nutrition index (no greater than 0.8).

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5.9 TABLES AND FIGURES

Table 1. Forage accumulation (kg DM ha^{-1}) in warm-season based, mixed-species pastures submitted to two different pre-grazing canopy heights under intermittent stocking management and three nitrogen rates (average of three experimental years).

Forage accumulation (kg DM ha^{-1})	Treatment			*P-value*		
Management height (cm)	N Rate (kg N ha^{-1})			Height	N rate	H*N
	50	150	250	0.70	0.01	0.19
17 cm	6000	6090	7080			
23 cm	5730	6730	6950			
Average	5870 b	6410 ab	7010 a			

Table 2. Botanical composition (% species in pre-grazing herbage mass) of multispecies pasture submitted to different management strategies during the warm season.

	Treatments					
Species	17/50	17/150	17/250	23/50	23/150	23/250
Kikuyu grass	42.6	49.9	53.7	38.6	48.9	42.4
Tifton 85	48.1	43.7	40.3	50.4	43.3	46.4
Forage peanut	1.5	0.9	2.2	0.7	1.8	2.6
Birdsfoot trefoil	3.0	2.7	1.7	6.9	3.8	5.7
White clover	4.8	2.8	2.1	3.3	2.2	3.0

Legend: Treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Table 3. Simpson's Index and Rao Index of multispecies pasture submitted to different management strategies during the warm season.

	Treatment						*P-value*		
	17/50	17/150	17/250	23/50	23/150	23/250	Height	N rate	H * N
Simpson Index	0.573	0.553	0.536	0.578	0.553	0.572	0.09	0.04	0.14
Rao Index	0.381	0.377	0.357	0.383	0.348	0.364	0.31	0.02	0.06
Grazing (n°)	4.6	4.6	5.8	3.3	4.0	3.7	0.01	0.01	0.01

Legend: Treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Figure 1. Monthly average weather conditions during the study period (Nov. 2019 – May 2022) relative to the mean long-term values of rainfall and temperature in Lages, Santa Catarina, Brazil.

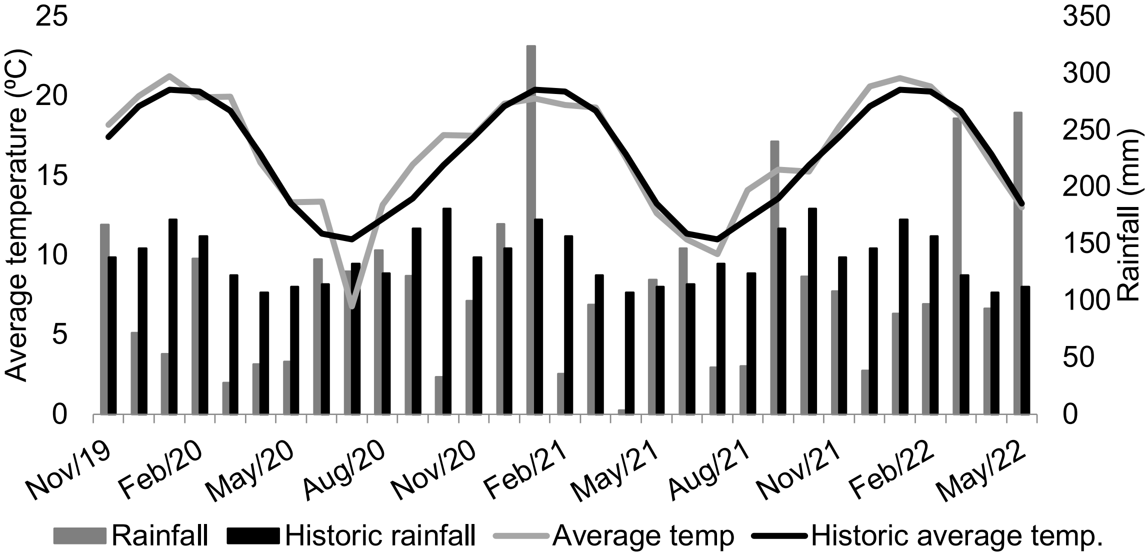
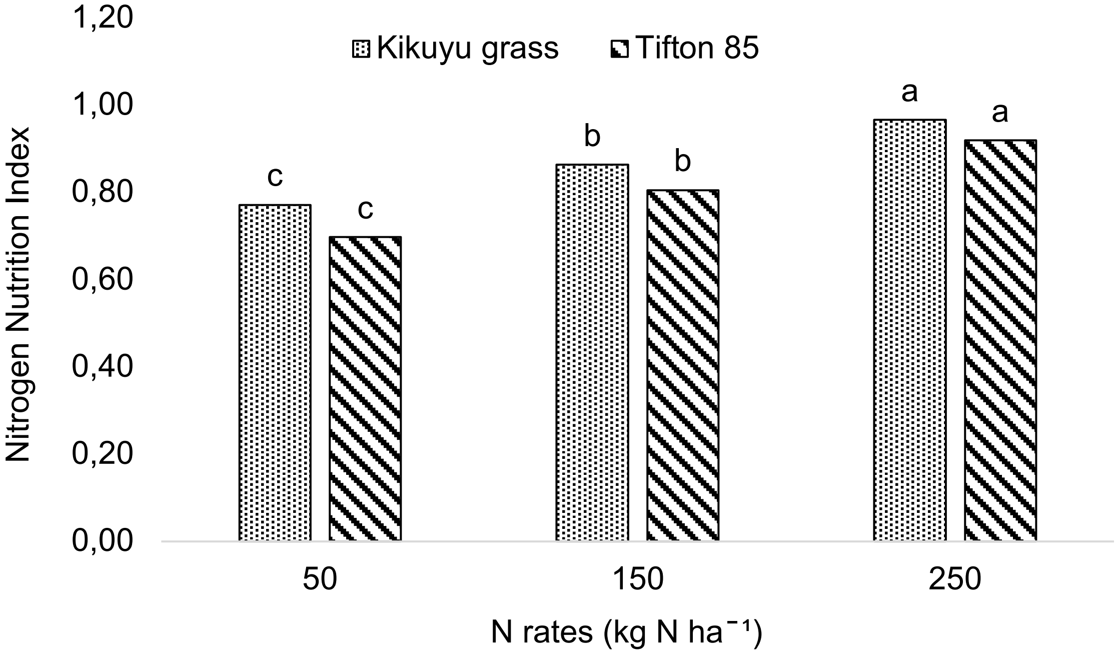
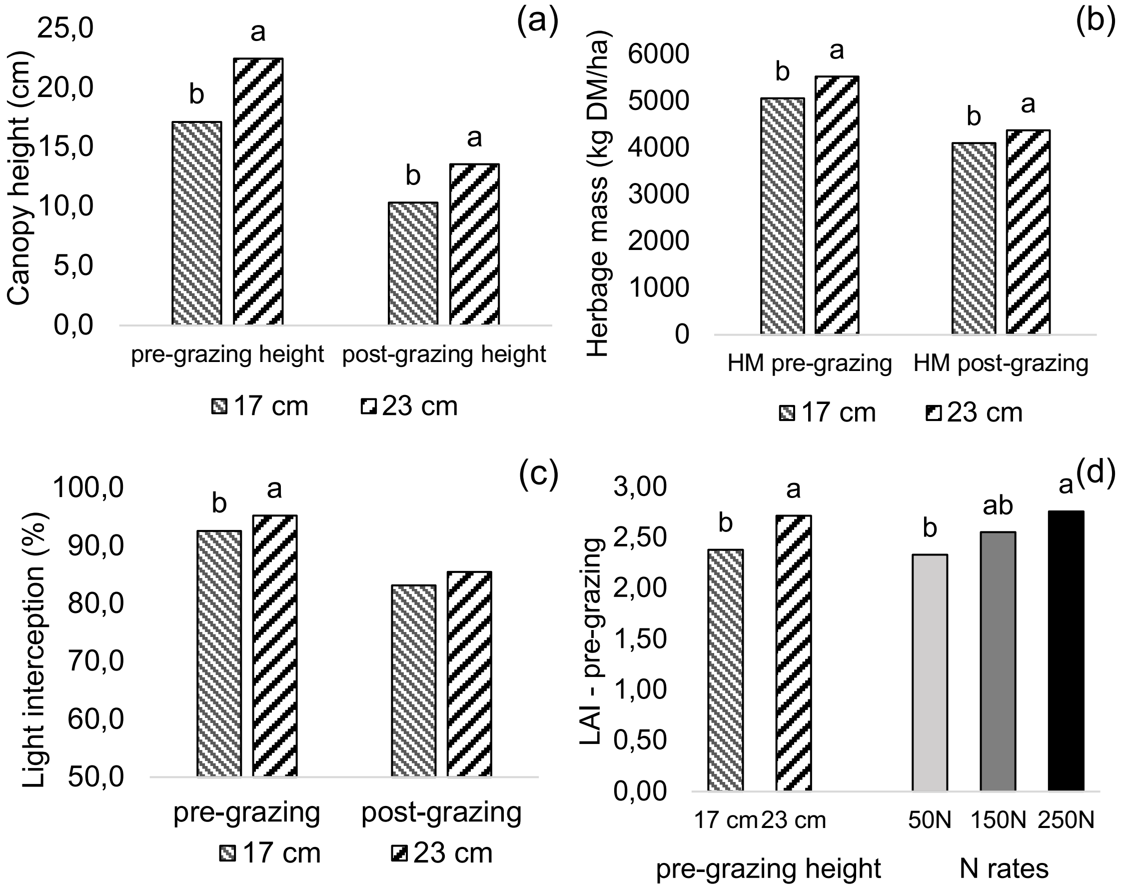


Figure 2. Nitrogen Nutrition Index (NNI) of kikuyu grass and Tifton 85 growing in a multispecies pasture submitted to different N rates applied in warm season.



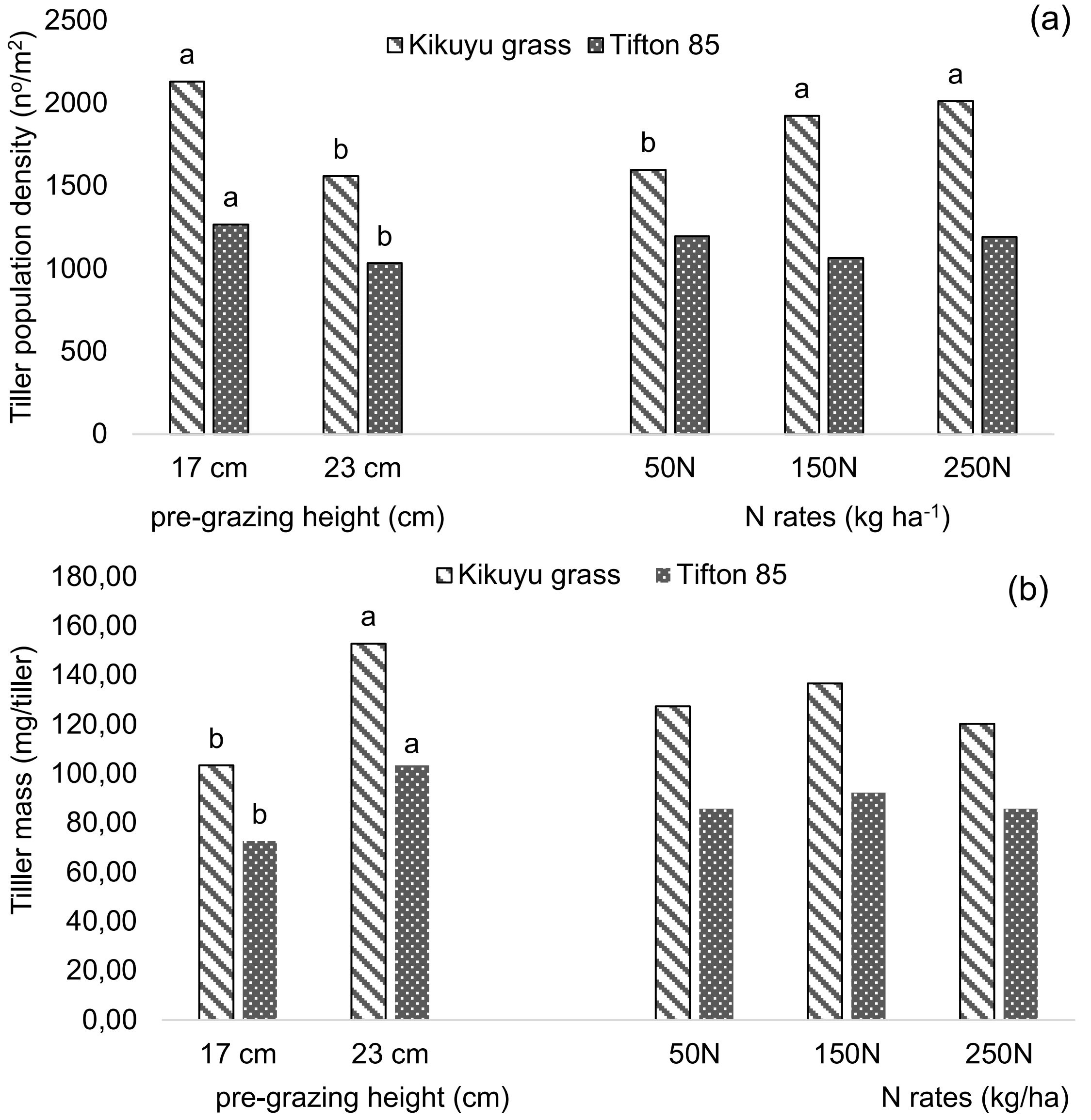
The letters compare the same species under three N rates, different letters meaning a significant difference between the N rates to Tukey Test with 5% of significance.

Figure 3. (a) Pre and post grazing height (cm), (b) herbage mass (HM, kg DM ha^{-1}), (c) light interception (LI, %) and (d) leaf area index (LAI) of multispecies pasture submitted to different management strategies during the warm season.



Bars with different letters have significant difference between the pre-grazing height targets and among N rates to Tukey Test with 5% of significance.

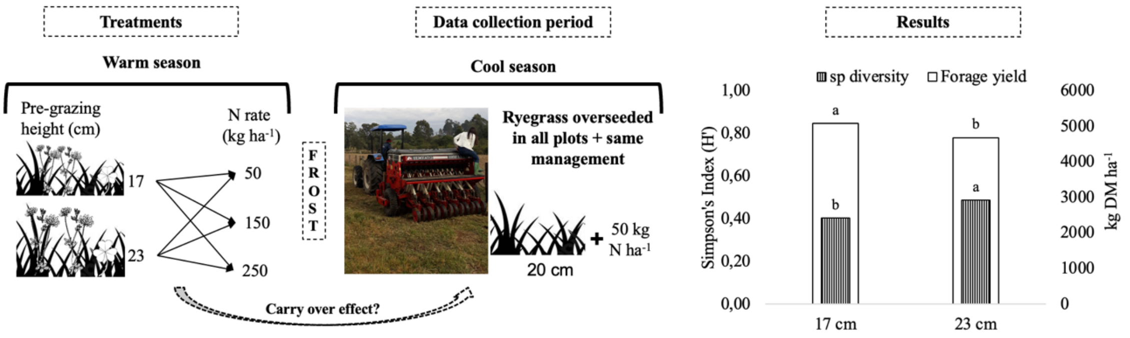
Figure 4. (a) Tiller population density (nº/m²) and (b) tiller mass (mg/tiller) of kikuyu grass and Tifton 85 under different pre-grazing height and N rates.



Bars with different letters compare the same species under different treatments (pre-grazing height or N rates) to Tukey Test with 5% of significance.

6 DOES WARM-SEASON MANAGEMENT OF PERENNIAL GRASS-LEGUME PASTURES AFFECT SUBSEQUENT COOL-SEASON PASTURE RESPONSES?

6.1 GRAPHICAL ABSTRACT



6.2 ABSTRACT

Overseeding cool-season annual grasses into a warm-season perennial pasture is a common practice, but little is known about the effect of warm-season pasture management on subsequent cool-season pasture responses. We hypothesized that management of a warm-season mixed pasture affects subsequent forage production and canopy diversity in the cool season. The experiment was conducted from 2020 through 2022. The treatments were applied during the warm season (November – April/May) to a mixed-species pasture containing grasses and legumes, and the evaluation was performed in the subsequent cool season (May – November) following overseeding with annual ryegrass (*Lolium multiflorum* L.). Treatments applied in the warm season were the factorial combinations of two pre-grazing canopy heights (17 and 23 cm) and three nitrogen (N) rates (50, 150, and 250 kg N ha^{-1}) replicated three times in a randomized complete block design. In the cool season, all plots were managed uniformly, with a pre-grazing canopy height of 20 cm and a single application of 50 kg N ha^{-1}. There was no residual effect of warm-season N fertilization on any cool-season response, but the 17-cm pre-grazing height in the warm season resulted in greater cool-season forage accumulation than the 23-cm height (5100 vs. 4780 kg DM ha^{-1}). In contrast, species diversity (Simpson's index of 0.49 vs. 0.40) and community functional diversity (Rao's index of 0.33 *vs*. 0.29) during the cool season were greater for swards with a warm-season pre-grazing height of 23 vs. 17 cm. Even though the 17-cm warm-season pre-grazing height increased forage production in the subsequent cool season, the magnitude of this effect was small. Therefore, a summer management height close to that corresponding to the critical LAI (23 cm) is recommended because it increased the proportion of legumes and enhanced pasture diversity in the cool season.

Keywords: functional diversity, legacy effect, legumes, species diversity

6.3 INTRODUCTION

Southern Brazil is dominated by a subtropical climate, which is characterized by a high intra-annual temperature range and the absence of a dry season. These characteristics allow cultivation of warm- and cool-season forage species on the same land during different seasons of the year (Sbrissia et al., 2017). Seeding annual grasses, such as Italian ryegrass (*Lolium multiflorum)* and oat (*Avena* spp.), into a warm-season perennial pasture, such as bermudagrass (*Cynodon* spp.), is a common practice for livestock production in such climates (Schmidt, 2022). Even though this is a typical practice, little is known about the impact of pasture management during the warm season on the dynamics of vegetation during the succeeding cool season. This carryover effect of a particular crop or management to a following crop/season can be described as a legacy effect, and it refers to effects that persist after the biotic interaction that caused them has ceased (Wurst & Ohgushi, 2015). Drought (Hahn et al., 2021), fertilization rate (Crème et al., 2018), and species diversity (Kostenko & Bezemer, 2020) are some of the treatments that have been applied, and the outcomes are typically measured in terms of subsequent crop yield. However, the effects of pasture management *per se* in promoting legacy effects are less studied (but see Grange et al., 2022).

Several management techniques can alter vegetation dynamics, but defoliation intensity and fertilization (especially nitrogen) are by far the most prevalent in managed grasslands (da Silva et al., 2019; Egan et al., 2018; Zanella et al., 2021). An increase in nitrogen fertilization typically increases forage production and the number of grazing cycles, particularly in grass monocultures (Enriquez-Hidalgo et al., 2016); however, this management can also increase treading, which is detrimental to legume persistence in grass-legume mixtures (Kleen et al., 2011). Furthermore, in these conditions, high levels of N fertilization can promote legume exclusion because grasses are better at capturing resources and can dominate the area (Soussana & Oliveira Machado, 1999).

Defoliation management can alter the canopy structure and may have legacy effects. In general, maintaining pastures at shorter heights stimulates tillering but reduces the leaf area index (LAI) (Bernardon et al., 2021; Sbrissia et al., 2020), whereas pastures grazed at taller heights exhibit less tiller population density and greater herbage mass (Sbrissia et al., 2018). Similarly, management height can also influence the botanical composition in mixed grasslands. For example, in a grass mixture of *Cenchrus clandestinus* and *Festuca arundinacea*, canopies managed at 12 cm had a lower proportion of tall fescue than canopies managed at 17 cm. Furthermore, one severe defoliation (7 cm) at the end of the warm season modulated the canopy structure (herbage mass, leaf, and dead material proportion) and delayed pasture regrowth in the following cool season (Miqueloto et al., 2020). In a grass-legume mixture, increasing canopy height over the range from 10 to 40 cm reduced the proportion of legume in the mixture due to increased light restriction to forage peanut, which occupies the stratum closest to the soil surface (Tamele et al., 2018).

Considering that N fertilization and defoliation management can alter the sward structure and species diversity in a multi-specific pasture, we hypothesized that the management applied in the warm season in a C_4 perennial-based canopy changes forage production and canopy diversity in the subsequent cool-season pastures.

6.4 MATERIAL AND METHODS

The study was carried out at Santa Catarina State University (UDESC) Lages, Santa Catarina, Brazil (27°47′ S, 50°18′ W). The soil at the experimental site is a typical Inceptisol (Cambissolo Húmico Alumínico Típico; EMBRAPA, 2006), and according to the Koppen classification, the region's climate is humid subtropical (Cfb) with mild summers, well-distributed rainfall throughout the year, and severe winters (10–15 freeze events per year). Figure 5 provides the weather conditions during the experimental period. The experiment was approved by the UDESC Ethics Committee (Number 1093021219).

6.4.1 Treatments

The experimental protocol lasted for three years (2020–2022). This trial was divided into two parts: warm season (November/December – April/May) and cool season (May – October/November). The treatments were applied during the warm season, and the measurements were taken during the subsequent cool season. The warm-season pastures were composed of kikuyugrass (*Cenchrus clandestinus*), Tifton 85 bermudagrass (*Cynodon* spp.), pintoi peanut (*Arachis pintoi* cv. Amarillo), birdsfoot trefoil (*Lotus corniculatus* L. cv. São Gabriel), and white clover (*Trifolium repens* L. cv. Zapican).

6.4.2 Warm season

The treatments were applied throughout the warm seasons of 2019–2020 (December to April), 2020–2021, and 2021–2022 (November to April/May) and corresponded to two canopy heights and three nitrogen (N) rates in a 2 × 3 factorial arrangement of a randomized complete block design with three replications. The two pre-grazing canopy heights employed were 17 and 23 cm, with grazing terminated at 10 and 14 cm, respectively. This was done to remove the same height proportion (40%) across treatments, and rotational stocking was employed with a one-day occupation period. The total nitrogen application rates were 50, 150, and 250 kg N ha^{-1} (urea 45%) split across two application events (November and January). Each paddock measured 315 square meters.

6.4.3 Cool season

In all years, at the end of the warm season (April), all plots were grazed down to 10 cm and the pastures were overseeded with annual ryegrass (*Lolium multiflorum* L.) (Figure 6-a). The seeding rate was 25 kg ha^{-1}, and 50 kg of N ha^{-1} was applied at the three-leaf stage of annual ryegrass (around 45 days after seeding, Figure 6-b). From June to the end of the experimental period (October), all eighteen plots were managed uniformly, with pre-grazing canopy heights of 20 cm and post-grazing canopy heights of 12 cm. Canopy height was measured with a ruler at 30 random points in the paddock and the grazing was done by dairy heifers weighing 330 kg live weight. All plots were grazed to 10 cm in early November to mark the end of the evaluation period and, in the case of year 1 and 2, the restart of the treatment application period (warm season). Because the same management criteria for all pastures were used during the entire cool season, differences in vegetation dynamics were attributed to differences in the previous (summer) management, i.e., a legacy effect caused by the warm-season management.

6.4.4 Response variables

Herbage mass was measured at pre- and post-grazing using a rising plate meter (Farmworks®; F200, New Zealand). The equations to calibrate the rising plate meter were developed by collecting three samples per experimental unit (pre- and post-grazing) at ground level in each grazing event in the first and second year. The determined equations were (1) pre-grazing: herbage mass = $89*x + 646$ ($r^2 = 0.60$); (2) post-grazing herbage mass = $106*x + 480$ ($r^2 = 0.54$). The forage accumulation (kg DM ha^{-1}) was calculated as the difference between the pre-grazing herbage mass of cycle $_n$ and the post-grazing herbage mass of cycle $_{n-1}$. However, during the first grazing, the post-grazing herbage mass was considered zero because all remaining warm-season vegetation was damaged by frosts that occurred at the beginning of the cool season (Figure 6-c).

Canopy light interception was measured using the canopy analyzer LAI 2000C (LI-COR, Lincoln, Nebraska, USA), and measurements were performed pre- and post-grazing in each experimental unit, in the middle of cool season. Readings were taken from 5 sampling areas per paddock, representative of the sward condition at the time of sampling. In each sampling area, 1 reading was taken above the canopy and 6 at ground level, for a total of 5 readings above the canopy and 30 at ground level per experimental unit.

The botanical composition of the canopy was determined at pre-grazing in the middle (August) and at the end (October) of the growing season in the first two experimental years only, due to logistical constraints. Three quadrats of 0.5-m² (0.5 × 1.0 m) were sampled per paddock to ground level. After harvesting, a subsample (~25%) of each sample was manually separated into species and then divided into their botanical components [leaves or stems (including reproductive parts)] and dead material. The number of ryegrass tillers was also counted.

Next, the detached leaf blades of each species were scanned with a LAI-3100C leaf area meter (LI-COR Biosciences Lincoln, NE, USA). Following these procedures, all components were placed in a forced-air circulation oven at 65 °C for 72 h before being weighed. Using the data from the subsamples and the dry matter (DM) weight of the samples and components, we calculated the canopy LAI, annual ryegrass tiller population density (TPD), tiller mass (g/tiller), and species diversity using the Simpson's Index (Simpson, 1949).

Additional measurements were taken in the second year. The ryegrass leaf spectral reflectance was measured in the middle of the cool season (August) using a portable narrow-bandwidth leaf spectrometer (CI-710; CID Bio-Science, Camas, WA, USA) in reflectance mode. Integration time was set to 300 ms, boxcar width was 10, and ten fully expanded leaves were scanned to generate average values per paddock. Because daily temperature and rainfall can affect the results, all measurements (paddocks) were taken on the same day, regardless of the number of days of regrowth. The calculated spectral indices were NDVI (Rouse et al., 1974), PRI (Gamon et al., 1992), MRESRI (Datt, 1999), and WBI (Penuelas et al., 1997).

In the second experimental year, we also sampled 35 fully expanded leaves of ryegrass (August) and 20 white clover (September) and birdsfoot trefoil (October) plants in pre-grazing conditions to determine the specific leaf area (SLA) and leaf dry matter content (LDMC) of species (Cornelissen et al., 2003). The legume sample collection followed the procedure adopted by Nölke et al. (2021). The months chosen for collection were selected to represent the major contribution of each species in the canopy (previous observations). Rao’s quadratic entropy (Rao, 1982) was used as a metric of community functional diversity.

6.4.5 Statistical analysis

The data analyses were performed using R environment (R Core Team, 2021). The analysis of variance was done using the mixed model of the lme4 package (Bates et al., 2015), considering block and year as random effects, while the height of defoliation, nitrogen rate, and their interaction were fixed effects. The Akaike Information Criterion was used to select the model (AIC). The Simpson's Index and Rao Index were calculated using the Rao.diversity function of the SYNCSA package (Debastiani & Pillar, 2012). The values were subjected to ANOVA, and when appropriate ($P<0.05$), the averages were compared using the Tukey Test with a 5% significance level.

6.5 RESULTS

The warm-season grazing height management affected cool-season forage accumulation. The pastures grazed to shorter heights in the summer had 7% (320 kg DM ha^{-1}) greater forage accumulation during the cool season than those grazed to taller heights (5100 vs. 4780 for 17- and 23-cm pre-grazing heights, respectively). However, the warm-season N rate did not affect cool-season forage accumulation (Table 4). The annual ryegrass tiller density was the same across the treatments (Table 4), but annual ryegrass tiller mass (mg/tiller) in the cool season increased with increasing warm-season N rates (48, 57, and 60 mg/tiller) (Table 4). There was no effect of summer grazing heights on annual ryegrass tiller mass in winter (Table 4). Herbage mass and LAI pre- and post-grazing were similar across treatments, and only post-grazing LAI was affected by previous management height (LAI: 1.16 vs. 1.00 for 17 and 23 cm, respectively) (Table 5).

Regardless of treatment, annual ryegrass predominated during the cool season. Summer grazing height influenced the presence of legumes during the cool season, particularly of birdsfoot trefoil, which comprised 3.4 and 8.6% of herbage mass in the 17- and 23-cm management heights, respectively. Furthermore, the combination of shorter summer grazing height and the greatest N rate reduced the presence of legumes (Table 6). Kikuyugrass contributed up to 10% of the herbage mass, and pintoi peanut made a negligible contribution during the cool season (Table 6).

Taller summer management heights increased species diversity compared with shorter heights (Simpson's index of 0.49 vs. 0.40 for 23 and 17 cm, respectively) (Table 7); however, summer N rates did not affect any diversity index during the cool season. Community functional diversity was not affected by N rates, but it tended to increase the functional dissimilarity in taller summer canopy heights (Rao's index of 0.29 *vs*. 0.33 for 17 and 23 cm, respectively) (Table 7).

Annual ryegrass SLA and LDMC were affected by summer management height (SLA: 254 vs. 211 and LDMC: 20.1 vs. 22.1, for 17 and 23 cm, respectively), with no effect of N rate (Table 8). Summer management did not affect annual ryegrass spectral index (Table 9).

6.6 DISCUSSION

6.6.1 Forage Accumulation

The forage accumulation of the cool-season pastures was greater for those canopies that were grazed to a shorter height in the previous summer season. A possible cause of this could be the difference in the sward botanical composition. The proportion of annual ryegrass in the pasture managed at a shorter height in the summer (17 cm) was greater than in the pasture managed at a taller height (76 vs. 69%), and this increase occurred at the expense of the proportion of legumes, especially birdsfoot trefoil. The replacement of birdsfoot trefoil by annual ryegrass affects forage accumulation because the sward then has a greater proportion of a species with greater forage production potential (Gierus et al., 2012). An increase in forage accumulation as a function of changes in the grass:legume ratio was previously reported by Payne et al. (2021) for a bermudagrass-white clover mixture.

Contrary to our second hypothesis, greater N rates applied during the warm season did not boost forage production of annual ryegrass-dominated pastures in the subsequent cool season. All available N applied during the summer may have been taken up by kikuyugrass and Tifton 85, two species well known for their capacity to take up and exploit large quantities of N (Cruz et al., 2002). This conclusion is supported by the similarities in the spectral index of annual ryegrass (Table 9), especially those components that have a close relationship with herbage N concentration, such as PRI. The PRI has been associated with numerous canopy features, including photosynthetic light use efficiency (Gamon et al., 1992; Patel et al., 2021). In addition, the litter remaining after summer grazing contained a significant amount of stems and old leaves; morphological components with a slower decomposition rate compared to combinations including a large proportion of legumes, which may have hindered N release throughout the winter (Kohmann et al., 2018).

Grange et al. (2022) established plots with 1 to 6 species from three functional groups (grasses, legumes, and herbs) that were fertilized with 150 kg N ha yr^{-1} and extra replicated plots of *Lolium perenne* (grass-only) fertilized with 300 kg N ha yr^{-1}. After two years (grassland phase), the plots were terminated (sprayed with herbicide) to establish a Lolium multiflorum (Italian ryegrass) monoculture (crop phase) to investigate the effect of grassland diversity on the performance of a follow-on crop. The authors found no indications of interspecific interactions (no overyielding caused by diversity) and recorded the lowest forage accumulation in plots where ryegrass was the preceding crop, regardless of N rate. The authors hypothesized that the added nitrogen from fertilizer applied to the monoculture was lost from the system (nitrate leaching and nitrous oxide emissions). The authors argue that the absence of a biodiversity effect (overyielding) in the succeeding forage accumulation was due to more effective resource use by the mixture in the grassland phase, which involved interspecific interactions (Grange et al., 2021). They suggested that the proportion of the legume functional group is critical for guaranteeing high-quality forage production and a robust legacy impact.

6.6.2 Botanical composition

Species diversity in the cool season was greater for pastures grazed to taller heights during the warm season, but there was no effect of warm-season N rate (Table 7). The lack of a N effect was attributed to the possible total use of available N applied during the summer by the grasses of that period, since that N rates and legumes typically have an inverse relationship (Lüscher et al., 2014).

The legacy of warm-season grazing height management in terms of biodiversity was more apparent in the percentage of birdsfoot trefoil in the canopy (Table 6). Birdsfoot trefoil is a crown-forming legume whose persistence under grazing is contingent on management to ensure natural reseeding and the establishment of new seedlings (Scheffer-Basso et al., 2011). In this regard, longer defoliation intervals (42 d) are better than shorter intervals (21 d) at preserving root total non-structural carbohydrates (TNC), an essential source of energy for birdsfoot trefoil regrowth (Alison & Hoveland, 1989). These authors also confirmed a positive correlation between root TNC during the warm season and forage accumulation in the following spring, indicating that the defoliation interval utilized during one period can affect future plant production. Similarly, Bologna et al. (1996) stated that management during autumn is crucial for birdsfoot trefoil persistence because most seedling recruitment occurs during this time, and a significant proportion of seedlings that were established early in the fall survived the following cool season. Therefore, we believe that shorter management heights and, as a result, shorter defoliation intervals during the warm season may have compromised their leaf area regrowth resulting in insufficient replenishment of reserves, thereby compromising the persistence of birdsfoot trefoil during the cool season.

On the other hand, the white clover maintained a proportion of approximately 10% in all treatments except for the 17/250 treatment (Table 6). This combination of reduced height and high N rate appeared to particularly damage the persistence of this legume. White clover is unaffected by shorter heights and can increase its proportion in the sward under these conditions (Phelan et al., 2013). However, the detrimental effects of a high nitrogen concentration on white clover are well known (Egan et al., 2018). In a three-year bermudagrass + white clover trial (mechanical cuts to 5 cm), N rates greater than 112 kg ha^{-1} led to a linear decrease in the proportion of white clover (Payne et al., 2021). Moreover, kikuyugrass comprised a larger proportion of the herbage mass than Tifton 85. This may be because kikuyugrass has a lower critical temperature than many other C4 grasses (Ivory & Whiteman, 1978). These qualities allow kikuyugrass to regenerate rapidly at the end of the cool season without suffering detrimental effects of ryegrass presence throughout the cool season (Botha et al., 2008).

6.6.3 Foliar traits and functional diversity

The SLA and LDMC reflect the plant-wide resource acquisition and utilization strategies. Thus, variation in these characteristics among dominant species due to management may affect plant functional overlap, coexistence, and ultimately community assembly (Siefert, 2012; Streit et al., 2022). The warm-season pre-grazing height of 17 cm shifted annual ryegrass to a more resource-acquisition role, which may have increased its dominance. Cruz et al. (2010) indicated that the capture strategy in this instance would correlate with maximizing light interception in the absence of N availability. The enhanced species diversity (reduced annual ryegrass dominance) observed in the 23-cm summer pre-grazing treatment (perhaps connected with trait species assembly) tended (P=0.06, Table 7) to increase the functional diversity of the community (Rao's Index). This index can be a better description of ecosystem functioning than the abundance of functional groups (Lepš et al., 2011) and relates to the supply of several ecosystem services (Gross et al., 2017). Therefore, if the objective is to create more sustainable grazing habitats, this taller warm-season grazing height seems to be more desirable.

6.7 CONCLUSIONS AND IMPLICATIONS

Warm-season management height influenced the forage production and species diversity of the subsequent cool-season pastures in a subtropical environment in southern Brazil. Furthermore, our data indicate the lack of residual effect of N fertilization applied during the warm season on the subsequent cool season. Although the warm-season pre-grazing height of 17 cm increased forage production in the subsequent cool season, this effect was negligible. Therefore, a summer management height close to that corresponding to the critical LAI (23 cm) is recommended because it increased the proportion of legumes and pasture species diversity.

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6.9 TABLES AND FIGURES

Table 4. Cool-season forage accumulation (kg DM ha^{-1}), annual ryegrass tiller population density (TPD – tillers/m²), and tiller mass (mg/tiller) following application of different management practices to a perennial pasture during the previous warm season.

	Warm-season treatments						*P-value*		
	17/ 50	17/ 150	17/ 250	23/ 50	23/ 150	23/ 250	Height	N rate	H * N
Forage accumulation (kg DM ha^{-1})	5140	5030	5130	4850	4760	4740	0.031	0.930	0.792
TPD (n°/m²)	2320	2340	2340	2590	2350	1780	0.623	0.236	0.209
Tiller mass (mg)	51.8	56.1	55.5	43.5	57.1	64.8	0.898	0.025	0.171

Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Table 5. Pre- and post-grazing height (cm), pre- and post-grazing herbage mass (HM, kg DM ha^{-1}), pre- and post-grazing light interception (LI), and pre- and post-grazing leaf area index (LAI) of cool-season pastures following different summer management strategies of warm-season perennial pastures.

	Warm-season treatments						*P-value*		
	17			23					
	50	150	250	50	150	250	Height	N rate	H * N
Pre-height (cm)	18.8	18.7	18.7	18.8	18.6	18.7	0.635	0.716	0.737
Post-height (cm)	11.4	11.6	11.4	11.4	11.3	11.8	0.762	0.613	0.313
HM (pre) (kg DM ha^{-1})	2730	2780	2750	2760	3110	2760	0.393	0.444	0.609
HM (post) (kg DM ha^{-1})	2330	2210	2180	2460	2460	2120	0.436	0.349	0.681
LI pre (%)	93.3	92.0	92.7	93.9	93.8	92.8	0.470	0.810	0.821
LI post (%)	81.5	76.0	79.0	77.0	81.9	76.3	0.880	0.844	0.284
LAI (pre)	2.38	2.20	2.38	2.33	2.55	2.13	0.930	0.804	0.318
LAI (post)	1.06	1.17	1.26	0.98	1.19	0.83	0.029	0.143	0.031

Means follow per different lower case in line and upper case in column differ statistically ($p<0.05$).

Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Table 6. Botanical composition (pre-grazing) of a cool-season pasture after different management practices were applied in the previous warm season.

	Warm-season treatments					
Specie (%)	17/50	17/150	17/250	23/50	23/150	23/250
Kikuyugrass	7.0	8.3	9.5	7.9	8.6	6.1
Tifton 85	5.9	3.0	5.8	3.2	4.1	5.7
Pintoi peanut	0.2	0.1	0.4	0.0	0.0	0.5
Annual ryegrass	73.7	74.0	79.2	66.7	71.2	67.6
Birdsfoot trefoil	4.0	4.8	1.3	11.5	7.6	6.8
White clover	9.2	9.8	3.8	10.6	8.4	13.3

Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Table 7. Simpson’s and Rao’s Index of cool-season pastures following different summer management strategies of warm-season perennial pastures.

	Warm-season treatments						*P-value*		
	17/ 50	17/ 150	17/ 250	23/ 50	23/ 150	23/ 250	Height	N rate	Inter.
Simpson Index	0.43	0.43	0.35	0.52	0.46	0.48	0.017	0.365	0.479
Rao Index	0.29	0.31	0.27	0.35	0.31	0.31	0.062	0.199	0.291

Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Table 8. Specific leaf area (SLA) and leaf dry matter content (LDMC) of species that compose a multi-specific pasture in cool-season pasture submitted to different managements in the previous warm season.

	Warm-season treatments						*P-value*		
Height (cm)	17			23					
N Rate (kg ha^{-1})	50	150	250	50	150	250	Height	N Rate	H*N
Species	SLA (cm^2/g)								
A. ryegrass	244	273	245	226	200	208	0.004	0.791	0.208
B. trefoil	225	254	225	207	215	206	0.013	0.149	0.544
W. clover	362	354	319	384	342	360	0.360	0.316	0.511
	LDMC (g g^{-1})								
A. ryegrass	20.8	19.3	20.1	21.7	23.0	21.6	0.009	0.873	0.219
B. trefoil	23.2	20.8	23.5	23.1	23.1	23.3	0.283	0.120	0.152
W. clover	17.2	18.2	19.8	16.4	18.7	17.3	0.296	0.210	0.384

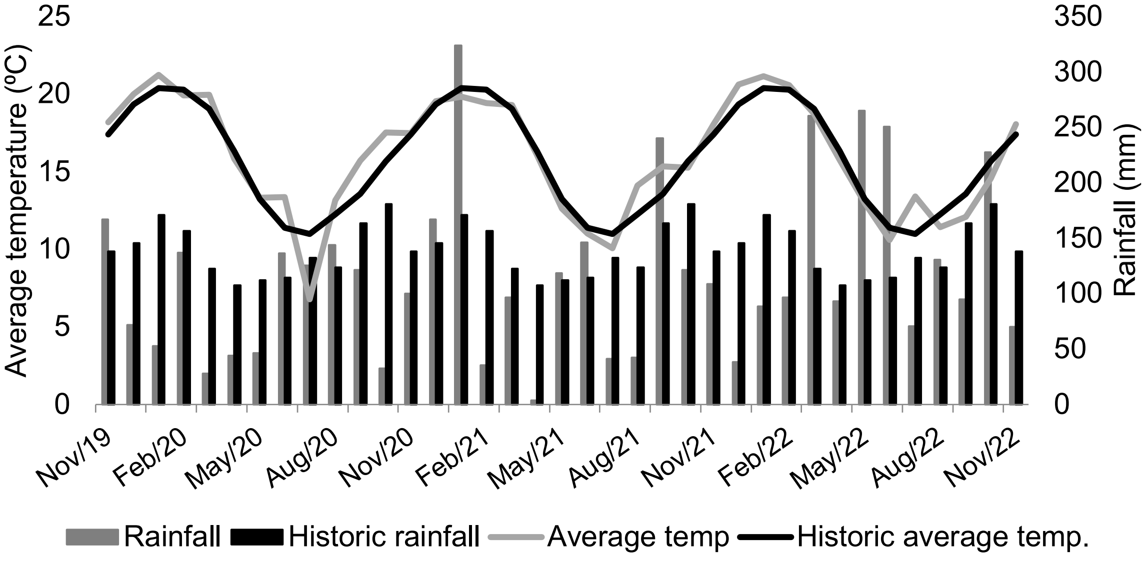
Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}. Species: A. ryegrass=annual ryegrass; B. trefoil= birdsfoot trefoil; W. clover= white clover.

Table 9. Spectral index of annual ryegrass in cool-season pastures submitted to different management practices during the previous warm season.

	Treatments						*P-value*		
Index	17/ 50	17/ 150	17/ 250	23/ 50	23/ 150	23/ 250	Height	N Rate	Inter.
ndvi	0.877	0.882	0.860	0.859	0.836	0.863	0.102	0.800	0.272
mresri	3.18	3.26	3.69	3.27	4.14	3.60	0.226	0.219	0.224
pri	0.036	0.039	0.026	0.029	0.028	0.030	0.134	0.276	0.12
wbi	1.71	1.61	1.47	1.61	1.33	1.47	0.164	0.166	0.42
psri	-0.032	-0.032	-0.031	-0.026	-0.034	-0.028	0.457	0.387	0.431

Legend: warm-season treatments: 17/50=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 17/150=pre-grazing height of 17 cm and 150 kg N ha^{-1}; 17/250=pre-grazing height of 17 cm and 250 kg N ha^{-1}; 23/50=pre-grazing height of 23 cm and 50 kg N ha^{-1}; 23/150=pre-grazing height of 17 cm and 50 kg N ha^{-1}; 23/250= pre-grazing height of 17 cm and 50 kg N ha^{-1}.

Figure 5. Monthly average weather variables during the study period (Nov. 2019 – Nov. 2022) and relative to the mean long-term values of rainfall and temperature in Lages, Santa Catarina, Brazil.

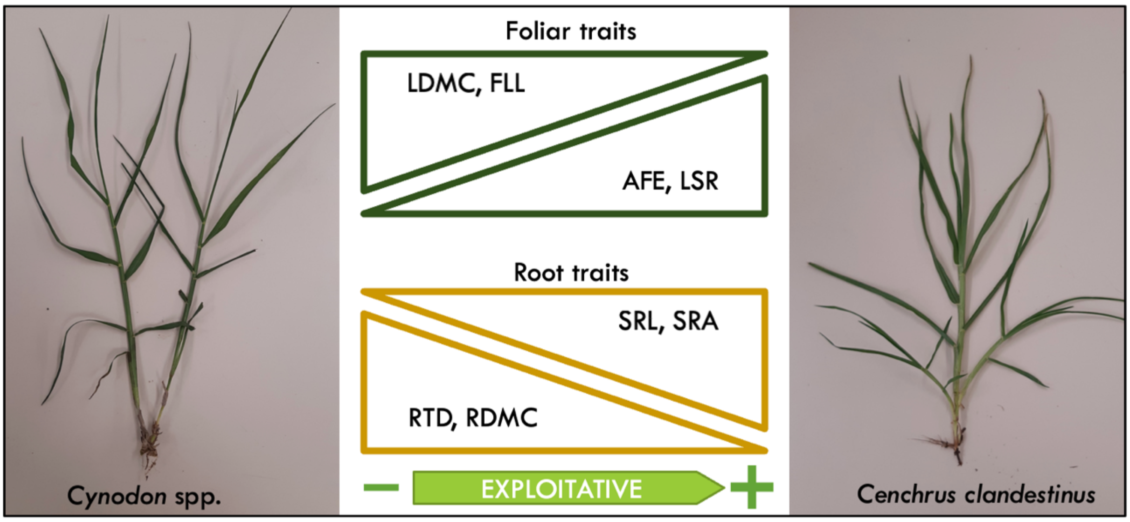


Source: Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, 2020. Historic average values (1948-2016).

Figure 6. (a) Annual ryegrass overseed in warm season perennial pasture; (b) annual ryegrass tillering stage (45 days after seeded); (c) remaining warm season pasture (parched) 10 days after the frost.

7 EXPLORING SOME FUNDAMENTAL DIFFERENCE BETWEEN THE EXPLOITATIVE TIFTON-85 AND KIKUYU-GRASS PLANTS TO EXPLAIN THEIR STABLE COEXISTENCE IN A GRAZING ENVIRONMENT

7.1 GRAPHICAL ABSTRACT



7.2 ABSTRACT

The ecological theory of limiting similarity proposes that species coexistence is facilitated when they are functionally dissimilar. Despite this, we identified in an experiment that Tifton 85 (*Cynodon* spp.) and Kikuyu grass (*Cenchrus clandestinus*), belonging to the same functional group of resource acquisition, coexisted stably in a pastoral environment after three years of management. Thus, we investigated various functional attributes of these species with the hypothesis that plants belonging to the same ecological spectrum present important functional dissimilarities that allow their coexistence in a wide variation of nitrogen fertilizer input. The experimental area was formed with the grasses as well as legumes *Trifolium repens*, *Arachis pintoi*, and *Lotus corniculatus*. The experiment was conducted for three years, and the stocking method used was intermittent with 40% defoliation severity. The applied treatments were three rates of nitrogen fertilization: 50, 150, and 250 kg N ha^{-1}, distributed in a randomized complete block design with six replicates per treatment. The functional markers were measured only in Tifton 85 and kikuyu grass and comprised various root and shoot traits. After three years of management, the grasses together composed 90% of the forage canopy, with balanced proportions between the species in all treatments. Tifton 85 was associated with traits related to more conservative resource use strategies, such as LDMC (leaf dry matter content) and RTD (root tissue density), while Kikuyu grass was associated with traits related to more resource-explorative plants, such as SRL (specific root length) and SLA (specific leaf area). The species were dissimilar from each other and maintained these differences independently of the nitrogen fertilizer doses. Despite the differences between the plants, both showed higher values of SLA at higher N doses. The use of species from the same functional group (resource explorers), but which maintain dissimilarities in traits, is an alternative for the construction of stable mixtures in multi-species pastoral environments.

Keywords: complementarity, limiting similarity, multifunctionality, multispecies grasslands

7.3 INTRODUCTION

Communities composed of functionally dissimilar species have been considered an alternative to increase the delivery of ecosystem services by pastures (Gross et al., 2017). Functional diversity has been considered more important than species diversity per se since dissimilarities promote lower competition and greater complementarity among plants (Díaz & Cabido, 2001; Komainda et al., 2020), resulting in many cases in overyielding of mixtures compared to monocultures (Wagg et al., 2017).

Complementarity among plants is typically associated with the strategy of capturing and using resources in space and/or time (Reich, 2014), but it can extend to encompass physiological traits, such as preference for different source molecules of the same nutrient (Maire et al., 2012). Thus, species occupy niches, defined as a hyper-volume in the multidimensional space of ecological variables, within which a species can maintain a viable population (Hutchinson, 1957). Therefore, the number of species that compose a community is proportional to the total range of the environment divided by the specificity of each species (Pacala & Tilman, 1994).

In this sense, plants with functionally dissimilar characteristics could coexist in the same environment, while those occupying a similar multidimensional ecological space are more competitive with each other, and once this limit of similarity is surpassed, species coexistence can be compromised (Macarthur & Levins, 1967). In addition, pastoral environments aimed at intensive forage production are subject to high doses of nitrogen fertilization and high frequency of defoliation, conditions in which only tolerant species can persist (Fischer et al., 2019), creating an environmental filter imposed by management (Keddy, 1992). Thus, in a multispecies pasture, both niche differentiation and environmental filtering explain species abundance (Maire et al., 2012).

Plants have the ability to alter their morphology and physiology in response to environmental conditions (Louault et al., 2017; Pontes et al., 2015). This intra-specific variation in traits occurs in root and canopy structures and can be mediated by fertilization (Leuschner et al., 2013) and intensity and frequency of defoliation (Lezama et al., 2014). Streit et al. (2022) observed greater intraspecific variation for species ecologically defined as "conservative" compared to resource "explorer" species. Thus, management mediates the arrangement of community traits, which can make species more dissimilar and theoretically favor their coexistence, or increase niche overlap and therefore competition among plants and functional redundancy of the community (Louault et al., 2017; Macarthur & Levins, 1967; Siefert, 2012).

Considering this approach, we observed in an experimental area that after three years, two grasses, Cenchrus clandestinus and Cynodon spp., composed about 45% of the canopy each, without a clear dominance signal, and under a considerable range of nitrogen fertilization (50 to 250 kg N ha-1). Both species are perennials with a stoloniferous growth habit, similar management height recommendations (Mezzalira et al., 2014; Sbrissia et al., 2018), C4 photosynthetic cycle, and ecologically considered resource explorers (Cruz et al., 2002). Thus, considering the limiting similarity hypothesis (MacArthur & Levins, 1967) and the current challenge of the ecological community to understand the underlying processes that explain species abundance and coexistence in communities (Lhotsky et al., 2016), we investigated several functional traits of plants with the hypothesis that plants belonging to the same ecological spectrum present some functional dissimilarities that allow their coexistence in a wide range of nitrogen fertilization levels. Considerando esta abordagem, observamos em uma área experimental que após três anos, duas gramíneas, *Cenchrus clandestinus* e *Cynodon* spp., compunham cerca de 45% do dossel cada uma, sem um sinal claro de dominância e sob uma considerável amplitude de adubação nitrogenada (50 a 250 kg de N ha^{-1}). Ambas as espécies são perenes, com hábito de crescimento estolonífero, com recomendações de altura de manejo semelhantes (Mezzalira et al., 2014; Sbrissia et al., 2018), de ciclo fotossintético C4 e ecologicamente consideradas plantas exploradoras de recursos (Cruz et al., 2002). Thus, considering the limiting similarity hypothesis (MacArthur & Levins, 1967) and the current challenge of the ecological community to understand the underlying processes that explain species abundance and coexistence in communities (Lhotsky et al., 2016), we investigated several functional traits of plants with the hypothesis that plants belonging to the same ecological spectrum present some functional dissimilarities that allow their coexistence in a wide range of nitrogen fertilization levels.

7.4 MATERIAL AND METHODS

The experimental area, consisting of 18 paddocks of 315 m2, was established during the hot season of 2019 with the perennial species kikuyu grass (*Cenchrus clandestinus*), Tifton 85 (*Cynodon* spp.), and pintoi peanut (*Arachis pintoi* cv. Amarillo). In May 2019, the area was overseeded with cool-season species: annual ryegrass (*Lolium multiflorum* L. cv. La Estanzuela 284), white clover (*Trifolium repens* L. cv. Zapican), and birdsfoot trefoil (*Lotus corniculatus* L. cv. São Gabriel).

The experimental treatments were applied in the following three years (2020, 2021, and 2022) during the hot season (November and April). The treatments used were three nitrogen rates, 50, 150, and 250 kg of N ha^{-1} arranged in a randomized complete block design with six replications per treatment. The grazing method used was intermittent, with a pre-grazing height of 20± cm and exit height corresponding to 60% of the animal's entry height. During the cool season (2020 and 2021), all paddocks were overseeded with annual ryegrass and managed equally, with the application of 50 kg of N ha^{-1} during the initial tillering stage.

The determination of canopy botanical composition was performed twice in each paddock during each experimental period (hot season). In the pre-grazing condition, three samples of 0.5 m² (0.5 x 1.0 m) were collected at ground level, of which one subsample (~20%) was manually separated into leaf and stem of Kikuyu grass, Tifton 85, and legumes. Subsequently, the samples were dried in a forced-air oven at 65°C for 72 hours to determine the dry weight of the fractions.

7.4.1 Determinação dos traits

All procedures described below refer exclusively to the measurement of traits for kikuyu grass and Tifton 85. Leaves from kikuyu grass and Tifton 85 separated for determination of pasture botanical composition in the three experimental years were analyzed using the Dumas combustion method 968.07

(Association of Official Analytical Chemists, 1990) through the FP 528 equipment (LC, Leco Corporation, Saint Joseph, MI) to determine leaf nitrogen content (LNC). During the summer of 2021, 30 fully expanded leaves were collected to determine the following traits: specific leaf area (SLA) and leaf dry matter content (LDMC) following the methodology proposed by Cornelissen et al. (2003). Leaves were scanned using the LAI-3100C leaf area meter scanner (LI-COR Biosciences Lincoln, NE, USA) and dried in a forced-air oven at 65°C for 72 hours before weighing.

Leaf spectral indices were measured using a narrow-bandwidth leaf spectrometer (CI-710; CID Bio-Science, Camas, WA, USA) in reflectance mode, with an integration time of 300 ms and a boxcar width of 10. Ten fully expanded leaves from each species were scanned to generate mean values per paddock. Despite the paddocks being in different regrowth conditions, measurements were made on the same day (March 2022) to minimize the influence of environmental conditions. The calculated spectral indices were NDVI (Rouse J W et al., 1974), PRI (Gamon et al., 1992), MRESRI (Datt, 1999), and WBI (Penuelas et al., 1997).

Morphogenic traits were evaluated in ten marked tillers per paddock. Measurements were taken at seven-day intervals throughout the interval between grazing events. From these measurements, the rates of leaf elongation (cm tiller day^{-1}) (LER), leaf senescence (cm tiller day^{-1}) (LSR), number of live leaves per tiller (NLL), final leaf length (cm) (FLL), phyllochron (degree-day) (Ph), and leaf lifespan (degree-day) (LLS) were determined. For calculations that used degree-days, a base temperature of 8°C was established for kikuyu grass (Bell et al., 2013) and 10°C for Tifton 85 (de Oliveira et al., 2000).

Determination of root traits was carried out in February 2022. For this purpose, two soil samples were collected in each paddock. Although the grasses were homogeneously distributed in the area, each sampling was done in a location where only one of the species was present within a 30 cm radius of the collection point, to ensure the absence of roots belonging to another species.

Samples were collected at a depth of 10 cm using an 8 cm diameter steel cylinder, corresponding to a collection volume of 500 cm^3 of soil. Roots were separated from soil by washing through sieves with 2- and 0.5-mm apertures before being scanned using a flat-bed scanner with a light transparency unit

(Epson Expression 12000XL). The WinRhizo root analysis program (Regent Instruments, Québec, Canada) was used to obtain root length, mean diameter (Diam), area, and volume. After this procedure, roots were dried for 72 hours at 65°C and weighed, which allowed us to calculate specific root length (SRL), proportion of root length with a diameter <0.1mm (% Fine roots) (Craine et al., 2002), specific root area (SRA) (Mokany et al., 2008), root tissue density (RTD) (Craine et al., 2002), and root dry matter content (RDMC).

7.4.2 Statistical analysis

A discriminant analysis was performed using the statistical software InfoStat (Di Rienzo et al., 2020), in which all morphogenic, root traits and spectral indices previously described were used. The classification criterion was the grasses under study and the nitrogen doses, identified as low dose (50 kg of N ha^{-1}), medium dose (150 kg of N ha^{-1}), and high dose (250 kg of N ha^{-1}). To evaluate the relationship between the species and nitrogen rates with the previously described traits, a principal component analysis was used with the statistical software InfoStat (Di Rienzo et al., 2020). The normality of the variables was confirmed before performing the multivariate analyses. The data analyses were performed using R environment (R Core Team, 2021). The analysis of variance was done using the mixed model of the lme4 package (Bates et al., 2015), considering block as random effects, while the nitrogen rate was fixed effects. The values were subjected to ANOVA, and when appropriate ($P<0.05$), the averages were compared using the Tukey Test with a 5% significance level.

7.5 RESULTS

The canopy botanical composition was predominantly composed of grasses (90%). Both quicuio grass and tifton 85 had similar proportions in the mixture across all treatments, ranging from 40 to 50% of the canopy for each species (Figure 7).

Principal component analysis showed that 75% of the data variation was explained by the first two axes (Figure 8). The first principal component explained 54% of the data variation and contrasted kikuyu grass with Tifton 85. In this sense, a strong correlation was observed between SRL, SRA, phyllochron, MRESRI, leaf senescence rate, and AFE with kikuyu grass, while Tifton 85 was more associated with RTD, RDMC, final leaf length, and CMSF. The second axis explained 22% of the data variation and distinguished the treatments with medium nitrogen doses from the treatments with high and low doses of both species. The variables most strongly associated with this separation in the second axis were the number of live leaves and root diameter.

The confidence ellipses generated from the discriminant analysis showed dissimilarity between the species regardless of the N dose (Figure 9). When considering the variation within each species, Tifton 85 showed slightly greater intraspecific variation with the three nitrogen doses applied, resulting in the formation of three different groups of individuals (Figure 10).

7.6 DISCUSSION

Kikuyu grass and Tifton 85, despite being species considered resource exploiters (Cruz et al., 2002), presented important differences in their traits. The dissimilarities observed between the species in some classic ecological markers, such as SLA and LDMC, were also maintained in some root traits, which supports the proposition of the existence of a root economics spectrum, as demonstrated by Roumet et al. (2016). These dissimilarities are fundamental to explain how Kikuyu grass and Tifton 85 coexisted after three years of management and maintained proportions between 41 and 50% each in all treatments (limiting similarity principle; MacArthur & Levins, 1967).

SLA is one of the main ecological markers for discriminating plant functionality, as its values rapidly change in response to environmental changes (Wilson et al., 1999). Higher values of SLA are typical of plants with resource acquisition strategy, while lower values in this characteristic are associated with more conservative plants. Although an unusual index of functionality, the spectral reflectance index MRESRI is associated with higher concentrations of leaf chlorophyll, as demonstrated by Haboudane et al. (2002). This set of foliar traits is associated with higher photosynthetic rates and typically increases the demand for nutrients and water by plants. These attributes were more associated with kikuyu grass plants, which also showed greater association with SRL and SRA, root traits that characterize greater root absorption surface (Mommer & Weemstra, 2012).

Tifton 85 was mainly associated with root system traits such as RTD, RDMC, FLL and LDMC. Similar to SLA, LDMC is a very effective marker in determining the ecological spectrum of plants in terms of resource use (Wilson et al., 1999). The same pattern of response was observed in the association of Tifton 85 with root traits of RDMC and RTD, also linked to resource conservation strategies (Mccormack et al., 2012). Species with higher RTD are more efficient in water use and can maintain absorption during periods of water shortage, unlike exploitative species that are more effective in water use (Fort et al., 2017; Moreno-Gutiérrez et al., 2012). In addition, higher RTD values are associated with lower turnover of roots and leaves, which provides a long-term advantage in environments with lower nutrient availability (Ryser, 1996).

Increases in N rates led to both plants becoming more resource exploitative, and these changes were mainly associated with LNC, RTD and SRA (particularly Tifton 85) (Table 10). Therefore, the variations within species illustrated in Figure 10 should be mainly attributed to variations in the plant root system in response to increases in N doses. Despite the variation in phenotypes due to N fertilization, the species maintained their dissimilarities, without niche overlap, which may explain the stable and balanced coexistence of grasses in the canopy (Siefert, 2012). Variation in traits in response to fertilization has also been reported by Niu et al. (2014), but in their case, greater fertilization increased the functional diversity of the community by making the remaining species more dissimilar. In our case, there is an indication (Figure 10) of greater dissimilarity under the lower N dose, which may be associated with a greater capacity for adaptation (increase RTD and decrease SRA) to low fertility environments by species with more conservative resource use characteristics (Siebenkäs et al., 2015).

Maintaining canopies where dominant plants are functionally dissimilar can bring several benefits to ecosystems, especially related to the maintenance of forage production during periods with fluctuations in management and climate (Grime, 1998). Experiments with grass mixtures have provided advantages over monocultures regarding greater production stability (Duchini et al., 2019), biomass production (Morcillo et al., 2019), and resilience to extreme weather events (Oram et al., 2020). Thus, the stable coexistence between Tifton 85 and Kikuyu grass under grazing conditions and a considerable range of fertilization qualifies these two species as candidates to compose, together, multi-species pastoral environments.

7.7 CONCLUSIONS

The use of species from the same functional group (resource exploiters), but that maintain their trait dissimilarities, is an alternative for the establishment of stable mixtures in multi-species pastoral environments.

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7.9 TABLES AND FIGURES

Figure 7. Botanical composition of a mixed pasture of grasses and legumes subjected to three nitrogen fertilization rates (50, 150, and 250 kg N ha^{-1}) during three experimental years.

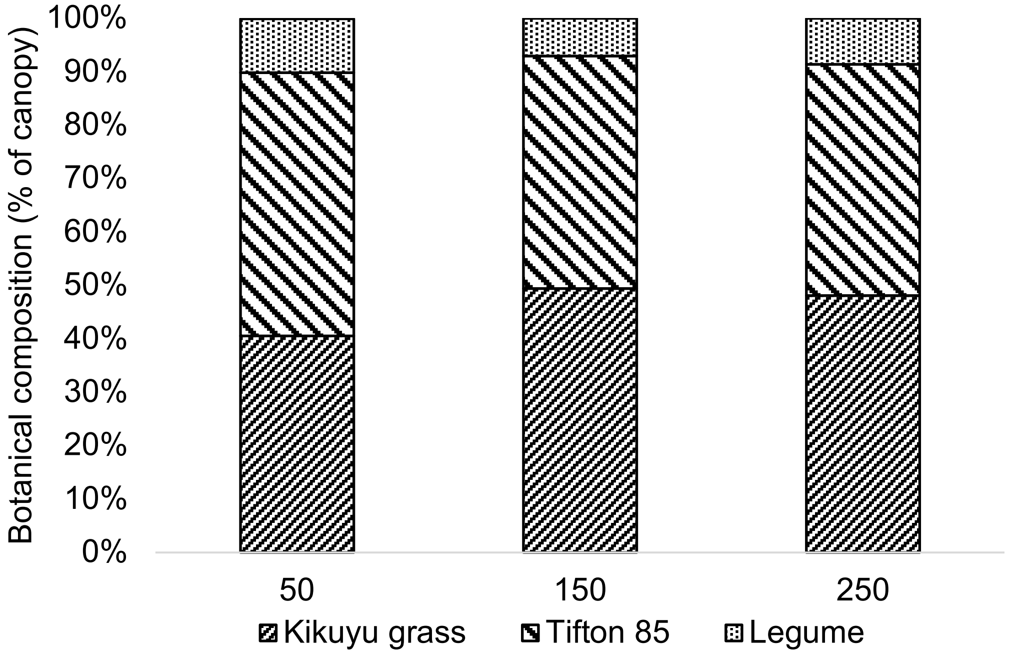
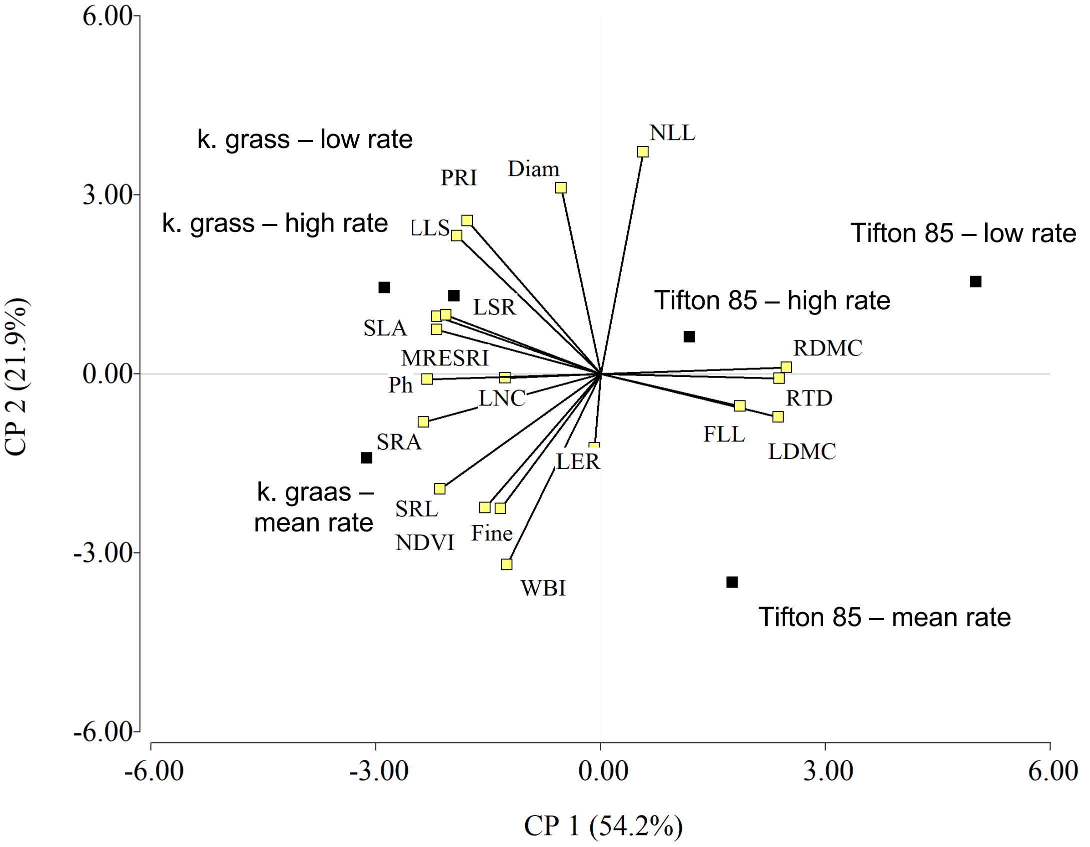


Figure 8. Principal component analysis (PCA) of traits of kikuyu grass and Tifton 85 intercropped and under increasing nitrogen fertilization doses.



Legend: number of live leaves (NLL), root dry matter content (RDMC), root tissue density (RTD), leaf dry matter content (LDMC), final leaf length (FLL), leaf elongation rate (LER), wbi, ndvi, % roots < 0,2 mm (Fine), specific root length (SRL); specific root area (SRA), leaf N content (LNC), phyllochron (Ph), mresri, leaf senescence rate (LSR), specific leaf area (SLA), leaf life spam (LLS), pri, average root diameter (Diam).

Figure 9. Image of the roots of kikuyu grass (A) and Tifton 85 (B) collected at a soil depth of 0-10 cm (500 cm^3) in a mixed pasture.

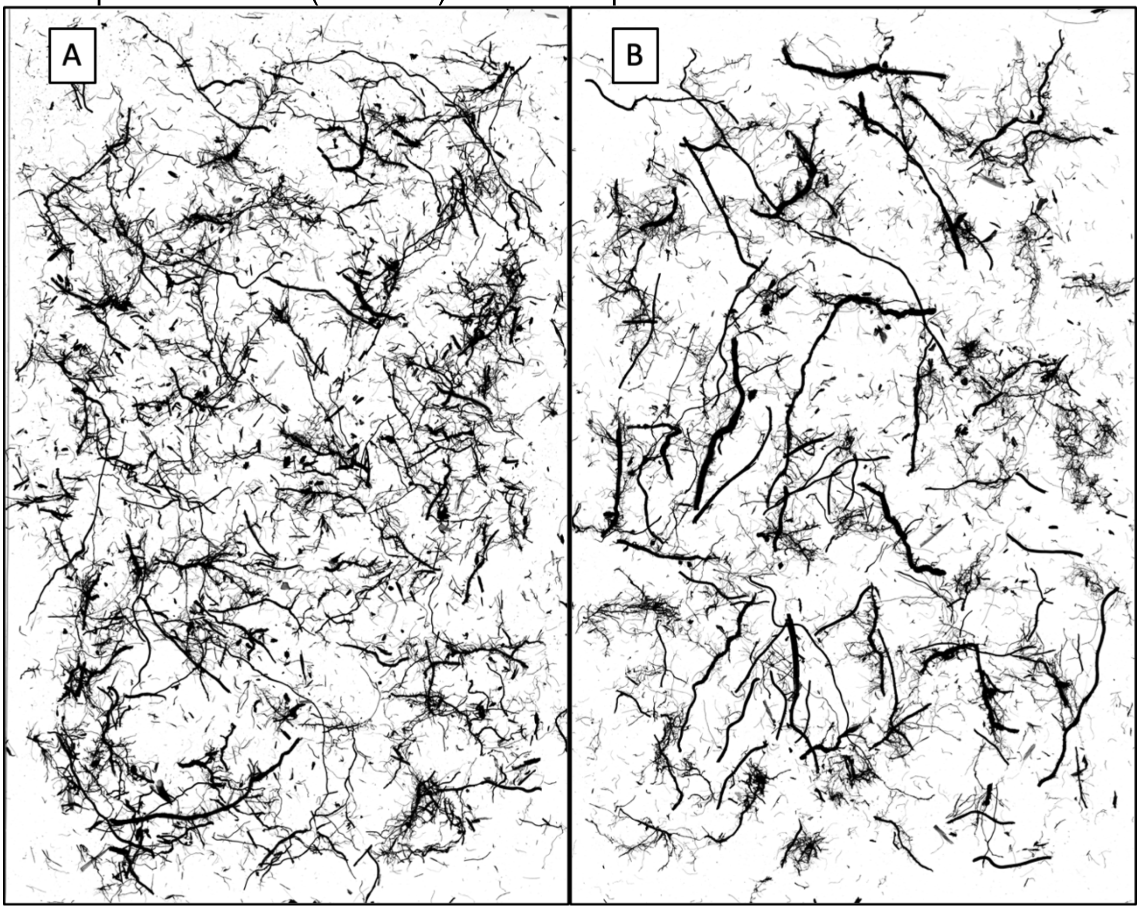
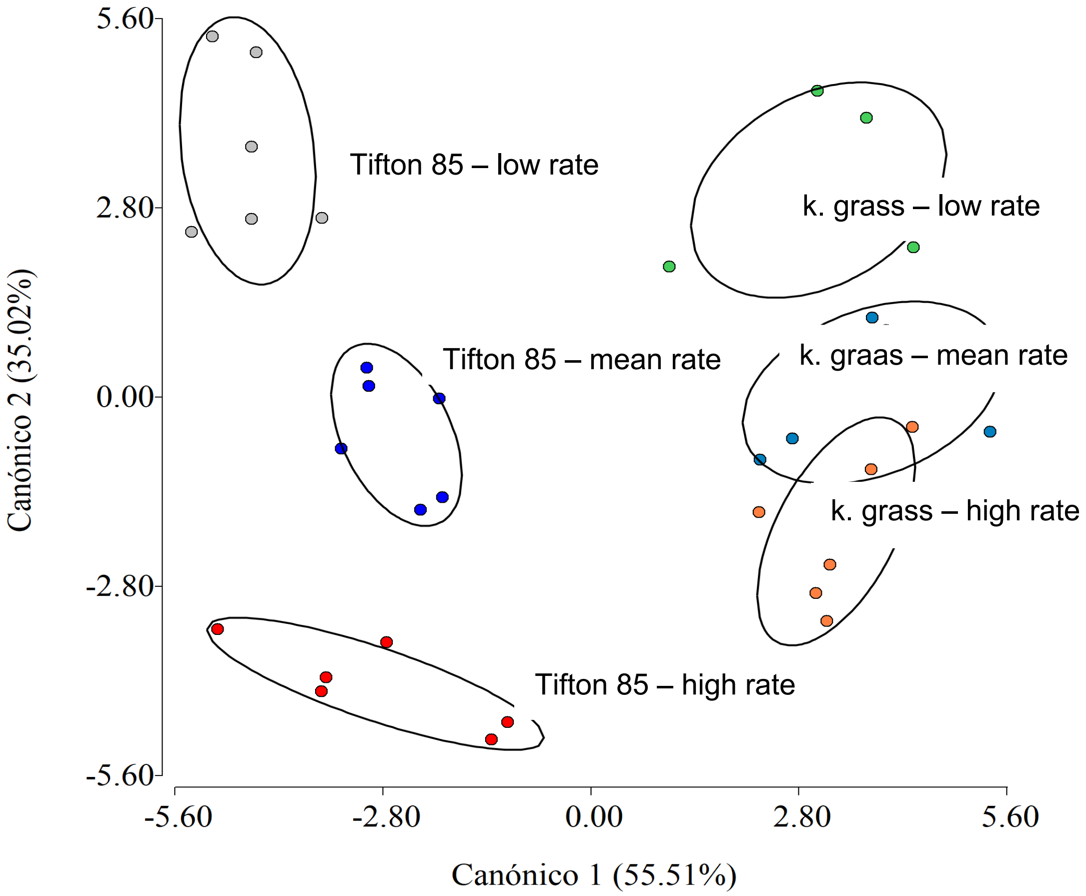


*Image dimensions are 20 x 30 cm.

Figure 10. Discriminant analysis of species kikuyu grass and Tifton 85 in a mixed pasture under increasing rates of nitrogen fertilization.



Legend: Ellipses indicate a 95% confidence interval. The traits used in this analysis were: root dry matter content (RDMC), root tissue density (RTD), leaf dry matter content (LDMC), final leaf length (FLL), leaf elongation rate (LER), wbi, ndvi, % roots < 0.2 mm (Fine), specific root length (SRL), specific root area (SRA), leaf N content (LNC), phyllochron (Ph), mresri, leaf senescence rate (LSR), specific leaf area (SLA), leaf life spam (LLS), photochemical reflectance index (pri), average root diameter (Diam), number of live leaves (NLL).

Table 10. Average trait values to kikuyu grass and Tifton 85 in a mixed pasture under increasing rates of nitrogen fertilization.

Trait	Species	N rates			
		50	150	250	*P*-value
LNC (%)	k. grass	2,6 c	3,0 b	3,3 a	<0.01
	Tifton 85	2,4 c	2,8 b	3,2 a	<0.01
LDMC (%)	k. grass	26,0	25,7	24,4	0.51
	Tifton 85	37,2	35,0	34,5	0.24
SLA (cm^2/g)	k. grass	212	209	212	0.98
	Tifton 85	176	175	170	0.88
RTD ($g\ cm^{-3}$)	k. grass	0,41	0,40	0,35	0.11
	Tifton 85	0,67	0,53	0,51	0.08
SRA (cm^2/g)	k. grass	319	340	346	0.74
	Tifton 85	194	291	273	0.10
RDMC (%)	k. grass	0,33	0,35	0,37	0.87
	Tifton 85	0,49	0,42	0,42	0.40
SRL ($m\ g^{-1}$)	k. grass	3340	3540	3250	0.83
	Tifton 85	2040	3350	2860	0.14

Legend: LNC= lean nitrogen content, LDMC= leaf dry matter content, SLA= specific leaf area, RTD=root tissue density, SRA= specific root area, RDMC=root dry matter content, SRL= specific root length.

8 FINAL CONSIDERATIONS

The general objective of this thesis was to determine management strategies to cope forage production and diversity in multispecies pastures. Despite no treatment had presented legume proportions greater than 20% during the warm season, we observed that some management strategies could mitigate species diversity losses in mixed pastures composed of grasses and legumes. The main findings can be summarized as follows:

1) Within the range of grasses management height in which the forage accumulation rate is constant, managing the mixture close to the upper limit (LAIc) is an alternative to promote the persistence of legumes less tolerant to frequent grazing, such as the birdsfoot trefoil.

2) Nitrogen fertilization is a key modulator of community species and functional diversity in multi-specific grasslands. Thus, providing N rates in which the grasses maintain a nitrogen nutrition index (NNI) around 0,7 - 0,8 seems to be an alternative to keep the diversity, mainly through the maintenance of legumes in the area.

Since the seminal papers of Bircham & Hodgson (1983) and Parsons et al., (1988), several authors (Pinto et al., 2001; Calsina el a., 2012; Sbrissia et al., 2018) have shown that forage accumulation in grass monocultures can be similar in a wide range of grazing heights (or herbage mass, LAI etc). In our work we could show that this same approach is valid for multispecies pastures managed in an amplitude of nitrogen supply conditions. However, one should be aware that in our experimental condition the pastures were dominated by only two grass species and maybe scenarios with more legume proportion should be considered.

The land model proposed here allowed for grazing in the area for approximately 270-290 days per year, with a period of forage shortage particularly at the beginning of the cool season. This indicates the need for alternative forage supply during this period of the year. The model was tested within a dairy system and may be adopted in subtropical climates as an alternative to providing good quality forage to dairy cows or heifers.

The results of our research demonstrate also that the utilization of N rates up to 250 kg ha^{-1} (NNI < 1,0) during the warm season was ineffective in promoting a legacy effect on forage yield in the following cool season pastures.

Finally, the data showed that Tifton 85 and kikuyu grass can coexist and compose stable swards in an amplitude of management conditions. Despite belonging to the same functional type (resource-exploitative), they differ in several functional traits related to resource use and acquisition and show moderate intraspecific trait variation in a range of N supply. However, management with high N rates and low grazing height, or low N rates and high grazing height acted to unbalance the proportion, but it seems that management could be an effective tool to reestablish species' parity in the mixture. Threshold

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