FEDERAL RURAL UNIVERSITY OF PERNAMBUCO

GRADUATE PROGRAM IN ANIMAL SCIENCE

PASTURE CHARACTERIZATION AND ANIMAL PERFORMANCE ON SILVOPASTORAL SYSTEMS USING TREE LEGUMES OR GRASS MONOCULTURE

IZABELA ALINE GOMES DA SILVA

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Dissertation submitted to the Coordination of the Graduate Program in Animal Science (UFRPE), area of concentration in Forage, as a requirement for Animal Science Doctoral Degree.

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Pasture characterization and animal performance on silvopastoral systems using tree legumes or grass monoculture

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IZABELA ALINE GOMES DA SILVA - daughter of Mr. Izaias Gomes da Silva and Mrs. Eliete Maria Ferreira da Silva, brazilian, born on December 19, 1988 in Recife-PE, Brazil.

Joined in March 2008 the undergraduate course in Animal Science at the Federal Rural University of Pernambuco – UFRPE, campus Dois Irmãos/Recife-PE. During her undergraduate studies, Izabela was a student of the Scientific Initiation Program –PIBIC conducted in the forage area. Izabela participated in a training program at University of Florida for a period of six months, where she accomplished the Mandatory Supervised Internship. Izabela received the Honors Merit Award, Prof. José Carlos Batista Dubeux and Prof. Lauro Ramos Bezerra, awarded by the Department of Animal Science and by the Undergraduate Coordination of Animal Science, respectively, and the Academic Laurea granted by the University President, Maria José de Sena. Izabela received her Bachelor degree (BSc) in February 2013.

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In March 2016, Izabela joined the Graduate Program in Animal Science at the same institution. She participated in a sandwich doctorate program at University of Florida for six months, defending her Dissertation in February 2020.

Our Father

Our Father in heaven,

hallowed be your name, your kingdom come,

your will be done on earth as it in heaven.

Give us today our daily bread.

Forgive us our debts,

As we also forgiven ou debtors.

And lead us not into temptation,

But deliver us from the evil one.

Amem!

Jesus Christ (Matthew, 6:9-13)

"Have I not commanded you? Be strong and courageous. Do not be afraid; do not be discouraged, for the LORD your God will be with you wherever you go". (Joshua 1:9)

#CarpeDiem

I OFFER

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"If I got here it was because I leaned on the shoulder of the giants"

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SUMMARY

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Chapter IV

General abstract: The world population is expected to grow by 2050 and also demand for food will increase, it is necessary to find alternatives for a sustainable livestock production. Silvopastoral systems-SPS with tree legumes are promising choices to adapt Agriculture to climate change and provide welfare for cattle, also increase forage nutritive value when the management is applied correctly. This 2-yr study evaluated animal performance and herbage responses in C4-grass monoculture or in SPS in the sub-humid tropical region of Brazil and evaluate productivity and nutritive value of signalgrass [*Urochloa decumbens* (Stapf.) R. Webster] subjected to shading from the tree legumes gliricidia [*Gliricidia sepium* (Jacq.) Steud] or mimosa (*Mimosa caesalpiniifolia* Benth.) under different tree canopy management (harvesting or not one of the rows in the double-row tree planting). The experimental design was randomized complete block with three replications. Treatments were: *Urochloa decumbens* (Stapf.) R. Webster (Signalgrass) + *Mimosa caesalpiniifolia* Benth (SPS-Mimosa); Signalgrass + *Gliricidia sepium* (Jacq.) Kunth ex Walp (SPS-Gliricidia); and Signalgrass monoculture (SM). Response variables included herbage and livestock responses. Cattle were managed under continuous stocking with variable stocking rate. There was interaction between treatment × month for herbage mass. Green herbage accumulation rate ranged from 20 to 80 kg DM $ha^{-1}d^{-1}$ across months, with SPS-Mimosa presenting lower rates. Average daily gain was greater in SPS-Gliricidia, followed by SM, and SPS-Mimosa, respectively (0.77; 0.56; 0.23 kg d^{-1}), varying across months. Stocking rate ranged from 0.86 to 1.6 AU ha^{-1}. Total gain per area during the experimental period was greater for SPS-Gliricidia (423 kg BW ha^{-1}), followed by signalgrass in monoculture (347 kg BW ha^{-1}), and SPS-Mimosa (50 kg BW ha^{-1}). The responses variables for different management included canopy height, herbage mass (green leaf blade, green stem, senescent leaves, and senescent stem), herbage accumulation rate, canopy bulk density, and soil moisture, bare soil and botanical composition. Total herbage mass, green herbage mass, and green leaf mass were affected by treatment × evaluation date and harvest management × evaluation date interactions. Herbage accumulation rate in SPS-Gliricidia was greater (55 kg DM $ha^{-1}d^{-1}$) than SPS-Mimosa (32 kg DM $ha^{-1}d^{-1}$). Soil moisture was lower at the Mimosa sites (16.2%) compared with the Gliricidia ones (17.2%), and it was greater between tree rows (21.9%) compared with full sun (11.5%), varying across the season. There were significant differences between harvest management for bare soil and litter percentage ($P \leq 0.05$). The botanical composition found 36 weed species, distributed in 19 botanical families and 36 of these species were dicotyledonous (53%) and 17 were monocotyledonous (47%). The silvopastoral systems have benefits for the environment, such as the increase of the carbon stock in the biomass and provide more comfort to the animal raised on pasture.

Keywords: condensed tannins, harvest, moisture, sustainable, tree spacing

Resumo geral: Prevê-se que a população mundial cresça até 2050 e também aumente a demanda por alimentos, é preciso encontrar alternativas para uma produção pecuária sustentável. Os sistemas silvipastoris-SPS com leguminosas arbóreas são escolhas promissoras para adaptar a agricultura às mudanças climáticas e proporcionar bem-estar ao gado, além de aumentar o valor nutritivo da forragem quando o manejo é aplicado corretamente. Este estudo de 2 anos avaliou o desempenho animal e as respostas da forragem em monocultura de grama C4 ou em SPS na região tropical subúmida do Brasil e avaliou a produtividade e o valor nutritivo do signalgrass [*Urochloa decumbens* (Stapf.) R. Webster] submetido ao sombreamento das leguminosas arbóreas gliricidia [*Gliricidia sepium* (Jacq.) Steud] ou mimosa (*Mimosa caesalpiniifolia* Benth.) sob diferentes manejos da copa das árvores (colhendo ou não em uma das fileiras no plantio de duas fileiras). O delineamento experimental foi em blocos casualizados com três repetições. Os tratamentos foram: signalgrass + mimosa (SPS-Mimosa); signalgrass + Gliricidia (SPS-Gliricidia); e monocultura de Signalgrass (SM). As variáveis de resposta incluíram as respostas à forragem e ao gado. O gado era manejado em lotação contínua com taxa de lotação variável. Houve interação entre tratamento × mês para massa de forragem. A taxa de acúmulo de forragem verde variou de 20 a 80 kg MS $ha^{-1}d^{-1}$ ao longo dos meses, com SPS-Mimosa apresentando taxas mais baixas. O ganho médio diário foi maior em SPS-Gliricidia, seguido por SM e SPS-Mimosa, respectivamente (0,77; 0,56; 0,23 kg d^{-1}), variando ao longo dos meses. A taxa de lotação variou de 0,86 a 1,6 UA ha^{-1}. O ganho total por área durante o período experimental foi maior para SPS-Gliricidia (423 kg PV ha^{-1}), seguido do capim-braquiária em monocultura (347 kg PV ha^{-1}) e SPS-Mimosa (50 kg PV ha^{-1}). As variáveis de resposta para diferentes manejos incluíram altura do dossel, massa de forragem (folha verde, caule verde, folhas senescentes e caule senescente), taxa de acúmulo de forragem, densidade do dossel e umidade do solo, solo descoberto e composição botânica. A massa total de forragem, a massa de forragem verde e a massa de folha verde foram afetadas pelas interações tratamento × data de avaliação e manejo de colheita × data de avaliação. A taxa de acúmulo de forragem em SPS-Gliricidia foi maior (55 kg MS $ha^{-1}d^{-1}$) do que SPS-Mimosa (32 kg MS $ha^{-1}d^{-1}$). A umidade do solo foi menor nas áreas de mimosa (16,2%) em comparação com as de gliricídia (17,2%), e foi maior entre as fileiras das árvores (21,9%) em comparação com pleno sol (11,5%), variando ao longo da estação. Houve diferenças significativas entre o manejo de colheita para solo descoberto e a porcentagem de serapilheira ($P \leq 0,05$). A composição botânica encontrou 36 espécies de plantas daninhas, distribuídas em 19 famílias botânicas, sendo 36 dessas espécies dicotiledôneas (53%) e 17 monocotiledôneas (47%). Os sistemas silvipastoris trazem benefícios ao meio ambiente, como o aumento do estoque de carbono na biomassa e proporcionam mais conforto aos animais criados a pasto.

Palavras-chave: taninos condensados, colheita, umidade, sustentabilidade, espaçamento entre árvores

INITIAL CONSIDERATIONS

Brazil has the largest commercial herd of cattle in the world and primarily uses tropical forages as a feed base for these animals. In 2018, it reached the record among all exporting countries in the volume of beef, further consolidating the country's leadership in this segment.

Although Brazil stands out in the international scenario, the average rates of Brazilian livestock are still far below their potential and one of the reasons is the fluctuations in nutrient content are closely correlated with the annual growth cycle of the forage and reduced use of mineral fertilizer on pastures. It is necessary to increase the productive capacity with the insertion of new alternatives and to worry about the animal welfare. Studies indicate that introducing legume trees into intercrop pastures promote soil nutrient returns, among other benefits in animal performance, for example.

However, in systems incorporating trees, management can represent a greater challenge. A balance between stocking rate and the amount of forage required to support livestock is necessary the knowledge of the state of the plant community, as well as its nutritional values and soil moisture content are fundamental in pasture management to monitor competition for water and nutrients. In this way, a botanical composition can measure the level of weed invasion, estimate or organize the space of the plants according to their occurrence, frequency and dry weight, which changes are strongly altered by grazing selectivity and plant succession. It is noteworthy that biological degradation, where vegetation is devoid of vegetation, can be measured by analysis of uncovered soil. The nutritional value of plants and their secondary compounds, such as condensed tannins, can be used for the planning of animal dietary intake and consequent supply of forage to be used.

The adoption of tree vegetation in areas previously intended exclusively for pasture brings environmental and economic advantages to the producer. These include mitigating carbon dioxide (CO_2) emissions, providing new green areas, increasing organic matter by litter deposition, improving soil physical aspects, and increasing the supply of feed (in use of forage plants) as well as an increase in income for the producer, when opting for the exploitation of trees with timber value. In the case of using leguminous species, the advantage of biological nitrogen fixation in the soil can also be attributed to the symbiosis of the roots of these plants with the bacteria of the genus *Rizhobium* and *Bradyrhizobium*.

This intercropping can lead to improved soil fertility, as well as upgrade in the economy the farmer when reducing the need to replace N to the soil. However, some aspects related to the effects of inter-species intercropping, economic increment and system sustainability need to be further studied.

Thus, the present thesis was organized in four chapters. The first one is a literature review. The second one refers to pasture characterization and animal performance in silvopastoral or monoculture systems. The third chapter is about botanical composition, forage mass and structural characteristics of pasture in two different area managements in two silvopastoral systems. The fourth chapter refers to isotopic composition, *in vitro* organic matter digestibility, soil moisture, condensed tannins in leaves of tree legumes under silvopastoral systems.

CHAPTER I

Review: Influence of silvopastoral systems on soil, animal performance, plant and environment

1.0 Review

1.1 Pasture characterization in Pernambuco Forest Zone

Pernambuco state has three distinct physiographic zones: Semiarid, Transition and Forest Zone. In the Semiarid and Transition, climate is defined by water deficits, negative soil factors besides inadequate management (NDMC, 2017). The major problems associated with the semiarid region include environmental degradation, poor quantitative and qualitative knowledge of its biodiversity, agriculture and livestock are limited by the low water supply and the quality of the water (Ramalho et al., 2009; Araújo et al., 2011).

The most frequent species *are Caesalpinaceae, Mimosaceae, Euphorbiaceae, Fabaceae* and *Cactaceae*, with the genus *Senna*, *Mimosa* and *Pithecellobium* with the largest number of species. The most abundant plants found in survey studies in the caatinga area are *Caesalpinia pyramidalis* Tul., *Mimosa* spp. and *Croton* spp., which are the pioneer species in the process of secondary succession resulting from anthropogenic action (Filho and Crispim, 2002). The caatinga is very rich and diversified, with a very high forage, wood, fruit, medicinal, and fauna potential.

In the Pernambuco Forest Zone, divided into Dry and Wet Forest, there is a strong presence of monoculture farming, predominantly sugarcane culture. Rainfall in this region is greater than in other zones, but still a period with shortage of rain. Cultivated pastures are more common in this area, but animal production may still be compromised by the low forage productivity if fertilizers or soil changes are not applied (Apolinário et al., 2013; Gomes da Silva, 2015).

In order to improve production conditions in this environment, many researches have been carried out at the Experimental Station of Itambé-PE, both for harvest forage (Pita, 2013) and forage for grazing animals (Viana et al., 2009; Apolinário et al., 2016). Species recommended for this area include *Urochloa* sp., *Pennisetum purpureum* Schum., *Mimosa* *caesalpiniifolia* Benth., *Gliricidia sepium* (Jacq.) Kunth ex Walp., *Leucaena leucocephala* (Lam.) DeWit.

However, the knowledge of the productive potential of different forages in each physiographic zone of the State is importance for the development and sustainability of livestock and agriculture in each area (Santos et al., 2003), must be based on criteria such as site potential, characteristics of native forage resources, training of the available labor force, input and equipment availability, and market conditions. The use of environmental-friendly alternatives must be considered in order to obtain optimal and sustainable production.

1.2 Nutritional characteristics of forage plants

Higher pasture mass and allowance can provide higher dry matter intake and animal performance (Delevatti, et al., 2019). The forage nutritive value (chemical composition and digestibility) is affected by abiotic factors such as light, water, temperature, and soil, as well as factors related to the management of the production system, such as height and frequency of defoliation (Abraham et al., 2009). Forage chemical composition and digestibility may vary according to tissue type, age, size and plant fraction (leaf, stem, trunk, fruits, seeds) as well as and place of harvesting (Kaplan et al., 2014).

Forage legumes tend to have greater nutritional value than grasses, as well as may increase decomposition and mineralization of organic materials in the soil, with faster nutrient cycling (Chalk et al., 2014); fix atmospheric nitrogen, use nitrogen fertilizers (Divito and Sadras, 2014), and synthesize CT that have anthelmintic effect and methane suppression (Naumann et al., 2013; Tedeschi et al., 2014).

In vitro techniques are precise and can help reducing costs at initial researches stages, however, they need to be validated later using animals models to obtain a representative sample of ruminal digestion. (DeFeo et al., 2019). Direct study of rumen fermentation is difficult and different systems have been designed to allow ruminal content to continue fermentation under laboratory-controlled conditions following normal animal organism standards. Notably the methodology used by Tilley and Terry (1963) modified by Goering and Van Soest (1970), applied to the fermenter models proposed by Hoover et al. (1976) and Holden (1999).

Nutrient digestibility is important because it reflects the nutritional value of a given feed, however, digestibility alone cannot explain changes in voluntary intake (Alstrup et al., 2014). Dry matter is the most important factor influencing animal performance, as it is the first determining point of nutrient intake, especially energy and protein, and requires the maintenance and production requirements (Gross et al., 2011; Safayi and Nielson, 2013). Thus, animals that consume high digestibility forages suffer less from low protein availability than low digestibility forages (Weisbjerg et al., 2010).

1.3 Signalgrass (*Urochloa decumbens* (Stapf.) R. Webster.) as widely cultivated in the Brazilian tropics

Grasses from the *Urochloa* genus are the most important forage source for grazing ruminants in warm climates (Jank et al., 2014). *U. decumbens* is a decumbent perennial grass, considered one of the major tropical forage C_4 grasses introduced from Africa and responsible for increasing the average stocking rate from 0.3 to 1.0 animal per ha over the last 40 years in Brazil (Valle et al., 2010).

Signalgrass is still used in beef cattle operations, owing to its high tolerance to acid, infertile soils and because it is well adapted to stockpiling management (Euclides et al., 2007), also tolerant to moderate shading (Paciullo et al., 2007; Guenni et al., 2008). Average daily gain of individual steers (approx. 200 kg live weight) on Signalgrass in monoculture and Signalgrass intercropped with Gliricidia or Mimosa did not vary with treatment, ranging from 0.3 kg AU^{-1} d^{-1} in the dry season to 1.1 kg AU^{-1} d^{-1}in the rainy season in Pernambuco Forest Zone-Brazil (Costa et al., 2016). In another study at the same prior area, the results obtained were considered satisfactory in a continuous stocking experiment with Signalgrass in monoculture and

Signalgrass intercropped with tree legumes, where average daily gain gained in the experiment were 0.65 kg UA^{-1} day^{-1} for all treatments (Santos et al., 2019).

Hepatogenic photosensitization (FTS) in Brazil has been diagnosed mainly in cattle and sheep and sometimes also in horses, goats and buffalo grazing Urochloa grasses (Tokarnia et al., 2012), which contain significant amounts of saponins - substances that cause this intoxication. It is important to be careful not to leave too much decomposing mass, as this is a favorable condition for the appearance of the fungus *Phithomyces chartarum*. This can be avoided by maintaining pasture at optimal grazing pressures.

If the initiation of grazing dictated by time intervals in excessively delayed under conditions of rapid growth, excessive accumulation of stem, known to be avoided by grazing animals (Benvenutti et al., 2008), can occur. At the same time, it is important to ensure sufficient leaf area after grazing for fast regrowth following defoliation. Forage growth rate is largely determined by leaf area, and when available light is limited by self-shading, plants respond by elongating their stems (Kutshera and Niklas, 2013).

1.4 Gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex Walp) as high-quality feed for ruminants during the dry season

Gliricidia sepium (Jacq.) Kunth ex Walp has been introduced to farmers but adoption is still low. Moreover, agronomic information and defoliation management of this plant were limited. Nevertheless, *G. sepium* was categorized as the second multifunction trees after the *Leucaena leucocephala* tree in the tropical humid areas (Kabi and Lutakome, 2013).

Gliricidia has the capacity to recompose the entire aerial part in about four months after some cut, causing greater mass availability as alternative for food in the drought season (Massafera et al., 2015), but according to Castro Filho et al. (2016), there is little information about the ideal density of gliricidia plants for the formation of selected legumes in the production of forage mass. Gliricidia is almost always cultivated as a consortium, mainly with grasses (Rodriguez, 2011) and its consumption occurs directly in the form of paste. However, due to the scarcity of quality food in some seasons and low acceptance for animals, forage conservation practices are also used in this legume.

The recommended defoliation interval for *G. sepium* is 6 to 12 weeks in tropical humid areas (Edwards et al., 2012); the forage nutritive value decreased gradually following the increasing maturation (Kabi and Bareeba, 2008). *Gliricidia sepium* is used as forage for cattle, goats and sheep, with the crude protein ranging from 18 to 30% and in vitro DM ranging from 60 to 65% (Lisson et al., 2010).

Due to the low content of tannins in gliricidia (Varón and Granados, 2012), with an average of 0.62% in the leaves, its ingestion has no inhibitory effect on digestibility (Vieira et al., 2001). Despite some limitations, recent research has yielded excellent results for animal performance in sylvipastoral systems with intercropping between Gliricidia and *U. decumbens*, with the peak of average daily gain of 0.84 kg LW day^{-1}in Forest Zone in Pernambuco (Santos et al., 2019).

1.5 Sabiá (*Mimosa caesalpiniifolia* Benth) as a multipurpose plant

Mimosa Caesalpiniifolia Benth belongs to the family *Fabaceae* subfamily *Mimosoideae* which comprises about 490 to 510 species, distributed in the paleotropical region (Indian subcontinent), New World (Central and South America), Mexico and the United States (Lewis et al., 2005; LPWG, 2017). In Brazil, an occurrence of gender is present in all regions, which, among the Angiosperms, is the second richest in the country, popularly known as "sansão-do-campo" or "sabiá", having greater diversity in the Cerrado and Caatinga (Silva, 2013; BFG, 2015).

Mimosa is one of the main sources of timber for fence in the Northeast of Brazil, being used for energy (firewood and coal), and its often used as hedge and windbreak (Ribaski et al., 2003). This tree legume has high forage potential recommend its use as hay (Alves et al., 2011), also is considered an important specie because of its great potential and multiple uses in traditional medicines (Silva et al., 2014). It has a great potential to fix atmosphere N in symbiosis with diazotrophic bacteria (Silva et al. 2006). The Mimosa leaf is nutritious, around 17% crude protein. It can be used during the rainy season and can be consumed when it comes off the branches after senescence, in the dry season of the year (Mendes, 2001; Maia, 2004).

Mimosa caesalpiniifolia has a variable growth habit, ranging from a shrub to an evergreen tree. The largest trees can reach up to 10 m in height and 30 cm in DBH (diameter at breast height, 1.30 m above soil). It produces a broad, sparsely branched canopy and multiple stems that are usually armed with prickles. It is a pioneer species that occurs in both primary and secondary formations, where it is common or frequent in old fields. It occurs in both Caatinga (dry forests) and Cerrado ecosystems (from savanna-steppe to woodland formations) in Brazil. This species begins to flower and produce fruit at an early age, usually at around two years (MMA, 2012).

The seeds are dispersed by gravity (barochory) (Carvalho, 2006) and the seeds are hard, measuring 5-8 mm in diameter (Brasil, 2013). It has high potential for Degraded Area Recovery (RAD), as it has good adaptation in rugged soils; It has reciprocal advantages in intercropping and some species are pioneers in RAD and reforestation.

The adaptability and utility of *M. caesalpiniifolia* for rehabilitating degraded soils has been demonstrated in other studies (Podadera et al., 2015) and is considered to be a useful candidate species for facilitating early successional processes. Although the litter produced by this species has a high nutrient content and high decomposition rate (Silva, 2015), the potential allelopathic effect of its litter on native seed germination should be considered (Ferguson et al., 2016).

Mimosa it is fast growing and high density, and is therefore cultivated by farmers and agricultural producers for charcoal and firewood production, and because it has a high carbon absorption index, and it is possible to provide utilities for the energy market and carbon credit. In addition to having flowers that are pollinated by bees, it is used in folk medicine; the seeds are also used in the use of galactic substances, which are used as antivirals against HSV1 (herpes simplex virus), yellow fever and dengue virus (Coradin, 2011).

1.6 Benefits provide by silvopastoral or silvopasture systems (SPS)

It is important to be clear about the definitions of silvopastoral and agroforestry systems.

The International Centre for Research on Agro-Forestry (ICRAF) defines agroforestry as collective name for land use system and technologies where wood perennials are deliberately used on the same land management unit as agricultural crops and/or animals, either in some form of spatial arrangement or temporal sequence. In agroforestry systems, there are both ecological and economical interactions between components (Mauricio et al., 2019). Agroforestry can thus be viewed more as an intervention, whereas silvopastoral systems provide the link between trees and livestock as system components (Toral et al., 2013; Dubeux Jr et al., 2015).

The advantages of SPS were addressed in four categories: provision, regulation, support and cultural services (Dubeux Jr et al., 2017). Due to the ecological and economic interactions between the different components, these are structurally and functionally more complex systems than monocultures (Liu et al., 2008). Benefits of enhance structure and function of forage species by integrating forage legumes into grass monoculture include better forage nutritive value than grass monocultures for grazing livestock (Muir et al., 2014) and sustainable intensification as the production of more output through the more efficient use of resources, while minimizing negative impact on the environment (Tedeschi et al., 2015).

The following interactions are common: beneficial effects of shade and available feed on livestock (Bussoni et al., 2015); draught animal power on land preparation and crop growth; dung and urine to increase soil fertility and crop growth (Lira et al., 2019); biological N_2 fixation

(BNF); carbon sequestration (Powlson et al., 2011; Parron et al., 2016); flowers for pollinators (Potts et al., 2010), reduction of greenhouse gas (GHG) emissions (Puchala et al., 2005); use of crop residues and agro-industrial by-products (AIBP) from trees *in situ*; use of native vegetation and effects on cost of weed control (Thompson et al., 2015), crop management and crop growth; type of animal production system (grazing, semi-intensive, and stall feeding or zero grazing) on tree crops, increased income and environmental integrity (Cubbage et al., 2012).

1.7 Silvopastoral systems under different tree canopy management

Silvopastoral systems can be implemented in pastures by adding trees or shrubs; in forested areas by adding forages; or on land that has neither the desired trees nor forages in sufficient quantity to meet the land use objectives. Appropriate establishment methods depend on site conditions, tree species and allowing their coexistence with animals, age, spacing, and existing pasture conditions (Giustina et al., 2017).

The consequent competition for light between trees and grass may promote reduced dry matter production under the canopy (Taiz and Zeiger, 2010) and this competition changes can affect positively or negatively the production and morphology of a variety of plants under shading. Some factors are related to increase in the quality of light under the tree canopy, such as tree height, tree vertical structure, number and distribution of branches, leaf density, area, leaf angle, and leaf reflectance (Fey et al., 2015), all of which are severely affected by the dry season.

The relationship between tree canopy height and the production of pasture species in a silvopastoral system based on alder trees was reported by Devkota et al. (2009) and they found pruning of alder can increase canopy height and has the potential to improve the productivity of the understorey pasture and its acceptability to sheep.

Another study reported by Rozados-Lorenzo et al. (2007) about the effect of six tree species planted at six different densities on pasture production seven years after establishment, realized that the tree effect became more noticeable over time, with the last sampling showing the inverse relationship between tree density and herbage production most clearly. The study also indicated that by the sixth growing season, annual pasture production under different tree species is inversely correlated with tree leaf area index.

Sward characteristics and performance of dairy cows in organic grass–legume pastures shaded by tropical trees was evaluated by Paciullo et al. (2014) and they found that in In the SPS, with moderate shade (19% shade relative to a full-sun condition), the grass crude protein was higher than in the monoculture and this result justified the better performance of the cows in silvopastoral areas during the 1st year.

1.8 Performance of cattle in silvopastoral systems

An important feature of Brazilian livestock is the majority of its herd raised on pasture, but the grassland degradation compromises the profitability, for this reason, sustainable intensification can optimize pasture restauration (Silva et al., 2017). In this way, the production of beef cattle through the opening of new pasture areas, however, a relationship between the animal and the environment, including all factors and their interactions, is quite complex and even greater understanding needed from producers.

Climate is one of the factors that has the greatest influence on animal production, for interfere, from forage production to animal welfare, promoting in the conversion of fodder into animal products. This influence is quite complex, when it comes to the tropical climate, as this type of climate is characterized by major environmental adversities (Hoffmann et al., 2014; Vale et al., 2019).

The silvopastoral system, when managed, presents advantages for use in animal performance when compared to traditional systems. In addition to being increasingly necessary for trees to increase production, quality sustainability of pastures, contribution to animal comfort, shading, attenuating as extreme temperatures, reducing the impact of gloves and in addition to promoting shelter for animals, productive and reproductive (Pezzopane et al., 2019).

Animal performance in grass monoculture (*Urochloa decumbens* (Stapf.) R. Webster**.**) or silvopastoral using tree legumes (*Mimosa caesalpiniifolia* Benth and *Gliricidia sepium* (Jacq.) Kunth ex Walp) was evaluated by Santos et al., (2019) and the authors found that animal performance tended to decrease in the SPS (411 kg LW ha^{-1} yr^{-1}) when compared to Signalgrass in monoculture (508 kg LW $ha^{-1}yr^{-1}$), especially in SPS-Mimosa. The total herbage mass in Signalgrass also was higher than SPS-systems with average of 5.057 kg DM ha^{-1} vs 3.292 kg DM ha^{-1}, respectively.

A long term of an evaluation of a long-established silvopastoral *U. decumbens* system to analyze plant characteristics and feeding value for cattle was performance by Lima et al., (2018) and they found that a total forage mass was reduced in SPS compared to the monoculture. Crude protein content was higher in the SPS (113 $g.kg^{-1}$) than in the monoculture (91 $g.kg^{-1}$). The liveweight gain of heifers was similar between systems since the higher crude protein content available in SPS contributed to improved forage nutritional value. The authors concluded than *U. decumbens* presents a high degree of phenotypic plasticity in response to changes in shade levels, which gives this species a high potential for use in SPS.

The productive potential of *U. decumbens* Stapf. and performance of cattle in silvipastoral systems with *M. caesalpiniifolia* Benth and *Gliricidia sepium* (Jacq.) Kunth ex Walp were evaluated by Costa et al., (2016) and the authors found that the total forage mass of monoculture of Signalgrass (5.091 kg ha^{-1} DM) was greater ($P \leq 0,05$) than the consortium (3.964 kg ha^{-1} DM), while for dry green forage mass, the consortium are greater (2.237 kg MSV ha^{-1}) in relation to the monoculture (1.934 kg MSV ha^{-1}). For forage accumulation and supply rate, stocking rate, weight gain daily weight gain they did not found significant difference for treatments, with 45.6 kg ha^{-1} day^{-1}; 2.9 kg MVS / kg PV; 1.8 UA ha^{-1}; 0.6 kg UA^{-1} day^{-1} and 33.1 kg ha^{-1} 28 $days^{-1}$, respectively.

The animal performance and gain per area can be improved by using the intensive silvopastoral systems-ISS (Cardona et al., 2013) that provides high fodder shrub densities (more than 10,000/Ha), i.e. the association of the leguminous shrub *Leucaena leucocephala* (Lam.) de Wit. with high biomass producing grasses and native or introduced timber trees, which are grazed under intensive rotational grazing with use of electric fences and provide a permanent supply of drinking water. Under these conditions, high stocking rates are achieved, with high milk and meat production. These systems increase biodiversity (compared to conventional production systems) and reduce vulnerability to extreme weather changes. In addition, intensive silvopastoral system can be a tool to help this sector mitigate and adapt to climate change (Murgueitio et al., 2011).

1.9 Condensed tannins (CT)

Tannins are substances produced mainly by some plants to protect them against attacks from bacteria, fungi, viruses, and also herbivores (predators), or in response to a limitation on plant growth, extensive researches have been done over the last couple decades to search for natural alternatives to in-feed antibiotics, and phytogenic compounds (Yang et al., 2015; Paes et al., 2010). Under conditions of environmental stress, plants can increase the production of tannins for storage of photosynthesis products that can be used in cold or dry temperatures (Prill, 2011).

Tannins are made up of polyphenols and classified according to their chemical structure into hydrolysable or condensed, are distinguished from other polyphenols by their ability to form complexes with and precipitate proteins (Hagermann, 2012). Hydrolysable tannins are glucose polyesters and are classified according to the acid formed after hydrolysis, in gallic tannins or ellagic tannins (Hagermann, 2011), not very often found in nature, but restricted to dicotyledonous angiosperm species (*Caesalpiniifolia chinata*, Oaks), and when consumptions are promptly metabolized, they can cause poisoning.

However, condensed tannins (CT) are catechin-like monomers known as flavonoids, and are larger molecules found in angiosperm and gymnosperm species; e.g. *Eucalyptus, Gliricidia sepium* and *Mimosa caesalpiniifolia* Benth (Naumann et al., 2013). Condensed tannins are located inside the plant cells and inside the seed coat and are released quickly during chewing or by manually cutting the forage. In this first moment, the tannins give a feeling of astringency, impairing the palatability of the forage and generally lead the animal to reduce dry matter (DM) consumption.

Condensed tannins can act by protecting the forage from ruminal degradation (by-pass) or acting as antinutritional factors. Some authors provided better understanding about anti-nutritional responses, for example, concentrations of CT exceeding 5% of dry matter (DM) must decrease diet palatability, depress intake at dietary, depress digestibility of nutrients and depress feed efficiency and production of animal products (Mueller-Harvey 2006; Naumann et al., 2017). However, not all CT bind protein equally (Naumann et al., 2014).

The characterization and biological activity of condensed tannins from tropical forage legumes was reported by Pedreira et al (2018) and they found extractable condensed tannin (ECT) fraction ranged from non-detected in *Cratylia argentea* and *Gliricidia sepium* up to 79 g kg^{-1} in *Mimosa caesalpiniifolia* leaves, which is significantly greater than the values found in *Flemingia macrophylla*, *Cajanus cajan*, and *M. caesalpiniifolia* bark: 41, 25, and 15 g kg^{-1}, respectively.

Initial interest in utilizing CT to improve ruminant N metabolism was based on work by Waghorn et al. (1987), who reported 50% greater post-ruminal flux of essential amino acids due to *L. corniculatus* CT followed by an average of 60% improvement in intestinal amino acid availability. There are potential interactions of CT with other plant constituents that can potentially reduce CH_4 production (Tedeschi et al., 2011), also can reduce gastro-intestinal parasites, and mitigate rumen methanogenesis, through improves energy efficiency, and change nitrogen route from urine to feces generating positive aspects on the environment (Desrue et al., 2016).

Other uses attributed to tannins are tanning and skin recovery (Liger, 2012), as well as its use in the petroleum industries as a dispersing agent to control the viscosity of clay in well drilling (Tanac, 2008). In addition, tannins are also used in water treatment and residential supplies, in the manufacture of paints and adhesives for wood and wood products and tested in the prevention of xylophages (Colli et al., 2007), in the pharmaceutical industry, in beverages, in the manufacture of plastics, used as flocculants and coagulants (Simões et al., 2010).

In Brazil there are several species of tannins, notably: The Cashew tree [*Anacardium occidentale*]; Jurema preta [*Mimosa tenuiflora*]; Jurema vermelha [*Mimosa arenosa* (Willd.)]; Algaroba [*Prosopis juliflora* (Sw.) D.C.] this last one is exotic, but all are found in the semiarid region. However, despite the diversity of tree and tree species occurring in the region, Angico vermelho [*Anadenanthera colubrina* (Vell.)] and [*Brenan var*. Cebil (Gris) Alts.] are most used source of tannins (Diniz et al., 2003). The northeastern region represents 100% of the production of tannins, with the largest producers being the states Bahia, Pernambuco and Alagoas (Moura, 2016).

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CHAPTER II*

Tree legume enhance livestock performance in silvopasture system

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Tree legume enhance livestock performance in silvopasture system

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Core ideas:

- Gliricidia-signalgrass systems enhanced livestock gains compared with signalgrass monoculture.
- Mimosa trees outcompeted signalgrass and reduced livestock gains.
- Silvopasture systems with tree legumes have potential to provide numerous ecosystem services and reduce C footprint of livestock systems in the tropics.
- If livestock production is the major desired ecosystem service, gliricidia is a better option to use with signalgrass in SPS compared with mimosa trees.

LIST OF ABBREVIATIONS: ADG, average daily gain; BW, body weight; CBD, canopy bulk density; CP, crude protein; DM, dry matter; ES, ecosystem services; GHAR, green herbage accumulation rate; HAL, herbage allowance; HM, herbage mass; SM, signalgrass monoculture; SPS, silvopasture system; SR, stocking rate

ABSTRACT

Tree legume enhance livestock performance in silvopasture system

Silvopastoral systems (SPS) can increase overall productivity and long-term income due to the simultaneous production of trees, forage, and livestock. This 2-yr study evaluated animal performance and herbage responses in C4-grass monoculture or in SPS in the sub-humid tropical region of Brazil. The experimental design was randomized complete block with three replications. Treatments were: *Urochloa decumbens* (Stapf.) R. Webster (Signalgrass) + *Mimosa caesalpiniifolia* Benth (SPS-Mimosa); Signalgrass + *Gliricidia sepium* (Jacq.) Kunth ex Walp (SPS-Gliricidia); and Signalgrass monoculture (SM). Response variables included herbage and livestock responses. Cattle were managed under continuous stocking with variable stocking rate. There was interaction between treatment × month for herbage mass. Green herbage accumulation rate ranged from 20 to 80 kg DM $ha^{-1}d^{-1}$ across months, with SPS-Mimosa presenting lower rates. Average daily gain was greater in SPS-Gliricidia, followed by SM, and SPS-Mimosa, respectively (0.77; 0.56; 0.23 kg d^{-1}), varying across months. Stocking rate ranged from 0.86 to 1.6 AU ha^{-1}. Total gain per area during the experimental period was greater for SPS-Gliricidia (423 kg BW ha^{-1}), followed by signalgrass in monoculture (347 kg BW ha^{-1}), and SPS-Mimosa (50 kg BW ha^{-1}). Silvopasture systems using signalgrass and gliricidia enhanced livestock gains compared with signalgrass in monoculture, and mimosa trees outcompeted signalgrass, reducing livestock gains. Silvopasture systems with tree legumes have potential to provide numerous ecosystem services and reduce C footprint of livestock systems in the tropics, however, the choice of tree species is key and determined by which ecosystem service is prioritized.

Keywords: ecosystem service, forage legumes, protein,

RESUMO

Leguminosas arbóreas melhoram o desempenho do gado no sistema silvipastoril

Os sistemas silvipastoris (SPS) podem aumentar a produtividade geral e a renda de longo prazo devido à produção simultânea de árvores, forragem e gado. Este estudo de 2 anos avaliou o desempenho animal e as respostas à forragem em monocultura de gramínea C4 ou em SPS na região tropical subúmida do Brasil. O delineamento experimental foi em blocos casualizados com três repetições. Os tratamentos foram: *Urochloa decumbens* (Stapf.) R. Webster (Signalgrass) + *Mimosa caesalpiniifolia* Benth (SPS-Mimosa); Signalgrass + *Gliricidia sepium* (Jacq.) Kunth ex Walp (SPS-Gliricidia); e monocultura de Signalgrass (SM). As variáveis de resposta incluíram as respostas à gramínea e ao gado. O gado era manejado em lotação contínua com taxa de lotação variável. Houve interação entre tratamento × mês para massa de forragem. A taxa de acúmulo de forragem verde variou de 20 a 80 kg MS $ha^{-1}d^{-1}$ ao longo dos meses, com SPS-Mimosa apresentando taxas mais baixas. O ganho médio diário foi maior em SPS-Gliricidia, seguido por SM e SPS-Mimosa, respectivamente (0,77; 0,56; 0,23 kg d^{-1}), variando ao longo dos meses. A taxa de lotação variou de 0,86 a 1,6 UA ha^{-1}. O ganho total por área durante o período experimental foi maior para SPS-Gliricidia (423 kg PV ha^{-1}), seguido do Signalgrass em monocultura (347 kg PV ha^{-1}) e SPS-Mimosa (50 kg PV ha^{-1}). Os sistemas silvipastoris usando signalgrass e gliricídia aumentaram os ganhos do gado em comparação com o signalgrass na monocultura, e as árvores de mimosa competiram com o signalgrass, reduzindo os ganhos do gado. Os sistemas silvipastoris com leguminosas arbóreas têm potencial para fornecer numerosos serviços ecossistêmicos e reduzir a pegada C dos sistemas pecuários nos trópicos; no entanto, a escolha das espécies de árvores é fundamental e determinada por qual serviço ecossistêmico é priorizado.

Palavras-chave: serviço ecossistêmico, leguminosas forrageiras, proteína

INTRODUCTION

In the tropics, livestock production is pasture-based using C_4-grass monoculture. These grasses typically deposit litter with high C:N ratio, which coupled with lack of fertilization and overgrazing, leads to pasture decline (Boddey et al., 2004). This process results in a progressive reduction in herbage accumulation, decrease in forage nutritive value (Nave, Sulc, Barker, & St-Pierre, 2014), change in botanical composition over time with increase in weeds, reduction in intake and animal performance, and decrease in the economic efficiency of livestock production systems (Boddey et al., 2004; Oliveira et al., 2004).

Integrating trees into grasslands (i.e. silvopasture systems - SPS) is an option to overcome these problems and increase sustainability of livestock systems. Benefits of SPS include the delivery of more ecosystem services from different categories such as provisioning, regulating, supporting, and cultural services (Dubeux et al., 2017a). Grazing animals in SPS improve nutrient cycling because of faster nutrient turnover and soil organic carbon (Wesp et al., 2016), increasing soil carbon stock (Aryal, Gómez-Gonzalez, Hernández-Nuriasmú, & Morales-Ruiz, 2019), and leading to a more efficient land use (Dubeux Jr et al., 2017b). Further benefits include decreased greenhouse gas emissions, such as nitrous oxide and methane (Gibbs et al., 2010; Foley et al., 2011), and the additional source of income to producers coming from livestock (Esperschuetz et al., 2017). Numerous challenges occur when incorporating trees into grassland systems due to interactions among their components (e.g. trees, herbaceous vegetation, soil, and livestock). Growth of tree canopies reduces light reaching the understory, affecting forage physiology and morphology (Nascimento et al., 2019). These changes in the light environment will act directly on forage production, nutritive value, and livestock responses (Geremia et al., 2018). However, there are also potential benefits resulting from the interaction among components of SPS. Therefore, managing these components to create positive interactions for the system is both the goal and the challenge.

We hypothesized that SPS with tree legumes promote animal performance by providing better quality forage and adding N to the system compared with grass monoculture, however, these responses vary with SPS and season of the year. The objective of this research was to evaluate pasture canopy structure and livestock responses in SPS and in grass monoculture in a sub-humid tropical region in Brazil.

MATERIALS AND METHODS

Site Description

The study was carried out at the Experimental Station of Itambé (7°23’ S and 35°10’ W and 190 m above sea level), Agronomic Institute of Pernambuco-IPA. The soil in the experimental area is classified as an Ultisol (Apolinário et al., 2016). Average annual rainfall is 1200 mm and annual average temperature is 25°C. Rainfall in 2017, 2018, and 2019 were 1010, 958, and 1295 mm, respectively, and the monthly distribution is described in Figure 1. The relative annual air humidity is 80% and the local climate is defined as As’ warm-humid rainy tropical with dry summer.

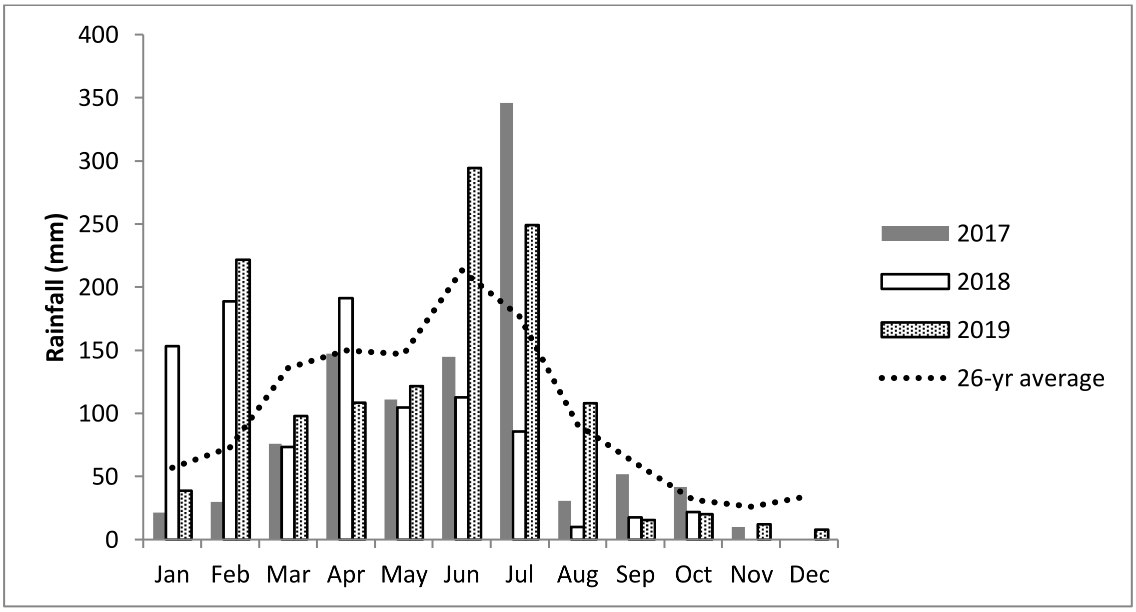

Figure 1. Monthly rainfall at the experimental site during 2017, 2018, and 2019, and the historical 26-yr average.

Treatments, Experimental Design, Establishment, and Management

Treatments consisted of two SPS and one grass monoculture, as follows: 1. Signalgrass (*Urochloa decumbens* Stapf) + Mimosa [*Mimosa caesalpiniifolia* Benth]; 2. Signalgrass + Gliricidia [*Gliricidia sepium* (Jacq.) Kunth ex. Walp]; 3. Signalgrass monoculture. Treatments were allocated in a randomized complete block design, with three blocks (n = 12). Each experimental unit (paddock) had 1 ha and 14 double rows of tree legumes. Tree spacing in the double-row system was 15 x 1.0 x 0.5 m as described in Figure 2.

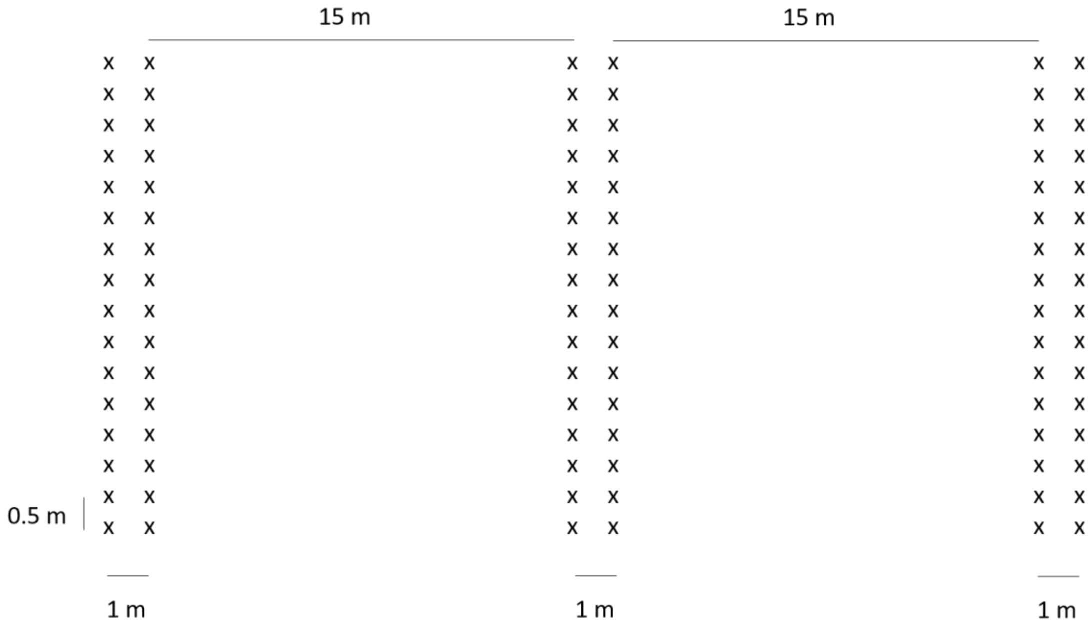

Figure 2. Tree spacing in the double row system. Each 1-ha plot had 14 double rows.

In July 2011, tree legume seedlings were planted in fourteen double rows in 1-ha paddocks (experimental unit), and tree population was 2500 trees ha^{-1}. Signalgrass was previously established in one of the blocks in 1969. In the other two blocks, signalgrass was established along with the tree legumes (July 2011), between the double rows. Signalgrass seeds were placed manually into shallow open holes (about 5-cm deep) spaced at 1.0 m x 0.5 m. Seeding rate was 10 kg ha^{-1} with 40% of pure viable seeds. Pastures were fully established by the end of the rainy season in 2011.

Gliricidia and mimosa were seeded into seedling trays (128 counts) in a greenhouse and inoculated with specific *Bradyrhizobium* strains obtained from the soil Microbiology Laboratory at Federal Rural University of Pernambuco (UFRPE). Legume seedlings (approximately 30-cm height) were transplanted into 20-cm-deep furrows in double-row spacing (10 m x 1 m x 0.5 m; Figure 2). All paddocks were fertilized in July 2011 with 44 kg P ha^{-1} (as single superphosphate) and 100 kg K ha^{-1} (as potassium chloride) on the entire area.

Herbage Responses

Signalgrass herbage mass was determined using the double-sampling technique, described by Haydock and Shaw (1975). Briefly, every 28 days, direct measurements were obtained by harvesting six 0.25-m^2 quadrats per paddock, at ground level. Indirect measurement, the average canopy height, was measured using a sward stick (Barthram, Elston, & Bolton, 2000) at 60 random points, every 28 days. The average of these 60 scores was used in the regression equation to estimate herbage mass. After harvesting, green and dry material were separated per treatment. Grass samples were separated into stem (green and dry) and leaf blade (green and dry). Forage samples were oven-dried at 55°C for 72 h to a constant weight. Herbage mass (kg ha^{-1}) was expressed as total herbage mass (dry and green fractions) and green herbage mass (green fraction only). Only the green fraction was chemically analyzed for crude protein (CP) using the Kjeldahl method. In a companion study in the same area (Herrera et al., 2020 *in review*), the average leaf biomass potentially reached by livestock (from ground level up to 1.5 m height) ranged from 0 to 94 kg DM ha^{-1} for gliricidia and from 0 to 27 kg DM ha^{-1} for mimosa. Trees were fully developed, and the major benefits were the nutrient cycling and provision of shade. Because of the limited amount of forage mass within the browsing height (0 to 1.5 m), the forage component of the tree legumes was not included in the current manuscript.

Green herbage accumulation rate (GHAR) was determined by placing four exclusion cages (1 m^2; 1 x 1 m) within each paddock. Cages were placed on sites representing the average pasture structure and relocated every 14 days to a new location within the paddock. This frequent change of cage site was used in order to minimize the effect of structural differences in the canopy that could happen if cages are left on site for longer periods. Green herbage mass inside the cages was determined using the double-sampling technique previously described. Differences between green herbage mass inside the cage at the beginning and at the end of 14 days, divided by the growth period, resulted in the green herbage accumulation rate. Canopy bulk density (CBD) of signalgrass was expressed in kg DM ha^{-1} cm^{-1} and it was obtained by dividing the green herbage mass by the average canopy height measured with a ruler.

Livestock Management and Responses

Grazing commenced in 2012 and management of the area was reported in previous studies performed from 2012 to 2016 (Costa et al., 2016; Santos et al., 2020). For the current study, a minimum of two crossbred Holstein x Zebu (193±70 kg) steers grazed paddocks under continuous stocking with variable stocking rate. Water and mineral mix were provided *ad libitum*. During the two years of evaluation, two groups of experimental animals were used. The first one was from June 2017 to Jan 2018 and the second one was from June 2018 to Jan 2019. Most of the rainfall in this region occurs typically from March/April until August/September. Therefore, the period from June to January represents both the rainy and dry seasons.

Cattle were weighed every 28 days after a 12-h fasting period. Animal performance was evaluated by assessing the average daily gain (ADG), estimated by the weight difference of the testers at the beginning and at the end of each cycle. Stocking rate was calculated based on the number of grazing days, estimated by multiplying the number of tester and “put and take” animals within each grazing cycle of 28 days. Stocking rate adjustment was performed every

28 days adopting the method described by Sollenberger, Moore, Allen, & Pedreira (2005). Briefly, herbage allowance was adjusted based on green herbage mass (on a DM basis) and cattle body weight (BW), with target herbage allowance of 3 kg green DM kg^{-1} BW. Gain per area was estimated by multiplying ADG by the stocking rate and time interval between sampling dates.

Statistical analyzes

The data were submitted to statistical analysis using the Mixed procedure of the statistical package SAS 9.4 (2012). Fixed effects included the type of system (signalgrass monoculture and the two SPS), grazing cycle, and their interaction. Year and block were random effects. Grazing cycle was analyzed as a repeated measurement and the covariance structure selected based on the least Akaike information criterion value. If there was a significant effect for consortium / monoculture, orthogonal contrasts were applied to compare them. Means were compared using PDIFF of the SAS adjusted by Tukey and statistical differences were considered significant when $P \leq 0.05$.

RESULTS

Herbage Responses

Total and Green Herbage Mass

There was an interaction for treatment × evaluation for herbage mass (Fig. 3). Signalgrass monoculture in general had greater total (Fig.3A) and green HM (Fig.3B) than both SPS, especially SPS-Mimosa, which overall had the lowest HM; however, the degree of these differences varied with month. Average total herbage mass for signalgrass in monoculture was 3496 kg DM ha^{-1}, and average total HM for both SPS was 2266 kg DM ha^{-1}. Likewise, average green herbage mass of signalgrass monoculture was 2106 kg DM ha^{-1}, which was greater ($P \leq 0.05$) than the SPS treatments, which averaged 1305 kg DM ha^{-1} (Fig. 3 A and B). Seasonal variations influenced the proportion of the green fraction (Fig. 3C), affecting green herbage mass as a result.

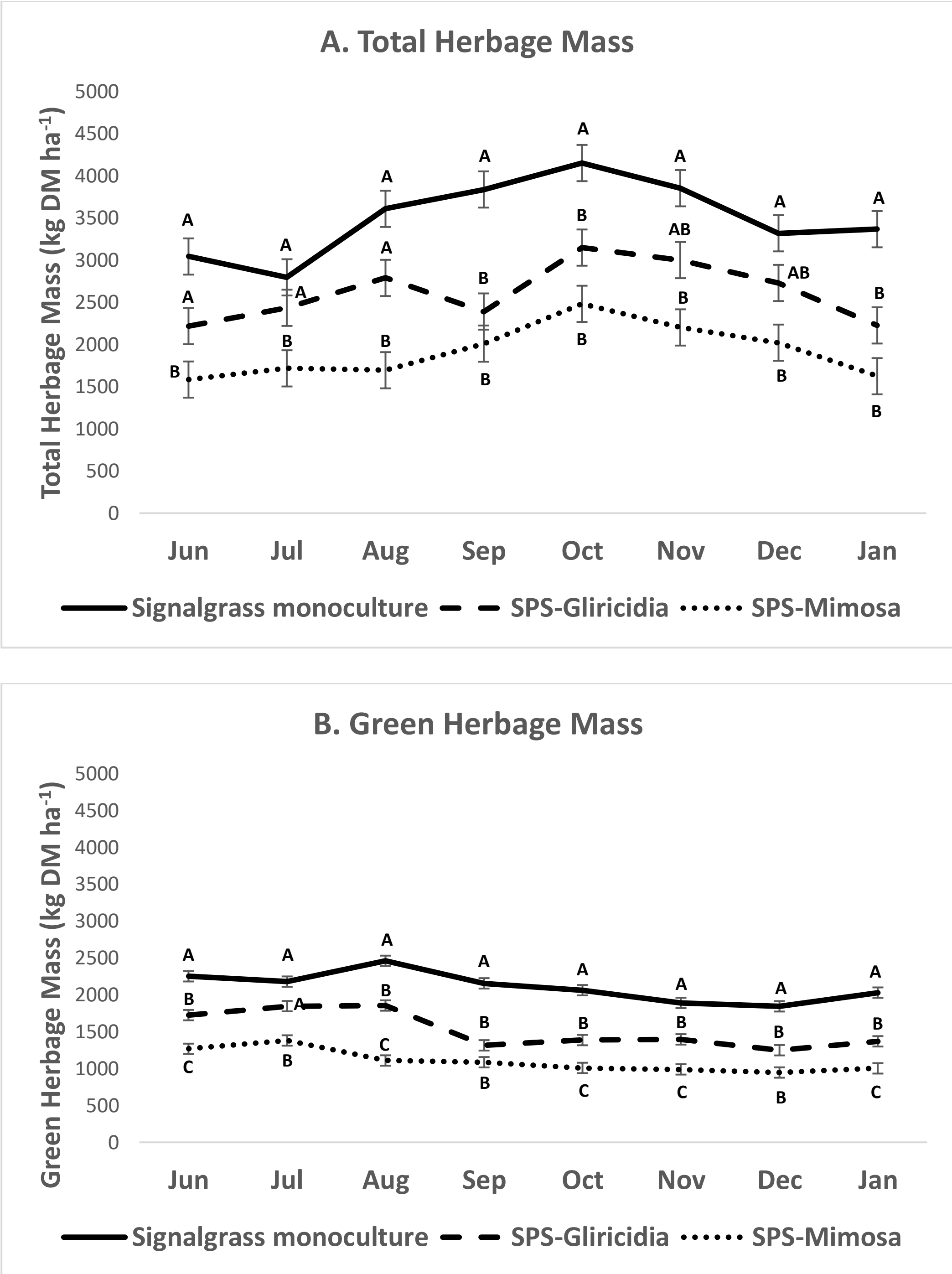

Figure 3. Total herbage mass (A) and green herbage mass (B) (kg DM ha^{-1}) in signalgrass monoculture, SPS-Gliricidia, and SPS-Mimosa during the experimental period. Data averaged across replications and years. Letters are comparing treatments within each month.

Canopy Green Fraction, Leaf Blade and Stem Proportions and CP (in the green fraction)

Treatment × month interaction occurred for canopy green fraction, proportion of green leaf and green stem (Table 1), and for CP concentration of these plant fractions in signalgrass (Table 2). During the rainy season (Jun – Sept), there was a greater proportion of the green forage fraction in the canopy, however, after September the average proportion of green forage was < 500 g kg^{-1} (Table 1). Signalgrass had lower proportions of green leaf tissues when grown in the SPS-Mimosa system (450 g kg^{-1}) than when grown in monoculture or SPS-Gliricidia systems (average 600 g kg^{-1}). However, these differences varied with month. Proportion of green stem had an inverse pattern, since it is a compositional data. Leaf CP was greater in the Gliricidia SPS and lower in the Mimosa SPS for most of the study period; grown in monoculture, signalgrass CP concentrations dropped from September and remained low during the summer. Stem CP concentrations were more variable among treatments and over time but generally declined for all treatments beginning in September (Table 1).

Table 1. Canopy green fraction and proportion of leaf blade and stem in the green fraction during the experimental period. Data averaged across replications and years.

Treatments*	**Canopy green fraction (g kg^{-1})**							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	740 aAB	780 aA	677 aABC	562 aCD	505 aD	497 aD	557 aD	605 aBC
SPS-Gliricidia	778 aA	763 aAB	667 aBC	552 aCD	463 aD	475 aD	477 aD	617 aC
SPS-Mimosa	805 aA	807 aA	655 aB	542 aBC	428 aC	468 aC	480 aC	618 aB
SEM	25							
	Proportion of leaf and stem in the canopy green fraction							
	Leaf Blade (g kg^{-1})							
Signalgrass	662 abA	652 abA	594 aAB	498 abBC	425 abCD	534 bBC	333 aD	323 bD
SPS-Gliricidia	742 aA	734 aA	612 aB	516 aBC	458 aCD	495 bBC	356 aD	515 aBC
SPS-Mimosa	588 bB	605 bAB	546 aBC	432 bCD	322 bD	300 aA	200 bE	350 bD
SEM	24							
	Stem (g kg^{-1})							
Signalgrass	338 abD	348 abD	406 aCD	502 abBC	575 abB	434 aC	667 bA	677 aA
SPS-Gliricidia	258 bE	266 bE	388 aD	484 aC	542 bB	505 aBC	644 bA	485 bC
SPS-Mimosa	412 aDE	395 aE	454 aD	568 bC	678 aB	700 bF	800 aA	650 aB
SEM	15							

†Means followed by the same lowercase letters in the columns (treatments) and upper case letter within rows (sampling dates), within each response variable (leaf blade or stem), do not differ by Tukey test (P≤0.05). *Signalgrass in monoculture; SPS: silvopastoral systems. SEM = standard error of mean.

Table 2. Crude protein of green fractions (leaf blade and stem) during the experimental period. Data averaged across replications and years.

Treatments*	**Crude protein (CP) of canopy green fractions**							
	Leaf CP (g kg^{-1})							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	65 aAB	68 aAB	69 aA	63 abB	50 bC	52 bC	52 aC	51 bC
SPS-Gliricidia	70 aAB	72 aA	69 aABC	69 aABC	62 aBCD	62 aD	58 aD	62 aCD
SPS-Mimosa	54 bA	57 bA	58 bA	56 bA	62 aA	52 bA	53 aA	51 bA
SEM	5							
	Stem CP (g kg^{-1})							
Signalgrass	10 bABC	15 aA	14 aAB	10 aABC	9 aBC	10 aABC	8 aC	10 aBC
SPS-Gliricidia	15 aA	13 aAB	13 aAB	11 aAB	11 aAB	9 aB	10 aB	11 aAB
SPS-Mimosa	12 abAB	13 aA	14 aA	8 aB	10 aAB	10 aAB	10 aAB	10 aAB
SEM	1							

†Means followed by the same lowercase letters in the columns (treatments) and upper case letter within rows (sampling dates), within each response variable (leaf blade or stem), do not differ by Tukey test (P≤0.05). *Signalgrass in monoculture; SPS: silvopastoral systems. SEM = standard error of mean.

Green Herbage Accumulation Rate and Canopy Bulk Density

GHAR was greatest for all systems during winter and spring (Jun - Aug) and declined beginning in Sept for signalgrass and SPS Mimosa systems in September (system by sampling date interaction; P < 0.05) (Fig. 4). Lower GHAR occurred in January with 47, 49, and 24 kg DM ha^{-1} d^{-1}, and greater GHAR occurred in July with 77, 76, and 49 kg DM ha^{-1} d^{-1}, for SM, SPS-Gliricidia, and SPS-Mimosa, respectively. There was no significant difference between SPS-Gliricidia and SM with an average of 61 kg DM ha^{-1} d^{-1}, however, it was lower in SPS-Mimosa in all months averaging 33 kg DM ha^{-1} d^{-1}.

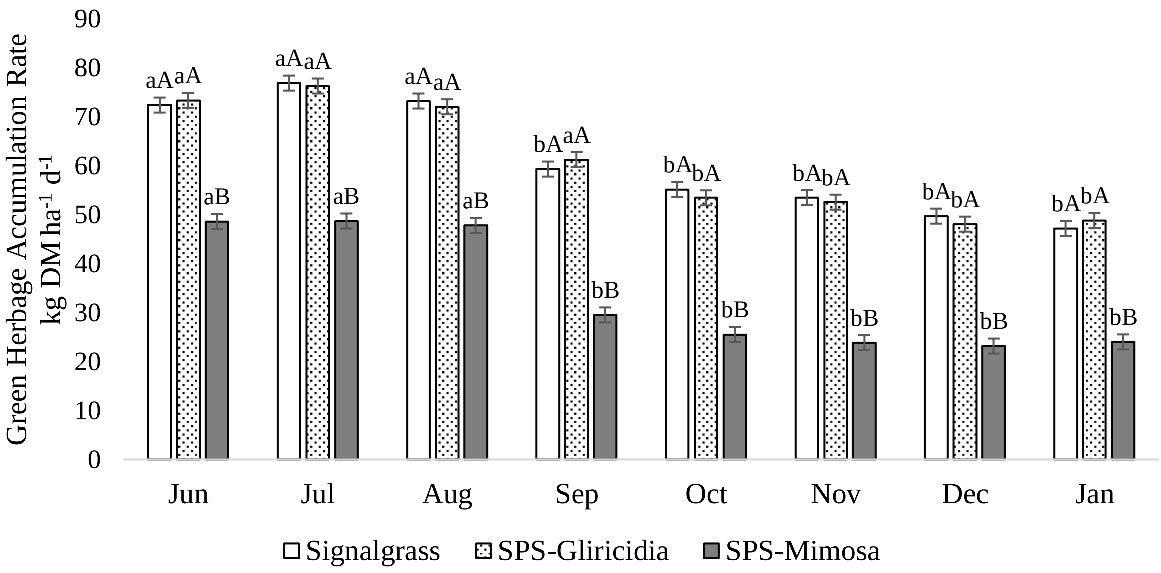

Figure 4. Green herbage accumulation rate (kg DM ha^{-1} d^{-1}) for signalgrass monoculture, SPS-Gliricidia, and SPS-Mimosa. Data averaged across replications and years. Lowercase letters show comparisons of months within treatments and uppercase letters show comparisons of treatments within each month.

The interaction treatment × month affected canopy bulk density ($P \leq 0.05$), which ranged from 40 to 80 kg ha^{-1} cm^{-1}. Signalgrass monoculture and SPS-Mimosa had greater canopy bulk density when compared with SPS-Gliricidia (Figure 5).

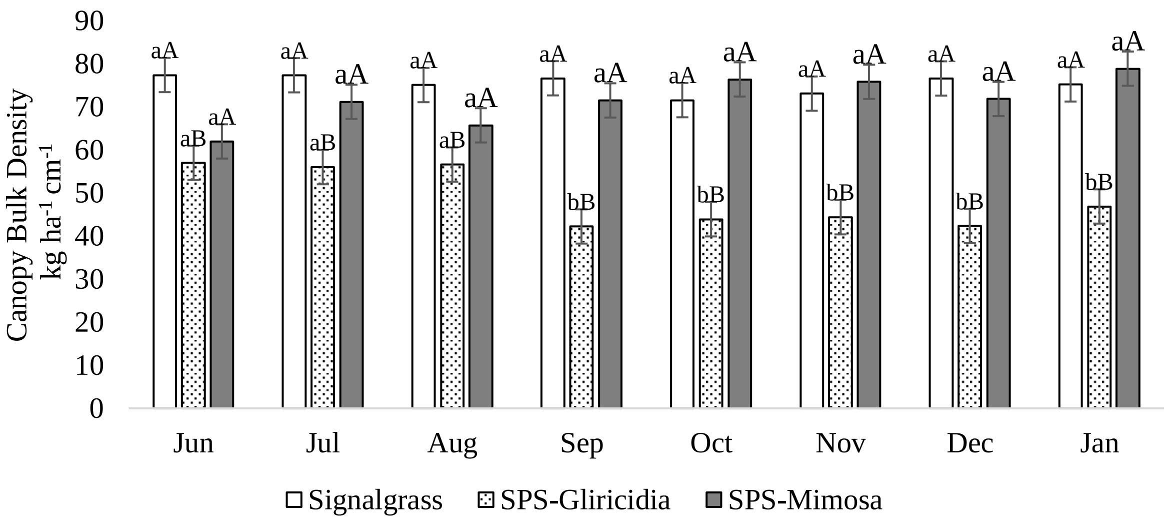

Figure 5. Canopy bulk density in signalgrass monoculture and SPS. Data averaged across replications and years. Lowercase letters show comparisons of months within treatments and uppercase letters show comparisons of treatments within each month.

Forage-Livestock Interface and Livestock Responses

Herbage Allowance

Herbage allowance varied during evaluations because of different herbage accumulation and senescence rates (Table 3). The main goal was to keep herbage allowance similar across treatments using the variable stocking rate technique. Similar herbage allowance occurred for signalgrass monoculture and SPS-Gliricidia. The SPS-Mimosa, however, had lower herbage allowance because of significant lower GHAR and the need to maintain the tester animals. From October to January, there were no grazing animals in SPS-Mimosa due to reduced herbage mass.

Average Daily Gain, Stocking Rate, and Gain per Area

Average daily gain (ADG) was greatest on SPS-Gliricidia during two out of eight evaluations when compared with signalgrass monoculture. Steer ADG for these two systems was always greater than SPS-Mimosa (Table 3). If considering the entire grazing period from June to January, ADG for cattle in SPS-Gliricidia was 0.77 kg head^{-1} d^{-1}, greater than for signalgrass monoculture (0.56 kg head^{-1} d^{-1}). The SPS-Mimosa had the lowest ADG (0.23 kg head^{-1} d^{-1}), not mentioning that cattle stayed in the paddocks for only four out of the eight months of evaluation due to reduced green herbage mass.

Stocking rate varied over the season, with SPS-Mimosa having the lowest SR at all evaluation dates from June to September, and because of reduced herbage mass, SPS-Mimosa did not have grazing animals from October to January. Signalgrass monoculture had greater SR than SPS-Gliricidia only in June, but no differences occurred at other months (Table 3).

Gain per area (GPA) followed a pattern similar to ADG, with animals grazing SPS-Gliricidia and signalgrass in monoculture having greater gains than those grazing SPS-Mimosa (Table 3). Over the two-year study, livestock gains were greater in the SPS-Gliricidia (423 kg BW ha^{-1}) compared with signalgrass in monoculture (347 kg BW ha^{-1}) and SPS-Mimosa (50 kg BW ha^{-1}).

Table 3. Livestock responses during 2-yr experiment comparing silvopastoral systems using tree legumes with Signalgrass monoculture.

	Herbage Allowance (kg DM kg^{-1} BW)							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	3.1 aA	3.1 aA	3.0 aA	3.0 aA	3.0 aA	2.9 aB	3.0 aA	3.0 aA
SPS-Gliricidia	3.2 aA	3.1 aA	3.0 aA	3.0 aA	2.9 aA	2.9 aA	3.0 aA	3.0 aA
SPS-Mimosa	3.0 bA	2.9 bAB	2.4 bB	2.3 bC	---	---	---	---
SEM	------------------------ 0.02 -----------------------				--------------------- 0.04 ----------------------			
	Average daily gain (kg head^{-1} d^{-1})							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	0.93 aA	0.94 bA	0.52 aAB	0.38 aB	0.40 bB	0.36 aB	0.38 aB	0.55 aB
SPS-Gliricidia	1.11 aA	1.28 aA	0.89 aB	0.70 aBC	0.61 aBC	0.49 aC	0.43 aC	0.63 aBC
SPS-Mimosa	0.50 bA	0.44 cA	-0.01 bB	-0.03 bB	---	---	---	---
SEM	--------------------- 0.07 ---------------------				---------------------- 0.10 ------------------------			
	Stocking rate (steers ha^{-1})							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	3.7 aA	3.5 aA	2.0 aB	2.0 aB	2.0 aB	2.0 aB	2.0 aB	2.0 aB
SPS-Gliricidia	3.2 aA	3.2 aA	2.0 aB	2.0 aB	2.0 aB	2.0 aB	2.0 aB	2.0 aB
SPS-Mimosa	2.0 bA	2.0 bA	2.0 aA	2.0 aA	---	---	---	---
SEM	---------------------- 0.2 ------------------------							
	Gain per area (kg BW ha^{-1} 28 d^{-1})							
	Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Signalgrass†	95 aA	94 aA	37 aB	26 aB	22 bB	20 aB	21 aB	31 aB
SPS-Gliricidia	100 aA	112 aA	50 aB	39 aB	34 aB	27 aB	24 aB	35 aB
SPS-Mimosa	28 bA	25 bA	-1 bB	-2 bB	---	---	---	---
SEM	---------------------- 10 ----------------------				------------------------ 6 -----------------------			

† Means followed by the same lowercase letters in the columns (treatments) and upper case letter within rows (sampling dates), within each response variable, do not differ by Tukey test (P≤0.05). *Signalgrass in monoculture; SPS: silvopastoral systems. SEM = standard error of mean.

DISCUSSION

Herbage Responses

Total and Green Herbage Mass

Herbage mass is the net result of plant growth, grazing, and senescence processes. Both total and green herbage masses decreased during the dry season, but this response occurred earlier in the season in SPS than in signalgrass monoculture. The SPS-Mimosa had less green herbage mass from August to January, and SPS-Gliricidia had less green herbage mass from September to January. Signalgrass in monoculture presented greater green herbage mass than SPS-systems, probably due to the lack of competition with legume trees for light, water, and nutrients. The lower herbage mass in SPS-Mimosa might have occurred due to greater competition for resources between the Mimosa tree and Signalgrass, considering that this Mimosa species is highly competitive for water (Mendonça et al., 2008). Lima et al. (2020) assessed signalgrass growth in SPS of gliricidia and mimosa, under full sun or shade. Signalgrass herbage accumulation rate was 56 and 27 kg DM ha^{-1} d^{-1} under full sun or under shade, respectively, and did not vary with tree species. According to Barreto, Lira, Santos, & Dubeux (2001) water deficit may cause reduction of basal and aerial tillering and leaf/stem ratio in forage grasses. Green herbage mass fluctuations during the growing season are in accordance with the reports of Aroeira et al. (2005), who verified that in signalgrass pastures, the green herbage mass varied between 800 and 1800 kg DM ha^{-1}, with the lowest value obtained in the dry season and the greatest in the rainy season.

Canopy Green Fraction, Leaf Blade and Stem Proportions, and CP (in the green fraction)

Canopy green fraction is directly correlated with animal performance (Santos et al., 2004; Euclides et al., 2018). Greater proportion of green fractions during the rainy months (June, July, and August) and resultant greater green herbage accumulation led to greater animal performance over this time period. Besides the role of forage species and plant maturity, abiotic factors have a key effect on forage nutritive value. Overall, there was a reduction not only in the size of the green forage fraction in the canopy, but within this fraction, there was a lower proportion of leaf blades (and corresponding greater proportion of stems) during the dry season (Table 1). The combination of the greater proportion of lower-CP stems (relative to leaves; Table 2) and the lower proportion of green fraction explains the overall lower CP concentration in the dry season.

Greater CP concentration observed in the green material for SPS-Gliricidia compared with SPS-Mimosa in six out of eight evaluations might relate to greater competition for water for this Mimosa species (Mendonça et al., 2008), compared with gliricidia. Frame et al. (1989) reported that botanical composition of grassland has an impact on herbage production and nutritive value, including mineral and CP concentrations. Signalgrass growing in the SPS-Gliricidia also had greater CP concentrations than signalgrass in monoculture in three out of eight evaluations. Nitrogen recycling from tree legumes might exert positive effect on forage nutritive value. In fact, Apolinário et al. (2016) indicated that gliricidia leaves have greater N concentration than mimosa leaves, and their enhanced litter decay rate, ultimately increases N available for signalgrass uptake. Cotrufo et al. (2013) indicated that up to 50% of the N present in a companion grass species might derive from a companion legume. Shade from gliricidia might have been another factor affecting signalgrass CP. Increase in forage CP concentration with increasing shade level is consistent with results from literature (Paciullo et al., 2017). Faria, Morenz, Paciullo, Lopes, & Gomide (2018) reported productive and qualitative responses of *U. decumbens* and *U. ruziziensis* to three levels of shade (0, 36, and 54%) and four N rates (0, 50, 100 and, 150 mg dm^{-3} soil). They observed a reduction in tillering for both species with increased shade and N levels, highlighting that forage under shade requires lower levels of N, unlike the response of forage grown in full sun. However, this result indicates the ability of signalgrass to adapt and maintain tillering, even under conditions of increasing light restriction.

Green Herbage Accumulation Rate and Canopy Bulk Density

The decline in GHAR during the dry season is likely a consequence of water deficit along with shading and senescence of leaves lower in the canopy (Costa et al., 2016). Mimosa trees on average were taller (7.2 m) than gliricidia trees (5.9 m) and that might have affected the light reaching the understory. However, soil moisture was lower in SPS-Mimosa compared with SPS-Gliricidia during this experimental period (Silva et al., 2020 *in preparation*), and it seemed to drive the process since signalgrass growing on the strips under full sun in the SPS-Mimosa was visually more water stressed than the ones growing under similar conditions in the SPS-Gliricidia. It is interesting to note that signalgrass growing in SPS-Gliricidia or in monoculture had similar GHAR across evaluation dates, indicating that gliricidia does not compete as much for water compared with Mimosa. Some trees have the ability to transfer water from deeper soil layers to the surface layers, a process called hydraulic redistribution, and that affects net primary productivity as well as water and vegetation dynamics (Prieto, Armas, & Punaire, 2012). In contrast, signalgrass growing in the SPS-Mimosa had always the lowest GHAR. Lower herbage accumulation might lead to frequent defoliation, reducing residual leaf area to support photosynthesis and depleting carbohydrate reserves, resulting in reduced growth (Chaparro, Sollenberger, & Quesenberry, 1996).

Canopy bulk density was similar between SPS-Mimosa and signalgrass monoculture, and both had greater CBD than signalgrass growing in the SPS-Gliricidia. This might have occurred due to lower herbage mass per unit of canopy height due to etiolation resulting from denser tree canopy under gliricidia trees. Lower CBD observed in SPS-Gliricidia would decrease bite mass and forage intake by animals, which would result in compromised animal productivity; however, this did not happen in this study, likely because the lower CBD was compensated by greater forage nutritive value and greater green leaf proportion for signalgrass in the SPS-Gliricidia. Santos et al. (2018) observed reductions of 40 and 60% in CBD of a SPS with increased shade to relative full sunlight. Greater CBD observed in signalgrass growing in the SPS-Mimosa is likely function of shorter canopy height, since CBD typically is greater in the lower canopy stratum (Sollenberger and Burns, 2001). Competition with mimosa trees likely reduced signalgrass growth, resulting in lower canopy height and increased CBD. Removal of mimosa trees in a succession study benefited the understory vegetation and accelerated the succession process (Podadera et al., 2015). Signalgrass growing in monoculture had greater CBD due to greater herbage mass per unit of canopy height. Lopes et al. (2017) observed a reduction of 18 and 58% in the canopy bulk density of Signalgrass grown with 20 and 70% sunlight, respectively.

Forage-Livestock Interface and Livestock Responses

Herbage Allowance

Herbage allowance was similar between signalgrass growing in monoculture or in the SPS-Gliricidia; however, it was always lower when growing in the SPS-Mimosa. Lower herbage allowance in SPS-Mimosa was a consequence of lower green herbage mass in that system. In fact, it was necessary to withdraw grazing animals from SPS-Mimosa pastures from October to January, in both years, because of reduced green herbage mass. Since herbage allowance is a forage/animal relationship, stocking rates consequently became a consequence of the amount of herbage mass (Conte et al., 2011). Herbage allowance peaked for all treatments in June, resulting from greater rainfall and faster green herbage accumulation rates. The challenge to maintain the same herbage allowance for all treatments was established for SPS-Gliricidia and signalgrass monoculture, but it was not possible to keep animals in SPS-Mimosa due to low signalgrass herbage mass. Similar herbage allowance is key when comparing treatments in grazing settings (Sollenberger et al., 2005), and this is possible by using variable stocking rate, using tester and put-and-take animals.

Average Daily Gain, Stocking Rate, and Gain per Area

Lower ADG in SPS-Mimosa might have occurred because of lower herbage allowance. Greater ADG in SPS-Gliricidia can be a consequence of the presence of younger leaves and the legume contribution to the system. In a companion work, legume leaf biomass within the reach of the grazing animals (<1.5 m) ranged from 0 to 94 kg DM ha^{-1} at any given evaluation (Herrera et al., 2020, *under review*). Therefore, although legume leaf biomass was not a significant component of total forage biomass, litter deposition and decay might have resulted in greater nutritive value for signalgrass growing in SPS-Gliricidia. Small contribution of gliricidia leaves could also support greater livestock gains in that system. In previous work at this same experimental area, Apolinário et al. (2015) observed biological fixed N contained in leaves of gliricidia and mimosa trees ranged from 30 to 121 kg N ha^{-1} at any given evaluation.

Average daily gains observed in this study (0.56, 0.77, and 0.23 kg head^{-1} d^{-1}, for cattle on signalgrass growing in monoculture, in SPS-Gliricidia, and in SPS-Mimosa, respectively), are in the range observed in the literature for growing animals grazing on signalgrass pastures (Santos et al., 2004). The gains observed for the animals in the SPS-Gliricidia, however, are in the upper range and likely reflect the contribution of the legume to the system, and potential benefit of shade coupled with greater herbage allowance vis-à-vis the SPS-Mimosa, improving the wellbeing of grazing animals (Paciullo et al., 2014). The ADG was driving the overall results from the systems since the stocking rate was similar between signalgrass in monoculture or in the SPS-Gliricidia. The arboreal component in silvopasture systems takes time to reach maturity; therefore, results might be different depending on the tree developmental stage. Animal gain was lower at this same site during an earlier stage of silvopasture development (2012-2015; Santos et al., 2020), likely due to differences in tree development and competition with the herbaceous component. Results from the current study highlight the potential of silvopastoral systems using tree legumes to improve animal performance in tropical regions. In addition to greater livestock performance, SPS provide a myriad of other ecosystem services that benefit the entire society (Dubeux et al., 2017a).

SUMMARY AND CONCLUSIONS

Silvopastoral systems using tree legumes are an option to develop sustainable livestock systems; however, tree legumes differ in their ability to provide ecosystem services. In this 2-yr study, we compared herbage and livestock responses in two SPS using either Gliricidia or Mimosa combined with signalgrass and contrasted with signalgrass in monoculture. Greater animal productivity (average daily gain and gain per area) occurred for the SPS-Gliricidia, followed by Signalgrass in monoculture, and then SPS-Mimosa. Competition between the Mimosa tree and the herbaceous signalgrass canopy reduced green herbage accumulation rate, decreasing stocking rate and gain per area as a result. In general, both SPS had lower herbage mass compared with monoculture; however, greater crude protein concentration in signalgrass growing in SPS-Gliricidia compensated the lower herbage mass translating into greater livestock gains. Silvopastoral systems are a sustainable option for warm-climate regions. They have potential not only to support greater livestock gains, but also to provide other ecosystem services that benefit the entire society. If livestock production is the major desired ecosystem service, gliricidia is a better option to use with signalgrass in SPS compared with mimosa trees.

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CHAPTER III

Tree canopy management affects dynamics of herbaceous vegetation in silvopasture systems using arboreal legumes

Tree canopy management affects dynamics of herbaceous vegetation in silvopasture systems using arboreal legumes

Abstract: Understanding ecological interactions between the arboreal and the herbaceous components is key to get the full benefits from silvopastoral systems. The objective of this 2-yr research was to evaluate productivity and nutritive value of signalgrass [*Urochloa decumbens* (Stapf.) R. Webster] subjected to shading from the tree legumes gliricidia [*Gliricidia sepium* (Jacq.) Steud] or mimosa (*Mimosa caesalpiniifolia* Benth.) under different tree canopy management (harvesting or not one of the rows in the double-row tree planting). Response variables included canopy height, herbage mass (green leaf blade, green stem, senescent leaves, and senescent stem), herbage accumulation rate, canopy bulk density, bare soil and botanical composition. Total herbage mass, green herbage mass, and green leaf mass were affected by treatment × evaluation date and harvest management × evaluation date interactions. Herbage accumulation rate in SPS-Gliricidia was greater (55 kg DM $ha^{-1}d^{-1}$) than SPS-Mimosa (32 kg DM $ha^{-1}d^{-1}$). There were differences between harvest management for bare soil and litter percentage ($P \leq 0.05$). The botanical composition found 36 weed species, distributed in 19 botanical families and 36 of these species were dicotyledonous (53%) and 17 were monocotyledonous (47%). Tree canopy management can affect forage quantity and quality; however, these effects are transient and vary with tree spacing. Signalgrass grew faster and had better nutritive value when growing in SPS-Gliricidia.

Keywords: *Gliricidia sepium*, legume, *Mimosa caesalpiniifolia*, mixture, pasture, nutrient cycling, shade, soil moisture, tree spacing

O manejo do dossel das árvores afeta a dinâmica da vegetação herbácea em sistemas silvipastoris usando leguminosas arbóreas

Resumo: Compreender as interações ecológicas entre os componentes arbóreo e herbáceo é fundamental para obter todos os benefícios dos sistemas silvipastoris. O objetivo desta pesquisa de 2 anos foi avaliar a produtividade e o valor nutritivo do signalgrass [*Urochloa decumbens* (Stapf.) R. Webster] submetido ao sombreamento da leguminosa gliricídia [*Gliricidia sepium* (Jacq.) Steud] ou mimosa (*Mimosa caesalpiniifolia* Benth.) Sob diferente manejo de copa de árvore (colheita ou não de uma das linhas no plantio de árvore de linha dupla). As variáveis de resposta incluíram altura do dossel, massa da forragem (lâmina da folha verde, caule verde, folhas senescentes e caule senescente), taxa de acúmulo de forragem, densidade do dossel, solo descoberto e composição botânica. A massa total de forragem, a massa de forragem verde e a massa de folha verde foram afetadas pelas interações tratamento × data de avaliação e manejo de colheita × data de avaliação. A taxa de acúmulo de forragem em SPS-Gliricidia foi maior (55 kg MS $ha^{-1}d^{-1}$) do que SPS-Mimosa (32 kg MS $ha^{-1}d^{-1}$). Houve diferenças entre o manejo da colheita para solo descoberto e porcentagem de serapilheira ($P \leq 0{,}05$). A composição botânica encontrou 36 espécies de plantas daninhas, distribuídas em 19 famílias botânicas, sendo 36 dessas espécies dicotiledôneas (53%) e 17 monocotiledôneas (47%). O manejo do dossel das árvores pode afetar a quantidade e a qualidade da forragem; no entanto, esses efeitos são transitórios e variam com o espaçamento das árvores. Signalgrass cresceu mais rápido e teve melhor valor nutritivo quando cresceu em SPS-Gliricidia.

Palavras-chave: *Gliricidia sepium*, leguminosa, *Mimosa caesalpiniifolia*, mistura, pastagem, ciclagem de nutrientes, sombra, umidade do solo, espaçamento entre árvores

1. Introduction

Shade affects productive, nutritional, and morphological traits of tropical forages (Coleman et al., 2004). Warm-climate C4 grasses growing under shade must self-adapt through phenotypic plasticity, such as increased leaf area and shoot-to-root ratio, as well as decreased tiller population density, and canopy bulk density (Lima et al., 2019). Moreover, shade might increase chlorophyll (Martuscello et al., 2009) and crude protein (CP) concentrations (Paciullo et al. 2017) of herbaceous vegetation in silvopastoral systems.

Silvopastoral systems with tree legumes have added benefit, which is the potential biological-N_2 fixation, and nutrient recycling (Dubeux Jr et al., 2007; Lemos et al., 2014). In addition, there have been reports that gliricidia and mimosa root systems are able to take advantage of the association with rhizobia symbionts, influencing the dynamics of soil nitrogen storage (Silva et al., 2017; Kaba et al., 2019).

Selecting the appropriate arboreal species for a silvopastoral system is key to enhance the sustainability of the system. Multi-purpose tree species with economic potential that also provides shading, soil protection, fire resistance, without toxic effect to animals, and forage potential must be selected (Oliveira et al., 2003). Tree spacing and canopy management affect the dynamics of the herbaceous vegetation, and it varies with species, environment, and management practices. Tree density affects the conditions of the light environment under the canopy altering the growth of forages. The greater the spacing between the lines of the trees, the greater the light penetration reaching the understory, favoring herbage accumulation (Ribaski et al., 2009). Most of the studies with tropical grasses have shown a reduction in forage production when shade levels exceed 50% of the incident radiation due to the acute decrease in photosynthetic rates of C_4 grasses (Paciullo et al., 2010).

The potential for commercial use of timber and firewood in these tree legume species, especially Mimosa, contributes significantly to generate revenue for the producer with the sale of wood and other products extracted from trees (Silva et al., 2004). Moreover, the changing perception of the consumer about food production practices in various parts of the world is very present and noticeable (Drouillard, 2018; Hocquette et al., 2018), which justifies the development of technologies or new alternatives to provide more comfort and welfare to production animals.

We hypothesized that the management of tree canopy would affect forage nutritive value and herbage accumulation of signalgrass in SPS, however, these responses vary with SPS and season of the year. This study evaluated canopy characteristic of *U. decumbens* Stapf. in two SPS under two tree canopy managements, in a sub-humid tropical region of Brazil.

2. Material and Methods

2.1 Site description and establishment

The study was carried out at the Experimental Station of Itambé (7°23’ S and 35°10’ W and 190 m above sea level), Agronomic Institute of Pernambuco-IPA. The soil in the experimental area is classified as an Ultisol (Apolinário et al., 2016). Average annual rainfall is 1200 mm and annual average temperature is 25°C. The relative annual air humidity is 80% and the local climate is defined as As’ warm-humid rainy tropical with dry summer. Soil chemical characteristics in 2017 were: $pH_{(water;\ 1:2.5)}$ = 5.2, Mehlich-I P = 7.2 mg dm^{-3}; Ca^{2+} = 3.0 $cmol_c\ dm^{-3}$; Mg^{2+} = 1.1 $cmol_c\ dm^{-3}$; K^+ = 0.17 $cmol_c\ dm^{-3}$; Al^{3+} = 0.17 $cmol_c\ dm^{-3}$ (Herrera et al., 2020).

In 2011, tree legumes were established in double rows in 1-ha paddock. Each paddock had 14 double rows, resulting in 2500 trees ha^{-1}. Legume seeds were planted in a greenhouse and inoculated with specific *Bradyhizhobium strains*, obtained from the soil Microbiology Laboratory at Federal Rural University of Pernambuco (UFRPE). All paddocks were fertilized in July 2011 with 44 kg P ha^{-1} (as ordinary superphosphate) and 100 kg ha^{-1} (as potassium chloride) on the entire area. Legume seedlings were transplanted to the field in June 2011 with approximately 30-cm height and planted in 20-cm deep furrows.

Signalgrass was planted between tree rows. Signalgrass was previously established in one of the blocks since 1969 (Lira et al., 1995). In the other two blocks, signalgrass was established along with the tree legumes, between the double rows. Briefly, the establishment of signalgrass occurred in open pits (about 5-cm deep), spaced 1.0 x 0.5 m; seeds were placed manually (10 kg of commercial seed ha^{-1} with 40% of pure viable seeds). Pastures were fully established by the end of the rainy season in 2011. In September 2016, in order to allow more light to reach the herbaceous layer, one of the tree rows was harvested in half of the plots, reducing the tree population to 1250 trees ha^{-1}. The harvested side was randomized in each experimental unit. All plots were managed under continuous stocking and specific details about livestock management are described by Gomes da Silva et al. (2020).

2.2 Treatments and experimental design

A 2-yr experimental period was adopted from January 2017 to December 2018. Treatments consisted of two SPS with two harvest regimes for the tree components. In one harvesting regime, the trees in the double-rows were not harvested, whereas in the other harvesting regime, the trees from one-row were harvested while keeping the trees from the other row. The hypothesis behind these management strategies was that harvesting one of the rows would enhance the light environment in the understory while providing cash flow for the producer by selling the harvested wood. The SPS were: (1) *Urochloa decumbens* Stapf. (signalgrass) + *Mimosa caesalpiniifolia* Benth (mimosa) → SPS-Mimosa; (2) signalgrass + *Gliricidia sepium* (Jacq.) Steud (Giricídia) → SPS-Gliricidia. Treatments were allocated in split-plot in a randomized complete block design. Main plot was the SPS and the split-plot was the harvest management. Therefore, half of the entire paddock was under a given harvest regime. Split plots were randomized across experimental units.

2.3 Herbage responses

Average canopy height (CH) of signalgrass was measured using a sward stick (Barthram, 1985) at 60 random points, and the average of these 60 scores were used in the regression equation to estimate herbage mass (Pedreira, 2002). Signalgrass herbage mass was determined using the double-sampling technique described by Haydock and Shaw (1975). Briefly, every 28 days, direct measurements were obtained by harvesting six 0.25-m^2 quadrats per paddock, at ground level. After harvesting the forage, botanical and morphological separations were performed.

Grass samples were separated into stem (green and dead) and leaf blade (green and dead). Forage samples were oven-dried at 55°C for 72 h to a constant weight. Herbage mass was calculated without considering the dead material. Laboratory analyzes (CP and DM concentrations) were performed only in the green forage fractions (leaf and stem). Dead material was used to calculate the proportion of leaf and stem and DM concentration.

Herbage accumulation rate (HAR) was determined by placing four exclusion cages within each paddock. The cage location was defined by assessing 60-point measurements with the sward stick. Cages were placed on an average location and relocated every 14 d to a new location within the paddock. This procedure was done in order to minimize the effect of structural differences in the canopy. Differences between mean values at the beginning and at the end of 14 d, divided by the growth period, estimated herbage accumulation rate (Sollenberger and Cherney, 1995).

Canopy bulk density of signalgrass was expressed in kg DM $ha^{-1}cm^{-1}$ and it was obtained by dividing the green herbage mass by the average canopy height, which was determined by using 60-point measurements with measuring sward stick. Sward stick was preferred over disk height to measure canopy height because the compressed disk height might over-estimate canopy bulk density (Arruda, 2009). The measurement was taken at the extended height of individual profiles, according to the recommendation of Frame (1981).

2.4 *Botanical composition and herbicide management*

The botanical assessment was carried out at in the beginning of June in 2016 and 3 d after the botanical assessment, herbicide Tordon (pyridine carboxylic-acid herbicide) was applied to control undesirable arboreal shrubs and dicot vegetation. To prevent the death of legumes in the study, the herbicide was applied locally to weeds when plants were actively growing.

Three months after the herbicide application, a new botanical survey was made, and every 3 mo thereafter until March 2019. In this manuscript, it will be reported botanical data from December 2016 to March 2019, totaling 10 evaluations (Evaluation 1-Dec 2016; Evaluation 2 Mar 2017; Evaluation 3 Jun 2017; Evaluation 4 Set 2017; Evaluation 5 Dec 2017; Evaluation 6 Mar 2018; Evaluation 7 Jun 2018; Evaluation 8 Set12018; Evaluation 9 Dec 2018 and Evaluation 10 Mar 2019). Herbicide application was performed three times during the experiment to try to suppress broadleaf herbaceous weeds.

The botanical composition was estimated based on the method proposed by Mannetje and Haydock (1963), adapted by Jones and ha (1979). In the same square used for forage mass, the species present were visually assigned ranks of 70, 21 and 9%, for those components whose participation in the pasture were in 1st, 2nd and 3rd place, respectively. In addition, 14 0.25-m^2 squares were randomly selected in Area 1 (harvesting) and 14 squares in Area 2 (no harvesting - in the double-row tree planting).

In cases of occurrence of species with high dominance in the sample, more than one class was assigned; that is, the species received a cumulative classification, corresponding to the first and second places.

The collected material was conditioned and dehydrated in a greenhouse at 40 ºC. After drying the material was sent in the form of exsiccates (Figure 1) to the Herbarium Dárdano de Andrade Lima at the Pernambuco Agronomic Institute in Recife to identify the scientific names.

Figure 1: Some weed species found during the botanical survey in the experimental area.

3.5 *Bare soil*

Estimates of bare soil and litter were visual (Figure 2), ranging from 0 to 100% (Gardner, 1986). This evaluation occurred in the same area of the square used for evaluation of forage mass and botanical composition. Litter was defined as all plant material deposited on the soil surface which were detached from plants.

Figure 2. Bare soil in a ranking from 0 to 100%. (A) 0%; (B) 25%; (C) 50%; (D) 100%.

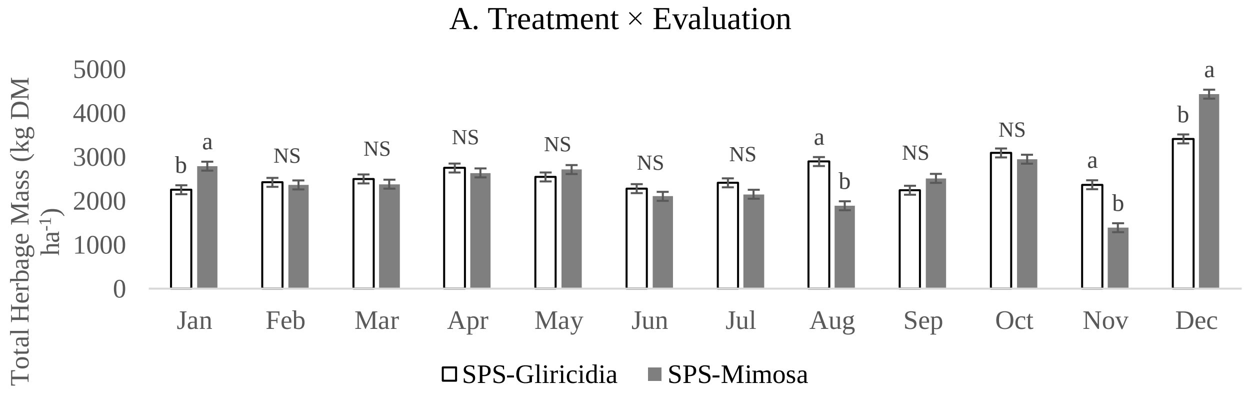
2.6 Data analyses

Data were submitted to analysis of variance using the mixed procedure of the statistical package SAS 9.4 (2012). Fixed effects included the type of system, harvest management, grazing cycle, and the interaction between them. Year and block were considered random effects. If there was a significant effect for consortium / monoculture, orthogonal contrasts were applied to compare them. Means were compared using PDIFF of the SAS adjusted by Tukey, and statistical differences were considered significant when P ≤ 0.05.

3. Results

3.1 Total herbage mass and total green herbage mass

There was a significant treatment × evaluation interaction for total herbage mass. In eight out of twelve months of the year herbage mass was similar between the two legume systems, with SPS-Mimosa presenting greater herbage mass in January and December, and SPS-Gliricidia with greater herbage mass in August and November (Figure 3A). Interaction also occurred between harvest management × evaluation date, and one-row area had greater total forage mass only in December (Fig. 3B).



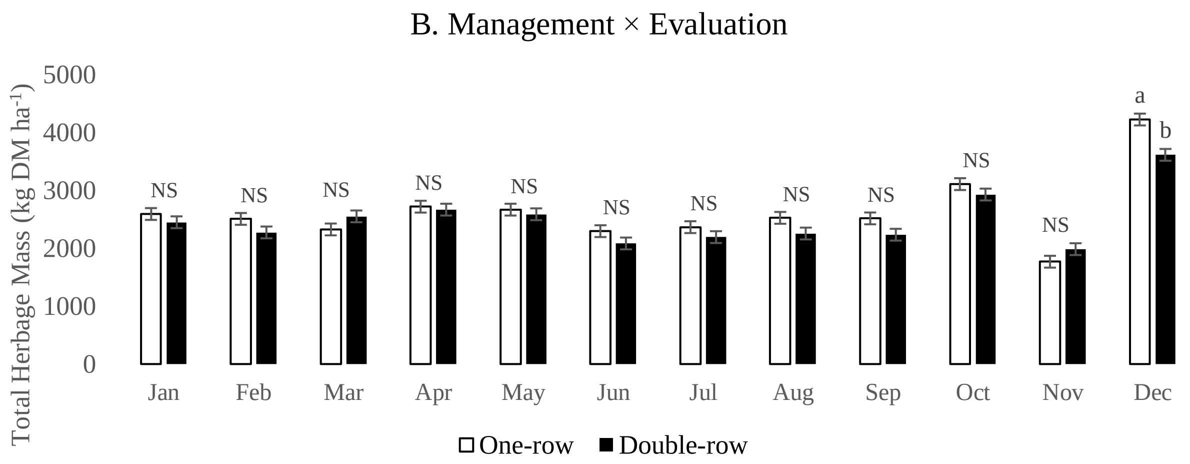


Figure 3. Total herbage mass of legume trees under different tree canopy management during the experimental period. Different letters between treatments within each month indicate significant difference using the PDIFF procedure adjusted to Tukey (P<0.05). NS = non-significant. Data averaged across two experimental years and three blocks.

There was a treatment × evaluation date interaction (P<0.05) for total green herbage mass and there was no significant difference between harvesting management for this response ($P \geq 0.05$). SPS-Gliricidia always had greater green herbage mass for the herbaceous vegetation than SPS-Mimosa, but the difference varied along the season. May to July were the months with greater proportions of green material to both systems, corresponding to the rainiest months (Table 1).

Table 1. Total green herbage mass (kg DM ha^{-1}) for both systems during the experimental period

	SPS-Gliricidia†	SPS-Mimosa	*P* value
January	1207 c	977 c	<.001
February	1287 c	979 c	<.001
March	1293 bc	949 c	<.001
April	1479 c	1103 bc	<.001
May	1491 b	1120 bc	<.001
June	1724 a	1254 ab	<.001
July	1814 a	1351 a	<.001
August	1864 a	1066 bc	<.001
September	1327 bc	1028 c	<.001
October	1332 bc	1008 c	<.001
November	1337 bc	988 c	<.001
December	1366 bc	925 c	<.001
SEM	54		

†Means followed by equal lowercase letters in the column do not differ by the PDIFF procedure adjusted by Tukey ($P<0.05$). SPS: silvopastoral systems. SEM = standard error of mean.

3.2 Green and dry plant fraction (leaf blade and stem) biomass

There was a treatment × evaluation interaction for green leaf blade mass and for green stem mass. Green leaf blade biomass was greater for SPS-Gliricidia in most of the evaluation dates, but the difference between SPS systems varied along the year (Fig. 4A; SE = 39 kg DM ha^{-1}). Green stem biomass varied between SPS systems along the year, with SPS-Mimosa showing greater green stem biomass in the first evaluation but declining along the year (Fig. 4B; 28 kg DM ha^{-1}).

There was treatment × evaluation interaction for senescent leaf (Fig. 4C; SE = 41 kg DM ha^{-1}) and senescent stem (Fig. 4D; SE = 68 kg DM ha^{-1}), which varied in both cases along the season. SPS-Mimosa had greater senescent stem biomass starting for the first five months of the year and in the last month. Rainy season (Jun-Sep) had greater green forage biomass for both treatments compared with the dry season.

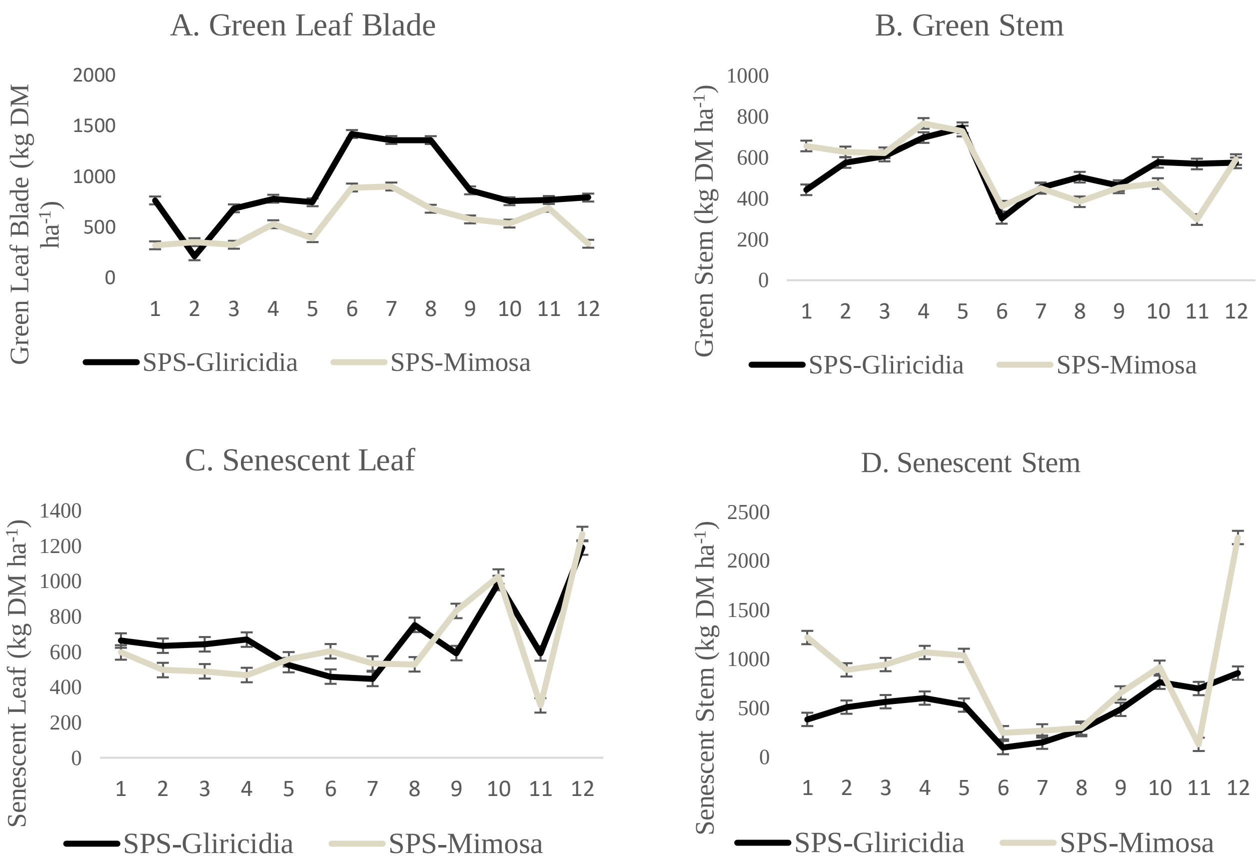


Figure 4. Green leaf blade (A), green stem (B), senescent leaf blade (C) and senescent stem (D) during experimental period.

There was a management × evaluation date interaction for green leaf mass (Table 2). Green leaf mass did not differ among management systems in any of the evaluation dates, but they did vary along the months. Interaction occurred because the variation along the months was not similar for both systems, as indicated by the *P* values comparing management systems within each month.

Table 2. Green leaf blade in one-row and in double-row during the experimental period

Evaluation	Green leaf mass (kg DM ha^{-1})		*P* value
	One row*	**Double row**	
January	534 ef	550 de	1.000
February	536 ef	527 e	1.000
March	474 f	538 de	0.9714
April	529 ef	589 cde	0.9875
May	557 def	580 cde	1.000
June	1192 a	1122 a	0.9341
July	1140 ab	1125 a	1.000
August	1049 b	994 a	0.9966
September	733 c	707 bc	1.000
October	627 cde	665 bcd	1.000
November	690 cd	771 b	0.7781
December	530 ef	598 cde	0.9545

†Means followed by equal lowercase letters within the same column do not differ by Tukey test ($P \leq 0.05$). *P* value compares within row. *One row = double-row planting with one row harvested; double-row = double-row planting with no rows harvested; SEM = standard error of mean.

3.3 Crude protein of green leaf blade and green stem

There was treatment × evaluation interaction for crude protein of green leaf blade (Fig. 5A). Signalgrass in SPS-Gliricidia had greater CP in the green leaf blade in nine out of ten evaluations, compared with the signalgrass growing in the SPS-mimosa (Fig. 5A). Crude protein of green stem varied along the evaluations; however, the results were not as affected by rainfall (Fig. 5B).

Crude Protein of Green Leaf Blade

B. Crude Protein of Green Stem

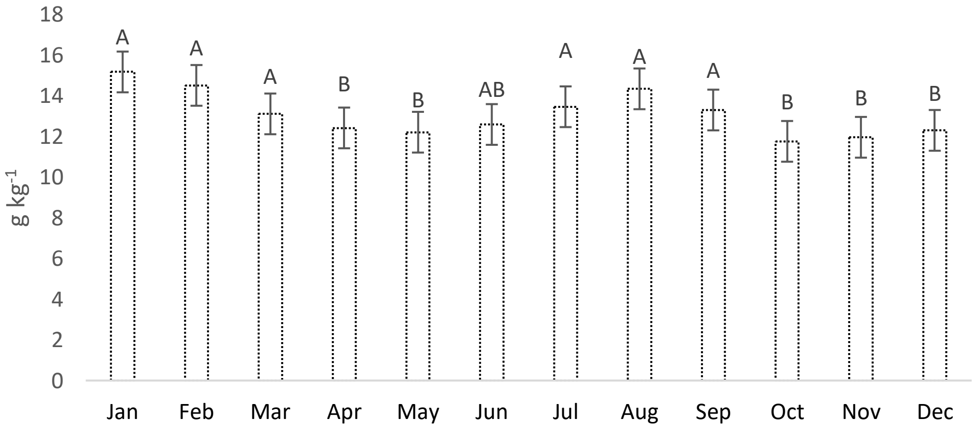


Figure 5. Crude protein of green leaf blade (A) and crude protein of green stem (B) (g kg^{-1}) in signalgrass growing under SPS-Gliricidia and SPS-Mimosa during the experimental period. Data averaged across replications and years. Small case letters are comparing treatments within each month in Fig. 5A and capital letters are comparing evaluations in Fig. 5B. In both cases, equal letters are not different by the PDIFF adjusted by Tukey (*P*>0.05).

3.4 Herbage Accumulation Rate

There was a treatment × evaluation interaction for herbage accumulation rate (HAR) (Figure 6). Herbage accumulation rate was always greater for signalgrass growing in the SPS-Gliricidia compared with SPS-Mimosa. The HAR peaked in July (71 kg DM ha^{-1}d^{-1} for SPS-Gliricidia and 48 kg DM ha^{-1}d^{-1} for SPS-Mimosa) and had its lesser growth rate in December to SPS-Mimosa (22 kg DM ha^{-1}d^{-1}), coinciding with greater and lower rainfall, respectively; however, SPS-Gliricidia did not follow rainfall pattern as mimosa with lower HAR occurring in May (41kg DM ha^{-1}d^{-1}).

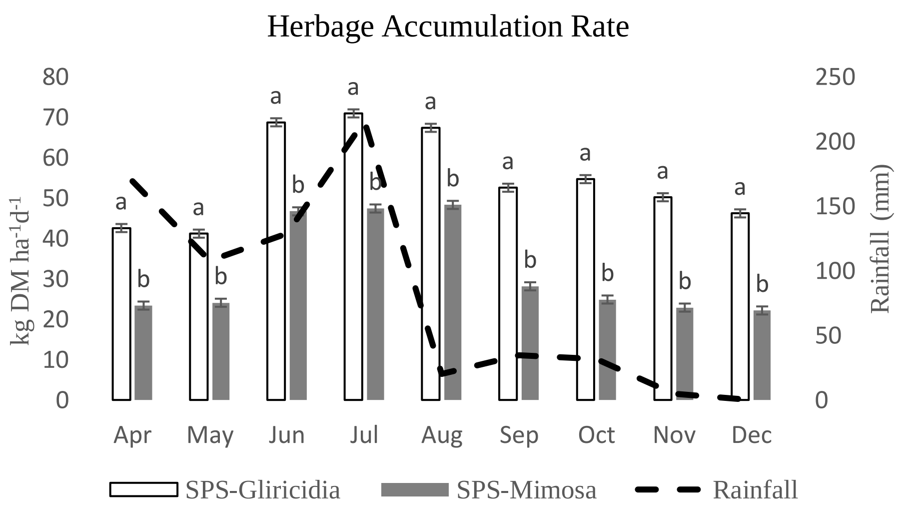


Figure 6. Herbage accumulation rate for SPS-Gliricidia and SPS-Mimosa during the experimental period (SE=1.5 kg DM ha^{-1} d^{-1}). Letters are comparing treatments within each

evaluation month. Similar letters are not different according to PDIFF adjusted by Tukey (P > 0.05).

3.5 Canopy Bulk Density (CBD) and Canopy Height

There was a treatment × evaluation interaction for CBD (Fig. 7). SPS-Mimosa had greater CBD in two out of 12 evaluations and SPS-Gliricidia had greater CBD in one out of 12 evaluations, with the remaining evaluation indicating similar CBD. Overall, the SPS-Mimosa treatment had greater heights (28 cm) throughout the experimental period when compared to the SPS-Gliricidia (19 cm).

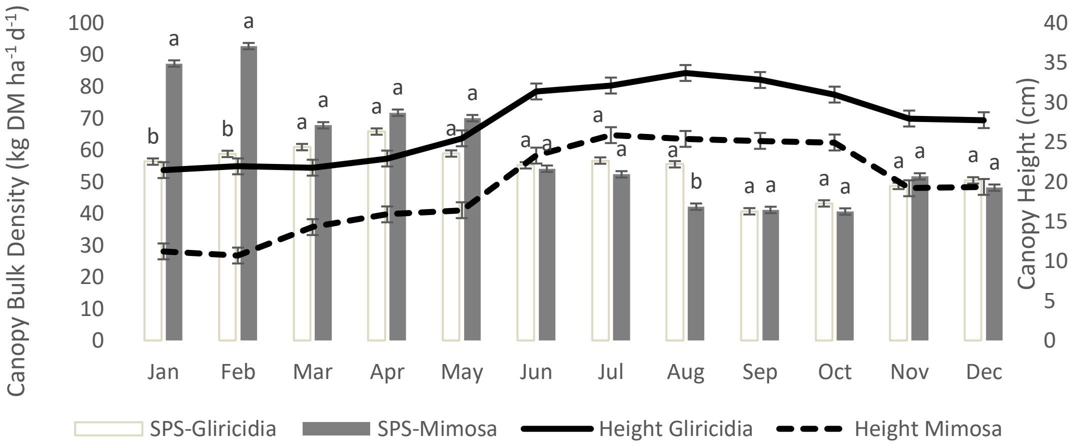


Figure 7. Canopy bulk density and canopy height of signalgrass growing in SPS-Gliricidia and SPS-Mimosa during the experimental period. Letters are comparing treatments within each evaluation month. Similar letters are not different according to PDIFF adjusted by Tukey (P > 0.05).

3.6 Bare soil

Bare soil and litter percentage in SPS-Mimosa averaged 30 ± 15.3%, being greater in the dry season (45 ± 9.3%). In SPS-Mimosa, the presence of litter corresponded on average to 11.6 ± 6.2% of the soil cover, and the remaining area, the rest of the contribution came from *U.decumbens* and weeds during periods of lower rainfall (Figure 8).

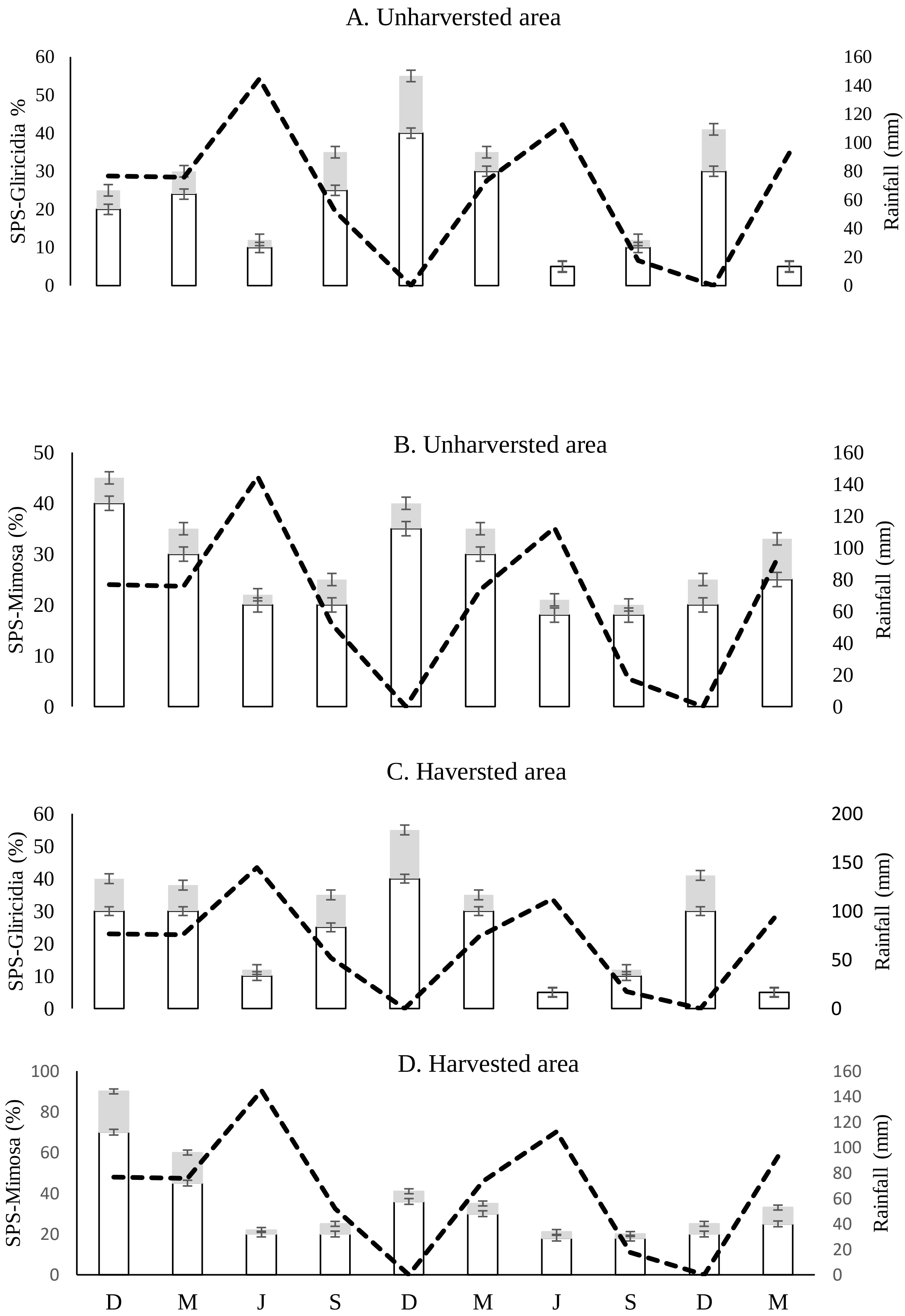


Figure 8. Bare soil and litter percentage in SPS in unharvested and harvested area.

3.7 Botanical composition

The species found during the botanical survey prior to herbicide application were classified under their common and scientific names (Table 3). The assessment resulted in 36 weed species, distributed in 19 botanical families. Of these 36 species, 19 were dicotyledonous (53%) and 17 monocotyledonous (47%).

Table 3: Plant species present in SPS-Gliricidia, SPS-Mimosa and Signalgrass in monoculture browsed by steers at Itambé-PE, Brazil.

Family	Common name	Scientific name
Herbaceous Stratum		
Boraginaceae	Crista de galo	*Heliotropium tiaridioides* Cham.
Poaceae	Barba de bode	*Aristida jubata* (Arechav.) Herter
Mimosoideae	Malícia	*Mimosa sensitiva* L.
Fabaceae	Orelha de onça	*Macropitilium martii* (Benth.) Marechal e Baudet
Rubiaceae	Vassourinha de botão	*Spermacoce verticilata* L.
Cucurbitaceae	Melão de São Caetano	*Momordica charantia*
Fabaceae	Mata pasto	*Senna uniflora (Mill.) H.S. Irwin & Barneb*
Malvaceae	Malva branca	*Waltheria macropoda Turez*
Amaranthaceae	Bredo	*Amaranthus viridis*
Poaceae	Graminha	*Paspalum Platycaulon*
Verbenaceae	Chumbinho	*Lantana camara* L
Malpighiaceae	Lava prato	*Mapighia* L.
Fabaceae	Estilosante	*Stylosanthes* sp
Malvaceae	Malva roxa	*Malva sylvestris*
Fabaceae	Aleluia	*Senna bicapsularis*
Araceae	Banana de macaco	*Mostera deliciosa*
Mimosideae	Malícia roxa	*Mimosa pudica*
Asteraceas	Fura capa	*Bidens cynapifolia*
Zengiberiaceae	Jengibre	*Zingiber officinale* (Wild.) Roscoe.
Cucurbitaceae	Maxixe	*Cucumis anguria* L.
Poaceae	Coloniao	*Panium maximum* Jacq.
Poaceae	Capim corrente	*Urochloa mosambicensis* (Hack.) Dandy
Shrub or Semi-shrub Stratum		
Cactaceae	Mandacaru	*Cereus jamacaru*. DC.
Solanaceae	Jurubeba	*Solanum paniculatum*
Fabaceae	Anil	*Indigofera microcarpa*
Fabaceae	Calopogônio	*Calapogonium mucunoides*
Rosaceae	Espinheiro	*Crataegus monogyna* Jacq.
Salicaceae	Caubim	*Salix* spp
Fabaceae	Pata de vaca	*Bauhinia variegata*
Flacourtiaceae	Limãozinho	*Caesearia mariquitensis*
Bigorneaceae	Jacarandá	*Jacaranda mimosifolia* D. Don.

Rosaceae	Marmeleiro	*Croton sonderianus* Müll. Arg.
Urticaceae	Embaúba	*Cecropia angustifolia*
Arecaceae	Macaíba	*Acrocomia aculeata*
Rubiaceae	Vassourinha de botao	*Spermacoce verticillata* L.
Anacardiaceae	Caju	*Anacardium occidentale* L.

Source: The Plant List taxonomic database (www.theplantlist.org).

Plant families with the largest numbers of species were: Fabaceae [*Senna bicapsularis; Senna uniflora (Mill.) H.S. Irwin & Barneb; Macropitilium martii* (Benth.) Marechal e Baudet; *Indigofera microcarpa; Calapogonium mucunoides*]; Poaceae [*Panium maximum* Jacq.; *Urochloa mosambicensis* (Hack.) Dandy; *Paspalum platycaulon; Aristida jubata* (Arechav.) Herter] and Rosaceae [*Croton sonderianus* Müll. Arg. And *Crataegus monogyna* Jacq.] representing 85 percent of the families found in this study for SPS-Systems (Fig. 9 A, B, C e D). In SM there were greater presence of *Indigofera microcarpa* and *Crataegus monogyna* Jacq, representing 90% of total of weeds (Figure 10).

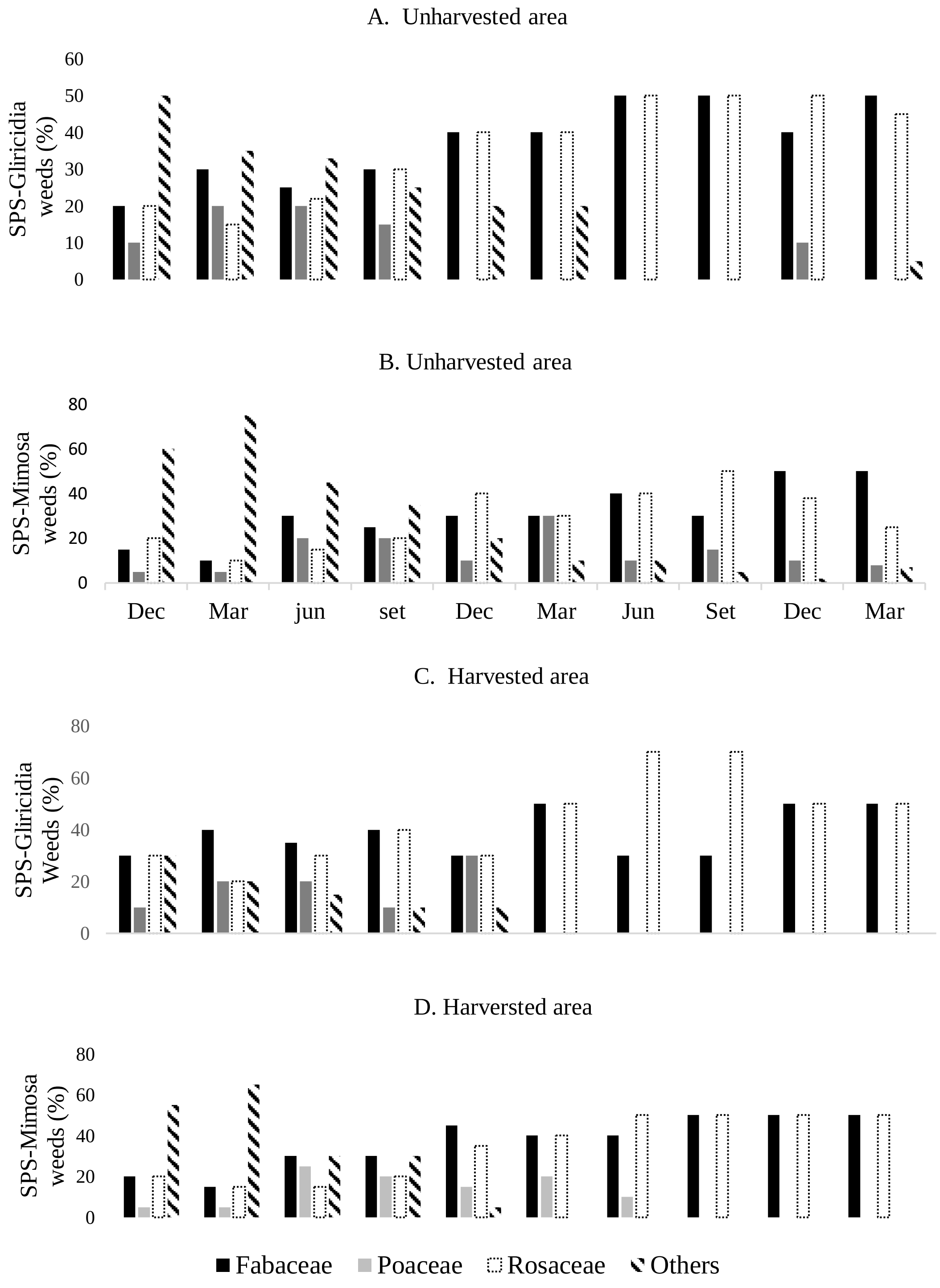


Figure 9. Weed percentage in unharvested and harvested area in silvopasture systems during ten evaluations.

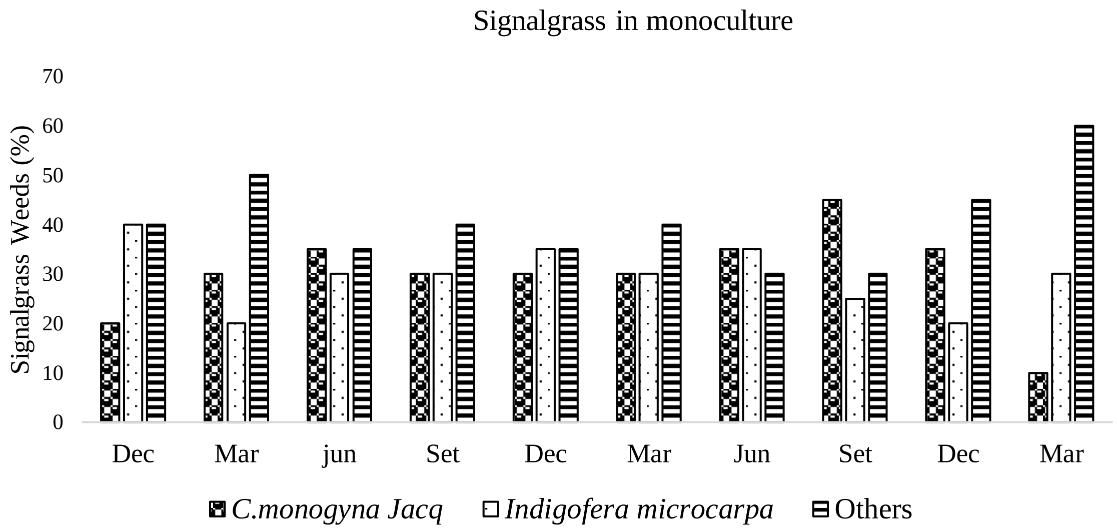


Figure 10. Weeds percentage in Signalgrass monoculture during ten evaluations.

4. Discussion

4.1 Total and Green Herbage Mass

Herbage mass is a net result of processes such as plant growth, senescence, and grazing. Some of these processes might benefit from shade in the occurrence of water deficit, due to the reduction of the evapotranspiration and conservation of the soil moisture (Abraham et al., 2014). However, this effect was not observed in the SPS-Mimosa in this trial as showed in previous experiments in the same area (Mello et al., 2014; Costa et al., 2016; Lima et al., 2020). The SPS-Gliricidia, however, had greater total herbage mass, likely due to lesser competition from water from gliricidia compared with mimosa, which provides more shade and less amount of forage under the crown.

There are several arrangement possibilities (tree species and planting density), which promote contrasting understory microclimate (Karvatte et al., 2016) and may affect pasture and livestock production. Although the drier months have greater forage mass, most of this material was not green forage. According to Santos et al. (2016), there was a reduction in cumulative dry mass and in herbage accumulation rate of *Brachiaria brizantha* cv. BRS Piatã in a silvopastoral system with *Eucalyptus grandis* × *E. urophylla* during the rainy season, with the grass suffering greater interference from trees during this season.

The one-row harvest management provided more space and light, which allowed the greater growth of signalgrass in most evaluations. Shading above 40-50% can affect the production of most forage grasses (Pandey et al., 2011), which was confirmed in this study by the lower herbage accumulation under double-row treatments, compared to one-row. Although signalgrass presents morphological and physiological adjustments as a strategy for shade tolerance (Guenni et al., 2008), in this trial such mechanisms were not enough to provide the same pasture productivity under denser tree canopy. At the same area of this trial, Lima (2019) reported greater forage production under full sun (3193 kg DM ha^{-1}) compared with the one that underwent excessive shade exposure (1502 kg ha^{-1}) (P = 0.0006).

For both treatments, green forage mass correlated well with the rainfall in the area, showing greater productivity during the rainy season and reduced productivity during the dry season. This response was also reported by Cavalcanti Filho et al. (2008) who evaluated signalgrass pastures in the same region of this experiment and observed that herbage mass decreased during the experimental period from 5625 kg DM ha^{-1} in May to 3701 kg DM ha^{-1} in November, according to the rainiest and least rainy season, respectively.

4.2 Proportions of green (leaf blade and stem) and senescent material (leaf blade and stem)

Signalgrass growing in SPS-Gliricidia had better nutritive value than the one growing in the SPS-Mimosa, likely because of its greater proportion of green material, even in the months with lower rainfall, indicating a better association between signalgrass and gliricidia. Trees can increase soil quality and water retention as well as carbon content in the soil (Haile et al., 2010; George et al., 2013). Gargaglione et al. (2014) observed that *Nothofagus antarctica*, despite not being a nitrogen-fixing tree legume, enhanced N uptake by grasses due to improved environmental conditions such as water availability, in addition to reduced competition for inorganic N between soil microorganisms and plants.

In SPS-Gliricidia, the signalgrass had taller tillers likely to support their greater weight. Furthermore, in these areas of the pasture, it was possible that there was a competition for light among tillers and, as a consequence, the stem lengthened, as a way of exposing the younger leaves in the upper part of the canopy where the light was more abundant, a mechanism suggested by Silva and Corsi (2003). These arguments justify or increase the mass of green stem in the areas where the forage was tallest.

4.3 Crude protein of green leaf blade and green stem

Competition between trees and grasses in the SPS-Mimosa contributed to the lowest values of CP in signalgrass leaves and stems. Signalgrass, however, might have benefited from the N fixed by the tree legumes present in the SPS-Gliricidia, resulting in greater CP. Cotrufo et al. (2013) reported that more than 50% of N in companion species might derive from N-fixing legumes. Furthermore, the greater proportion of green material in the double-row management contributed with this result, because the shade improved forage nutritive value. The effect of moderate and dense shade on the quality and nutritive value of 22 forages, including 16 grass species and 6 legumes was evaluated by Pang et al. (2019) who showed that most grass and legume forages had quality equivalent or greater when grown in silvopasture compared to monoculture.

4.4 Herbage Accumulation Rate

Herbage accumulation rate was not influenced by harvesting management, opposite to results found by Lopes et al. (2017) who showed differences between herbage accumulation of signalgrass growing under full sun (3150 kg ha^{-1}) and under shade (1280 kg ha^{-1}). Herbage accumulation was greater in the rainiest months for both treatments, with growth changing seasonally, slowing down during the dry season. Herbage accumulation rate for signalgrass growing in SPS-Mimosa followed the same behavior of previous trials at this same experimental area, as reported by Santos et al. (2019) who found lower herbage accumulation rate in December (15 kg DM $ha^{-1}day^{-1}$), and greater in June (56 kg DM $ha^{-1}day^{-1}$).

4.5 Canopy Bulk Density – CBD and Canopy Height

Greater CBD observed in the SPS-Mimosa might have occurred due to lower canopy height measured with a ruler when compared with greater values of canopy height for SPS-Gliricidia. In general, the canopy bulk density decreases with the height of the plants in the pasture. These results corroborate reports by Molan (2004) that the CBD decreases with the increase in the average height of the *Urochloa brizantha* cv. marandu under continuous stocking.

4.6 Bare soil and litter percentage

These results for litter and soil cover are probably due to water competition among other species within SPS. Such a result may be associated with plants losing their leaves to reduce respiratory losses as an adaptation mechanism (Silva et al. 2001). In this sense, Santos (2007) also found more litter in the dry period of Caatinga, in Sertânia-PE.

4.8 Botanical composition

There were differences in the percentage of weeds between treatments throughout the evaluations (P<0.05). The weed percentage across time was lower in the harvested area. The most important species in density corresponded to *Indigofera microcarpa* (anil), *Senna uniflora* (mata pasto), *Cratoegeus monogymia* (espinheiro), *Calopogonium mucunoides* (Calopogônio), *Waltheria macropoda* Turez (malva branca), *Panicum maximum* (colonião), *Salanum paniculatum* (jurubeba), and *Acrocomia aculeata* (macaíba). All these weeds represented 85% of all weed species.

One alternative to decrease weeds in pasture is integrated management of weed plants in pastures using cultural control (Fontes et al., 2001), which takes advantage of the characteristics of the crop and its cultivation system to increase the plant competitive capacity against invasive species. Performing a survey of weeds in a agroforestry system, Sousa et al. (2008) used a 50 x 50 cm^2 quadrat to collect six points per plot. They identified 55 species, distributed in 23 botanical families. Some invasive control methods were described Machado et al. (2014) as wider planting spacing and some forest species's canopy favor the passage of solar radiation. These weeds can be mechanically controlled, since most of them are shrubby dicots.

5. Conclusions

Signalgrass grew faster and had better nutritive value when growing under gliricidia compared with mimosa silvopasture systems. Competition of mimosa with signalgrass, mostly for water, as indicated by the soil moisture data, is the likely explanation for changes in growth and quality of the grass. The competition between *Mimosa caesalpiniifolia* Benth and the herbaceous vegetation reduced not only *U. decumbens* biomass, but also increased weeds presence. Trees also kept soil moisture better compared with grass strips fully exposed to sunlight. Because of lesser competition, signalgrass pastures had greater proportion of green herbage mass and greater crude protein concentration in green leaf blades. Combination of faster growth and better nutritive value of signalgrass in the SPS-Gliricidia indicates that this system is more recommended if livestock production is the major goal of the operation. Mimosa does produce other products, such as timber and firewood that might become an important source of revenue; however, livestock production is reduced. These aspects might be considered when deciding which system to adopt.

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CHAPTER IV

Nutritive value and condensed tannins of tree legumes in silvopasture systems

Abstract: Condensed tannins (CT) have economic, ecological, social and nutritional potential, but there is scarce information about the effect of external factors on their concentration in plants. The objective of this 2-year study was to sample tree legume leaves from gliricidia [*Gliricidia sepium* (Jacq.) Kunth ex Walp] and mimosa [*Mimosa caesalpiniifolia* Benth] to assess the seasonal variation on CT, nutritive value, and isotopic $\delta^{13}C$ and $\delta^{15}N$ composition. The research was carried out at Itambé Experimental Station located in the sub-humid tropical region of Brazil. Crude protein (CP) and *in vitro* digestible organic matter (IVDOM) differed ($P \leq 0.05$) between legume leaves and varied along the year. In general, CT concentration was greater during the rainy season and in mimosa leaves. Mimosa CT concentrations in the dry and rainy season were 106 and 160 mg/g DM, respectively, while gliricidia CT concentrations were 20 and 48 mg/g DM for the same seasons, respectively. Gliricidia CP ranged from 24 to 28 g/kg DM and Mimosa CP from 16 to 22 g/kg DM. There was a treatment × season interaction affecting IVDOM during the dry period, when they also, had the lowest CT concentrations. Gliricidia had, on average, 16% greater in IVDOM ($P \leq 0.05$) when compared with mimosa. There were differences in foliar $\delta^{15}N$ between tree legume leaves, with an average of 0.58 to 0.66 ‰ for gliricidia and 0.03 to 0.62 ‰ for mimosa. Gliricidia was more depleted of $\delta^{13}C$ (-30.6 ‰) compared with mimosa (-29.8 ‰), with fluctuations along the seasons, with some enrichment occurring during the dry season. Soil moisture was lower at the mimosa sites (16.2%) compared with the gliricidia ones (17.2%), and it was greater between the rows (21.9%) compared with full sun (11.5%), varying across the season. The complete role of CT in the grassland ecosystem require further investigation. From the forage perspective, mimosa had greater CT than gliricidia, reducing its nutritive value, but it was alleviated during the dry season due to reduction in CT concentration.

Keywords: digestibility, moisture, secondary compounds, stable isotopes

Resumo: Taninos condensados (CT) possuem potencial econômico, ecológico, social e nutricional, mas existem poucas informações sobre o efeito de fatores externos sobre sua concentração nas plantas. O objetivo deste estudo de dois anos analizar folhas de leguminosas arbóreas de Gliricidia [*Gliricidia sepium* (Jacq.) Kunth ex Walp] e Mimosa [*Mimosa caesalpiniifolia* Benth] para avaliar a variação sazonal na TC, valor nutritivo e composição isotópica ($\delta^{13}C$ e $\delta^{15}N$). A pesquisa foi realizada na Estação Experimental de Itambé, localizada na região tropical sub-úmida do Brasil. A proteína bruta (PB) e a digestibilidade da matéria orgânica *in vitro* (DIVMO) diferiram ($P \leq 0{,}05$) entre as folhas das leguminosas e variaram ao longo do ano. Em geral, a concentração de TC foi maior durante a estação chuvosa e nas folhas de Mimosa. As concentrações de TC da mimosa na estação seca e chuvosa foram de 106 e 160 mg / g MS, e as concentrações de Gliricidia TC foram de 20 e 48 mg / g MS nas mesmas estações, respectivamente. Gliricidia CP variou de 24 a 28 g / kg MS e Mimosa CP de 16 a 22 g / kg MS. Houve uma interação tratamento × estação que afetou o DIVMO durante o período seco, quando também apresentaram as menores concentrações de TC. Gliricidia teve maior DIVMO ($p \leq 0{,}05$) em comparação com Mimosa. Houve diferenças significativas no $\delta^{15}N$ foliar entre as folhas das leguminosas arbóreas, com média de 0,58 a 0,66 ‰ para Gliricidia e 0,03 a 0,62 ‰ para Mimosa. A gliricídia apresentou maior $\delta^{13}C$ (-30,6 ‰) em comparação com Mimosa (-29,8 ‰), com flutuações de $\delta^{13}C$ ao longo das estações, com algum enriquecimento ocorrendo durante a estação seca. A umidade do solo foi menor nos locais de Mimosa (16,2%) em comparação com os de Gliricidia (17,2%), e foi maior nas entrelinhas (21,9%) em relação ao sol (11,5%), variando ao longo da estação. O papel completo da CT no ecossistema de prados exige uma investigação mais aprofundada. Do ponto de vista forrageiro, Mimosa apresentou maior TC que Gliricidia, reduzindo seu valor nutritivo, mas foi aliviado durante a estação seca devido à redução na concentração de TC.

Palavras-chave: digestibilidade, umidade, compostos secundários, isótopos estáveis

1.0 Introduction

Rapid technological advancement and globalization of agriculture markets demand efficiency in the production systems to stay competitive. Sustainable livestock systems require alternative feed options to reduce costs and enhance delivery of ecosystem services (ES).

Shortage of quality forage and feed resources is a major limitation for ruminant production in developing countries. Integrating tree legumes into grasslands might be a viable option for increasing production per area. Benefits include biological N_2 fixation (BNF), increase protein supply for livestock, and provision of shade (Muir et al., 2014). Deep-rooted-tree legumes can also serve as a fodder reserve for the dry season or droughty years, besides supplying N to the pasture ecosystem via N recycling of litter and cattle excreta, thereby decreasing the dependence on expensive fertilizers, in comparison to grass monoculture (Nepomuceno et al., 2018; Dubeux et al., 2017; Lira et al., 2019).

Plant secondary compounds have ecological roles in grasslands ecosystems. Among the secondary compounds, condensed tannins (CT) are known for their role in ruminant and non-ruminant nutrition, bypassing protein in the rumen, and reducing parasite infestation (Min and Hart, 2003; Williams et al., 2014). Condensed tannins are complex polyphenolic compounds made of oligomers or polymers of polyhydroxyflavan-3-ol that are linked through carbon bonds between flavanol subunits (Santos and Scalbert, 2000) and have great economic and ecological interest.

Condensed tannins are widely found in dicotyledons, especially warm-season or tropical legumes and browse plants (Muir et al., 2009) and are produced by plants for protection against pathogens and herbivores (Aerts et al., 1999). The phenolic hydroxyl groups of CT can bind to proteins, metal ions, and polysaccharides (Schofield et al., 2001). Because of their ability to bind to these nutrients, CT are usually considered anti-nutritional factors for ruminants when exceeding 6% of DM by reducing DMI, protein, phosphorus, and fiber digestibility, and overall animal performance (Mcallister et al., 2005; Pagán-Riestra et al., 2010).

The biological activities of condensed tannins, are intrinsically related to a combination of factors, including molecular weight, stereochemistry, hydroxylation and functional groups contained in polyphenolic compounds (Naumann et al., 2013). In addition, they are related to their ability to complex proteins, lipids and carbohydrates, based on which they become structured depending on the pH of the environment (Smith et al., 2005)

Forage CTs are grouped into three fractions: CT bound to protein, CT bound to fiber and extractable CT (Wolf et al., 2008), according to the methodology of Terril et al., (1992). The CT concentration is dependent on several factors, including biotic and abiotic stresses (Veteli et al., 2007), the age and anatomical origin of the plant tissue, and the variation between both, but also within each plant species. The relative abundance of these three fractions can affect CT biological activity.

Potential benefits of CT include improved livestock gain (Hu et al., 2006), wool production (Panzella et al., 2019), reproductive efficiency and reduced gastrointestinal parasitism, N pollution, and CH_4 emissions (Waghorn, 2008; Klevenhusen et al., 2011). Methods for CT determination have been discussed by Terrill et al. (1992) and Wolfe et al. (2008), recommending the use of self-standards to determine relative concentrations.

The concentration of CT is a highly plastic trait; it varies with plant genotype, tissue developmental stages and environmental conditions, tree position within the stand (edge vs. interior), tree size, and time of year (Dettlaf et al., 2018). The mechanisms of action by which CT reduce CH_4 production include the indirect effect of reducing fiber fermentation in the rumen, thus decreasing acetate and H_2 formation, and the direct effect of inhibiting the growth of methanogens (Carulla et al., 2005; Tavendale et al., 2005).The suppressing of fiber fermentation might be caused by reducing cellulolytic bacteria (Barry et al., 1986; Bhatta et al., 2002; Mcsweeney et al., 2001), formation of complexes between CT and cellulose (Makkar et al., 1995), or possible impairment of microbial attachment by CT (Bento et al., 2005).

The dynamics of these complexes, however, are greatly affected by CT molecular weights which can vary from 500 to greater than 60,000 Daltons (Santos-Buelga and Scalbert, 2000; Sarni-Manchado et al., 1999). The heavier the CT, the more reactive it is (Huang et al., 2010) which affects astringency to ruminants (Sarni-Manchado et al., 1999) and binding strength to fibers (Mcallister et al., 2005). Because molecular weights of CT are specific to plant species (Huang et al., 2010; Mcallister et al., 2005), source of CT as well as percentage in the diet should be considered when feeding them to ruminants. As a result, in vitro studies have shown a weak negative correlation between CT concentration and rumen gas production when making interspecific comparisons (Abdulrazak et al., 2000) but not when comparing intraspecific concentrations (Animut et al., 2008).

Phytogenic substances such as plants, plant parts or extracts of secondary plant compounds (e.g. tannins, saponins, organosulfur compounds, essential oils) have gained attention in animal nutrition. Major uses include the replacement of antibiotics and other feed additives such as copper and zinc to reduce CH4 emissions.

Some studies have dealt with the antinutritional aspect of cultivars with high tannin content and their resistance to pests (Rodrigues et al., 1992; Magalhaes e Rodriguez, 1997). Other studies assessed the variation in CT concentration in species of economic interest (Teixeira, 1990; Caldeira, et al., 1998). Seasonality was pointed as an interesting factor for some species. Collections performed with a difference of one year showed quantitative variation between tannins in Quercus species (Simon et al., 1999).

Many substances have been examined using ruminal fluid *in vitro* and for only a few hours or days (Garcia-Gonzales et al., 2008; 2010; Wallace et al., 2008) and some have been examined *in vivo* (Kamra et al., 2008). A range of additional ecological functions at both the organismic and ecosystem level have been proposed. These include the role of CT as antioxidants, mediators of nutrient availability in soils, and regulating factors of litter decomposition. In addition, as Zucker (1983) pointed out more than two decades ago, that the chemical structure of CT suggests there is tremendous scope for specific chemical interactions of CT both within organisms and in ecosystems. This view of multiple ecological roles for CT is now widely accepted but data that would allow assembling a clear overall picture of CT function are still limited.

There is evidence, moreover, that CT interact with microbial decomposers (Kraus et al. 2003), indicating that there is significant potential for them to affect litter decomposition in both terrestrial (Horner et al. 1988) and aquatic environments (Stout 1989, Ostrofsky 1993, Campbell & Fuchshuber 1995). Tannin concentration thus could be an important indicator of chemical litter quality when addressing a variety of ecological questions relating to litter use and turnover.

We hypothesized that forage nutritive value and CT vary along the year, affecting *in vitro* digestible organic matter, but this variation differs among tree legume species. Another hypothesis is that there are greater concentrations of CT in leaves during the rainy season, since during the dry season the production of secondary compounds is limited by reduced photosynthetic activity. However, there is also a strong body of work that indicates that stress, including heat and drought, increase CT concentration (Kaplan et al., 2008)

1.0 Material and Methods

2.1 Local and climate

The study was carried out at the Experimental Station of Itambé (7°23’S and 35° 10’W and 190 m above sea), Agronomic Institute of Pernambuco-IPA. The soils of the region are classified as RED-YELLOW TB UTISOLS (Santos et al., 2018). Average annual rainfall is 1200 mm and annual average temperature is 25°C. The relative annual air humidity is 80% and the local climate is defined as As’ warm-humid rainy tropical with dry summer. The monthly rainfall during experimental period can be observed in Figure 1.

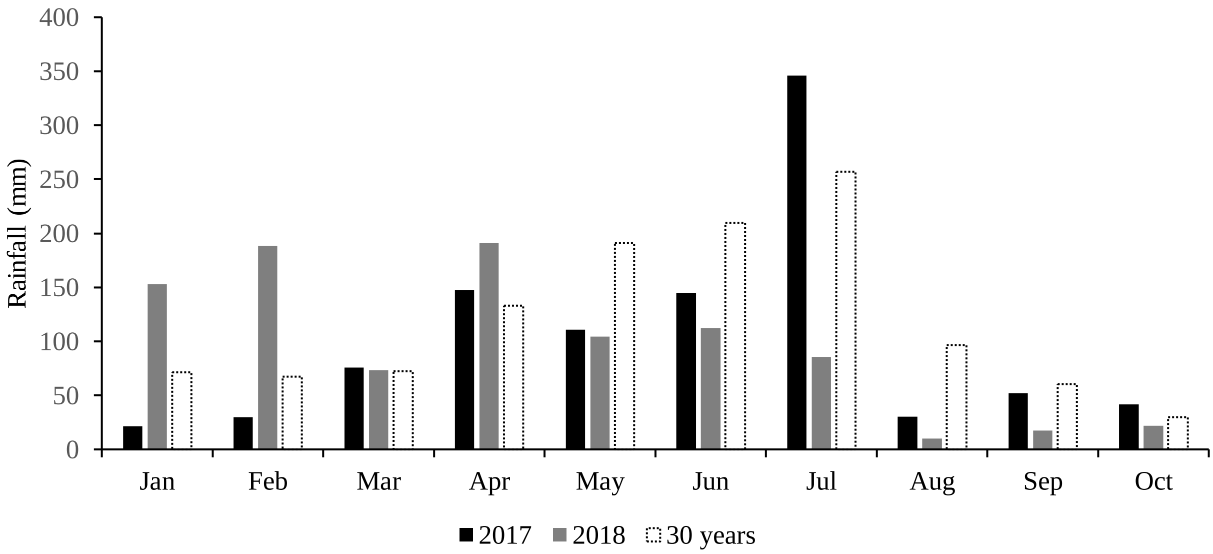

Figure 1. Rainfall during the experimental period in Itambe-PE, Brazil. Source: Pernambuco State Agency for water and climate (Agritempo, 2019)

2.2 Treatments, experimental design, establishment, and management

Treatments consisted of two silvopasture systems: 1. signalgrass (*Urochloa decumbens* Stapf) + mimosa [*Mimosa caesalpiniifolia* Benth]; 2. signalgrass + gliricidia [*Gliricidia sepium* (Jacq.) Kunth ex. Walp]. These systems were arranged in a randomized complete block design, with three blocks. Each experimental unit (paddock) had 1 ha and 14 double rows of tree legumes.

In July 2011, tree legume seedlings were planted in 14 double rows in 1-ha paddocks (experimental unit), and tree population was 2500 trees ha^{-1}. Signalgrass was previously established in one of the blocks in 1969 (Lira et al., 1995). In the other two blocks, signalgrass (cultivar Basilisk) was established along with the tree legumes, between the double rows. Briefly, the establishment of signalgrass occurred in open pits (about 5 cm deep), spaced 1.0 x 0,5 m; seeds were placed manually (10 kg of commercial seed ha^{-1} with 40% cultural value). Pastures were fully established by the end of the rainy season in 2011.

Legume seed were planted in a greenhouse and inoculated with specific *Bradyrhizobium* strains obtained from the soil Microbiology Laboratory at the Federal Rural University of Pernambuco (UFRPE). All paddocks were fertilized in July 2011 with 44 kg P ha^{-1} (as ordinary superphosphate) and 100 kg K ha^{-1} (as potassium chloride) on the entire area. Legume seedlings were transplanted to the field in June 2011 with approximately 30-cm height and planted in 20-cm deep furrows.

In 2017, a minimum of two crossbred Holstein x Zebu (193±70 kg) steers were used to graze under continuous stocking with variable stocking rate. The stocking rate was calculated based on the herbage allowance, using green herbage mass and the metabolic weight of the tester and "put and take" animals (Del Claro et al., 2012). Cattle were weighed every 28 d after a 12-h fasting period. The first group of animals started grazing this area in February 2017 to January 2018 and, the second group of animals grazed from June 2018 to May 2019.

2.3 Response variables

Leaves from both legume species were collected monthly from January to October 2017 and from January to October 2018. Samples were analyzed for CP, $\delta^{13}C$, $\delta^{15}N$ at the University of Florida Forage Laboratory - North Florida Research and Education Center (NFREC), located in Marianna, Florida. The samples were dried in circulation oven at a temperature of 55 ° C to constant weight. After drying, samples were ground to pass a 1-mm screen using a Wiley Mill

(Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Sub-samples were taken and ball-milled in a Mixer Mill MM 400 (Retsch, Newton, PA, USA) at 25 Hz for 9 min. Ball-milled samples were analyzed for total N and $\delta^{15}N$ using a CHNS analyzer and the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK). Crude protein concentration was estimated by multiplying the total N by 6.25 (Azevedo et al., 2014).

Samples ground through a 1 mm screen prior to determining IVDOM using the methodology described by Moore and Mott (1974), a modification of the original procedure developed by Tilley and Terry (1963), including an ashing step to remove any contamination/contribution from soil. Briefly, ruminal fluid was collected from two steers. Samples (0.7000 ± 0.0009 g) were placed into pre-labeled 100-mL plastic centrifuge tubes (Nalgene), with recorded weight. The calculation of IVDOM was:

IVDOM (%) = substrate OM incubated – (substrate OM remains – blank OM remains) x 100

2.4 Sample collection and preparation for CT analysis

Leaves from three tree legumes in each paddock were collected every 28 d from the sample plant. The criterion for choosing the tree was the diameter at breast height (DBH) greater than 30 cm and the average Lorey height (hL) of 6 m. Approximately 300 g of plant material was collected.

Standards for CT determination were prepared from the plant material of each species, according to Wolfe et al. (2008). Thus, the values presented are based on the true concentrations of CT. The CT analyzes in individual plants were defined according to methodology described by Hagermann & Butler (1978). Briefly, we used 10-mg aliquot sample in duplicate to extract the soluble CT using a mixture of 2.5 mL of 70% aqueous acetone with 0.1% ascorbic acid and

2.5 mL of diethyl ether. After removing the solvents by evaporation, the extract was adjusted to 5 ml with distilled water, centrifuged and separated from the solid residue. Then, 1.8 mL of 5% butanol-HCL were added in 0.3-mL aliquots of the extract and taken to a water bath at 95 °C for 70 minutes.

In both cases, the absorbance was read on a 550 nm spectrophotometer (Fento 600-Plus) and the result converted to percentage CT in legumes, based on the regression equation of the standards used from the legume CT. Total CT concentration was soluble fractions and CT bound to the residue (Mupangwa et al., 2000). The standard curve was determined using the Butanol-HCL method, using purified CT from each legume species. The CT concentration was calculated based on the standard curve of the legumes.

In order to assess the potential ecological interaction affecting CT in tree legumes, we determined the soil moisture. Soil moisture was calculated by the difference between wet (m_1) and dry (m_2) masses, divided by dry mass and multiplied by 100 (Klein, 2004), using the following formula: m1-m2 / m2 * 100. Soil samples were collected from 0 to 20-cm soil layers, at two sites, i.e. between tree legume rows and at full sun (i.e middle of the grass strip). Samples were weighed and placed in a greenhouse at 105 °C for 24 h.

2.6 Soil moisture

Soil moisture was calculated by the difference between wet (m_1) and dry (m_2) masses, divided by dry mass and multiplied by 100 (Klein, 2004). Soil samples were collected from 0 to 20-cm soil layers, at two sites, i.e. between tree legume rows and at full sun (i.e middle of the grass strip), every 56 days. Samples were weighed and placed in a greenhouse at 105 °C for 24 h.

2.7 Statistical Analyses

Data were submitted to analyses of variance using the Mixed procedure of the statistical package SAS 9.4 (2012). Fixed effects included silvopastoral systems and evaluation dates.

Block and year were considered random effects. Least square means were compared using PDIFF of the SAS adjusted by Tukey, and statistical differences were considered significant when P ≤ 0.05.

3.0 Results and Discussion

3.1 Condensed tannin concentration

There was a treatment × season effect (P≤0.05, SE=1.9223) on CT concentration. Greater levels occurred in mimosa (133 mg TC/g) when compared to gliricidia (32 mg TC/g) in all evaluation dates. During the rainy season, there were greater CT concentrations for both species, 161 mg CT/g for mimosa in July and 45 mg CT/g for gliricidia in June (Figure 2).

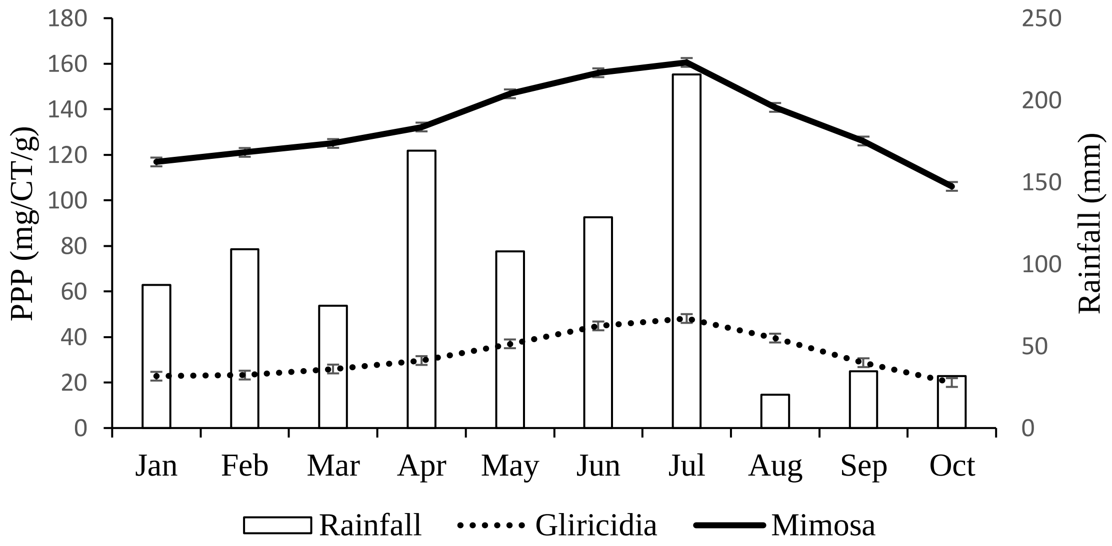

Figure 2. Condensed tannin concentration in gliricidia and mimosa and average rainfall during 2-yr.

This response/behavior indicates that secondary metabolic compounds in deciduous plants are often limited by lack of photosynthesis (carbon fixation) since most of the deciduous vegetation is leafless in the dry season, with a lower photosynthetic rate, resulting in great leaf CT concentration during the rainy season. Azevedo et al. (2017) indicated that sampling period affects CT concentration in the bark of *Mimosa tenuiflora* (Willd.) Poir.

The litter from tree legumes presents different CT concentrations over time, mainly during the dry season. Condensed tannins can strongly affect litter decomposition, but their fate during the decomposition process, in particular as influenced by detritivore consumption, is not well understood (Coulis et al., 2009).

Tree litter deposition reduces soil water loss and decreases evapotranspiration. In general, low fertility soils results in plants with greater total phenols and CT-concentration compared to more fertile soils (Jacobson et al., 2005; Chini, 2013).

In the current study, lower soil moisture was associated with Mimosa, which in turn had greater leaf CT concentration. This might not have a cause-effect explanation, but the literature correlates biotic and abiotic stress with tannin production (Poyer et al., 2015). Jacobson et al. (2005) studied two species [*Stryphnodendron adstringens* and *S.polyphyllum]* and found greater total phenol and CT concentrations during the rainy season. The same pattern was observed by Azevedo et al. (2017), who indicated that the best time to collect the bark of *M.tenuiflora* for tannin extraction is during the rainy season.

Forages of tropical and temperate climate containing CT were researched for their probability of decreasing CH_4 production. The authors conclude that CTs act both by reducing the number of protozoa and by a direct toxic effect on methanogens (Cieslak et al., 2013). A CT action on ruminal methanogenesis can be attributed to an indirect effect on the reduction in H_2 production, as a consequence of less fiber digestibility, and by a direct inhibitory effect on the methanogenic population (Gemeda; Hassen, 2015).

In a study carried out with 100% substrate of tropical tannin-rich forages (*Stylozobium aterrimum, Stylozobium deeringianum, Leucaena leucocephala* and *Mimosa caesalpiniifolia* Benth), Longo (2007) observed that these vegetables were able to reduce CH_4 production and alter the populations of microorganisms.

3.2 IVDOM

In vitro digestible organic matter concentration followed the same pattern for both species. CT level increased, IVDOM decreased (P≤0.05, SE=1.2358). Average IVDOM was 63% for Gliricidia and 53% for Mimosa (Figure 3).

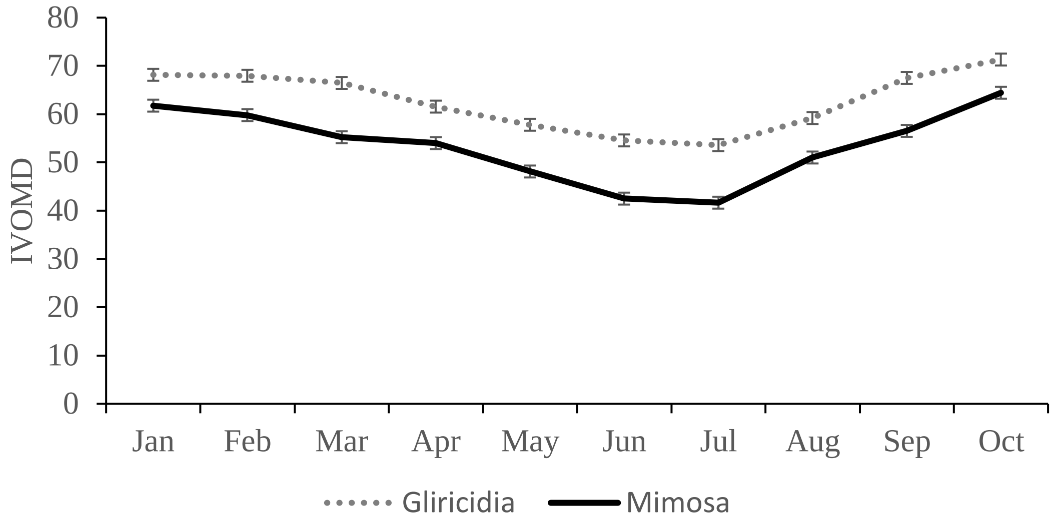

Figure 3. *In vitro* digestible organic matter in gliricidia and mimosa leaves across different evaluation dates.

Lower leaf digestibility in mimosa can possibly be explained by its greater CT and lignin concentrations when compared with Gliricidia (Silva et al., 2013; Apolinário et al., 2015). indicated that lignin compound reducing forage digestibility.

However, in the case of CT influence on leaf digestibility, this is not all directly correlated due to lack of CT biological activity (Kariuki & Norton 2008). Further research in which CT is neutralized with polyethylene glycol prior to IVDMD (Fagundes et al., 2013) can determine whether lower IVDMD is a result of CT interference or simply greater lignin content.

The negative correlation between CT and degradability characteristics suggests a negative role of tannins on fodder digestibility potential (Rubanza et al., 2003). Nevertheless, the presence of moderate levels of CT in the rumen is related to the protection of dietary protein against degradation by ruminal microorganisms, increasing the flux of dietary protein to be absorbed in the intestines (Min et al., 2003). In some legumes, the presence of biologically-active CT in moderate quantities may limit animal nutrition (Grainger et al., 2009) or may contribute to overall animal health (Muir, 2011). Depending on the content present in the plant, these phenolic compounds may increase or decrease palatability (Adams et al., 2013; Provenza et al., 2015).

Condensed tannin in jurema preta [*Mimosa tenuiflora*], sabiá [*Mimosa caesalpiniifolia* Benth] and mororó [*Bauhinia cheilantha*] were studied by Beelen (2002). She observed that the elevated CT concentration negatively affected DM, CP, and NDF ruminal degradation, decrease in consumption, rumen microbial adhesion to forage leaves, as well as reduced enzymatic activity in rumen content of goats.

The IVDOM observed in our study is similar to the one reported by Lima (2019), 430 g kg^{-1} in gliricidia leaves and 213 g kg^{-1} in mimosa leaves at the same location of the current trial. Pereira (2016) found greater leaf digestibility for gliricidia leaves (592 g kg^{-1}) and lower for mimosa leaves (121 g kg^{-1}) and Silva (2011) found grater IVDOM concentration than those found in this research, for both species, being 692 g kg^{-1} for gliricidia leaves and 405 g kg^{-1} for mimosa leaves.

Rumen microbial population is affected by ingested CT, causing inhibition of enzymatic activity due to the emergence of tannin-enzymatic enzymes (Béelen et al. 2006), resulting in loss of digestibility. This enzymatic transformation can lead to the increase of lignin, reducing the action of cellulase on cellulose, resulting in decreased digestibility (Brito et al., 2003). Rumen bacteria penetrate easily into tissues with lower CT content, but this action is inhibited when CT levels are high (Chiquette et al., 1988). Van Soest (1994) stated that both CT and other polyphenols tend to protect cellulose and protein degradation driven by ruminal bacteria.

3.3 Crude protein

There was difference for CP between gliricidia and mimosa's leaves ($P \leq 0.05$, SE=0.5834). The three legumes of this study showed crude protein content above 16%, it was greater in gliricidia (26%) when compared with mimosa (18%), which indicates the potential of these species for use in the feeding of small ruminants (Figure 4). According to Van Soest et al. (1994), protein levels below 7% in the diet can impair ruminal fermentation, and result in negative balance of nitrogen.

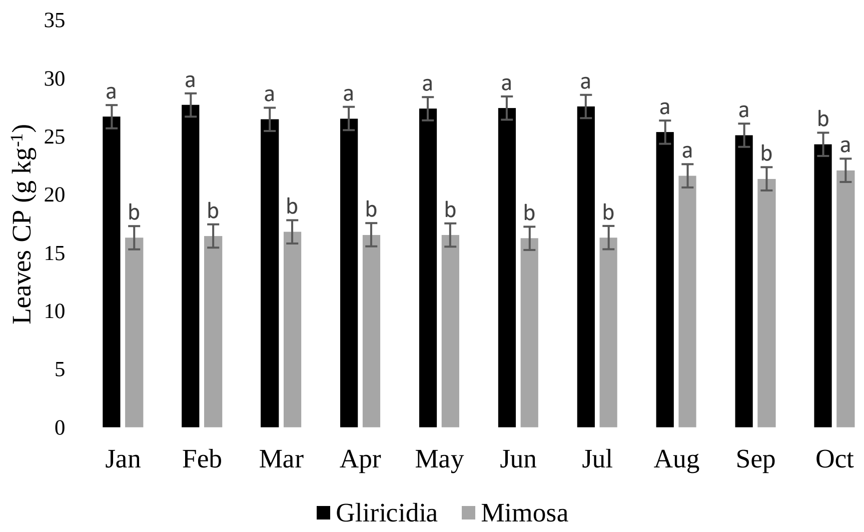

Figure 4. Crude protein in gliricidia and mimosa leaves across different evaluation dates.

3.4 Isotopic composition

Leaf $\delta^{15}N$ varied ($P \leq 0.05$) among tree legume species, evaluation dates, and there was a species × evaluation date interaction. Gliricidia was, in general, more enriched 0.63‰) compared with mimosa (0.24‰), but fluctuations occurred along the year with similar $\delta^{15}N$ occurring in the beginning of the dry season (Figure 5). This fact might be linked with the onset of the dry season, reducing BNF from legumes. A relative enrichment of the leaves can be a consequence of nitrate reductase or glutamate synthetase activity (Yoneyama et al., 1991). The reverse situation may be related to ammonia volatilization, exudation of organic N from the roots (Robinson et al., 1998; Kolb et al., 2002), or association with mycorrhizae colonization (Hogberg et al., 1999).

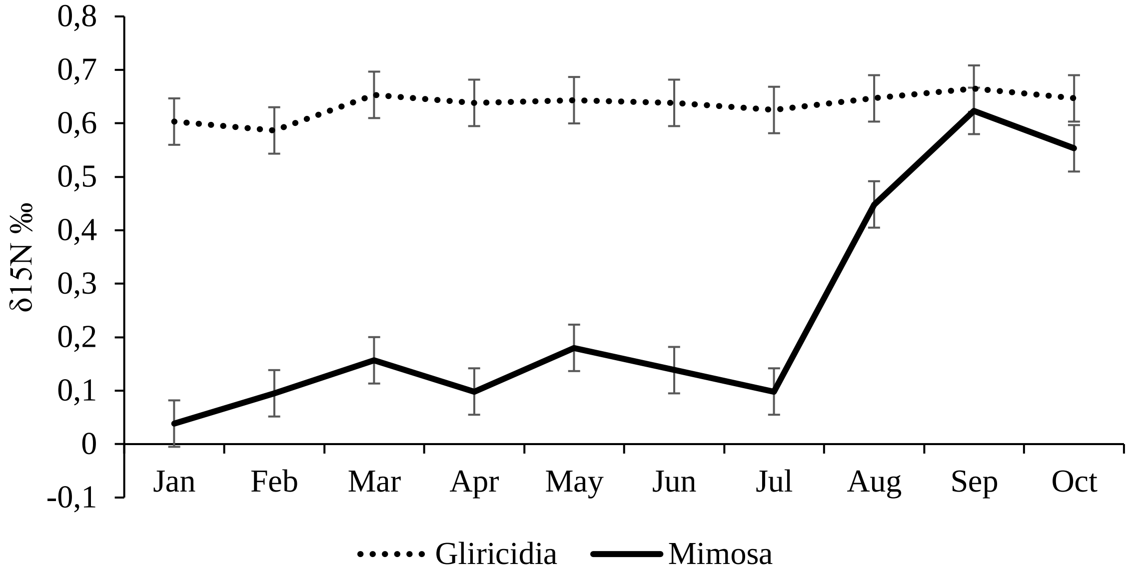

Figure 5. Foliar δ15N across evaluation dates for gliricidia and mimosa. Data averaged across replications and years.

Leaf $\delta^{13}C$ varied with species and evaluation date ($P \leq 0.05$). On average, gliricidia leaf was -30.63 ‰ and mimosa leaf was -29.8 ‰ (Figure 4). Leaf $\delta^{13}C$ was more enriched during the dry season, likely indicating stomatal closure and internal recycling of CO_2. These results are consistent with other reports (Leffler and Evans 1999; Leffler and Enquist, 2001) indicating a negative correlation with annual rainfall versus $\delta^{13}C$ in tropical sites. Consequently, $\delta^{13}C$ is correlated with water content in drier tropical forests.

Gliricidia leaf $\delta^{13}C$ ranged from a low of -31.09 up to −29.43, while mimosa ranged from −30.1‰ to −29.9‰, respectively (Figure 6). Wet tropical ecosystems range from a low of −32.1‰ (Martinelli et al. 1998) to −30.6‰ (Von Fischer and Tieszen 1995). This suggests that leaf $\delta^{13}C$ could be a potential indicator to asses water use efficiency (WUE, the ratio of carbon gained to water lost during gas exchange) (Farquhar et al. 1988). Generally, plants with more enriched $\delta^{13}C$ have greater WUE, while those with low $\delta^{13}C$ have less WUE. Therefore, it can be an indicator of water stress, as was related by Fisher and Toit (2019), who conclude that $\delta^{13}C$ in tree rings has potential to measure water availability and drought stress in *Pinus radiata stands* in South Africa.

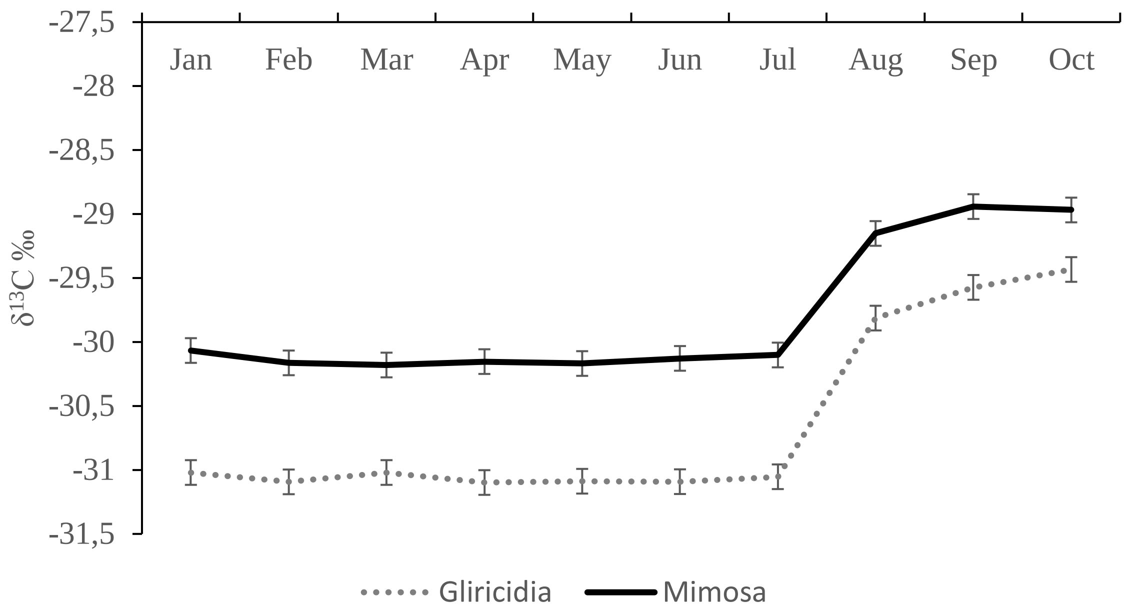

Figure 6. Foliar $\delta^{13}C$ overtime for gliricidia and mimosa

3.5 Soil moisture

Soil moisture was lower in the mimosa pasture compared with gliricidia (Figure 7). There was also an interaction for area management × evaluation × point that affected soil moisture ($P \leq 0.05$). There was no difference in soil moisture between trees when comparing harvested and unharvested area. However, soil moisture under full sun was greater for the unharvested area in two evaluations, and greater for the harvested area in one evaluation with no differences found in two other evaluations (Figure 8) ($P \leq 0.05$).

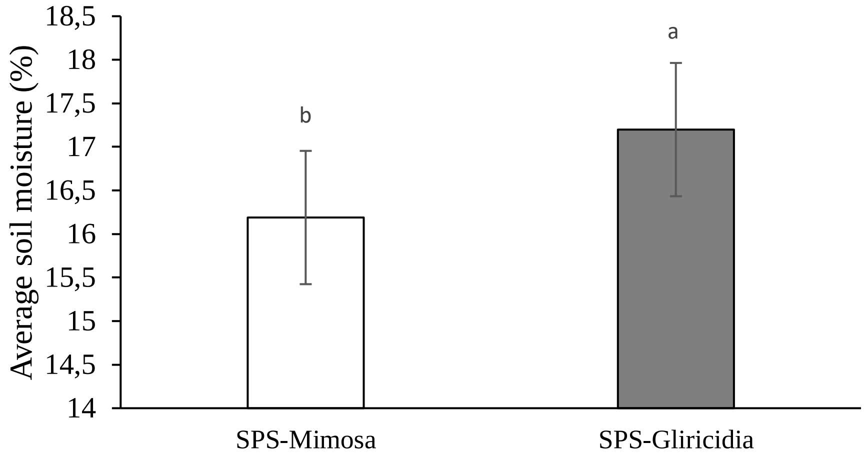

Figure 7. Average soil moisture in mimosa and gliricidia SPS silvopasture (SPS) systems during period of evaluation. Values are averages of different evaluation dates, replications, collection points, and harvesting management.

Similar results were found by Bosi et al. (2019), who evaluated a pasture system of *Piatã palisadegrass* in monoculture and a SPS with Eucalyptus rows. Soil water availability (SWA) until 1-m depth was greater at the inter-row than under the trees, which indicates a faster water uptake by the trees; however, when the inter-row was shaded, SWA was lower in the open pasture than at the inter-row. This occurred because of the shading and windbreak effects on evapotranspiration. Soil water recharge, during rainy days, was faster near the trees, as a result of large water interception by trees and its subsequent deposition into the soil, increasing the amount of SWA at this position.

Soil physical properties in a SPS in the central northern region of Piaui State, Brazil, were different between two SPS (SPS andropogon grass- *Andropogon gayanus* Kunth and SPS mombaça grass - *Panicum maximum*), however both SPS differed from the native forest (Lima et al., 2013). Presence of trees increases ground cover, thereby reducing runoff.

Rainfall was greater in June and March, resulting in greater soil moisture during these periods. Tree might have opened macrospores, increasing infiltration rate, affectting soil moisture under the canopy, in addition to the tree evapotranspiration (Silva and Amaral, 2014).

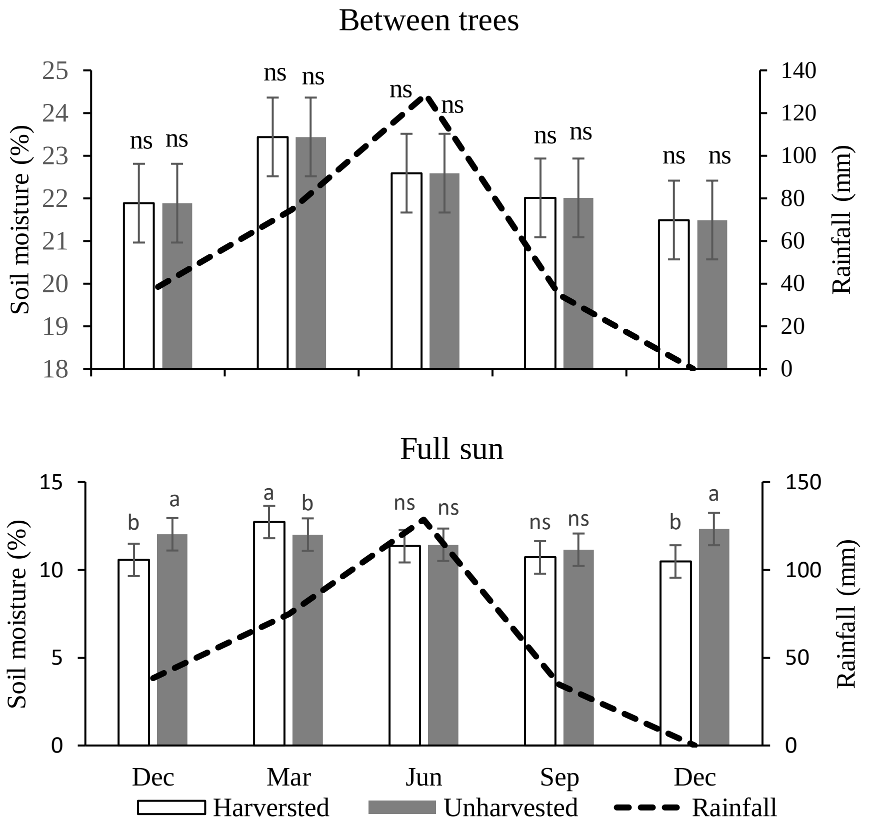

Figure 8. Soil moisture between trees and in full sun for harvested and unharvested area.

4.0 Conclusions

Mimosa leaves had greater condensed tannin and lower IVDOM concentration than gliricidia. Seasonal variation for both CT and IVDOM occurred, with lower CT occurring in the dryer months, and those were the months when leaves had greater IVDOM, likely resulting from a reduction in CT. Whether there was a direct negative biological correlation between CT and IVOMD needs further study because lignin concentration was also negatively correlated to IVOMD.

Seasonal variation occurred for leaf $\delta^{13}C$ and leaf $\delta^{15}N$, with more enriched $\delta^{13}C$ occurring for mimosa and in dryer months, reflecting an increase in water use efficiency and internal C recycling. Mimosa had lower leaf $\delta^{15}N$ for most of the year, except during the dry season when approached the gliricidia values, becoming more enriched likely due to reduction in biological N_2 fixation activities.

Soil moisture was lower under mimosa than under gliricidia canopy, and it varied with sampling point, harvesting management and evaluation date. Lower soil moisture under mimosa reflects the competition of this tree legume species, which might negatively affect herbaceous vegetation in silvopastoral systems. Further studies looking at more compatible grasses for the understory might be necessary to reach the full potential of SPS with mimosa.

FINAL CONSIDERATIONS

The silvopastoral systems promote environmental services and are used as an alternative to diversify and increase food production, in addition to exploring different activities, providing other sources of income for the producer.

In cattle, direct benefit includes animal welfare and thermal comfort and this result improves the image of the livestock business, an excellent marketing opportunity for improving production, as well as, the product and its derivatives. It is also environmentally appropriate, socially beneficial and economically viable products. Cattle can also grazing pastures that integrate tree legumes due to the intake of forage with better nutritional value and, in some cases, the presence of secondary compounds in beneficial amounts that improve protein digestibility (bypassing) and even in the decrease methane to atmosphere, due to the action of these secondary compounds in the rumen.

Soils are also beneficiaries of this system: maintenance or increase of soil organic matter through carbon fixation through photosynthesis and transfer due to the fall of leaves, branches and, rotting of old roots; nitrogen fixation by tree legumes and also by some non-tree legumes; protection of the soil against erosion, making it possible to reduce losses of organic materials and nutrients; nutrient recovery, fixation and recycling of nutrients that may otherwise be lost; by the lower rate of mineralization of organic matter resulting from the presence of shade. However, the complexity of this system is an obstacle to its adoption. More research is needed to find grass and legume species adapted to grow together without high competition for water, light and nutrients.

In this context, one of the most important decisions in establishing silvopastoral systems, is the definition of tree spacing and arrangements. This decision will determine the influence of the solar radiation on the growth of forages from planting to the harvest of the trees. The greater the spacing between the tree lines (rows), the greater the penetration of radiation into the forage substrate, favoring the accumulation of biomass. In countries with tropical climates such as Brazil, a large part of pastures is degraded a few years after establishment. In the current scenario of climate change and the need to increase food security, tree legumes provide a key component for the sustainable intensification of livestock systems in warm-climate regions.

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