Maize-tropical forages intercropping: an evaluation above and below the soil surface

Lucas Freitas Nogueira Souza

Thesis presented to obtain the degree of Doctor in Science. Area: Crop Science

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Maize-tropical forages intercropping: an evaluation above and below the soil surface
versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. JOSÉ LAÉRCIO FAVARIN

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I dedicate this work to my parents, Marisa and João Luiz, who have always loved and supported me.
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“Eia, pois, esalqueanos, sem guerra!
Co’ a bandeira da Escola na mão,
ensinai que plantar nesta terra
é lutar pela grande Nação!”.  

Salvador de Toledo Piza Jr.
RESUMO

Consórcio entre milho e forrageiras tropicais: uma avaliação acima e abaixo da superfície do solo

Os solos tropicais, que cobrem 80% das terras agrícolas do Brasil, são suscetíveis à erosão, levando à perda de biodiversidade e à redução da sequestração de carbono, fertilidade e conteúdo de matéria orgânica. O uso de resíduos vegetais ou cobertura de culturas, particularmente de espécies forrageiras tropicais como Urochloa spp. e Megathyrsus spp., foi identificado como um meio eficaz de prevenir a erosão do solo. Essas espécies, quando consorciadas com o milho, produzem resíduos que se degradaram lentamente, proporcionando uma proteção mais longa ao solo. No entanto, gerenciar a competição entre espécies consorciadas provou ser um desafio. Fatores como condições climáticas, características do solo, taxa de nitrogênio, manejo de herbicidas e padrão de semeadura em consórcio afetam o crescimento e a produtividade do milho e forragem consorciadas. Este estudo teve como objetivo realizar uma metanálise dos dados do consórcio milho-brachiaria, avaliar o desempenho do consórcio de maneira integrada e analisar o crescimento e as características do sistema radicular de sistemas de consórcio e monocultura. Os sistemas de consórcio, demonstraram desempenho superior ao monocultivo em vários aspectos. Em relação ao monocultivo, o consórcio reduziu a produção de grãos de milho em 5,6% e a produção de biomassa da grama Brachiaria em 64%. No entanto, sob condições específicas, como clima subtropical, semeadura de verão e certas taxas de adubação, essas penalidades de rendimento foram minimizadas. Apesar da redução, os sistemas de consórcio mostraram melhor utilização de recursos e produtividade, com 25% a mais de biomassa total na colheita do milho. A eficiência de uso do solo (LER) do consórcio foi consistentemente maior, indicando otimização da utilização de recursos. Os sistemas de consórcio aumentaram significativamente a biomassa, volume e comprimento radicular do sistema, contribuindo para a agregação do solo, proteção contra erosão, ciclagem de nutrientes e fixação profunda de carbono. Os sistemas de consórcio aumentaram em média 92,1% os lucros em comparação com o monocultivo de forrageiras tropicais. Em conclusão, os sistemas de consórcio ofereceram uma estratégia robusta para diversificação e intensificação simultânea da produção, com pequeno ou nenhum comprometimento em relação ao rendimento de grãos de milho. Apesar de algumas penalidades de produção, os sistemas de consórcio, com práticas de manejo específicas e sob certas condições climáticas, podem superar o monocultivo em termos de produção de biomassa, desenvolvimento radicular, utilização de recursos, rentabilidade e sustentabilidade.

Palavras-chave: Consórcio, Zea mays, Urochloa, Panicum
ABSTRACT

Maize-tropical forages intercropping: an evaluation above and below the soil surface

Tropical soils, which cover 80% of Brazil’s agricultural land, are susceptible to erosion, leading to biodiversity loss and reduced carbon sequestration, fertility, and organic matter content. The use of plant residues or crop cover, particularly from tropical forage species like Urochloa spp. and Megathyrsus spp., was identified as an effective means of preventing soil erosion. These species, when intercropped with maize, produces residue that degraded slowly, providing longer soil protection. However, managing the competition among intercropped species proved challenging. Factors such as climatic conditions, soil characteristics, nitrogen rate, herbicide management, and intercropping sowing pattern affected the growth and productivity of intercropped maize and forage. This study aimed to perform a meta-analysis of the maize-brachiaria intercropping data, evaluate the performance of the intercropping in an integrated way, and analyze the growth and characteristics of the root system of intercropping and monoculture systems. Intercropping systems, demonstrated superior performance over monocropping in several aspects. Relative to monocropping, intercropping reduced maize grain yield by 5.6% and Brachiaria grass biomass production by 64%. However, under specific conditions such as subtropical climate, early season sowing, and certain fertilization rates, these yield penalties were minimized. Despite the reduction, intercropping systems showed better resource utilization and productivity, with 25% higher total biomass at maize harvest. The land equivalent ratio (LER) of intercropping was consistently higher, indicating optimized resource utilization. Intercropping systems significantly increased root biomass, volume, and length, contributing to soil aggregation, protection against erosion, nutrient cycling, and deep carbon fixation. Intercropping systems boosted profits by an average of 92.1% compared to tropical forages monocropping. In conclusion, intercropping systems offer a robust strategy for simultaneous diversification and intensification of land use, with minor or no trade-off in maize grain yield, highlighting their potential in sustainable agriculture. Despite some production penalties, intercropping systems, with specific management practices and under certain climate conditions, could outperform monocropping in terms of biomass production, root development, resource utilization, profitability, and sustainability.

Keywords: Intercropping, Zea mays, Urochloa, Panicum
INTRODUCTION

Tropical soils, which cover about 80% of Brazil’s agricultural land, are particularly susceptible to erosion due to the typical characteristics of tropical climates (Alvares et al., 2013). In Brazil, these soils are vulnerable to water erosion. This leads to a loss of biodiversity, a reduction in sequestered carbon, fertility, and organic matter content (Sakuno et al., 2020). The use of plant residues or crop cover has been identified as an effective means of preventing soil erosion (Flumignan et al., 2023).

Due to the climatic conditions of tropical regions, the accumulation of biomass or plant residues on the soil in the tropics is a difficult task to achieve, compared to the subtropical environment. In this environment, the difficulty is not limited to the accumulation of residues in the soil, but extends to their intense degradation, since the temperature throughout the year favors biotic activity (Franzluebbers, 2002).

Tropical forage species, as Urochloa spp. (syn. Brachiaria) and Megathyrsus spp. (syn. Panicum), intercropped with maize produce homogeneous residue, well distributed and with high lignin content and carbon and nitrogen ratio, which provides a slower degradation compared to residues from legume species. Consequently, the longer the residues remain on the surface, the greater the protection of the soil against the erosive process (Cecon et al., 2013; Costa, da et al., 2016; Pereira et al., 2016). In addition, the tropical forage remains in the area after the maize harvest, a dry off-season period which limits plant growth and the implementation of other traditional cover crops (Alvares et al., 2013).

The competition for resources among intercropped species is the main factor to be managed (Vandermeer, 1989). Several factors, including the nitrogen rate, the herbicide management, and the intercropping sowing pattern, affect the competition and growth among intercropped cultures (Almeida et al., 2017; Borghi et al., 2012; Souza et al., 2020). Generally, maize tends to have a competitive advantage over forage species when both are intercropped (Almeida et al., 2018; Oliveira et al., 2018). This occurs especially due to the rapid growth in height of maize in the initial phase of the intercropping, while there is slow development of the forage species until the tillering phase, ensuring corn as the dominant species and the forage as the dominated species during the coexistence of the cultures (Zhang and Li, 2003).

Climatic conditions and soil characteristics are equally decisive to the management factors of the system in determining the intensity of competition among intercropped species (Cagna et al., 2019; Salas Méndez et al., 2019). Unlike maize, which divides photoassimilates
between reproductive drains and roots, forages seem to favor root growth (Gifford et al., 1984), providing greater resistance to drought periods, and greater sequestration of carbon in depth (Baptistella et al., 2020). Therefore, there is a need to know and quantify the development and accumulation of root biomass of maize intercropped with tropical forages, which have a deep and vigorous root system (Baptistella et al., 2020; Huot et al., 2020; Rosolem et al., 2017).

However, there is no consensus in the literature about the effects of the environment and the management of the intercropping on the growth and productivity of intercropped maize and forage. In this context, a meta-analysis of a robust database can guide the understanding of the factors and performance of intercropped production systems (Li et al., 2020; Rodriguez et al., 2020; Yu et al., 2016). Meta-analyses are systematic and quantitative reviews of the literature, which apply statistical treatments for the analysis of the data set obtained (Philibert, Loyce and Makowski, 2012). In the intercropping between maize and tropical forages, grouping the knowledge generated in a meta-analysis would help to consolidate the potential benefits and knowledge gaps in this system.

Thus, the present work aimed to: (i) Perform a meta-analysis of the data from the maize-brachiaria intercropping derived from the literature, to quantify the effect of this system on maize grain yield and brachiaria grass biomass. (ii) Evaluate in an integrated way the performance of the intercropping, to investigate the interaction between maize and tropical forage in intercropping systems, assessing its impact on grain yield, biomass accumulation, biological energy content, land equivalent ratio, and economic analysis. Finally, this work also (iii) analyzes the growth and characteristics of the root system of intercropping and monoculture systems, in order to deeply understand the interactions and potentials of the corn-tropical forages system for maintaining soil quality and intensifying the production system in Brazil.

References


MAIZE-BRACHIARIA GRASS INTERCROPPING: A META-ANALYSIS OF MAJOR PRODUCTIVITY DRIVERS IN BRAZIL

Abstract

The intercropping between cash crops and tropical forages such as maize and brachiaria in Brazil is an important strategy in tropical regions. This method improves soil cover and prevents erosion, contributing to soil conservation. However, the effects of this intercropping on the performance of each individual crop are still not agreed upon. Thus, a meta-analysis was conducted to quantify the effect of intercropping on maize grain yield and Brachiaria grass biomass. The literature search included scientific articles concerning maize-brachiaria intercropping. The database included 429 data points extracted from 56 published manuscripts from all regions of Brazil. Relative to monocropping, intercropping reduced maize grain yield (-5.6%) and Brachiaria grass biomass production (-64%) in all scenarios. The intercropped maize grain yield was less affected under the following conditions: i) in the subtropical climate zone (-3.6%), ii) sowing the intercrop early in the season (-5.8%), iii) applying monocot-selective postemergence herbicides (-4.4%), iv) relay temporal arrangement (-2.8%), and v) with N and P fertilization rates above 150 and 35 kg ha\(^{-1}\), respectively. In relative terms, Brachiaria grass performance was more affected by intercropping adoption, with a reduced biomass penalty under a i) tropical climate (-63.9%), ii) late sowing time for the intercrop (-59.5%), iii) application of N fertilizer at a rate above 50 kg ha\(^{-1}\) (-70.5%), and iv) non-use of postemergence herbicides (-21.9%). Maize-Brachiaria grass intercropping was an effective alternative to produce residue (no-till system adoption) or forage (crop-livestock integrated system adoption) with reduced maize grain yield penalties, diversifying cropping systems in Brazil. Despite the production penalties for both crop species, considering different relative values, specific management practices and climate conditions could minimize yield penalties.
1.1. Introduction

There are several barriers to achieving long-term global food security in agriculture (Godfray et al., 2010; Beddington et al., 2012). Some of the major obstacles are the loss of 35 to 43 billion tons of soil surface annually (Borrelli et al., 2020; 2017) as a result of erosion. Although soil erosion is a recurring global problem, it is often more significant in tropical regions (Lai, 1989; Lal, 1990; Labrière et al., 2015) due to the warm climates and rainy seasons. Brazil encompasses 223 million hectares of arable land area, of which 81% is located in tropical zones and 19% is located in subtropical zones (Alvares et al., 2013). Estimates based on the universal soil loss equation indicate that worldwide, tropical and subtropical agricultural regions lose approximately 847 million tons of soil annually due to erosion (Merten & Minella, 2013; Gomes et al., 2019) and face serious issues related to carbon and nutrient losses. To minimize these problems, no-tillage systems have been adopted (Landers, 2001), which show a potential reduction of soil losses of up to 90% (Thiagalingam et al., 1996; Merten & Minella, 2013; FAO and ITPS, 2015).

The no-till system not only involves minimal soil disturbance but also takes into account crop diversification and leaving crop residues on the soil surface (Derpsch et al., 2014). In Brazil, soybean \( \textit{[Glycine max (L.) Merrill]} \) and maize \( \textit{(Zea mays L.)} \) are the major row crops, and double-cropped soybean–maize systems are commonly adopted (Goldsmith & Montesdeoca, 2018; Battisti et al., 2020). These cropping systems do not provide an adequate quantity or quality of residues for proper soil cover (Gregory, 1982; Kumar, 2000), particularly given the heterogeneity of the residues (Tarkalson et al., 2008; Xu et al., 2019) and their accelerated decomposition rate (Nearing et al., 2004; Ranaivoson et al., 2017). Moreover, soil tillage and low crop biomass inputs are closely related to soil erosion, a contributing factor to Brazil reaching 100 million hectares of pasture under moderate to severe levels of degradation (Feltran-Barbieri and Féres, 2021).

In current agricultural systems, the lack of crop diversity results in yield losses and environmental impacts, especially in tropical areas (Doran, 2002; Archer et al., 2020; Mota et al., 2020; Wang et al., 2020). The inclusion of intercropping (also referred to as multicropping, mixed cropping, or interseeding) systems has been considered a way to grow cover crops (Crusciol et al., 2015; Queiroz et al., 2016; Cagna et al., 2019; Silva et al., 2020). Maize–Brachiaria grass \( \textit{(Urochloa spp.)} \) intercropping is the most commonly adopted intercropping system in Brazilian agriculture. Brachiaria grass is often grown as a pasture in monocropping, but when intercropped with maize, it also serves as a cover crop. Following maize harvest, intercropped Brachiaria grass provides biomass to form a uniform soil cover and/or serve as animal feed (Ceccon et al., 2013; da Costa et al., 2016; Pereira et al., 2016) by remaining in the system or being dried for the sowing of a new crop.

Overall, intercropped maize leads has a competitive advantage over Brachiaria grass during the cogrowth period (Almeida et al., 2018; Oliveira et al., 2018). Maize-Brachiaria grass intercropping
is practiced across a wide range of environments and management practices in Brazil with different levels of positive (facilitation) and negative (competition) interactions, as reported in the scientific literature (Cagna et al., 2019; Méndez et al., 2019).

Over the past years, maize-Brachiaria grass intercropping has become an important alternative in grain and forage systems, but it has been adopted under different management, labor, and field scale levels. Previous studies have assessed the effect of climate and management factors on the overall performance of intercropping systems. However, these studies have not yet quantitatively summarized the effects of these factors on the overall performance of intercropping systems. Therefore, a meta-analysis was executed with the main goal of examining the effects of maize-Brachiaria grass intercropping on cereal grain yield and grass biomass production with studies covering many agricultural landscapes of Brazil and measuring the influence of the main environments and adopted management practices on intercropping.

1.2. Materials and Methods

1.2.1 Literature search

A literature search was conducted to retrieve peer-reviewed studies carried out on maize-Brachiaria grass intercropping under field conditions. Studies published in Portuguese and English were searched in three platforms: Web of Science, Scopus, and Google Scholar. The search equation was composed of the terms maize, Brachiaria, Urochloa, intercropping, intercropped, intercrop, consortium, and the respective Portuguese terms. Studies were scrutinized and included in this meta-analysis when they met the following criteria: (i) analysis of maize grain yield and Brachiaria grass biomass under intercropping, (ii) inclusion of a control (monocropping) for at least one of the species, and (iii) field trials with at least three randomized replications. The total number of studies that met the inclusion criteria was 56. The majority of studies included only one species as a control. Therefore, we conducted the meta-analysis on the effects of intercropping on maize and Brachiaria grass by group, which resulted in two sets of studies (i.e., one for each species). More information about the studies, references, years, number of observations, locations, and crop species can be found in Figure 1 and Supplementary Table 1.
Figure 1. Geographical distribution of 56 experimental sites around Brazil with data on maize-Brachiaria grass systems included in the meta-analysis. The numbers associated with the geographical sites are the study identification numbers, and the complete details are provided in Supplementary Table 1.

1.2.2 Data analysis

The response ratio (RR) between monocropping and intercropping was estimated as the effect size (Hedges et al., 1999) in a random-effects model calculated using the *metafor* package (Viechtbauer, 2010) in R software (RStudio Team, 2020).

\[
RR = \ln \left( \frac{\bar{X}_{\text{Intercrop}}}{\bar{X}_{\text{Monocrop}}} \right) \tag{1}
\]

where \(\bar{X}_{\text{Intercrop}}\) is the mean of the investigated group (intercropping), and \(\bar{X}_{\text{Monocrop}}\) is the mean of the control group (monocropping). The results were exponentially transformed and presented as the percentage of change \([\text{RR} - 1] \times 100\) in a forest plot. Means and confidence intervals computed in the R program were used to build forest plots with GraphPad Prism software.

We assigned weights to the studies using the inverse of the variance of each study (Hedges et al., 1999):
\[ \text{Variance} = \frac{1}{\text{Weight}} = \frac{(SD_{\text{Monocrop}})^2}{n_{\text{Monocrop}} \times (\text{Yield}_{\text{Monocrop}})^2} + \frac{(SD_{\text{Intercrop}})^2}{n_{\text{Intercrop}} \times (\text{Yield}_{\text{Intercrop}})^2} \]  

(2)

where SD is the standard deviation, and \( n \) is the number of observations of the study included in each cropping model group.

The significance of effect sizes was assessed based on whether the 95% confidence intervals (CIs) overlapped with zero. The 95% CI in our study was calculated using a resampling test from 10,000 interactions with the \textit{boot} package (Canty and Ripley, 2016). The heterogeneity among studies was calculated using the I-square (\( I^2 \)) index (Higgins and Thompson, 2002), with an overall value exceeding 75%. Thus, due to the high \( I^2 \) index among studies, the inclusion of moderator variables was pursued to further study the source of variation among studies (Higgins and Thompson, 2002).

To evaluate the publication bias, the effect sizes measured were plotted against their standard error in a standard funnel plot (Egger et al., 1997). We used the rank correlation test (Beeg et al., 1994) to measure the degree of funnel plot asymmetry. No significant value \( (p > 0.05) \) was found, indicating no publication bias.

The following information was extracted from the studies: 1) site of the experiment, 2) soil pH, 3) amount of N fertilizer applied, 4) time of the season in which the experiment was carried out, 5) data related to the maize crop (hybrid, plant population, yield), 6) data related to Brachiaria grass (species, plant population, biomass production), and 7) management practices adopted in both monocropping and intercropping (herbicide spraying, date of maize sowing, and spatial arrangement of the intercropping component crops in the field). The data were grouped into moderator variables as indicated in Table 1. The qualitative groups were defined according to the scientific literature, and the quantitative variables were formulated to balance the number of observations in each group. Only moderator variables that indicated significant differences will be presented in this study, and the remaining data are available in the supplementary material (Supplementary Figures 1, 2 and 3).
Table 1. Moderator variables evaluated in the meta-analysis.

<table>
<thead>
<tr>
<th>Moderator variable</th>
<th>Description</th>
<th>Units</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climate zone</td>
<td>Tropical / Subtropical</td>
<td>-</td>
<td>(Alvares et al., 2013)</td>
</tr>
<tr>
<td>Soil pH¹</td>
<td>(Low) &lt;5&gt; (High)</td>
<td>-</td>
<td>(Raij et al., 1997)</td>
</tr>
<tr>
<td>N application rate</td>
<td>&lt;50, 50-100, 101-150, 150&gt; kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorous rate</td>
<td>&lt;35&gt; kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium rate</td>
<td>&lt;41&gt; kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sowing time in the season</td>
<td>Spring (Early) / Summer (Late)</td>
<td>-</td>
<td>(Andrea et al., 2018)</td>
</tr>
<tr>
<td>Maize row distance</td>
<td>(Narrow) &lt;0.7&gt; (Large) m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbicide management</td>
<td>Selective for monocots / Selective for dicots / No herbicide</td>
<td>-</td>
<td>(Souza et al., 2020)</td>
</tr>
<tr>
<td>Brachiaria grass species</td>
<td>U. brizantha / U. ruziziensis / others²</td>
<td>-</td>
<td>(Baptistella et al., 2020)</td>
</tr>
<tr>
<td>Brachiaria grass seed rate</td>
<td>&lt;5&gt; kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporal arrangement</td>
<td>Simultaneous/ Relay</td>
<td>-</td>
<td>(Baptistella et al., 2020)</td>
</tr>
<tr>
<td>Spatial pattern</td>
<td>Strip row / Broadcast</td>
<td>-</td>
<td>(Baptistella et al., 2020)</td>
</tr>
<tr>
<td>Maize yield³</td>
<td>- kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachiaria grass biomass⁴</td>
<td>- kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Classified according to the recommendation for each region

² U. decumbens, U. spp. Hybrids and U. humidicola

³ Grain yield from all studies was adjusted to a standard moisture basis of 130 g kg⁻¹

⁴ Dry basis, moisture of 0 g kg⁻¹

1.3. Results

1.3.1 Yield performance of sole crops and intercrops

On average, the grain yield of intercropped maize was 6.8 Mg ha⁻¹, while that of monocropped maize was 7.3 Mg ha⁻¹. Grain yield decreased by 5.6% when maize was intercropped with Brachiaria grass (Figure 2). Maize-Brachiaria grass intercropping decreased maize grain yield in 72% of the studies and increased maize grain yield in only 24% (with 4% of studies showing no effect on yield). Individual studies representing a wide range of agronomic management and environmental conditions reported a maize grain yield in intercropping ranging from -29% to 20% relative to monocropping.
Figure 2. Effect size on maize grain yield (%) of maize intercropping and monocropping. The calculated weight for all studies is indicated as a percentage, numbers in brackets give the confidence intervals, error bars represent 95% CIs of the means, “Q” indicates values of Cochran’s test, and “I^2” indicates values of the heterogeneity test.
On average, the yield of intercropped Brachiaria grass was approximately 64% less than that of the monocropped grass (Figure 3). The biomass production of Brachiaria grass when intercropped was 3.9 Mg ha\(^{-1}\), while that when monocropped was 8.9 Mg ha\(^{-1}\). Intercropping systems led to lower Brachiaria grass biomass in all studies, with biomass reductions ranging from -93% to -2.7% compared to monocropping systems.

**Table:**

<table>
<thead>
<tr>
<th>Author(s) and Year</th>
<th>Weight</th>
<th>Effect [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oliveira et al, 2020</td>
<td>4.82%</td>
<td>-92.68 [-93.74, -91.45]</td>
</tr>
<tr>
<td>Mateus et al., 2020</td>
<td>4.84%</td>
<td>-90.72 [-91.81, -89.48]</td>
</tr>
<tr>
<td>Freitas et al., 2005</td>
<td>4.81%</td>
<td>-90.33 [-91.93, -88.42]</td>
</tr>
<tr>
<td>Wroniak et al., 2021</td>
<td>4.77%</td>
<td>-83.47 [-86.83, -79.25]</td>
</tr>
<tr>
<td>Ikeda et al., 2013</td>
<td>4.82%</td>
<td>-82.39 [-85.00, -79.31]</td>
</tr>
<tr>
<td>Dan et al., 2011</td>
<td>4.76%</td>
<td>-80.56 [-84.67, -75.34]</td>
</tr>
<tr>
<td>Cruz et al., 2009</td>
<td>4.72%</td>
<td>-76.72 [-82.41, -69.20]</td>
</tr>
<tr>
<td>Pittelkow et al., 2009</td>
<td>4.77%</td>
<td>-74.65 [-79.81, -68.18]</td>
</tr>
<tr>
<td>Torino et al., 2020</td>
<td>4.79%</td>
<td>-70.25 [-75.56, -63.77]</td>
</tr>
<tr>
<td>Baldé et al., 2011</td>
<td>4.76%</td>
<td>-63.89 [-71.05, -54.69]</td>
</tr>
<tr>
<td>Brambilla et al., 2009</td>
<td>4.72%</td>
<td>-48.50 [-51.08, -43.12]</td>
</tr>
<tr>
<td>Silva et al., 2014</td>
<td>4.84%</td>
<td>-47.55 [-53.18, -41.93]</td>
</tr>
<tr>
<td>Pariz et al., 2010, 2011</td>
<td>4.83%</td>
<td>-44.10 [-51.37, -35.75]</td>
</tr>
<tr>
<td>Araujo et al., 2011</td>
<td>4.59%</td>
<td>-40.00 [-53.53, -27.00]</td>
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**Figure 3.** Effect size on Brachiaria grass biomass yield (%) of Brachiaria grass intercropping and monocropping. The calculated weight for all studies is indicated as a percentage, numbers in brackets give the confidence intervals, error bars represent 95% CIs of the means, “Q” indicates values of Cochran’s test, and “I\(^2\)” indicates values of the heterogeneity test.
1.2.3 Effect of climatic zones and management practices on intercrop yield

The first moderator variable was the climate, which was divided into tropical and subtropical climate. The tropical climate with a dry season is characterized by a mean temperature between 20° and 26 °C and a dry period in the off-season, while the subtropical climate without a dry season presents an annual average temperature between 14° and 20 °C. Maize yield reductions were larger in the tropical (10%) than in the subtropical (3.6%) zone for intercropping relative to monocropping (Figure 4a).

![Figure 4](image)

Figure 4. Effect size on maize grain yield (%) of intercropped and monocropped maize grouped by climate zone (a), sowing season (b), nitrogen (N) application rate (c), and phosphorus (P) application rate (d). Error bars represent 95% confidence intervals (CIs) of the means, and numbers in parentheses represent the number of independent observations.

In Brazil, maize can be sown either early in the rainy season (early season) or late at the end of the rainy season (late season) following soybean harvest. Our results showed that the yield of intercropped maize significantly differed when maize was sown at different times of the season (Figure 4b). Intercropped maize sown in the early season showed decreased yields of only 5.8% relative to monocropping. When sown late, the yield of intercropped maize decreased by almost 12% compared to that of monocropping.

When cultivated using a fertilizer application rate below 50 kg N ha⁻¹, the grain yield of intercropped maize decreased by 14% (Figure 4c) compared with monocropping. The yield penalty declined slightly with increased N fertilization, ranging from -6.2% to -5.9% across different N application rates. Higher P application rates also decreased the intercropped maize grain yield penalty.
relative to monocropping (-3.6%), while low P application rates penalized the intercropped maize grain yield more than monocropping (-11%).

Herbicide management was classified as post-emergence and selective for monocots or selective for dicots. The management with monocot-selective herbicide resulted in a smaller impact on intercropped maize grain yield (Figure 5a), decreasing it by only 4.4% relative to the control. The intercropped maize grain yield was -10% and -11% less than that of monocropped maize when dicot-selective herbicide and no-herbicide management strategies were adopted, respectively.

**Figure 5.** Comparison of the effect size on maize grain yield (%) between maize intercropping and monocropping grouped by herbicide management (a), soil pH (b), temporal arrangement (c), and spatial pattern (d). Error bars represent 95% confidence intervals (CIs) of the means, and numbers in parentheses represent the number of independent observations.

A soil pH above 5 reduced the intercropped maize yield by 9.5% relative to monocropping (Figure 5). In soils with noncontrolled acidity (pH<5), the disadvantage of intercropping was a reduction of 5.4% in grain yield relative to monocropping. The sowing time of the intercrop was classified as ‘simultaneous’ when Brachiaria grass was sown with maize or ‘relay’ when the grass was sown after maize (Baptistella et al., 2020). Simultaneous sowing led to a larger effect on intercropped maize when compared with monocropping (-8.9%). Nonetheless, when Brachiaria grass sowing was delayed, the effect of intercropping on grain yield was smaller (-2.8%) relative to the control.

The intercropping spatial pattern was classified as “row” when the Brachiaria grass was sown in rows between maize rows and as “broadcast” when the grass was broadcast-sown among maize rows (Baptistella et al., 2020) The spatial pattern of Brachiaria grass when intercropped affected maize yield (Figure 5d). Intercropped maize with Brachiaria grass sown between maize rows decreased grain
yield by 8.9%, while intercropped maize growing with broadcast Brachiaria grass decreased maize grain yield by 3.0%, both relative to monocropping.

**Figure 6.** Effect size on biomass (%) of intercropping and monocropping Brachiaria grass grouped by climate zone (a), N application rate (b), Brachiaria grass sowing method (c), and herbicide management (d). Error bars represent 95% confidence intervals (CIs) of the means, and numbers in parentheses represent the number of independent observations.

In both tropical and subtropical climates, intercropping reduced the Brachiaria biomass production by 64% and 81%, respectively, compared to the monocropping scenario (Figure 6a). Sowing time was a relevant moderator variable (Figure 6b); intercropped Brachiaria grass sown in the early season produced 75% less biomass than monocropped Brachiaria, while for the late season, the biomass reduction was only 59%.

The N fertilizer rate and herbicide management had a significant effect on Brachiaria grass biomass (Figures 6c and 6d). The lower (50 kg ha\(^{-1}\)) N fertilizer rate decreased the biomass of intercropped Brachiaria grass by 82% relative to monocropping. No difference was found among N fertilizer rates above 50 kg ha\(^{-1}\), which reduced intercropped Brachiaria grass biomass by an average 70% relative to monocropping. Postemergence herbicide management revealed that the monocot- and dicot-selective herbicides did not differ, with similar reductions (between 75% and 73%) in the intercropped grass biomass compared with monocropping. No-herbicide postemergence led to a lower effect on intercropping, reducing grass biomass by 22% under intercropping compared to monocropping.

Finally, other management practices were investigated, such as P and K fertilizer rates for Brachiaria grass, Brachiaria grass sowing density, Brachiaria grass species, maize row spacing, soil
pH, temporal arrangement, and spatial pattern (Supplementary Figures 1, 2 and 3). However, no effects of these management factors were documented.

1.4. Discussion

This meta-analysis provides new insights into the low yield penalty for maize when intercropped with Brachiaria grass. Critical plant-to-plant competition occurs during the early growing stages (Fayaud et al., 2014; Zhu et al., 2014) due to competition for light, which may lead to a reduction in leaf area (Page et al., 2010), alteration in the partition of photoassimilates (Afifi & Swanton, 2011) and yield losses (Page et al., 2012). Thus, the slow emergence rates of Brachiaria grass seeds (ISTA, 2009) provide an advantage to emerging maize until the three-to-four leaf (V3-V4) stages (Portes et al., 2000), especially in simultaneous sowing arrangements. The climate is another critical factor, with tropical conditions favoring Brachiaria grass production and providing a similar habitat to the native natural habitat of *Urochloa* (Eastern Africa). In addition, temperatures during the late season in subtropical areas often reach values below the base temperature for *Urochloa* growth (17 °C) (Cruz et al., 2011). Brachiaria grass has superior drought tolerance (linked to the vigorous root system) compared to maize (Baptistella et al., 2020; Sarto et al., 2020), benefiting Brachiaria in climates with poorly distributed rainfall. These situations led to an intercropping system with less grain yield and more grass biomass yield that is ideal for either improved soil cover or more forage in integrated farming systems.

Soil N availability is closely correlated with maize grain yield and Brachiaria grass biomass production (Guenni et al., 2008). Intercropping with non-N-fixing species and under low N fertilizer (<50 kg N ha\(^{-1}\)) rates enhanced crop competition for N (Crusciol et al., 2020; Oliveira et al., 2020), affecting overall intercropping performance. Low N fertilizer rates limited maize grain yield and grass biomass production. Moreover, medium-to-high N (50-150 kg N ha\(^{-1}\)) fertilizer rates reinforce early maize dominance over Brachiaria grass, reversing negative effects on maize grain yield due to interspecific competition. From the perspective of P fertilization, the lack of effect of this factor on Brachiaria grass biomass under intercropping could be linked to the adaptation of Brachiaria grass to P-limited soils (Baptistella et al., 2021). Furthermore, under low pH (<5), which is characteristic of tropical soils, Al and Fe can fix the fertilizer P into low-solubility compounds (HSU, 1965). However, maize crops present a yield response to low P levels (Baldé et al., 2011; Galdos et al., 2020; Sarto et al., 2021). Soil acidity was also a significant factor in the response of maize to P fertilization in acidic soils. Controlled soil acidity is associated with higher soil fertility and nutrient uptake (Tinker & Barraclough, 1988; Sparling & Lowe, 1996). At low soil pH (<4.5), P and Al may form stable low-solubility compounds (Weng et al., 2011), increasing the response of maize to P fertilization (Nolla et al., 2013).
The temporal arrangement and spatial pattern of intercropping sowing affected the competition level with maize. In the early season, the favorable weather (temperature and water) for maize growth (Alvares et al., 2013) could explain the differences between maize and Brachiaria grass related to the sowing time in the season. Furthermore, restrictive maize growth conditions in late-season sowing (less precipitation and solar radiation) could delay crop development (Andrea et al., 2018), reducing the dominance of maize over Brachiaria. Overlaps in early growth may lead to larger grain yield penalties for maize (Vandermeer, 1989; Brooker et al., 2015). In addition, sowing without incorporation usually confers poor soil cover to the seeds (Brennan and Leap, 2014), slowing Brachiaria grass germination (Timossi et al., 2018). Thus, Brachiaria grass broadcast sowing may be prioritized due to the lower risk this management poses to maize grain yield and its lack of effect on grass biomass production.

The current meta-analysis lays a foundation for pursuing the development of more specific management guidelines for complex decisions aimed at grain, residue, or forage production through maize-Brachiaria grass intercropping. Future research should focus on studying plant competition for resources (e.g., water, light, nutrients) and root interactions in intercropping under different sowing times, different management practices, and different environments.

1.5. Conclusions

Intercropping maize and Brachiaria grass can decrease the productivity of both crop species (by -5.6% for maize and -63% for Brachiaria grass), but these penalties can be mitigated by the implementation of certain management practices and by certain climatic conditions.

Intercropping maize in a subtropical climate zone with early sowing led to reduced penalties, while Brachiaria grass showed greater biomass production when sown late in the season and in a tropical climate. Low fertilizer N application rates of 50 kg N ha\(^{-1}\) impaired intercropping yield.

In summary, maize-Brachiaria grass intercropping is an effective alternative to increase biomass production for soil cover in no-tillage systems or for forage production to implement crop-livestock systems, with minimal maize yield penalties, diversifying current cropping systems in Brazil.

References


Hsu, O. H., 1965 Fixation of phosphate by aluminum and iron in acidic soils. Soil science 99(6), 398-402.


References of the meta-analysis


Supplementary Figures

Supplementary figure 1. Effect size (%) comparison between intercropped and monocrop maize yield grouped by brachiariagrass species (a), maize row width (b), seed sowing rate (c) and K application rate (d). Error bars represent 95% CIs of the means and numbers in parenthesis represent the number of independent observations.

Supplementary figure 2. Effect size (%) comparison between intercropped and monocrop brachiariagrass biomass productivity grouped by P application rate (a), soil pH (b), temporal arrangement (c) and spatial pattern (d). Error bars represent 95% CIs of the means and numbers in parenthesis represent the number of independent observations.
**Supplementary figure 3.** Effect size (%) comparison between intercropped and monocrop brachiariagrass biomass productivity grouped by brachiariagrass species (a), maize row width (b), seed sowing rate (c) and K₂O rate (d). Error bars represent 95% CIs of the means and numbers in parenthesis represent the number of independent observations.
### Supplementary Table

List of the included studies with experimental years, number of observations, city and state localization in Brazil and the available monocrop yield for each intercrop species.

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2. ENHANCING LAND EFFICIENCY THROUGH MAIZE-TROPICAL FORAGES INTERCROPPING IN BRAZIL: AN ASSESSMENT OF YIELD, BIOMASS, ENERGY AND ECONOMIC PERFORMANCE

Abstract

Brazil, a leading food producer and exporter, has witnessed a significant rise in the adoption of maize-tropical forages intercropping, aiming the soil protection and enhance the land productivity. This study intends to investigate the interaction between maize and tropical forage in intercropping systems, assessing its impact on maize yield, biomass accumulation, biological energy content, land equivalent ratio, and economic analysis compared to monocropping systems. The experiment, employing a randomized block design with four replicates, contrasted monocultures and intercropping systems of maize with two forage species, *Urochloa brizantha* and *Panicum maximum*. The findings revealed that maize yield and biomass remained stable in intercropping compared to monocropping. Intercropping added 4.5 Mg ha\(^{-1}\) of biomass during the offseason compared to maize monocropping. At the time of maize harvest, tropical forages contributed 25% more biomass in intercropping, although with just 5% of biological energy. The land equivalent ratio (LER) of intercropping was consistently higher during species co-growth period. Remarkably, intercropping systems boosted profits by an average of 92.1% compared to tropical forages monocropping. In conclusion, both intercropping systems offer a robust strategy for simultaneous diversification and intensification of land use, optimizing resource utilization with a minor or no trade-off in maize grain yield.

2.1. Introduction

Brazil is one of the world's largest food producers and exporters (USDA, 2020). The tropical climate accounts for approximately 81% of Brazilian arable land (Alvares *et al.*, 2013). This climatic region covers approximately 50 million hectares of annual crops, primarily grains (PAM-IBGE, 2019),...
and recent estimates show that agricultural practices related to soil rotation emit approximately 1 Gt year-1 of C annually (Lal, 2003; Lal et al., 2004; Oost, Van et al., 2007; Quinton et al., 2010). The impact of C emissions on climate change is acknowledged (IPCC, 2014). Thus, intercropping maize (Zea mays L.) and tropical forages such as brachiaria grass species (Urochloa spp, syn. Brachiaria spp.) and guinea grass cultivars (Megathyrsus spp. syn. Panicum spp,) is one method used to reduce erosive processes and C emissions, especially in the tropical grain production areas in Brazil.

The main cropping rotation utilized in Brazil is double-cropped soybean-maize [Glycine max (L.) Merrill; Zea mays L.] (Battisti et al., 2020; Goldsmith e Montesdeoca, 2018), which takes advantage of the long growing season in the tropical climate. However, this cropping system lacks the required amount and quality of harvest residues for adequate soil cover (Gregory, 1982; Kumar, 2000). Maize is sown late in the season, and after its harvest becomes impractical to grow cover crops due the water deficit period typical of tropical environments (Alvares et al., 2013). Thus, the absence of crop residues for soil cover results in soil exposure to erosion at the beginning of the next rainy season. In this scenario, tropical forages could be intercropped with maize, for soil cover biomass production, or implementing integrated crop-livestock systems after maize harvest until the next crop season (Ceccon et al., 2013; Costa, da et al., 2016; Pereira et al., 2016).

The plant's interaction in an intercrop is important to determine the success of the intercropping system. A high crop initial growth rate frequently leads to resource capture dominance and hence the potential of increased biomass growth and production (Fukai e Trenbath, 1993). The slow emergence rates of brachiaria and guinea grass (ISTA, 2009) give an advantage to maize plants at early period of co-growth during intercropping. Tropical forages have their main photoassimilates sinks in their leaves, steam and roots, which represents low metabolically costs compared to the proteins and amid sinks in the maize grains (Shakya e Lal, 2018). Thus, the lower total calorific value, indicative of the reduced biological energy content in tropical forages compared to maize plants (Alluvione et al., 2011; Barbero et al., 2015; Jetana et al., 2000), could enable the forages to slowly continue their growth even in a maize-dominated environment (Portes et al., 2000).

Tropical forages, such as brachiaria and guinea grasses, are known for their ability to adapt to poor soil conditions and efficiently use available resources, which allows them to grow in limited energy environments (Capstaff e Miller, 2018). In this condition, tropical forages can produce biomass simultaneously with maize, and continue to grow after maize harvest, enabling soil cover during the dry season until the beginning of rainy season. On such wise, the biological efficiency in biomass accumulation in this intercropping could be reached through its land equivalent ratio (LER), that measures the intercropping production against their monocropping counterparts (Mead e Willey, 1980). However, this biomass production could have an agricultural cost, as reported by (Souza et al., 2024), with maize grain yield losses between 3 and 7%. This maize grain yield losses also could imply
direct economic losses in a short time. Nevertheless, few studies have examined the economic impacts of the maize-tropical forages intercropping system compared to its monocropping counterpart (Pariz et al., 2009; Richart et al., 2015; Simili et al., 2023; Wenneck et al., 2021). Thus, an integrated analysis can help with the systemic view of this intercropping.

Under these circumstances, the objectives of this work are to investigate the species interaction in maize-tropical forage intercropping and to determine how this intercropping affects (i) maize grain yield, (ii) species dry mass accumulation, (iii) balance between total biomass and biological energy equivalent produced, (iv) the land equivalent ratio in relation to each species monocropping systems over the growing season and offseason, and (v) an economic analysis of the intercropping and monocropping system during its growing periods, in order to perceive a general efficiency view of this system.

### 2.2. Material and Methods

**Site description**

The field trial was conducted over the late season of 2021 and 2022 in Torrinha (22° 10' 23" S 47° 13' 55" W, 620 m altitude) located in São Paulo State, situated in a Cwa Köppen climate classification (Alvares et al., 2013). The precipitation and average air temperature data are shown in Figure 1. The field soil is classified as Oxisol Ustox (Embrapa, 2013), and the soil chemical and physical properties of the experimental area at 0-20 cm depth are: 5.2 pH (H₂O), 30.9 g dm⁻³ of soil organic matter, 27 mg dm⁻³ of resin phosphorus, 13 mg dm⁻³ sulfur, 3.1 mmol c dm⁻³ aluminum, 4.3 mmol c dm⁻³ potassium, 27 mmol c dm⁻³ calcium, 11 mmol c dm⁻³ magnesium, 51.4% base saturation, 1.39 kg dm⁻³ bulk density, 515, 130 and 485 g ka⁻¹ of sand, silt and clay content respectively. Before the trial, the area had been cultivated with soybeans in the early season and maize in the late season for the past five years.
2.2.1 Experimental design

The experimental design was a complete randomized blocks with four replicates, composed of 5 m width and 20 m length plots. The treatments consisted of the three species cultivated in monoculture and the intercrop systems between maize and each forage species, as described: (i) Maize monoculture, (ii) Brachiariagrass monoculture, (iii) Guineagrass monoculture, (iv) Intercropping Maize-Brachiariagrass and (v) Intercropping Maize-Guineagrass.

2.2.2 Treatments and management

The maize hybrid Feroz Viptera3 and the forages *Urochloa brizantha* cv. Marandu and *Panicum maximum* syn. *Megathyrsus maximum* cv. Mombasa were sown on January 14th, 2021 and on February 2nd, 2022, both maize and tropical forage monocultures and their related intercropping systems were sown at the same time. In the case of intercropping systems, the sowing of the maize and each forage was simultaneous, the scheme presented in Figure 2 illustrates the treatments sowing information. Maize was sown at population of 60,000 plants ha\(^{-1}\), and both brachiaria and guinea
grasses were sown at 120,000 plants ha\(^{-1}\) population. Maize harvest occurred on June 18\(^{th}\), 2021 and on July 21\(^{th}\), 2022. Both brachiaria and guinea grasses were desiccated at October 1\(^{st}\), 2021 and October 25\(^{th}\), 2022.

Figure 2: The layout of the evaluated monocropping and intercropping systems, along with the respective row-spacing used.

The experimental area was fertilized at crop sowing in all plots, supplying 50, 80, and 100 kg ha\(^{-1}\) of N, P\(_2\)O\(_5\), and K\(_2\)O respectively. When maize reached the V4 phenological stage 100 kg ha\(^{-1}\) of N was side-dressed in all plots (Raij et al., 1997), at the same stage the weed control was conducted using 2500 g ai ha\(^{-1}\) of atrazine and 28 g ai ha\(^{-1}\) of nicosulfuron.

2.2.3 Sampling description

The above-ground biomass was sampled every 15 days after sowing until the maize harvest, and every 50 days after the maize harvest (offseason) until the forage desiccation before the new crop growth season. The photosynthetic active radiation above and under the maize canopy and the leaf area were sampled simultaneously with above-ground biomass as well, using FluorPen FP 110\(^{®}\) and Licor LI-3100C\(^{®}\) equipment respectively. After sampling, the above-ground biomass was partitioned into stover and grains for maize and leaves and stems for forage. The fresh material was subjected to forced drying at 65 °C for 72 hours.
2.2.4 Energy equivalent, land equivalent ratio, and economic analysis

For biological energy equivalent, the total calorific value embodied in the materials was considered (Maclean et al., 2003) found in the literature. The corn energy equivalent was divided between grains and the remaining biomass (stover). Calculations for forage species (brachiaria and guinea grass) value was adopted for total aboveground biomass. The values used were 18.92 MJ kg\(^{-1}\) of dry mass for corn grains, 18.67 MJ kg\(^{-1}\) for the remaining corn biomass (Alluvione et al., 2011), 16 MJ kg\(^{-1}\) for brachiaria grass biomass (Barbero et al., 2015), and 17.3 MJ kg\(^{-1}\) for guinea grass biomass (Jetana et al., 2000).

The land equivalent ratio (LER) was calculated independently on each subplot considering the total biomass using the following equations:

\[
LER_m = \frac{M_{b\text{int}}}{M_{b\text{mon}}} \\
LER_f = \frac{F_{b\text{int}}}{F_{b\text{mon}}} \\
LER_{\text{int}} = LER_m + LER_f
\]

Which \(L ER_m\) refers to the maize LER, \(M_{b\text{int}}\) to intercropped maize biomass, \(M_{b\text{mon}}\) to monocropped maize biomass, \(L ER_f\) to forage (brachiaria or guinea grass) LER, \(F_{b\text{int}}\) to intercropped forage biomass, \(F_{b\text{mon}}\) to monocropped forage biomass. Finally, \(L ER_{\text{int}}\) refers to the LER of the whole intercropping system, taking into account both species.

The economic analysis considered the mean gross operating cost, revenue, and profit of both years for each farming system over the growing period. The operating costs included plant protection, seeds, fertilizers, machinery (sowing, application, harvest), transport, storage, insurance, and other variable costs according to Brazilian National Supply Company (CONAB, 2020). For maize, grain value was used as an annual mean for each year according to the Center for Advanced Studies on Applied Economics of the University of São Paulo (CEPEA). For both brachiaria and guinea grass, biomass revenue was converted using a mean feeding conversion ratio for cattle of 29.7 kg of biomass for 1 kg of living animal weight, as reported by Aranha et al. (2022) and Meo-Filho et al. (2022) in their studies on cattle performance in integrated crop-livestock systems in Brazil. Total revenue per ha in the field trial period was calculated by the formula:

\[
Total\ revenue\ ha^{-1} = (price\ per\ kg \times maize\ yield) + (price\ per\ kg \times estimated\ meat\ production) \tag{4}
\]

Net return per ha in the field trial period was calculated by the formula:

\[
Profit\ ha^{-1} = (total\ revenue - operating\ cost\ ha^{-1}) \tag{5}
\]

2.2.5 Statistical analysis

The biomass, biological energy equivalent and land equivalent ratio data were subjected to three spatial correlation models (mixed linear effect models), which permitted modeling temporal
correlation between the observations. Moreover, the models were adjusted separately for each year taking the variability of field conditions into account, allowing a better understanding of how the effects of treatments may vary from year to year. Thus, the model with the lowest Akaike Information Criterion (AIC) (Cavanaugh e Neath, 2019) was chosen for the analysis. For maize grain yield and economic data an analysis of variance and comparison of means by the Tukey test at a 5% probability level was conducted. The *lem4* (Bates et al., 2015), *emmeans* (Searle, Speed e Milliken, 1980), and *multcomp* (Hothorn, Bretz e Westfall, 2008) packages for the R software (R Core Team, 2020) were used for this analysis.

### 2.3. Results

The mean maize grain yield 7.6 Mg ha$^{-1}$ in 2021, the grain yield did not differ between treatments as illustrated in Figure 3. The mean grain yield for the 2022 season was 8.9 Mg ha$^{-1}$, not differing between them, as well. The statistical analysis results of maize grain yield and all other variables for both years are summarized in Table 2.

![Figure 3: Maize grain yield in maize monocropping, maize-brachiaria grass intercropping, and maize-guinea grass intercropping systems for year 1 and year 2.](image-url)
### Table 2: Statistical analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>Treatment*Sample Timing</th>
<th>StdDev</th>
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</thead>
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<tr>
<td><strong>Year 1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maize Yield</td>
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<td>-</td>
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<tr>
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<td>&lt;0.001</td>
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<td>Guinea grass Biomass</td>
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<td>&lt;0.001</td>
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</tr>
<tr>
<td>LER maize-guinea</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LER integrated maize-brachiaria</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>LER integrated maize-guinea</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Accumulated PAR</td>
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</tr>
<tr>
<td><strong>Year 2</strong></td>
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<td></td>
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</tr>
<tr>
<td>Maize Yield</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maize Biomass</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brachiaria grass Biomass</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Guinea grass Biomass</td>
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<td>&lt;0.001</td>
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</tr>
<tr>
<td>Total Biomass</td>
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</tr>
<tr>
<td>Energy Equivalent</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>LER maize-brachiaria</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LER maize-guinea</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LER integrated maize-brachiaria</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LER integrated maize-guinea</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Accumulated PAR</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1Standart Deviation

The maize biomass accumulation curves show no difference between monocropping and intercropping systems in year 1, accumulating around 15.3 Mg ha\(^{-1}\) of dry mass (Figure 4a). However, in year 2 both intercropping systems differed from maize monocropping biomass accumulation at 75 days after sowing (10 Mg ha\(^{-1}\) and 11.2 Mg ha\(^{-1}\) for intercropped and monocropped maize respectively), but recovering at maize harvest (150 days after sowing). Even in year 2, the maize biomass dry mass accumulation did not differ at harvest, reaching a mean of 16.5 Mg ha\(^{-1}\).
Figure 4: Total system biomass for (a) maize, (b) brachiaria, and (c) guinea grass for both the years 2021 and 2022 in monocropping and intercropping systems.

The brachiaria grass biomass accumulation follows the same response in both years in the monocropping system, reaching 8.8 Mg ha\(^{-1}\) of dry mass (Figure 4b). In the intercropping system, brachiaria grass accumulated 3.2 and 5.2 Mg ha\(^{-1}\) of biomass at 2021 and 2022 season respectively. At 75 days after sowing, the monocropping system exceeded the brachiaria grass intercropping
biomass, and went through a period of marked growth until 135 days after sowing. Then, brachiaria grass monocropping maintained the biomass accumulation over time until the end of the trial (250 days after sowing). Intercropped brachiaria grass reached the maximum biomass accumulation at 120 days after sowing, in year 1, maintaining the biomass until the trials end. Contrarily, in year 2 the intercropped brachiaria grass still accumulated biomass until the trials end.

As well as brachiaria grass, guinea grass monocropping follows the same biomass accumulating pattern in both years, reaching 10.5 Mg ha\(^{-1}\) of dry mass (Figures 4c). At 75 days after sowing, the monocropping system exceeded the guinea grass biomass in intercropping, stabilizing its biomass accumulation just at 150 days after sown. In year 1, the intercropped guinea grass also stabilized its growth at 150 days after sowing, accumulating 3.9 Mg ha\(^{-1}\). However, in year 2, the intercropped guinea grass had a continuous biomass accumulation until the trial ends, reaching 5.5 Mg ha\(^{-1}\).

In both years, the intercropping systems accumulated more biomass than the monocropping systems. Specifically, they accumulated an average of 4.1 Mg ha\(^{-1}\) more biomass than monocropped maize and 10.1 Mg ha\(^{-1}\) more than tropical forages monocropped. This was observed in both years. Furthermore, at the time of maize harvest, the intercropping systems had more biomass than both brachiaria and guinea grass monocropping systems. After the maize harvest, at 250 days after sowing, the intercropping systems, as well as the grasses monocropped, continued to accumulate biomass. The average accumulation was 4.7 Mg ha\(^{-1}\) for the intercropping systems and 9.7 Mg ha\(^{-1}\) for the grasses monocropped.
Figure 5: The (a) total system biomass (Mg ha\(^{-1}\)) and (b) biological energy equivalent (TJ ha\(^{-1}\)) at maize harvest period (inseason) and at forage desiccation period (offseason) over all monocropping and intercropping systems in both 2021 and 2022 years.

Both intercropping systems had a greater energy equivalent accumulation compared with monocropping ones at maize harvest, reaching 35.7 TJ ha\(^{-1}\) (on average across both years), against 29.9 TJ ha\(^{-1}\) for monocrop maize, 12.9 TJ ha\(^{-1}\) for monocrop brachiaria and 17.35 TJ ha\(^{-1}\) for monocrop guinea grass (Figure 5b). After the maize grain harvest, the intercropping systems decreased their energy equivalent accumulation to 7.6 TJ ha\(^{-1}\) on average at 250 days after sowing, while monocropping brachiaria grass and guinea grass reached 14.1 and 18.2 TJ ha\(^{-1}\) at 250 days after sowing (trials end), respectively.

The LER was higher in both maize-brachiaria and maize-guinea intercropping, from the first days after sowing until the maize harvest, compared to monocropping maize, brachiaria and guinea grass LER (Figure 6a). Intercropping LER decreased from 1.95 to 1.36 over time, on both year and intercropping average. As well as intercropping, brachiaria and guinea grass monocropping LER decreased over the period, going from 1 to 0.37 at the maize harvest time. Maize LER was constant through time, except for a slight reduction 30 days after sowing regarding maize-brachiaria grass intercropping. The LER integration of intercropping systems was higher compared to sole crops. Intercropping alternatives achieved 223, while maize reached 139, brachiaria grass 88 and guinea grass 78 on average across both years (Figure 6b).
Figure 6: The (a) land equivalent ratio, and the (b) integration of the land equivalent ratio over the species co-growth period for both maize-brachiaria grass and maize-guinea grass intercropping systems, as well as their respective monocropping systems, for the years 2021 and 2022.

In the economic analysis the intercropping systems performed better than monocropping systems (Table 3). The total mean revenue of both intercropping were 2,963 US$ ha$^{-1}$, while for maize was 2,700 US$ ha$^{-1}$, 1,283 US$ ha$^{-1}$ for brachiaria grass and 1,524 US$ ha$^{-1}$ for guinea grass. Intercropping alternatives had an annual profit of 1,435 US$ ha$^{-1}$, while monocropping maize, brachiaria grass and guinea grass reached 1,346 US$ ha$^{-1}$, 651 US$ ha$^{-1}$ and 875 US$ ha$^{-1}$ respectively.

Table 3: Mean annual gross revenue, costs, and profits for each farming systems

<table>
<thead>
<tr>
<th>System</th>
<th>Operating cost (US$ ha$^{-1}$)</th>
<th>Revenue (US$ ha$^{-1}$)</th>
<th>Profit (US$ ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maize grain</td>
<td>Forage biomass$^1$</td>
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<tr>
<td>Monocropping</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>1354.6</td>
<td>2,700.7 ± 37.7</td>
<td>2,700.7 ± 37.7</td>
</tr>
<tr>
<td>Brachiaria grass</td>
<td>632.1</td>
<td>1283.3 ± 37.8</td>
<td>1,283.3 ± 37.8</td>
</tr>
<tr>
<td>Guinea grass</td>
<td>648.5</td>
<td>1524.4 ± 13.8</td>
<td>1,524.45 ± 13.8</td>
</tr>
<tr>
<td>Intercropping</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize-Brachiaria</td>
<td>1,520.2</td>
<td>2656.4 ± 18.7</td>
<td>309.1 ± 2.18</td>
</tr>
<tr>
<td>Maize-Guinea</td>
<td>1,536.5</td>
<td>2617.8 ± 18.5</td>
<td>343.9 ± 2.43</td>
</tr>
</tbody>
</table>

$^1$Estimated using cattle feeding conversion ratio
2.4. Discussion

Maize grain yield potential is defined over the entire plant growth cycle, by the number of grains per cob line, and the number of lines in the cob (Hawkins & Cooper, 1981). After the end of the juvenile stage (V4-V5), the maize plants present a fast growth pattern (Danilevskaya et al., 2008), and when intercropped with brachiaria or guinea grasses, which present a slow emergence period (ISTA, 2009), maize plants take the advantage in these intercropping systems. This fast growth can lead to a lower interspecific competition, which may explain the lack of yield and biomass accumulation reduction in these maize intercropping regarding the monocropping one (Figures 3 and 4).

Tropical forages growth and biomass accumulation are negatively impacted by shade (Moreno et al., 2022; Wong & Stür, 1996). The shaded environment provided by maize to tropical forages in the intercropping explain the lower biomass accumulation, regarding brachiaria and guinea grasses monocropping. The biomass accumulation resumption observed in the second year (Figure 4) could be related to the high mean temperature of the period between June and October (maize harvest until forage dissection), and to the higher precipitation over all the growing cycle, given the negative effect that water deficit and low temperatures can have in brachiaria and guinea grasses (Araujo, De et al., 2018; Gaspari Pezzopane, de et al., 2015).

The maize dominance over the grasses in the intercropping corroborates with Souza et al. (2024), and despite this maize control, the spatial and functional resources partitioning (Vandermeer, 1992) in the intercrop environment supports the grasses growth. Thus, the maize surplus resources enable a system biomass increase in the intercropping, with the brachiaria or guinea grasses exploring this niche. This also results in an increase in the energy equivalent output in the system, compared to monocropping ones, as can be seen in Figure 5. Singh et al. (2016) evaluating potatoes-base intercropping systems, and by Firouzi et al. (2017) studying groundnut-base intercropping, also found higher energy equivalent outputs in intercropping systems, compared to their monocropping, correlating this to the resources exploration gap of the dominant crop and the potential niche access of the dominated crop.

The land equivalent ratio (LER) is a widely used indicator of yield advantage in intercropping systems over sole-crop farms (Deb & Dutta, 2022). It is usually measured using crop biomass yield per unit area (Mead & Willey, 1980). The mean LER for maize-brachiaria and maize-guinea at the maize harvest was 1.36, suggesting that intercropping could produce the same yield as separate monocrops of maize and tropical forages on 36% less land. A similar LER was reported by Tan et al. (2020) evaluating analogous grass-grass intercropping. However, Crusciol et al. (2020) and Pariz et al. (2017) found smaller, but also higher than 1, LER in maize -tropical forages intercropping, linking the success of the system to the nitrogen management adopted, given the high dependency of these C4 species to the nitrogen available in the soil (de Oliveira et al., 2020; Gheith et al., 2022; Nakamura et al., 2005).
The economic analysis conducted in this study adds one more layer on the integrated view of this intercropping system. Besides the maize grain yield in the field, farmers can also use the tropical forage biomass for animal fodder during the offseason period (August to October). An economic analysis carried out by Crusciol et al. (2020) found a profit increase in the same intercropping system of US$ 500–600, more than the US$ 100 increase found in this work. However, even with the smaller profit gain, maize-tropical intercropping presented consistent economical, agricultural, energetic and biological advantage, regarding their monocropping counterparts (Figure 7).

Figure 7: Radar plots displaying both years mean percent of: maize grain yield, forage biomass production, total biomass production, total energy equivalent production, land equivalent ratio (LER) integration, and annual profit for all monocropping and intercropping systems.

A comprehensive analysis of the multifunctionality of each cropping system was conducted in accordance with Hodgdon et al. (2016). Upon evaluating all variables, the radar chart (Figure 7) reveals multifunctionality gaps across all systems. Tropical pastures, in general, exhibit a high potential for biomass accumulation (Silva, da, Sbrissia e Pereira, 2015) in full sunlight conditions. However, they possess a lower biological energy content in their biomass compared to cereals such as maize (Jetana et al., 2000; Alluvione et al., 2011; Barbero et al., 2015). In contrast, maize outperforms tropical forages in terms of multifunctionality, boasting higher total biomass accumulation and superior biological energy content. Which results in a greater net income. Intercropping systems address some of the shortcomings of maize monocropping, such as land use efficiency (LER), which is enhanced by the surplus of forage biomass. Despite a decrease in forage biomass within the intercropping system, both maize-brachiaria and maize-guinea systems demonstrate superior multifunctionality compared to their monocropping counterparts. This allows for an increase in food
2.5. Conclusion

This study adopts an integrated approach to the analysis of farming systems over time, considering not only yield and economic factors but also ecological, energetic, and land use efficiency aspects. The study found that the maize grain yield remained consistent between intercropping and monocropping systems across both years. Moreover, intercropping contributed an additional 4.5 Mg ha⁻¹ of biomass during the offseason compared to maize monocropping. At maize harvest time, tropical forages in the intercropping system added 25% more biomass, although with a modest 5% increase in energy.

Maize-tropical forages intercropping presented itself as a robust method for diversifying and intensifying land use simultaneously. Throughout the co-growth period of all species, the land equivalent ratio (LER) for intercropping consistently outperformed that of monocropping. Notably, the maize-brachiaria and maize-guinea intercropping demonstrated higher profits compared to their monocropping counterparts. This suggests that intercropping could be a viable strategy for farmers aiming to maximize their return on investment while also enhancing ecological sustainability.

The intercropping system exhibits a synergy that has the potential to diversify farmers’ income, boost land biomass productivity, and improve soil conservation, particularly when considering soil cover and the adequate association of two plants. In conclusion, this work highlights the potential benefits of maize-tropical grasses intercropping systems. These systems present a solid strategy for diversifying and intensifying land uses simultaneously, utilizing resources in a more efficient manner, with only a minor trade-off with grain yield.

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3. ROOT TRAITS AND BIOMASS ACCUMULATION IN MAIZE-TROPICAL FORAGES INTERCROPPING SYSTEMS: A COMPARATIVE STUDY

Resumo
Características das raízes e acúmulo de biomassa em sistemas de consórcio de milho-forrageiras tropicais: um estudo comparativo

Sistemas de consórcio estão surgindo como uma abordagem promissora para a agricultura sustentável, especialmente aqueles que contêm culturas de cobertura, como o consórcio entre milho e forrageiras tropicais. Este consórcio pode otimizar a exploração de recursos e aumentar a eficiência no uso do solo. Particularmente em ambientes tropicais como o Brasil, esses sistemas também podem contribuir para o aumento do carbono orgânico do solo e prevenir a erosão do solo por meio dos seus sistemas radiculares. No entanto, há pouca informação disponível na literatura científica sobre as características e acúmulo de biomassa do sistema radicular do consórcio entre milho e forrageiras tropicais. Assim, um experimento com parcelas subdivididas e quatro repetições foi projetado para explorar as características radiculares, distribuição no solo e biomassa radicular e total dos sistemas de monocultivo e consórcio de milho, braquiária e panicum. O estudo revelou que os sistemas de consórcio milho-braquiária e milho-panicum superam o monocultivo de milho na produção de biomassa e desenvolvimento radicular. Sistemas de consórcio tiveram 25% a mais de biomassa total na colheita do milho, indicando melhor utilização de recursos. A biomassa radicular em ambos os sistemas de consórcio e no monocultivo de forrageiras tropicais foi significativamente maior, com aumentos de 90% e 270% respectivamente, em comparação com 1,2 Mg ha\(^{-1}\) no monocultivo de milho. Até 40% da biomassa radicular nos sistemas de consórcio vieram da linha de plantio das forrageiras tropicais. O volume e comprimento radicular nos sistemas de consórcio foram 26% maiores e a área radicular foi 40% maior do que no monocultivo de milho. Isso sugere que o consórcio de milho e forrageiras tropicais aumenta a produção de biomassa radicular no sistema, podendo contribuir para a agregação do solo, proteção contra erosão, ciclagem de nutrientes e a fixação profunda de carbono. Sistemas de consórcio oferecem claras vantagens na produtividade acima e abaixo do solo, destacando seu potencial na agricultura sustentável.

Abstract

Intercropping systems are emerging as a promising approach to sustainable agriculture, especially those containing cover crops, such as the intercropping of maize and tropical forages. They may optimize resource exploration and enhance soil use efficiency. Particularly in tropical environments like Brazil, these systems can also contribute to increasing soil organic carbon and preventing soil erosion through their root systems. However, there is limited information available in the scientific literature about the traits and biomass accumulation of the root system in the intercropping of maize and tropical forages. Thus, an experiment with a split-plot design with four replications was designed to explore the root traits, distribution in soil, and root and total biomass in monocropping and intercropping systems of maize, brachiaria grass, and guinea grass. The study reveals that maize-brachiaria and maize-guinea grass intercropping systems outperform maize monocropping in biomass production and root development. Intercropping systems have 25% higher total biomass at maize harvest, indicating better resource utilization and productivity. Root biomass in both the intercropping and tropical forages monocropping systems was significantly higher, with increases of 90% and 270% respectively, compared to 1.2 Mg ha\(^{-1}\) in maize monocropping. Up to 40% of the root biomass in intercropping systems comes from the sowing row of tropical forages. The root volume and length in intercropping systems were 26% higher and the root area was 40% higher than that in maize monocropping. This suggests that intercropping maize and tropical forages increases root biomass production in the system, potentially contributing to soil aggregation, protection against erosion, nutrient cycling, and for deep carbon fixation. Intercropping systems offer clear advantages in above-ground and below-ground productivity, highlighting their potential in sustainable agriculture.
3.1. Introduction

Tropical regions, which make up 81% of Brazil’s land area (Alvares et al., 2013), are often more susceptible to soil erosion, and carbon and nutrient losses due to high weathering (Gomes et al., 2019; Merten e Minella, 2013). These regions are characterized by intense rainy seasons and a lack of plant residues to protect the soil, largely due to high degradation rates (Nearing, Pruski e O’Neal, 2004; Ranaivoson et al., 2017). These factors can significantly impact agricultural productivity and contribute to environmental degradation, making it a pressing issue. One potential solution is the intercropping between maize (Zea mays L.) and tropical forages, which has been recognized as an effective alternative for sustainable intensification of food production, particularly in tropical environments such as the Brazilian Cerrado (Andrea et al., 2018; Oliveira et al., 2019; Silva et al., 2020).

Intercropping systems have been established as being more efficient than monocropping in terms of land use efficiency, optimizing the allocation of resources across time and space (Fan et al., 2013; Romero et al., 2013; Vandermeer, 1989; Yin et al., 2015). The productivity benefit in intercropping systems is often achieved through the management of interspecies interactions (Li et al., 2006). For instance, well-managed intercropping, which combines species with compatible growth rates and physical characteristics, could lead to positive results due to improved resource sharing and temporal optimization (Bourke et al., 2021). This is particularly true for maize-tropical forages intercropping, where the fast growth and tall pattern of maize plants (MacLaren et al., 2023) providing severe shading and growth retardation in tropical forages during the establishment period (Portes et al., 2000).

Once maize enters the reproductive phase, its leaf area starts decrease, as well as the density of its canopy (Maddonni e Otegui, 1996; Muchow e Carberry, 1989), increasing solar radiation below the crop canopy (Makino et al., 2019). This change in light availability can alter the dynamics of above-ground competition. Simultaneously, below ground, intricate physiological, physical, and chemical processes come into play as plants compete for nutrients and water (Schenk, 2006). The understanding of these complex above and below ground interactions, including the competition for nutrients and water, is not only crucial for effectively combining species in cropping systems but also for optimizing resource use, improving yield, and enhancing system resilience (Wilson, 1988). This knowledge becomes particularly relevant in the context of tropical agriculture, where the unique challenges of the environment require innovative solutions.

Tropical soils, which are distinguished by their pronounced acidity, limited fertility, and susceptibility to erosion (Gomes et al., 2019; Merten e Minella, 2013), could significantly benefit from plants with deep and vigorous root systems. These root systems enhance soil exploration, enabling plants to access water and nutrients from deeper soil layers (Chen et al., 2018). The traits of these
roots, such as length, superficial area, and volume, directly affect the soil exploration capability of plants (Kaysar et al., 2022). Therefore, a comprehensive understanding of these root system traits can provide valuable insights into optimizing resource acquisition and improving agricultural productivity in these challenging tropical environments (Cardoso, Nogueira e Zangaro, 2017; Lynch, 2019).

Beyond the potential of maize-tropical forages intercropping to produce crop residues for no-tillage systems and pasture for offseason livestock production (Souza et al., 2024), the combination of maize with these cover crops could increase soil organic carbon (Gorfu Tessema et al., 2021; Saraiva et al., 2014). This is largely due to the vigorous root system characteristic of species like brachiaria (*Urochloa* spp, syn. *Brachiaria* spp.) and guinea grass (*Panicum* spp. syn. *Megathyrsus* spp.) (Baptistella et al., 2020; Gurgel et al., 2020). Recent literature suggests that root carbon inputs contribute equally or more to SOC than aboveground carbon inputs despite a smaller mass (Amsili e Kaye, 2021; Bispo et al., 2017; Chirinda, Olesen e Porter, 2012; Poeplau, 2016; Puget e Drinkwater, 2001; Zomer et al., 2017). Moreover, vigorous root systems contribute to soil aggregation (Demenois et al., 2018), due to organic compounds that bind soil particles, enhancing soil structure (Nikolaidis e Bidoglio, 2013). This process not only improves the soil’s water-holding capacity but also helps prevent soil erosion (Zhang et al., 2021). By anchoring the soil, deep roots can reduce the displacement of topsoil during heavy rains, a common issue in tropical regions (Abdallah et al., 2021; Chauhan et al., 2023).

Despite the potential benefits of maize-brachiaria grass and maize-guinea grass intercropping, the scientific literature currently lacks comprehensive information about the traits and biomass accumulation of their root systems. This gap in knowledge presents an opportunity for further research. Therefore, this study aims to contribute to this field by focusing on three key topics: (i) the root traits of maize-tropical forages intercropping, (ii) the vertical and lateral distribution of their root systems, and (iii) the total biomass and root biomass accumulated by each cropping system. By exploring these topics, this work information hope to provide valuable insights that could help these crops adapt to changing environmental conditions, improve soil carbon sinks, and provide helpful information to promote sustainable agricultural practices in tropical regions.

### 3.2. Material and Methods

#### 3.2.1 Site description

The field trial was carried out during the late seasons of the 2021 and 2022 in São Paulo State (22° 10’ 23” S 47° 13’ 55” W, 620 m altitude) following soybean harvest, characterized by a Cwa Köppen climate classification (Alvares et al., 2013). Data on precipitation and average air temperature are depicted in Figure 1. The field soil is classified as Oxisol Ustox (Embrapa, 2013), and the soil chemical and physical properties of the experimental area at 0-20 cm depth are: 5.2 ph (H2O), 30.9 g
dm-3 of soil organic matter, 27 mg dm$^{-3}$ of resin phosphorus, 13 mg dm$^{-3}$ sulfur, 3.1 mmolc dm$^{-3}$ aluminum, 4.3 mmolc dm$^{-3}$ potassium, 27 mmolc dm$^{-3}$ calcium, 11 mmolc dm$^{-3}$ magnesium, 51.4% base saturation, 1.39 kg dm$^{-3}$ bulk density, 515, 130 and 485 g ka$^{-1}$ of sand, silt and clay content respectively. In the five years preceding the study, the area was used for the cultivation of soybeans during the early season and maize during the late season.

![Figure 1: Precipitation, and mean temperature throughout months for the years of 2021 and 2022.](image)

3.2.2 Experimental design and crop management

The experimental arrangement utilized a split-plot design, organized into completely randomized blocks. Each block, replicated four times, consisted of plots measuring 5 m in width and 20 m in length. The treatment involved the cultivation of three species in monocropping and the intercropping arrangements between maize and each forage species, detailed as follows: (i) maize monocropping, (ii) brachiaria grass monocropping, (iii) guinea grass monocropping, (iv) intercropping of maize and brachiariagrass, and (v) intercropping of maize and guineagrass.

The maize hybrid Feroz Viptera3 were sown with population of 60,000 along with the forages Urochloa brizantha cv. Marandu and Megathyrsus Maximum cv. Mombasa. Both forage species were sown using population of 120,000 plants per ha. The maize crop inter-row space was 0.9m, and in both intercropping systems the tropical forage was sown in the middle of inter-row space between maize plants. Tropical forages monocropping were sown with 0.45m inter-row space. Maize and tropical forage monocultures, as well as their associated intercropping arrangements, were sown simultaneously on January 14th, 2021, and February 2nd, 2022. The maize was harvested on June 18th,
2021 and July 21st, 2022. Both brachiaria and guinea grasses were desiccated on October 1st, 2021 and October 25th, 2022 when assessing finished.

The experimental area was fertilized during crops sowing in all plots, supplying 50, 80, and 100 kg ha⁻¹ of N, P₂O₅, and K₂O respectively. When maize reached the V4 phenological stage, an additional 100 kg ha⁻¹ of N was side-dressed in all plots (Raij et al., 1997), and at the same stage, weed control was performed using 2500 g ai ha⁻¹ of atrazine and 28 g ai ha⁻¹ of nicosulfuron. The scheme presented in Figure 2 illustrates the treatments sown, and the sampling methodology information.

![Diagram](image)

**Figure 2:** The layout of the evaluated monocropping and intercropping systems, along with the transect representation adopted across these systems for root sampling.

### 3.2.3 Sampling and analyses methodology

The sampling of the above-ground and below-ground biomass was carried out at 30, 105, 150, and 250 days after sowing, matching the following periods, respectively: V4, VT, and R6 at grain harvest (maize phenology), and just previous of the new crop season (equivalent to the offseason end). As illustrated in Figure 2, the root system was collected at intervals of 0.2 m, up to a depth of 1 m, using a root sampling probe that was 1.2 m long and 0.055 m in internal diameter, with a collected volume of approximately 0.048 m³ (Otto et al., 2009). The sampling points were located on a transect starting from the crop row (0.0m), passing through an intermediate point (0.225 m) and ending at 0.45 m (Figure 2). After the collections were made, the soil samples were sieved and divided into soil and roots. Roots were scanned using a flatbed scanner (STD4800; Regent Instruments Inc., Canada) with a
resolution of 600 dots per inch (dpi). The images obtained were used to calculate the roots volume, surface area, and lengths for each probe core sample, using the WinRhizo software (Regent Instruments, Canada). Subsequently, they were subjected to forced drying at a temperature of 65 ºC for a duration of 72 hours and them weighed. The weights of the core probe samples were converted to area weight according to (Frasier et al., 2016).

3.2.4 Statistical analyses
The total and root biomass, root length, surface area and volume data were subjected to three spatial correlation models (mixed linear effect models), which allowed for the modeling temporal correlation between the observations. Thus, the model with the lowest Akaike Information Criterion (AIC) (Cavanaugh e Neath, 2019) was chosen for the analysis. For the root origin data, depth and position in the row, an analysis of variance and comparison of means were conducted using the Tukey test at a 5% probability level. The lem4 (Bates et al., 2015), emmeans (Searle, Speed e Milliken, 1980), and multcomp (Hothorn, Bretz e Westfall, 2008) packages for the R software (R Core Team, 2020) were used for this analysis. Contour maps were generated with the root biomass data obtained from probe core samples, and a Loess model was used for data interpolation before mapping.

3.3. Results
3.3.1 Roots length, superficial area and volume
At 30 days after sowing (equivalent to the V4 phenological stage in maize) the root length remained around 2,500 m ha⁻¹ for monocropping tropical forages (brachiaria and guinea grass), and around 4,000 m ha⁻¹ for both intercropping systems and monocropping maize, along the entire soil profile (Figure 3). At 105 days after sowing (equivalent to the VT phenological stage in maize), the root length in monocropping brachiaria increased at the first 0.2 m of soil depth, and both intercropping systems and monocropping maize increased their root length until 0.4 m soil depth. The tropical forages monocropping overlapped the root length of the other systems at 150 days after sowing (equivalent to the harvest stage in maize). At 250 days after sowing (equivalent to the off season end) the tropical forages monocropping maintained a higher root length at 0.2 m soil depth (around 7,500 m ha⁻¹), followed by intercropping systems (6,000 m ha⁻¹) and the smallest root length was recorded in the monocropping maize (4,500 m ha⁻¹). In deeper soil layers (0.8-1 m), the root length of the intercropping systems and monocropping maize was higher than that of the tropical forages monocropping.
Figure 3: (a) Roots length (m ha$^{-1}$), (b) superficial area (m$^2$ ha$^{-1}$), and volume (m$^3$ ha$^{-1}$) in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at 30 days (equivalent to the V4 phenological stage in maize), 105 days (equivalent to the VT phenological stage in maize), 150 days (equivalent to the harvest stage in maize), and 250 days after sowing (equivalent to the offseason end).

The root surface area remained around 1,500 m$^2$ ha$^{-1}$ for intercropping systems and monocropping maize, and around 1,000 m$^2$ ha$^{-1}$ for both brachiaria and guinea grass, throughout the entire soil profile (Figure 4) at 30 days after sowing. As the systems reached 105 days after sowing, the root surface area in both the monocropping maize, and intercropping systems increased, while that of brachiaria and guinea monocropping showed a decrease. By the time of 150 days after sowing, the
root surface area of brachiaria and guinea grass monocropping overlapped with that of the other systems at a soil depth of 0.6m. At 252 days after sowing, brachiaria and guinea monocropping maintained the largest root surface area at a soil depth of 0.2m (around 4,800 m² ha⁻¹), followed by intercropping systems (3,500 m² ha⁻¹) and the smallest root surface area was observed in monocropping maize (2,600 m² ha⁻¹).

Observing the period of 30 days after sowing, the root surface area was around 57 m³ ha⁻¹ for maize and approximately 38 m³ ha⁻¹ for both brachiaria and guinea grass monocultures. By 105 days, the root surface area in the monoculture brachiaria and both intercropping systems increased up to the soil depth of 0.4 m, surpassing that of the tropical forages in monoculture by 150 days. At 250 days, the largest root surface area at a soil depth of 0.2 m was maintained by the tropical forages monoculture (around 121 m³ ha⁻¹ for brachiaria and 118 m³ ha⁻¹ for guinea grass), followed by the intercropping systems (approximately 91 m³ ha⁻¹ for both maize-brachiaria and maize-guinea) and the smallest root surface area was observed in the monoculture maize (about 77 m³ ha⁻¹). However, in the deeper soil layers (0.8-1 m), the root surface area of the intercropping systems and monoculture maize was larger than that of the monoculture of tropical forages.

3.3.2 System total biomass and root biomass

Throughout the maize growth season, cropping systems including cereal consistently exhibited higher total biomass compared to those using just brachiaria and guinea grass (Figure 4). By 105 days after sowing, total biomass for maize monocropping was significantly higher (18.8 Mg ha⁻¹), compared to brachiaria and guinea grass monocropping (7.8 and 7.3 Mg ha⁻¹ respectively). However, the maize-brachiaria and maize-guinea intercropping systems presented the highest total biomass (around 21 Mg ha⁻¹) at this time. At 150 days after sowing, the total biomass of the guinea grass monocropping (14.7 Mg ha⁻¹) overlapped with brachiaria grass monocropping (12.9 Mg ha⁻¹), while the maize monocropping remained in an intermediary production (17.6 Mg ha⁻¹), and both intercropping systems persisted with the higher total biomass production (22.5 Mg ha⁻¹). By 250 days, the total biomass of maize monocropping was significantly lower than all other treatments, with tropical forages monocropping (14.5 and 12.6 Mg ha⁻¹ for guinea and brachiaria grass respectively) overlapping with the intercropping systems (7.7 Mg ha⁻¹).
Figure 4: (a) System total biomass (Mg ha\(^{-1}\)), and (b) root system biomass (Mg ha\(^{-1}\)) in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at 30 days (equivalent to the V4 phenological stage in maize), 105 days (equivalent to the VT phenological stage in maize), 150 days (equivalent to the harvest stage in maize), and 250 days after sowing (equivalent to the offseason end). Letters compare treatments at the same sampling time.

The root system biomass reveals a distinct pattern across different cropping systems (Figure 7). Initially, at 30 days after sowing, there was no difference in the root system biomass (0.06 Mg ha\(^{-1}\) respectively). Progressing to 105 days, maize monocropping witnessed an increase in total biomass to 1.8 Mg ha\(^{-1}\), outperforming brachiaria and guinea grass monocropping (0.95 and 0.53 Mg ha\(^{-1}\) respectively), while the intercropping systems did not differ from each other, and maize-brachiaria did not differ from maize monocropping (1.745 and 1.638 Mg ha\(^{-1}\) maize-brachiaria and maize-guinea respectively). By the 150 days after sowing, a significant increase was noted in the root biomass for brachiaria and guinea grass monocropping systems to 4.2 and 4.5 Mg ha\(^{-1}\) respectively, exceeding the other systems. At this time intercropping system recorded an intermediary root biomass (2.2 Mg ha\(^{-1}\)).
and maize monocropping had the small mean (1.2 Mg ha\(^{-1}\)). However, a decline was observed in all monocropping systems by 250 days after sowing, with maize monocropping recording the lowest root system biomass of 1 Mg ha\(^{-1}\), and tropical forages monocropping recording 3.8 Mg ha\(^{-1}\) of root biomass. The intercropping systems had a slight increase in root biomass at 250 days after sowing, recording 3.8 Mg ha\(^{-1}\).

3.3.3 Root biomass partitioning in the system

The root samples were collected at every 0.2 m interval of soil depth, down to a 1.0 m depth. The percent allocation of root system biomass across soil depth showed that in all cropping systems approximately 50% of root biomass was located in the initial 0.2 m layer (Figure 5). About 26% was situated in the 0.2-0.4 m layer, and 13% in the 0.4-0.6 m layer. The deeper layers altered their contribution over time. The 0.6-0.8 m layer accounted for 3% at 30 days after sowing, escalating to 6% at 250 days after sowing. The 0.8-1.0 m layer contributed 0.4% at 30 days after sowing, increasing its participation to 3.1% at 250 days after sowing.

At 30 days after sowing, the maize monocropping recorded the highest root biomass allocation at soil layers deeper than 0.6 m (7.7% and 1.4% respectively). At 105 days after sowing monocropped brachiaria grass recorded the highest root biomass in the 0.0-0.2m layer (64%), and monocropped guinea grass recorded the highest root biomass contribution at layers deeper than 0.4 m (12.5%, 5.2%, and 1.3% respectively). At 150 and 250 days after sowing, monocropped tropical forages increased their root biomass contribution in the 0.8-1.0m layer, reaching 5.6% and 4.6% for brachiaria and guinea grass monocropping, respectively.
Figure 5: Root system biomass origin (%) across soil layers in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at 30 days (equivalent to the V4 phenological stage in maize), 105 days (equivalent to the VT phenological stage in maize), 150 days (equivalent to the harvest stage in maize), and 250 days after sowing (equivalent to the offseason end).

Root biomass sampling in the cropping systems was conducted using a transect that originated at the main crop sowing row and was repeated at 0.225m and 0.45m intervals, corresponding to an intermediary point and the midpoint of the inter-row spacing of the main crops. The distribution of root biomass across the transect exhibited a pattern over time (Figure 6, with maize monocropping recording the highest root biomass accumulation (mean of 68% over time) at 0.0m (main crop row), and the tropical forages monocropping registering the highest root biomass accumulation (mean of 40% over time) at 0.45m (inter-row midpoint). The intermediary point in the transect (0.225m) began with a higher allocation in maize monocropping (43% and 45%) at 30 and 105 days after sowing respectively, while the intercropping system surpassed maize monocropping at 150 and 250 days after sowing, recording the highest biomass accumulation (36% and 39% respectively).
Figure 6: Root system biomass origin (%) across the sampling transect position in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at 30 days (equivalent to the V4 phenological stage in maize), 105 days (equivalent to the VT phenological stage in maize), 150 days (equivalent to the harvest stage in maize), and 250 days after sowing (equivalent to the offseason end).

3.4. Discussion

The relationship between root traits and the origin and accumulation of biomass in these cropping systems is a novel data contribution, not previously reported in literature. These root traits, such as length, volume, and surface area, as highlighted by (Kaysar et al., 2022), are known to exhibit a strong correlation with the root biomass in the system. This correlation was consistently observed across all treatments throughout the field trial (Figures 3 and 4). According to (Collins, Wright e Wurzburger, 2016), these root functional traits are related to the resource acquisition capacity of plants in the environment. This correlation explains why a higher total root length, surface area, and volume in the cropping system leads to a positive feedback relationship in biomass accumulation.

Early plant growth was significantly influenced by maize, resulting in the highest total biomass for maize monocropping and both maize-brachiaria and maize-guinea grass intercropping until harvest. Which could be related to the fast growth and biomass accumulation of maize plants,
compared to tropical forages (Portes et al., 2000; Zhou et al., 2023). However, both brachiaria and guinea grass begin to overlap with the maize monocropping and intercropping total and root biomass at 150 days after sowing. At maize flowering, the plant reaches its peak of root growth (Gao et al., 2022), and starts to mobilize photoassimilates to its reproductive organs (Lal et al., 2022), creating strong sinks that compete with roots at this maize phenological stage point. While tropical forages mainly direct their sinks to vegetative organs (Shakya e Lal, 2018), focusing more energy on root system development.

The root plant biomass decreases with increasing distance from root crown (Sultan et al., 2020), helping explain the root distribution across the systems (Figures 5 and 6). According to Barber (1995) and Laboski et al. (1998), most of maize roots are distributed in the top 0-0.2m layer, which corroborates the results found in this research. In contrast, brachiaria is known for its aggressive root growth and significant biomass production (Merloti et al., 2023), explaining the high density of biomass distribution in the soil of this species (Figure 7 and 8). Among tropical forages, guinea grass presented the highest biomass contribution at soil layers deeper than 0.4m at 105 days after sowing, and the highest total biomass accumulation at 250 days after sowing, showing the potential of this species to accumulate biomass in tropical climates with seasonal moisture deficits, corroborating the results found by (Huot et al., 2020).

The variability in spatial and temporal distributions of plant roots across different crop species (Liu et al., 2011) contributes to the complexity of intercropping systems, making their responses more intricate compared to those observed in monocropped systems. The total biomass of intercropping systems surpassed that of maize monocropping after 105 days. This could be due to the minimal decrease in maize biomass when intercropped with tropical forages, and the addition of the tropical forages biomass in the system, despite their lower biomass input due to light competition with maize (Crusciol et al., 2020; Makino et al., 2019). Intercropping of crops with contrasting growth habits can improve primary resource use in both spatial and temporal contexts due to improved root distribution and interactions between the species (Neykova et al., 2011). Furthermore, the exploration of a higher soil volume in intercropping systems could enhance nutrient cycling and water use efficiency, thereby potentially increasing crop productivity (Kaysar et al., 2022). In the context of maize monocropping, the root system presented a consistent distribution over the transect, primarily at the crop row, barely surpassing the 0.225m point and not reaching the inter-row middle point (Figures 7 and 8).
Figure 7: Root system biomass density (g 0.00048 m$^{-3}$) across the sampling transect position in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at (a) 30 days (equivalent to the V4 phenological stage in maize), and (b) 105 days (equivalent to the VT phenological stage in maize).
Figure 8: Root system biomass density (g 0.00048 m$^{-3}$) across the sampling transect position in maize, brachiaria and guinea grass monocropping and maize-brachiaria and maize-guinea grass intercropping at (a) 150 days (equivalent to the harvest stage in maize), and (b) 250 days after sowing (equivalent to the offseason end).

This distribution pattern leaves a biological gap in the system, considering the soil volume that could be explored by the plant, where other species could potentially grow, thereby exploring a larger total soil volume (Hauggaard-Nielsen, Ambus e Jensen, 2001). This could significantly contribute to soil carbon sequestration. The root system interacts with soil microorganisms, playing a crucial role in the process of carbon fixation (Jiang et al., 2022). An increase in root biomass and the exploration of a
larger soil volume enhances the soil’s ability to sequester carbon (Yang et al., 2024). This is especially pertinent in the context of intercropping systems. Thus, diversity of root traits among different crop species can lead to a more efficient utilization of the soil volume (Bardgett, Mommer e Vries, De, 2014).

Contrary to maize plants, which predominantly contribute to the above-ground biomass in both intercropping systems, tropical forage species notably make their significant contribution to the intercropping biomass via their root system. The exploration of a higher soil volume in intercropping systems could also enhance nutrient cycling and water use efficiency, while also protecting the soil against erosion through the residues over the soil and the aggregation promoted by the root system in tropical environments (Cagna et al., 2019). Understanding the complex interactions between different crop species and their root systems is crucial for optimizing agricultural practices and enhancing crop productivity (Villordon, Ginzberg e Firon, 2014). Optimizing root growth and distribution can be an effective strategy for increasing carbon stocks in the soil and promoting sustainable agricultural practices (Heinemann et al., 2023).

**3.5. Conclusion**

The study indicates a superior performance of maize-brachiaria grass and maize-guinea grass intercropping systems over maize monocropping in terms of biomass production and root development. The total biomass was found to be 25% higher in intercropping systems at maize harvest. This suggests that intercropping systems are more efficient in utilizing resources below-ground and enhancing biomass productivity. In terms of root biomass, monocropping maize accumulated 1.2 Mg ha$^{-1}$, while intercropping and tropical forages monocropping systems showed significantly higher accumulations, with increases of 90% (2.3 Mg ha$^{-1}$) and 270% (4.4 Mg ha$^{-1}$) respectively. Moreover, up to 40% of the root biomass in intercropping systems comes from the maize inter-row where tropical forages were set using a single row, indicating the substantial contribution of these forages to the overall root biomass of the systems.

Root volume, area, and length in intercropping systems were all considerably higher than in monocropping maize at the off-season end. Specifically, the root volume and length were 26% higher, and the root area was 40% higher in intercropping systems. Horizontal and vertical distribution of roots of two intercrops patterns investigated indicated that intercropping was an effect pathway to improve root distribution relative to the monocrop maize. These findings underscore the potential of maize-tropical forages intercropping in contributes to the aggregation and protection of the soil against erosion process, promoting nutrient cycling and enhanced potential for deep carbon fixation in the soil.

In conclusion, intercropping systems demonstrate a clear advantage over monocropping maize in terms of both above-ground and below-ground productivity. This highlights the potential of these
systems in increase deep soil biomass, offering opportunities for improved carbon fixing and soil health. Further research could explore the specific mechanisms behind the interaction of both species root systems, and the contribution of each species to the production of roots in this intercropping system.

References


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