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# Maize Morphophysiological Changes Modulated by Cover Crops Rotation in Northeast Brazil

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**Abstract:** Cover crops have gained attention due to their potential benefits for the soil and physiological performance of subsequent crops. This study aimed to evaluate the physiological and productive aspects of maize grown in succession to cover crops in northeastern Brazil. A randomized complete block design with four repetitions was employed, in which the treatments consisted of the following cover crops: sunn hemp, spectabilis, pigeon pea, *Brachiaria* sp., jack bean, millet, and fallow. Physiological aspects and production components of maize were evaluated at the tasseling (VT) and smooth grain (R3) phenological stages. Millet cover increased carotenoid content in maize leaves by up to 78% at R3. Maize grown after pigeon pea, millet, and *Brachiaria* sp. showed up to 42% greater $CO_2$ assimilation efficiency compared to jack bean. Carboxylation efficiency increased by up to 34% in maize grown after millet and *Brachiaria* sp., while water use efficiency improved by up to 76% in maize after sunn hemp and pigeon pea at R3. Sunn hemp, spectabilis, and jack bean reduced soil temperature by 2 °C compared to fallow. The highest maize yield was observed after jack bean, with an 8% increase over fallow. These findings demonstrate the benefits of incorporating cover crops into maize cultivation systems in the semi-arid region of Brazil.

**Keywords:** *Zea mays* L.; Poaceae; Fabaceae; *Crotalaria juncea*; sustainability; agricultural; yield

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## 1. Introduction

Maize (*Zea mays* L.) is one of the world's most important agricultural crops, holding significant socio-economic relevance due to its role in generating income in rural communities and its utilization in human and animal nutrition [1]. In Brazil, green maize cultivation is predominantly undertaken by small-scale and medium-scale farmers, who utilize the produce for fresh market consumption and silage for animal feed.

In 2022, Brazil produced 109.4 million tons of maize, with an average yield of 5.2 Mg ha$^{-1}$. This achievement highlights the country's agricultural prowess. However, within Brazil, there are significant regional disparities in maize yield. In the Northeast Region, maize production reached 8.8 million tons, with an average yield of 3.1 Mg ha$^{-1}$. Notably, despite being part of the Northeast Region, the state of Alagoas only managed to produce 48.1 thousand tons of maize in 2022, with an average yield of a mere 2.3 Mg ha$^{-1}$ [2]. This stark contrast in productivity results from the distinct agricultural practices employed in Alagoas state compared to other regions. In Alagoas, maize crops are traditionally carried out by small-scale farmers whose lack of access to modern agricultural technologies promote a lacking of proper mechanization, fertilization, and irrigation. This reliance on outdated methods has hindered productivity gains, resulting in significantly lower yields compared to other regions that have embraced technological advancements in agriculture.

The lack of fertilization and persistent water stress during green maize crops are major hindrances to achieving potential crop yields in Northeast Brazil. These factors contribute significantly to the region's overall low maize yield [3]. A promising solution lies in cultivating green maize in succession with cover crops (CCs), particularly legumes. This practice can enhance soil organic carbon, improve soil structure, and reduce nitrate leaching, leading to better water retention and nutrient availability for crops, ultimately boosting agricultural productivity [4] (Figure 1).

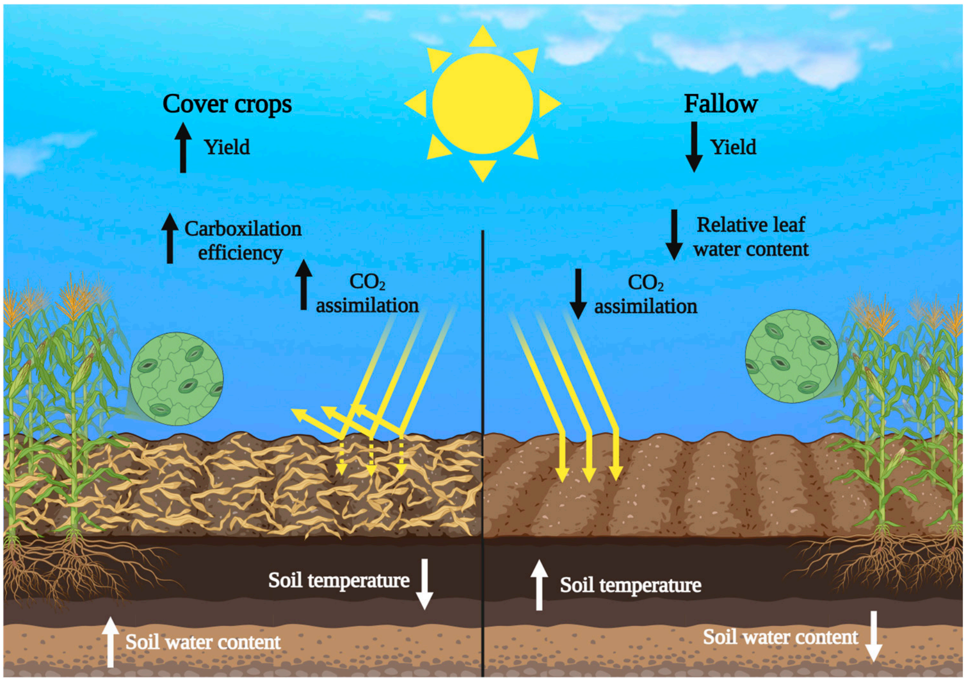


**Figure 1.** Impact of cover crops vs fallow on corn ecophysiology. Black upward arrows indicate the positive effect of cover crops on maize compared to fallow (black downward arrows). White arrows compare the impact of the two cultivation systems (with cover crops versus fallow) on the soil, showing improvements in the system with cover crops through a reduction in soil temperature (downward white arrow) and greater water availability (upward white arrow). This happens because less solar radiation is intercepted (yellow arrow) due to the protection provided by the cover crops.

The crops species most used in crop rotation systems in Northeast Brazil include *Mucuna aterrima*, *Crotalaria juncea*, *Cajanus cajan*, *Pennisetum glaucum*, and *Crotalaria spectabilis* [4], with positive effects on soil microbial attributes, organic matter quality, biomass production, and nutrient cycling [5].

Like other agricultural crops, maize exhibits a rapid response to unfavorable conditions that limit nutrient availability. When essential nutrients are scarce, $CO_2$ assimilation plummets, hindering plant growth. Conversely, under optimal nutrition and sound management practices, maize retains high leaf chlorophyll levels, promoting a vigorous $CO_2$ assimilation and assimilating production, ultimately leading to greater biomass accumulation. This highlights the crucial role of adequate plant nutrition and management strategies in maximizing nutrient utilization and optimizing maize [6].

While the existing literature has explored the benefits of growing maize in succession to cover crops (CCs), there remains a significant knowledge gap regarding the physiological and productive responses of green maize in Brazil's semi-arid region, particularly in the State of Alagoas. Previous studies [7,8] have focused on different regions or agricultural contexts, failing to specifically address how crop rotation practices impact the performance of green maize in environments with water limitations and low fertilization. Most research has not considered the diversity of locally used CCs and their combined effect on improving maize productivity. Furthermore, few studies have investigated the interaction between the adoption of cultivation technologies and the traditional practices of small farmers, who are predominant in the region. By examining these aspects, this study aims to fill this gap, providing detailed information on how CCs can optimize the physiology of green maize and ultimately enhance yields under semi-arid conditions, which is crucial for sustainability and food security in the region.

This research hypothesizes that CCs cultivated in rotation enhance the physiological attributes of green maize, leading to improved yield. To test this hypothesis, physiological aspects, and production components of green maize grown in succession to CCs in the semi-arid region of Brazil.

## 2. Results

### 2.1. Cover Crop Yield

There was significance ($p < 0.0001$) observed for the fresh and dry mass yield of CCs. The sunn hemp and spectabilis species achieved the highest fresh mass yield and were statistically equal, with a general average yield of 22.9 Mg ha$^{-1}$. Meanwhile, pigeon pea and *Brachiaria* sp. generated the lowest fresh mass yield, with a general average of 2.8 Mg ha$^{-1}$, a yield 8 times lower compared to the highest value obtained. The highest dry mass yield was obtained with sunn hemp, with an average value of 6.0 Mg ha$^{-1}$, and lower dry mass values were obtained with pigeon pea and *Brachiaria* sp., 0.8 Mg ha$^{-1}$, which is 7.5 times lower compared to the highest dry mass productivity (Figure 2).

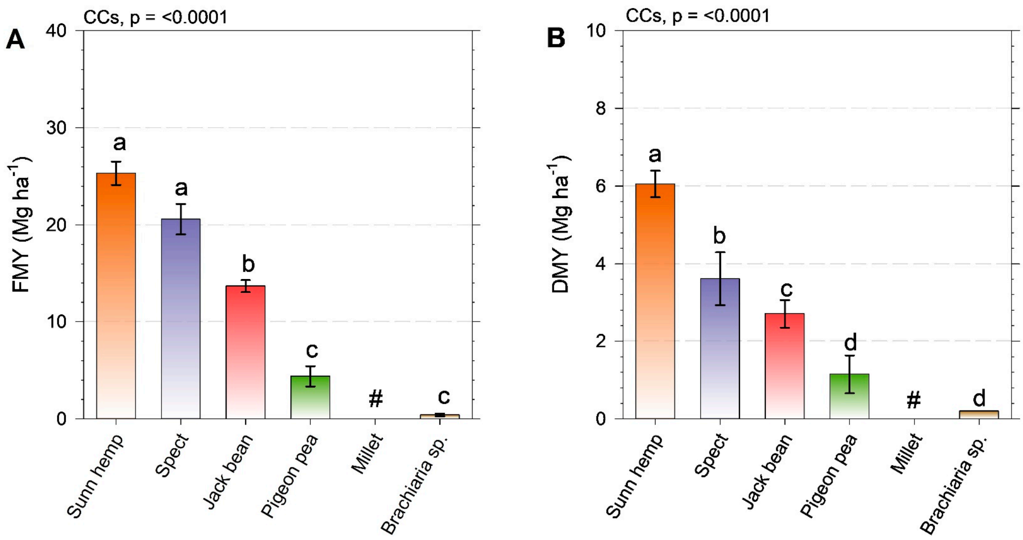


**Figure 2.** (**A**) Fresh mass yield (FMY) and (**B**) dry mass yield (DMY) of cover crops. # millet did not complete the cycle. Means followed by the same letter do not differ statistically from each other by the HDS test ($p \leq 0.05$).

### 2.2. Physiological Aspects and Yield of Green Maize

In this study, significance ($p \leq 0.05$) was observed for the variables: *Chlorophyll b*, a/b and total, carotenoids, relative water content in the leaf, $CO_2$ assimilation rate, transpiration, stomatal conductance, internal $CO_2$ concentration, carboxylation efficiency, water use efficiency, and soil temperature.

The concentration of chlorophyll *a* ($C_A$) in maize leaves was not influenced by CCs regardless of the maize phenological stage (St). This might be due to this pigment being highly resilient, remaining stable and effective in photosynthesis even when exposed to different environmental conditions, including those introduced by CCs (Figure 3A).

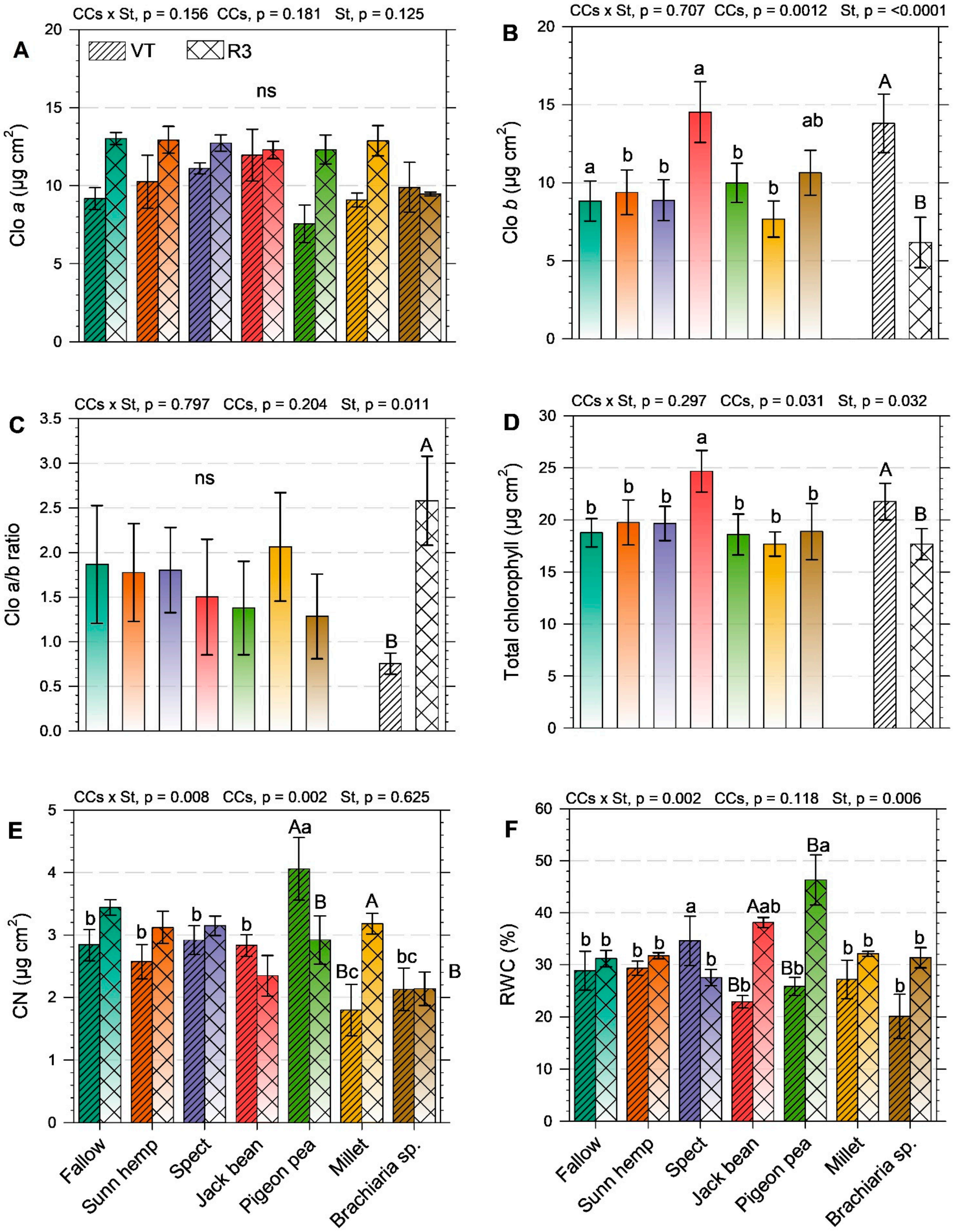


**Figure 3.** (**A**) Clorofila a ($C_A$), (**B**) clorofila b ($C_B$), (**C**) ratio clo a/b ($C_{AB}$), (**D**) clorofila total ($C_T$), (**E**) carotenoid content ($C_N$), and (**F**) leaf relative water content (RWC) in maize leaves under different cover crops. Means followed by the same letter do not differ statistically from each other by the HDS test for CCs and t-test for stage ($p \leq 0.05$). Letters were only shown where the effects were significant. ns: not significant by Fisher's test.

Maize leaves grown under jack bean cover presented the highest chlorophyll *b* ($C_B$) content, with 14.5 (µg $cm^2$), surpassing other species. Significant differences were observed between the $C_B$ means in the VT and R3 stages, with VT showing an average of 13.8 and R3 6.2 (µg $cm^2$) (Figure 3B).

The chlorophyll *a*/*b* ratio showed a significant difference only between the St, with the average in the VT stage being 2.4 times lower compared to R3 (Figure 3C). Total chlorophyll ($C_T$) concentrations varied significantly between both CCs treatments (p = 0.031) and St stages. Maize following jack bean had an average of 25.6 µg $cm^2$, statistically differing from the other CCs. The VT stage was statistically better than R3, with an average 23.1% higher than the R3 stage. The interaction between CCs and St was not significant (Figure 3D).

Carotenoid ($C_N$) levels showed a significant interaction (CCs × St). Maize following pigeon pea had the highest $C_N$ average in VT, with 4.05 µg $cm^2$, compared to other CCs. However, in R3, there was a 28.15% decrease compared to the VT stage. Plants in succession to millet had the lowest $C_N$, with 1.76 µg $cm^2$ in VT, compared to the other CCs. However, in R3, there was an increase of 77.65% (Figure 3E).

Relative water content in the leaf (RWC) varied significantly between CCs and St stages, with pigeon pea and jack bean in the VT stage having the highest RWC average, with 46.3 and 38.1 µg $cm^2$ compared to 25.8 and 22.9 µg $cm^2$ in R3, meaning there was a reduction of 44.2 and 39.8%, respectively, between the phenological phases (Figure 3F).

The $CO_2$ assimilation rate (*A*) was influenced by the interaction of CCs × St. Maize in VT had higher *A* compared to R3. Maize following jack bean had the lowest average *A*, with 12.8 µmol $CO_2$ $m^{-2}$ $s^{-1}$, statistically differing from the other CCs. Greater $CO_2$ assimilation was observed in maize plants grown in succession to pigeon pea, millet, and *Brachiaria* sp., with an increase of up to 42% compared to those grown in succession with jack bean (Figure 4A).

The transpiration rate (*E*) showed significant differences between the simple effects of CCs and St, although there was no significant interaction. Maize following millet, fallow, and *Brachiaria* sp. had the highest average E, ranging from 3.2 to 3.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$. These results highlight the importance of treatments and development stages in regulating plant transpiration (Figure 4B).

Stomatal conductance ($g_s$) varied only with the simple effects of CCs and St. Maize following fallow and millet were superior, followed by *Brachia Ria* sp., with the others being lower and not differing from each other. Overall, conductance was higher in the tasseling stage (VT) than in the milk grain stage (R3) (Figure 4C).

Internal $CO_2$ concentration varied significantly only among the CCs, with *Brachiaria* sp. showing the highest internal $CO_2$ concentration (98.3 µmol $CO_2$ $m^{-2}$ $s^{-1}$), 81% lower than jack bean (178.8 µmol $CO_2$ $m^{-2}$ $s^{-1}$) (Figure 4D). Carboxylation efficiency (EC) was higher in the VT stage (0.21 µmol $CO_2$ $m^{-2}$ $s^{-1}$ $mol^{-1}$) compared to R3. For the effects of CCs on EC, *Brachiaria sp*. and millet were statistically equal and had the highest averages, with values of 0.23 and 0.19 µmol $CO_2$ $m^{-2}$ $s^{-1}$ $mol^{-1}$, respectively, and an increase of up to 34% in relation to fallow (Figure 4E).

Water use efficiency (WUE) was strongly influenced by CCs ($p$ = 0.003) and the interaction between CCs × St. The results suggest that both sunn hemp and pigeon pea exhibit significantly higher WUE in VT compared to R3. The WUE of sunn hemp showed an increase of 39% in VT compared to R3, while pigeon pea showed an even more pronounced increase, with 76% higher in VT. In the R3 stage, the highest WUEs were observed under *Brachiaria* sp., millet, and fallow, with 6.98, 6.60, and 6.22 µmol $CO_2$ $mmol^{-1}$ $H_2O$, respectively (Figure 4F).

Soil temperature ($T_{SOIL}$) was altered only by the simple effects of CCs and St. For the effects of CCs, the average temperature ranged from 29.5 to 31.5 °C. Sunn hemp, spectabilis, and jack bean, on average, reduced $T_{SOIL}$ by ~2 °C compared to fallow, while the other CCs had no effect. Among the Stages, $T_{SOIL}$ was lower in R3, with a reduction of 3.7 °C from VT to R3 (Figure 5A).

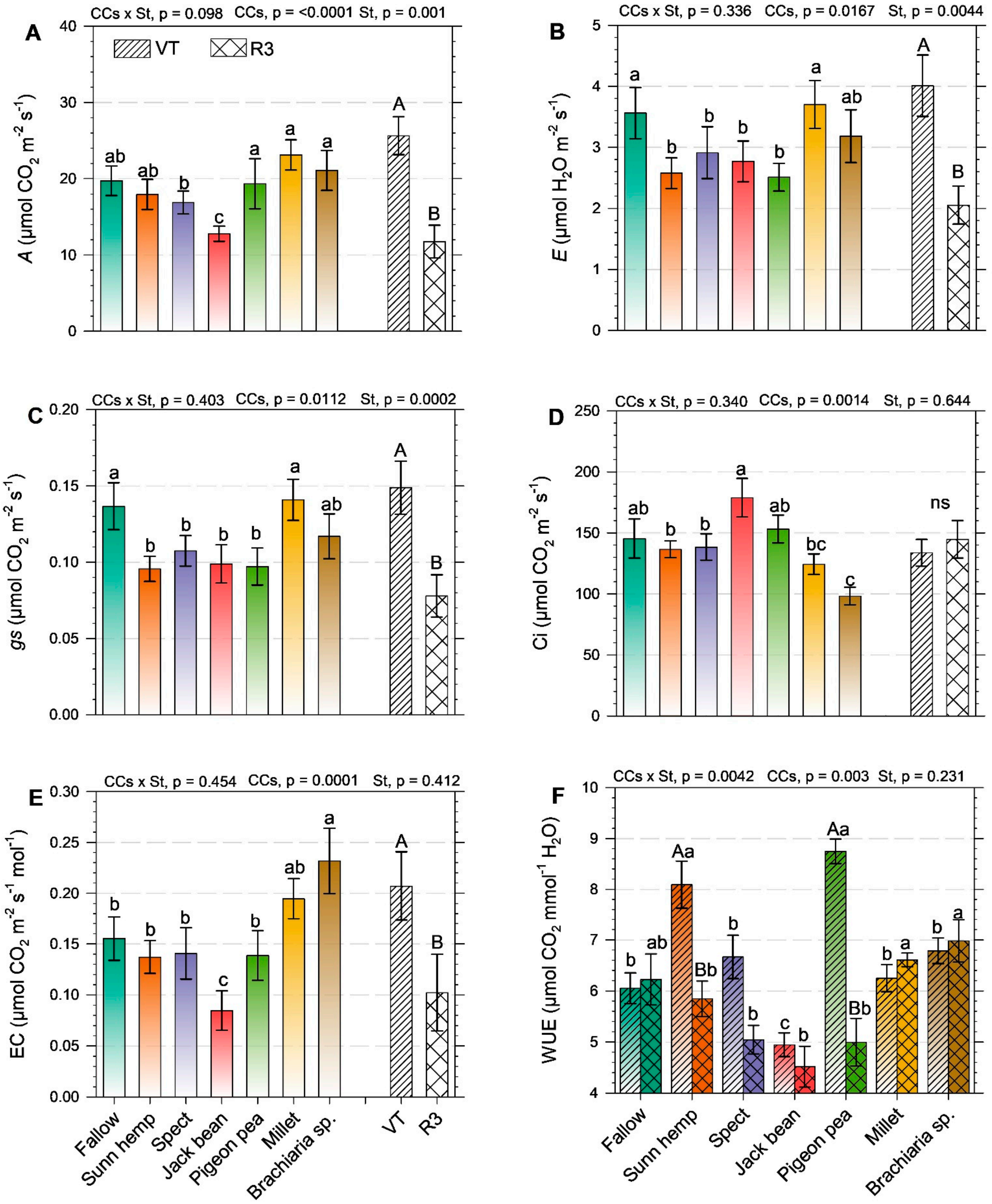


**Figure 4.** Gas exchange: (**A**) $CO_2$ assimilation (*A*); (**B**) transpiration rate (*E*); (**C**) stomatal conductance ($g_s$); (**D**) intercellular $CO_2$ concentration ($C_i$); (**E**) carboxylation efficiency (EC); and (**F**) instantaneous water use efficiency (WUE) in leaves of maize grown in succession to cover crops in Arapiraca, Alagoas. Means followed by the same letter do not differ statistically from each other by the HDS test for CCs and t-test for stage ($p \leq 0.05$). Letters were only shown where the effects were significant.

Maize productivity was significantly influenced by the CCs. Jack bean showed the highest productivity, with 15.5 Mg ha$^{-1}$, an 8% increase compared to fallow (14.3 Mg ha$^{-1}$). Spectabilis and *Brachiaria* sp. showed similar responses, though with a smaller effect size (~2%).

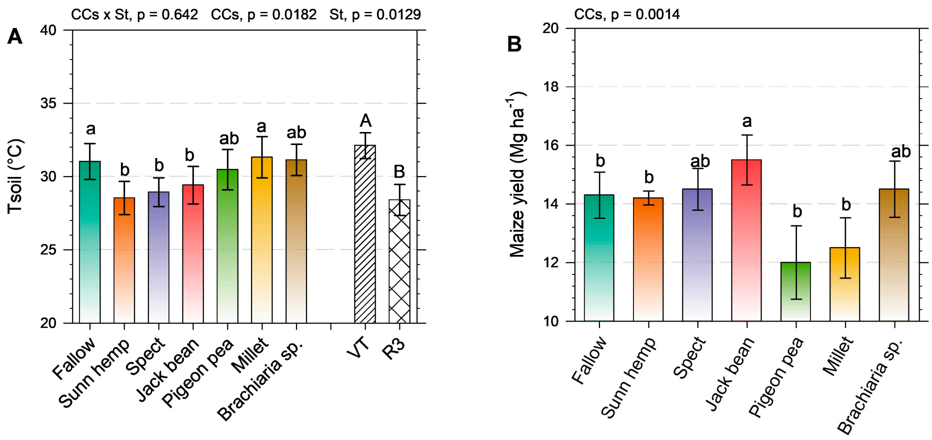


**Figure 5.** (**A**) Soil temperature ($T_{SOIL}$) during the VT and R3 stages of maize, and (**B**) maize yield in succession to cover crops in Arapiraca, Alagoas. Means followed by the same letter do not differ statistically from each other by the HDS test for CCs and t-test for stage ($p \leq 0.05$). Letters were only shown where the effects were significant.

## 2.3. Multivariate Comparisons

Dimensionality reduction using principal component analysis (PCA) showed slight adjustments promoted by the CCs in the dissimilarity of treatments between maize stages. In VT, only millet and *Brachiaria* sp. did not differ from fallow. These treatments were positively associated with higher $T_{SOIL}$, gs, $E$, chlorophyll $a/b$ ratio, and electrolyte leakage in maize, which were linked to the low biomass inputs from the CCs. The greatest Mahalanobis distances from fallow were observed with sunn hemp (5.1) and jack bean (4.6). Sunn hemp was associated only with the high biomass inputs from the CCs, which was essential for reducing the expression of variables that were high in fallow. However, in VT, these gains did not directly influence maize productivity. This latter variable was associated with chlorophyll $a$, $b$, and total content, all of which were strongly linked to jack bean (Figure 6A).

In R3 (the green maize harvest point, milk grain stage), jack bean continued to be associated with higher maize productivity and had the greatest Mahalanobis distance from fallow (4.1), followed by spectabilis (3.8) and sunn hemp (3.7). Here, only *Brachiaria* sp. was associated with $T_{SOIL}$. Millet and fallow exhibited higher gas exchange ($A$, $E$, $g_s$, EC, and WUE), especially fallow with carotenoids and chlorophyll $a/b$ ratio, but were negatively associated with maize productivity. This suggests that part of the photoassimilates produced at this stage were spent on secondary metabolism to mitigate the effects of stress that occurred in VT in these treatments, which may have resulted in lower net photosynthesis for translocation to the grains. These findings were only clearly observed with the use of PCA.

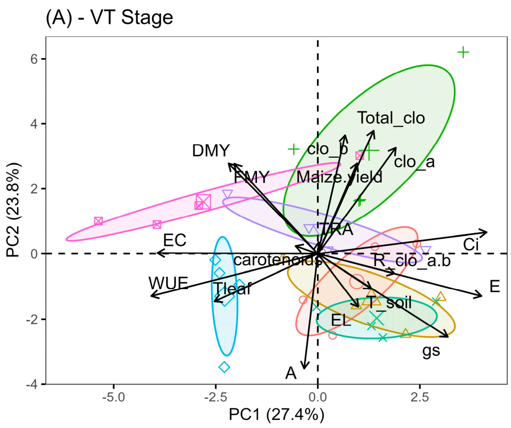


**Figure 6.** *Cont.*

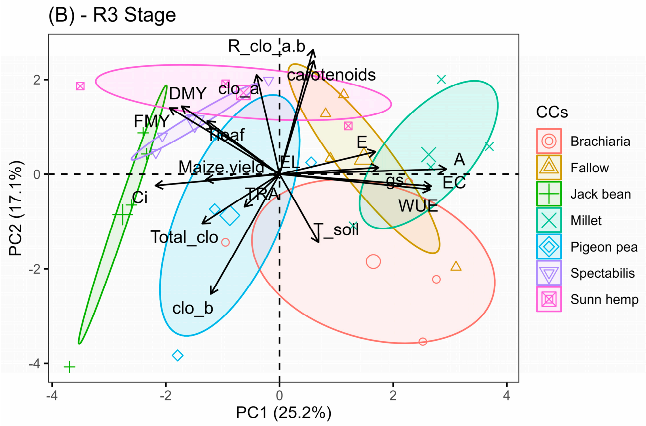


**Figure 6.** Biplot of the first two main components (PC1 and 2) that explain the variation in morphophysiological responses in two phenological stages of corn (VT in (**A**), and R3 in (**B**)) grown in succession to different cover crops. The ellipses function as confidence limits ($p \leq 0.05$). Tsoil: soil temperature; Tleaf: leaf temperature; *A*: $CO_2$ assimilation rate; *E*–transpiration rate; Ci: intercellular $CO_2$ concentration; EC: carboxylation efficiency; WUE: instantaneous water use efficiency; RWC: leaf relative water content; clo_a: chlorophyll a; clo_b: chlorophyll b; R_clo_a.b: Ratio chlorophyll a/b; Total_clo: Total chlorophyll; FMY: Fresh mass yield; DMY: dry mass yield.

## 3. Discussion

Cover crops (CCs) can have a significant impact on nutrient cycling, which can influence the growth and development of commercial crops [9]. They can increase water availability for the following crop by reducing evaporation and improving soil water storage [10]. However, the effect on soil water content varies depending on the type of cover crop species and the prevailing climatic conditions. This variability can potentially lead to a decrease in water storage for primary agricultural production in arid regions [11].

Regions with air temperatures ranging from 10 to 35 °C are classified as suitable for green maize cultivation [12]. Therefore, the Arapiraca region has sufficient thermal availability to meet the demand for green maize. In a study on the fresh and dry mass productivity of CCs under the edaphoclimatic conditions of the semi-arid region of Alagoas, [13] found that jack bean performed superiorly with 24 Mg ha$^{-1}$ compared to other species used as CCs, the arboreal pigeon pea had a yield of 15.7 Mg ha$^{-1}$, and the common pigeon pea had a yield of 18.2 Mg ha$^{-1}$. However, the results obtained in this research indicate a lower yield of pigeon pea among the cover crops analyzed and indicate a greater potential for adaptation of sunn hemp.

CCs differ in their vegetative cycle, which impacts the accumulation of fresh and dry mass, as well as the amount of residual straw in the soil [14]. The study mentions that jack bean has a short cycle, which favors biomass accumulation when CCs need to be cultivated in a shorter period. Therefore, it is evident that jack bean has characteristics that favor biomass accumulation in a shorter period, surpassing other species in our study, except for sunn hemp, in relation to fresh mass yield (FMY). This trend of jack bean can result in greater straw cover on the soil for successive crops.

Our results and others in the literature [4,13,15] confirm that jack bean achieves higher dry mass yield (DMY) compared to other species. This response can increase straw cover on the soil, consequently contributing to increased water retention and reduced soil temperature. These mechanisms contribute to increased water use efficiency (a key limiting factor in semi-arid regions) for commercial crops, as observed in the study.

Additionally, Oliveira et al. [16] stated that Poaceae species preceding maize increased fertilizer use efficiency, improved soil water retention, and maximized photosynthetic capacity. This, in turn, facilitates the development of plants with larger vegetative organs and higher carbohydrate content. Taiz et al. [17] affirms that these characteristics are desirable and contribute to increased biomass due to carbohydrate accumulation and assimilation, which can result in higher agricultural yields.

In studying the impact of CCs on maize's physiological attributes in succession, Aker [18] observed that *chlorophyll b* levels significantly decreased throughout the maize development cycle in all treatments (CCs), with the greatest reductions occurring in the final stages, especially between the dough grain stage and physiological maturity, where losses reached 55.1% compared to the initial levels observed in the tasseling stage (VT).

The increase in the chlorophyll $a/b$ ratio in the R3 stage may indicate selective degradation of chlorophyll *b*, without impacting maize yield. The reduction in total chlorophyll observed in R3 vs. VT suggests degradation due to senescence and nutrient retranslocation during the phenological cycle, explaining the reduction observed between VT and R3. The phenological progression of maize tends to decrease chlorophyll levels after the VT stage, as reported by Shashishekhar et al. [19].

Our results suggest that the higher total chlorophyll content obtained by maize following jack bean may be related to the decomposition rate of this plant. [20] found that the millet + jack bean consortium showed faster decomposition compared to millet straw alone, attributing this speed to the lower C/N ratio of jack bean. Although urea was applied in all plots, the increase in maize chlorophyll under jack bean may be justified by the contribution via biological nitrogen fixation (BNF), as well as the plant containing more soluble organic compounds that can stimulate the microbiota and nitrogen uptake mechanisms in maize [21].

The higher concentration of carotenoids in maize under pigeon pea may be attributed to a beneficial interaction between the two crops. When cultivating maize in succession to Poaceae species, Oliveira et al. [16] observed increased soil water retention and maize photosynthetic capacity, resulting in plants with larger vegetative organs and higher carbohydrate content. Taiz et al. [17] reinforces these benefits, highlighting the increase in biomass and agricultural yield. Therefore, cultivating green maize on Poaceae straw emerges as a promising strategy to enhance both photosynthetic efficiency and crop yield. [22] observed that maize cultivated in succession to CCs showed improvements in photosynthesis and transpiration rates, as well as variations in these variables at different growth stages, corroborating our results. The similarity between fallow, millet, and *Brachiaria* sp. may be related to the low or nonexistent biomass production, as in millet, since Santos et al. [23] states that the benefits of the no-tillage system become more visible from 6 t ha$^{-1}$ of residue on the soil.

One way to determine the performance of maize in sustainable systems is through the analysis of chlorophyll and carotenoid levels. Our research demonstrated that differences in chlorophyll and carotenoid levels have significant biological implications for the growth of maize in succession to cover crops. Chlorophyll is essential for photosynthesis, allowing plants to capture sunlight and convert it into energy, while carotenoids act as antioxidants and help protect leaves from damage caused by excessive light and environmental stress [17]. When maize is cultivated after cover crops, as in this study, and chlorophyll levels increase, the photosynthetic capacity of the crop can be optimized, resulting in higher biomass production. Additionally, adequate levels of carotenoids can enhance resistance to biotic and abiotic stresses, promoting more robust growth.

The intermediate $g_s$ values in corn following *Brachiaria* sp. compared to other cover crops can be attributed to the moderate adaptation of *Brachiaria* sp. to water variation [24]. Maize following *Brachiaria* sp. had the lowest internal carbon concentration, attributed to lower nutrient recycling efficiency, according to Dias et al. [25]. Oliveira et al. [26] suggest that this is due to increased soil organic matter and nutrient availability.

Gas exchange in plants plays a crucial role in agricultural production. The rate of $CO_2$ assimilation is a fundamental indicator of a plant's ability to convert sunlight into chemical energy, directly influencing the growth and productivity of crops. Crops with higher photosynthesis rates tend to produce more biomass, consequently increasing yields. In this study, we observed that certain cover crops favored photosynthesis in maize more than others, indicating that factors such as soil moisture availability provided by a thicker ground cover, along with greater nutrient availability for subsequent crops, can become determining factors for the adoption of cover crops by producers. On the other hand, water use efficiency refers to the plants' ability to absorb and utilize the available water for growth and production [17]. In water-scarce environments, such as the Brazilian semi-arid region, crops that optimize water usage are essential for ensuring sustainability and productivity. The interaction between these parameters is also important, as conditions that favor photosynthesis, such as adequate water availability, can enhance production, while abiotic factors, such as water stress, can limit photosynthetic efficiency.

The implementation of the no-tillage system has been associated with an increase in soil organic carbon (SOC) stocks, which is particularly evident in soils with initially low SOC concentrations. This increase in SOC stocks can have a beneficial impact on maize carboxylation efficiency [27,28]. In studying the use of CCs in the semi-arid region of Brazil, [4] observed that soils cultivated with millet had higher SOC averages compared to soils cultivated with spectabilis and pigeon pea. These results suggest that the choice of CCs can significantly influence SOC levels and, possibly, carboxylation efficiency (EC), due to the nitrogen present in the straw mainly in the form of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), responsible for assimilating atmospheric $CO_2$ [29].

Indeed, CCs contribute to organic matter storage, improving soil hydrology, which can potentially increase soil water retention and availability for plants [4,30]. In areas with sequential jack bean cultivation, [4] reported a greater increase in soil organic carbon compared to the species studied in this research. According to Vendig et al. [28], this increase in organic carbon content directly influences soil quality, increases fertility, and ultimately leads to higher maize yields. These findings validate the results obtained in this research, showing that green maize cultivation in areas previously planted with jack bean resulted in higher maize yield.

Here we demonstrate that cultivating green maize in succession to CCs has physiological and productive benefits. However, it is important to note that these benefits can vary depending on the CC species. Therefore, more studies are needed to explore in greater detail the specific interactions and interference of these CC species in maize cultivation, particularly in the semi-arid region of Brazil. These studies would provide valuable insights for optimizing maize production in semi-arid regions.

The varied effect of different cover crops on maize physiological parameters can be attributed to factors such as roots depth and density, nutrient competition capacity, and the release of bioactive compounds into the soil. Some cover crops promote a more favorable soil environment by increasing water and nutrient availability, resulting in greater photosynthetic efficiency and $CO_2$ assimilation in maize. The long-term implications for agricultural management include the need to strategically select cover crops, considering the specific characteristics of each plant and the soil conditions, to optimize crop yield.

## 4. Materials and Methods

### *4.1. Research Site*

The experiment was conducted from May to October 2023, in Arapiraca, situated in the Agreste region of Alagoas State, Northeast Brazil (9°46′07″ S, 36°33′41″ W, 324 m above sea level). The local climate is classified as type AS, tropical, with an average annual rainfall of 800 mm and an average air temperature of 25 °C. The rainy season in the region occurs from March to August, and the dry season from September to February [31].

The same CCs species had been cultivated during the rainy season for the past three years in the experimental area where the soil is classified as Argissolo Vermelho-Amarelo [32], which is equivalent to Ultisol [33], with a sandy loam texture. Chemical and physical-hydraulic characteristics of the soil in the 0.0 to 0.20 m layer are summarized in Table S1 (see Supplementary Data), and the local climatic conditions during the execution of the experiment are shown in Figures 7 and S1–S3 (see Supplementary Data).

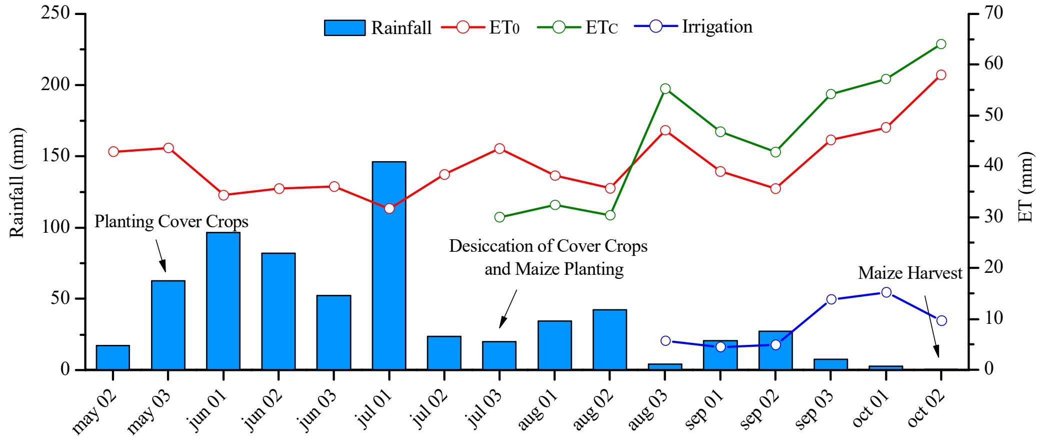


**Figure 7.** Minimum temperature ($T_{MIN}$), average temperature ($T_{AVE}$), maximum temperature ($T_{MAX}$), basal temperature ($B_T$), upper temperature ($U_T$), and average relative humidity ($RH_{AVE}$) during the cultivation of CCs and green maize.

### *4.2. Experimental Design and Crop Management*

A randomized complete block design with four replications was employed for this study. The experimental treatments consisted of CCs cultivation followed by maize over the residue. The CCs evaluated were as follows: sunn hemp (*Crotalaria juncea*); spectabilis (*Crotalaria spectabilis*); pigeon pea (*Cajanus cajan*); A mixture of *B. decumbens* and *B. ruziziensis*–*Brachiaria* sp.; jack bean (*Canavalia ensiformis*); millet (*Pennisetum glaucum*); and fallow without CCs as a control. The seeds used were obtained from commercial suppliers. The selection of the analyzed treatments was based on the potential adaptability of these cover crops to the study region. The CCs were grown during the rainy season, from May to July. Row spacing was set at 50 cm for Fabaceae CCs and 25 cm for Poaceae CCs. Sixty days after seeding, the CCs were desiccated using a glyphosate-based herbicide (3.0 L ha$^{-1}$ c.p., 200 L of solution ha$^{-1}$). Each experimental plot measured 4 m x 5 m (20 m$^2$). The maize cultivar used was DKB 177 TRECEPTA after CCs.

Maize seeding was carried out right after desiccating CCs, with a spacing of 80 cm between rows and 20 cm between plants in the row (62.500 plants ha$^{-1}$). Initial fertilization was 44 kg ha$^{-1}$ of phosphorus (P) and 50 kg ha$^{-1}$ of nitrogen (N). In the split-cover fertilization, performed at the V3 and V6 phenological stages, a total of 70 and 40 kg ha$^{-1}$ of N and potassium (K) were applied, respectively. The NPK sources used were urea (45% N), single superphosphate (18% $P_2O_5$), and potassium chloride (57% $K_2O$), respectively.

Irrigation was carried out following the meteorological parameters cited by [34] Pereira et al. (2002).

### *4.3. Experimental Evaluations*

Experimental design: Harvest area of CCs from each experimental plot: 1.0 m$^2$; evaluation of CCs: 60 days after sowing (DAS). Following the sampling harvest of 1.0 m$^2$, the shoot material was weighed. Biomass measurement: Fresh mass yield (FMY, Mg ha$^{-1}$): measured on all harvested plant material; dry mass yield (DMY, Mg ha$^{-1}$): measured on a 200 ± 0.1 g sample of the harvested plant material. Drying method for DBP determination: Oven type: forced-air circulation oven, drying at 65 °C for 72 h. Sampling Issues: Millet plants: FMY and DMY could not be determined due to sampling issues.

The physiological evaluations of maize were performed at the stages: VT (tasseling) at 60 DAS, and R3 (smooth grain) at 79 DAS. The final evaluation involved the harvest of green maize. The evaluated physiological variables included *A*–$CO_2$ assimilation (µmol $CO_2$ m$^{-2}$ s$^{-1}$), $g_s$–stomatal conductance (mmol m$^{-2}$ s$^{-1}$), $C_i$–intercellular $CO_2$ concentration (µmol mol$^{-1}$), *E*–transpiration rate (mmol m$^{-2}$ s$^{-1}$), and $T_{LEAF}$–leaf temperature (°C). These assessments were performed between 8 and 10 a.m. on three representative plants, on the third fully expanded leaf from the top. In addition to the directly measured variables, two derived physiological parameters were calculated: instantaneous water use efficiency (WUE) = $A/E$ and carboxylation efficiency (EC) = $A/\ C_i$. An infrared gas analyzer (IRGA, ADC model LCi, Hoddesdon, UK) with a coupled light source of 1800 µmol $m^{-2}$ $s^{-1}$ was used to obtain all physiological measurements. The soil temperature ($T_{SOIL}$) was measured using an infrared thermometer (Itti 1000 Infrared Thermometer, Instrutemp, São Paulo, Brazil). Two measurements were taken per experimental unit.

The chloroplastic pigments were quantified in fresh leaf tissues through extraction in N-dimethylsulfoxide (DMSO) solution and subsequent determination by spectrophotometry. The content of chlorophyll *a* ($C_A$), chlorophyll *b* ($C_B$), the chlorophyll $a/b$ ratio, total chlorophylls ($C_T$), and carotenoids ($C_N$) were determined by removing a disk, with an area of 0.5662 $cm^2$, from the leaf located in the middle third of the maize plants, immersed in DMSO solution for 24 h in the dark. Absorbance was determined by a spectrophotometer (UV-M51, BEL, Piracicaba, Brazil) at wavelengths of 665 nm, 649 nm, and 480 nm [35].

The relative leaf water content (RWC) was obtained by determining the fresh, turgid, and dry masses of ten leaf discs with an area of 0.5662 $cm^2$, taken from the leaves in the middle third of the maize plants. The dry masses were calculated based on the equation proposed by [36].

$$\text{RWC} = \frac{(F_M - D_M)}{(T_M - D_M)} \times 100 \quad (1)$$

In which:

RWC = relative leaf water content (%); FM = fresh mass of leaf discs (g); DM= dry mass of leaf discs (g); $T_M$ = turgid mass of leaf disc tissue (g).

The integrity of cell membranes was analyzed through electrolyte leakage ($E_L$) by removing 10 leaf discs, each with an area of 0.5662 $cm^2$, from the leaves in the middle third of the maize plants. These discs were kept for 24 h in 10 mL of deionized water. An electrical conductivity reading was taken 24 h after incubation, considered as the initial conductivity. Subsequently, the discs were placed in a water bath at 60 °C for three hours, and then a new electrical conductivity reading of the solution was taken, considered as the final conductivity. The $E_L$ was expressed as the percentage of initial conductivity relative to the final conductivity after heating for 3 h at 60 °C, according to the equation described by [37].

$$E_L = \frac{C_i}{C_f} \times 100 \quad (2)$$

In which:

$E_L$ = electrolyte leakage (%);

Ci = initial electrical conductivity (µS $cm^{-1}$);

Cf = final electrical conductivity (µS $cm^{-1}$).

The following growth and production variables were evaluated: LAI–Leaf area, or the ratio of leaf area to the area occupied by the plant. Leaf area was determined by multiplying the width and length of each leaf, and the resulting value was multiplied by the number of leaves per plant.

### *4.4. Statistical Analysis*

The data collected for the studied variables were subjected to the Shapiro–Wilk normality test ($p \geq 0.05$). After confirming normality, mixed models ($p \leq 0.05$) for repeated measures over time were applied. The model accounted for CCs, growth stages, and the interaction between CCs and stages (time) as fixed effects, while incorporating the random effects of stages (time) and blocks. When the interaction was significant, the effects were further partitioned. Means were compared using a HDS test ($p \leq 0.05$) for the effects of CCs and a *t*-test ($p \leq 0.05$) for the effects of phenological stages. Only FMY, DMY, and grain yield were analyzed using one-way ANOVA ($p \leq 0.05$). Principal component analysis (PCA) was used to reduce the dimensionality of the variables and determine the changes promoted by the CCs at each crop stage. Analyses and graphs were generated using R software version 4.4.1 (R Core team, Viena, Áustria), with the lme4 (Douglas Bates, EUA, Madison, WI, USA) and Factorextra (Alboukadel Kassambara, HalioDx, Marseille, France) packages.

## 5. Conclusions

(1) Cover crops were favorable for green maize cultivation due to the reduction in soil temperature and improvements in the plants physiological metabolism, thus supporting the initial hypothesis.

(2) The most prominent cover crop was sunn hemp, with positive effects on the water use efficiency of maize and a reduction in soil temperature. Under jack bean, there was the highest corn yield.

(3) Practical recommendations for farmers include adopting sunn hemp (*Crotalaria juncea*) as the preferred cover crop in maize-growing areas of Brazil's semi-arid regions or similar environments, due to its proven benefits in water use efficiency and reducing soil temperature. To maximize yield, jack bean can also be used as an alternative, especially in regions where productivity demand is high. However, when scaling up implementation, farmers should consider potential limitations, such as climatic variability, which may affect the efficiency of cover crops, and the initial adoption costs, particularly in seed acquisition.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/stresses4040045/s1.

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