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Article

Biomass and Biogas Yield of Maize (*Zea mays* L.) Grown under Artificial Shading

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Abstract: Agroforestry, as an improved cropping system, offers some advantages in terms of yield, biodiversity, erosion protection or habitats for beneficial insects. It can fulfill the actual sustainability requirements for bioenergy production like food supply, nature conservation, stop of deforestation. However, competition between intercropped species for water, nutrients and light availability has to be carefully considered. A field trial with shading nets was conducted in Southwest Germany to evaluate the influence of different shading levels (−12, −26, and −50% of full sunlight) on biomass growth, dry matter yield and biogas quality parameters of maize (*Zea mays* L., cv. 'Corioli CS'). Shading the plants causes a delayed development, a reduction in height and leaf area index and a slower senescence. Dry matter yields were reduced about 18%, 19%, and 44% compared to 21.05 Mg ha^{-1} year^{-1} at full sunlight. Biogas and methane yields were also significantly reduced, the 50% shading treatment showed a reduction of 45% for both parameters. Further, shading led to higher crude protein and crude ash contents. If silage maize is grown under shade, the yields of dry matter, biogas, and methane are nearly halved under 50% shade. Cultivation up to 26% shading could be possible.

Keywords: maize (*Zea mays* L.); agroforestry; biogas; shade; yield; growth; quality

1. Introduction

By 2020, 20% of the produced energy in Europe must be generated from renewable sources [1]. Hence, the area of plants grown for biogas production is steadily increasing. In Germany, the most commonly used crop for biogas production is silage maize (*Zea mays* L.) with a cultivation area of one million ha in 2016 [2]. Maize is a highly attractive crop for biogas production due to high biomass yields and methane yields between 7500 to 10,200 m^3 ha^{-1}, which has not been achieved by any other crop used for biogas production so far [3].

The increased cultivation of maize in monocropping systems has led to several environmental problems including a loss of biodiversity, increased nitrate levels in water bodies and soil degradation [4]. The implementation of maize intercropping systems could alleviate some of the problems related to maize monocropping. Intercropping systems could have several advantages; such as increased productivity; reduced pressure by pests, diseases, and weeds; higher carbon sequestration; and reduction in nutrient losses [5–7]. One particular type of intercropping system are silvoarable agroforestry systems (AFS) which combines annual crops with perennial, woody plants. Widely used crops for AFS are fast growing trees like willow (*Salix* spp. L.) or poplar (*Populus* spp. L.) as a short rotation coppice [8–12]. Alternatively, trees for high-value timber production can be used as shown in trials conducted in Canada, France, and the Philippines [13–17].

Within an AFS, interactions between the intercropped species occur in terms of competition for light, water, and nutrients [18,19]. The competition for light could lead to a considerable reduction in plant growth and yield, especially for maize, which is known to be a shade-intolerant C_4 species [16]. Further, delayed development and lower leaf emergence rates have been observed for maize when shaded at different growth stages. In an intercropping of maize and wheat, the tassel initiation and silking was delayed compared to the monocrop equivalent. Additionally, the interval between anthesis and silking was prolonged by shading [20]. This effect was also observed by Early et al. [21], who showed a delay in the emergence of tassel, anther, and silk; when the plants were shaded at levels of 30%, 60%, 70%, 80%, and 90%. In addition, shading reduced the plant height of maize [21]. A study by Syafrullah et al. [22] revealed that some light-sensitive maize genotypes intercropped with oil palms showed a decreasing plant height with shading from 22%, 37%, to 76%. Also, significant height reductions were observed at a distance of 2 m from the trunks of 10 to 12 m high maple (*Acer* spp. L.) and poplar trees (*Populus* spp. L.) compared to measurements at a distance of 6 m. Furthermore, shading can also reduce the whole plant leaf area of maize [16]. In a dense plant stand (9 to 12 plant m^{-2}) in which maize plants shade each other more than compared to a stand with 3 plants per m^{-2}, maize plants showed a significant decrease in leaf length and leaf width and, in turn, in leaf area [23]. Another study showed that unshaded leaves were larger than shaded leaves [24]. These growth changes will ultimately impact biomass. Under 20% of full sunlight, a reduction in total dry weight of maize was observed [25]. Growing maize at 1.0 to 2.5 m distances from 3 m high walnut (*Juglans regia* L.) and plum trees (*Prunus salicina* Lindl.) resulted in a reduction of total aboveground biomass by 29 and 41%, respectively [26].

Besides the impact of shade on maize growth and, thus, final biomass, a reduction of light also has an impact on plant composition components. The individual composition components contribute differently to biogas and methane formation. From one kilogram of carbohydrates 790 L of biogas with a quantity of approximately 50% methane can be obtained. For fat the values are 1250 L and 68%, and for proteins 700 L with 71% methane [27]. If the plant composition is changed under shade this will affect biogas and methane yields. For instance, Jia et al. [28] observed an increase in protein and fat content in maize grains under a 55% light reduction. Fat (also named ether extracts because it contains plant parts that are soluble in ether) consisting of fats and fatty acids, have the longest hydraulic retention time in the biogas process, but apart from protein, they contribute the most to the methane yield [29]. Studies with shaded soybeans and maize also observed an increased protein content with increasing shade [30,31]. A study by Singh [25] observed a rise in protein content as well as a decrease in cellulose by 80% shade. There could have been a poor synthesis of cellulose in cell walls due to shade. He also observed that sugar and starch, which could be grouped as nitrogen-free extractives, increased under shade in maize stems, due to a poor translocation. High contents of sugar and protein can cause acidification, resulting in an inhibition of biogas and methane production [32]. Under the shade of ponderosa pines (*Pinus ponderosa* P. Lawson & C. Lawson), Kentucky bluegrass (*Poa pratensis* L.) showed higher contents of crude ash [33]. High contents of ash reduce methane production due to lower amounts of biodegradables [34].

Shading can also affect the concentration of macronutrients in plant tissue. For instance, shading of 63% on cotton (*Gossypium hirsutum* L.) led to an increase of phosphorus and sulfur in leaves, buds, and bracts [35]. Two forages, crested wheatgrass (*Agropyron cristatum* L.) and basin wild rye (*Leymus cinereus* Scribn. & Merr.) showed a higher content of potassium, calcium, and magnesium under 75% of shade [36]. Potassium is needed for oxidative activity of the methanogenic archaea, but at high concentrations, the increase of osmotic pressure can lead to stress. The high salt content of the fermenter medium causes water diffusion out of the archaea cells. The cells dehydrate wherefore the archaea die, causing a decline in biogas production [37]. High contents of calcium can lower the protein and fat removal and inhibit the anaerobic digestion [38]. For normal functioning of the biogas and methane formation, there are critical values for some of these macronutrients, such as 3 mg m^{-3} potassium, 2.8 mg m^{-3} calcium ($CaCl_2$), 2.400 mg m^{-3} magnesium ($MgCl_2$), and 20–50 ppm for sulfur (H_2S) [39,40].

If solar radiation is one of the main growth factors for plant growth and some maize plants for biogas production are shaded while others remain unshaded, then the plant growth, yield, quantity, and quality of biogas yield from shaded plants will be lower than in plants grown under full solar radiation. To the best of our knowledge, there has been no published study investigating the effects of shade on maize and, ultimately, the impact of biogas and methane yields. Therefore, the objectives of this study were to: (i) evaluate the impact of three shade levels (12%, 26%, and 50 %) on maize growth and biomass yield; (ii) determine the effect of shade on biogas and methane forming parameters; and, (iii) determine the effect of these shade levels on the final biogas and methane yield.

2. Materials and Methods

2.1. Study Site and Experimental Design

The experiment was conducted in Southwest Germany in the lower Rhine valley at the experimental station of the Centre for Agricultural Technology Augustenberg (LTZ) in Rheinstetten-Forchheim (48°58′ N, 8°18′ E, 117 m above sea level (a.s.l.)). The mean long-term precipitation is 742 mm $year^{-1}$ with an average temperature of 10.1 °C. During the growing season (March–October), the average long-term precipitation is 509 mm. The year 2015 was drier than 2016 with only 223 mm of precipitation compared to 361 mm between April and August (Figure 1). In 2016, high rainfalls occurred in early summer (April–June); however, July and August were rather dry. Similar to 2016, rainfalls in 2017 were more evenly distributed.

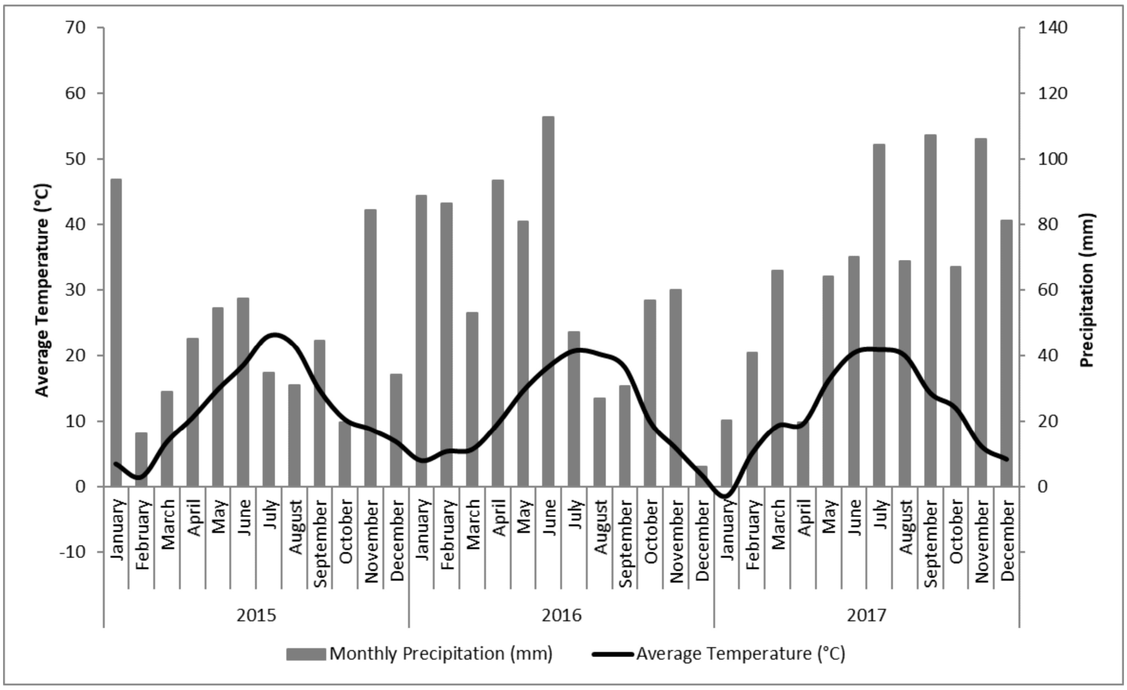


Figure 1. Average temperature (°C) and monthly sum of precipitation (mm) during the three experimental years 2015 to 2017.

The experimental soil was a Luvisol with a texture of 13.7% clay, 60.2% sand, and 26.1% silt in 0–90 cm. Additional soil characteristics are listed in Table 1.

Table 1. Soil characteristics (30 cm depth) of the experimental site.

Humus Content (%)	1.4
Soil N_t (%)	0.064
pH ($CaCl_2$)	5.8
Available Total P (mg 100 g^{-1}) [1]	18
Available Total K (mg 100 g^{-1}) [1]	22
Available Total Mg (mg 100 g^{-1}) [2]	8

[1] calcium acetate-lactate extraction; [2] calcium chloride extraction.

Before the establishment of the experiment, the soil was prepared with a mouldboard plough to 25 cm depth on 20 September 2014. A secondary tillage was done by a chisel plow (15 cm depth) on 24 April 2015, 21 April 2016, and 21 April 2017, respectively. On the same day that tillage occurred, maize (*Zea mays*, cv. 'Corioli CS') was sown with a row distance of 0.75 m and a planting density of 10 plants m^{-2}. Each plot consisted of eight rows of maize with a length of 10 m (Figure 2). The row orientation was east-west. The shading treatments (Figure 2, explained later in detail) were randomized in three complete blocks. The harvest was performed at the dough stage on 7 September 2015, 24 August 2016, and 24 August 2017 with a plot harvester (type 'BAURAL SF 2000', Zürn Harvesting GmbH & Co. KG, Schöntal-Westernhausen, Germany, cutting width = 1.50 m). The trial did not consist of repeated measurements. The plots rotated each year into a new strip with winter-sown barley as the previous crop.

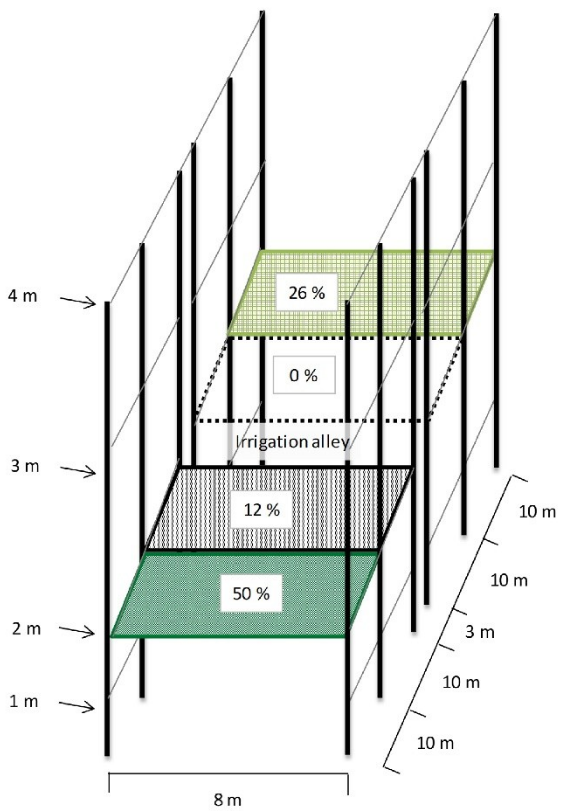


Figure 2. Construction outlines of the artificial shading system in East-to-West-View, exemplarily shown for one repetition. In this example, the nets are installed at a height of 2 m. The numbers in the boxes are the reduction values of solar radiation. The 2nd (12%) and the 3rd (0%) plot were separated by an irrigation alley of 3 m width.

Fertilization and plant protection were done according to the guidelines of Best Agricultural Practices. Fertilizer was applied as ALZON (46% N) at a rate of 151.8 kg N ha^{-1} in 2015, 142.6 kg N ha^{-1} in 2016, and 179.4 kg N ha^{-1} in 2017. In 2015, plant protection comprised of 1.5 L ha^{-1} Clio Super (32 g L^{-1} *topramezone* and 538 g L^{-1} *dimethenamid-p*, BASF SE) applied on 13 May 2015 and, due to strong weed infestation, a mixture of 1 L ha^{-1} Dash EC (345 g L^{-1} *FAME*, 205 g L^{-1} *fatty alcohol alkoxylates*, 46 g L^{-1} *oleic acid*, BASF SE) and 200 g ha^{-1} Arrat (250 g kg^{-1} *tritosulfuron*, 500 g kg^{-1} *dicamba*, BASF SE), which was applied on 15 June 2015. In 2016, a mixture of 0.75 L ha^{-1} Motivell forte (60 g L^{-1} *nicosulfuron*, Belchim Crop Protection NV/SA), 1.4 L ha^{-1} Spectrum (720 g L^{-1} *dimethenamid-p*, BASF SE), and 2 L ha^{-1} Laudis (44 g L^{-1} *tembotrione*, 22 g L^{-1} *isoxadifen-ethyl*, Bayer SE) was applied on 20 May 2016. A mixture of 1.25 L ha^{-1} MaisTer Power (30 g L^{-1} *foramsulfuron*, 9.77 g L^{-1} *thiencarbazone*, 0.85 g L^{-1} *iodosulfuron*, 15 g L^{-1} *cyprosulfamide*, Bayer SE) and 1.25 L ha^{-1} Spectrum was applied on 29 May 2017. Additionally, applications of *Ichneumonidae* spp. (*Trichogramma brassicae* Latreille) were utilized on 19 June 2015, 7 July 2015, 29 June 2016, 14 July 2016, 16 June 2017, and 30 June 2017 as protection against the European corn borer (*Ostrinia nubilalis* Hübner).

During the growing season, all plots were irrigated based on the 'Agrowetter' irrigation device to prevent water stress [41]. Irrigation was done by an irrigation gun at a rate of 30 mm at each irrigation event (29 May 2015, 29 June 2015, 7 July 2015, 16 July 2015, 3 August 2015, 7 July 2016, 13 July 2016, 29 July 2016, 12 August 2016, 31 May 2017, 20 June 2017, and 4 July 2017).

2.2. Shade Levels

Shading was realized by nets, which were stretched between wooden posts linked by steel wires (Figure 2). The relevant characteristics of the shading nets are given in Table 2. These nets reduced the incoming radiation by 12%, 26%, and 50%, respectively. These quantities of light reduction were chosen based on measurements in distances of 4.5, 7.5, and 11 m to the trunk of an 11-year old cherry tree, which was pruned up to 4 m. The control measurements were done at a distance of 20 m from the trunk corresponding to open-field conditions [42]. The height of the shading nets was adapted with increasing plant height to keep the distance between nets and plant canopy during the growing period at 0.5 m. The nets were installed after sowing and removed at harvest. From sowing to harvest, the average incoming solar radiation was 19.96 MJ m^{-2} day^{-1} in 2015, 19.11 MJ m^{-2} day^{-1} in 2016, and 19.73 MJ m^{-2} day^{-1} in 2017. In comparison, the incoming solar radiation below the shading nets was reduced to 17.56 (12%), 14.77 (26%), and 9.98 MJ m^{-2} day^{-1} (50%) in 2015, 16.82 (12%), 14.14 (26%), and 9.56 MJ m^{-2} day^{-1} (50%) in 2016, and 17.36 (12%), 14.60 (26%), and 9.87 MJ m^{-2} day^{-1} (50%) in 2017, respectively.

Table 2. Characteristics of the nets used for light reduction.

	Light Reduction (%)		
	12	**26**	**50**
Manufacturer	AGROFLOR Kunststoff GmbH, Wolfurt, Austria		
Color	black	green	green
Material	polyethylene	polyethylene	polyethylene
Mesh Size (mm)	3 × 8	12 × 12	3 × 3
Original Use	hail protection net	anti-bird net	shade sheet

2.3. Data Collection

2.3.1. Plant Growth

Non-destructive plant growth measurements were only conducted in 2016 and 2017 on six plants located in rows three and six of the plots. These measurements were done two times per week from the 3rd-leaf stage until two weeks after silking (8.5 total weeks in 2016 and 8 total weeks in 2017). From each plant, the growth stage (GS) was determined according to the BBCH scale [43]. Then, canopy height (soil surface to leaf tip or later tip of tassel) was measured. The number of senescent leaves was counted. A leaf was counted senescent when less than 50% was green. Leaf length and maximum width (at the widest part of the leaf) of every fully unrolled leaf were determined. Leaf area (LA) was calculated by the technique of Mokhtarpour et al. [44] using the following Equation:

$$\mathbf{LA = leaf\ length \times leaf\ width \times 0.75} \quad (1)$$

The leaf area index (LAI) was calculated by summing up the area of all leaves of one plant multiplied by the plant density.

2.3.2. Yield

Yield and quality measurements were done in all three growing seasons. At harvest all plants of the two center rows—leaving three border rows on each site—were cut by a crop chopper. The determination of dry matter yield (DMY) and dry substance (DS) were done gravimetrically after oven drying 2 kg of fresh material at 105 °C for two days.

2.3.3. Chemical Analysis and Calculation of Biogas and Methane Yield

For chemical analysis in all three years, an additional 2 kg of the harvested material was dried at 60 °C for two days and milled to 1 mm. Then, P, K, Ca, Mg and S were determined by spectrometry [45–47]. The determination of crude protein (XP), crude fiber (XF), ether extracts (EE), crude ash (XA), and nitrogen-free extractives (NfE) was done according to the Weender analysis [45,48]. With the results of the Weender analysis, the digestibility values of EE, XP, XF, and NfE of fresh maize silage and the DMY, the theoretical yields of biogas and methane were calculated by the Schattauer & Weiland technique [49,50]. For simplification and direct comparability whether the DMY or the quality of the DMY have a greater impact on biogas and methane yield, the formulas have been simplified to the multiplication of DMY with the conversion factors for biogas and methane (C_{biogas} and $C_{methane}$) in Equation (2) and (3). The equations can be found in detail in Schattauer & Weiland [50].

$$\mathbf{Biogas\ yield\ [m^3\ ha^{-1}] = DMY \times C_{biogas}} \quad (2)$$

$$\mathbf{Methane\ yield\ [m^3\ ha^{-1}] = DMY \times C_{methane}} \quad (3)$$

2.4. Data Analysis

The experimental design was a randomized complete block design with three replicates. For the annual analysis of growth data, statistical analysis was done using the PROC MIXED procedure of the SAS system. The fitted model was as follows:

$$y_{ij} = \mu + r_i + s_j + (rs)_{ij} + e_{ij}$$

where y_{ij} is the response, μ the general effect, r_i is the fixed effect of the i-th replicate, s_j is the fixed effect of the j-th shading level, rs_{ij} is the random plot effect where the j-th shading level is used in the i-th replicate, and e_{ij} is the residual error of y_{ij} which corresponds to the plant effect. After finding significant differences via F-test, differences between treatments were compared at $p < 0.05$ using Fisher's LSD test. For creating the letter displays the method of Piepho [51,52] was used.

For multi-year analysis of yield and quality data, an analysis was done using Residual Maximum Likelihood of the PROC MIXED procedure of the SAS system to fit a linear mixed model. The fitted model was:

$$y_{ijl} = \mu + a_l + s_j + (ra)_{il} + (as)_{lj} + e_{ijl}$$

where y_{ijl} is the response, μ the general effect, a_l is the effect of the l-th year, s_j is the fixed effect of the j-th shading level, ra_{il} is the effect of the i-th replicate in the l-th year, as_{lj} is the random interaction effect between the l-th year and the j-th shading level, and e_{ijl} is the residual error of y_{ijl}. If necessary, meaning if the AIC decreases, year-specific error variances were fitted.

Note that year (and replicate) effects are assumed as random, but the main effects were treated as fixed in the model because only three-year data (with nine replicates) were analyzed [53]. Furthermore, for balanced data as it is the case for the data in this study, no inter-year (and inter-replicate) information exists, thus models assuming random and fixed effects results in identical relevant results [53].

The variance of year effect is normally large. So, we assume year as random effect. Otherwise the variance component for the small number of years had to be estimated. However, this is only possible with more than 10 degrees of freedom, for which there should be more than four replications [53]. To make sure that no information is weighted incorrectly, the year is set as fixed effect in the model.

Again, after finding significant differences via F-test, differences between treatments were compared at $p < 0.05$ using Fisher's LSD test. For both models, normality and homogeneous variances were checked graphically.

3. Results

3.1. Growth Stages

No differences in emergence date were observed for maize plants grown at different shading levels in 2016 and 2017. Emergence took place 18 and 23 days after sowing, respectively (DAS, Table 3). In 2016 tassel initiation was observed after 74 DAS in the control and the 12% shading treatment. Fifty percent shading led to a delay of 11 days compared to the control. Increasing the shading to 50% further delayed silking to about 9 days to a total of 90 DAS. The end of flowering was delayed for only two days (99 DAS vs. 97 DAS) under 50% shade when compared to the control and the 12% shading treatment. Dough stage (harvest) was reached in the control after 126 DAS. Increasing shade to 50% delayed the dough stage about 7 days. In 2017, between the control and the 50% shade, there was a delay of 11 days in silking. The end of flowering was observed 91 DAS at 50% shade. The other three treatments reached this stage at 87 DAS. There was a difference of 16 days between the control and 50% shade reaching the dough stage.

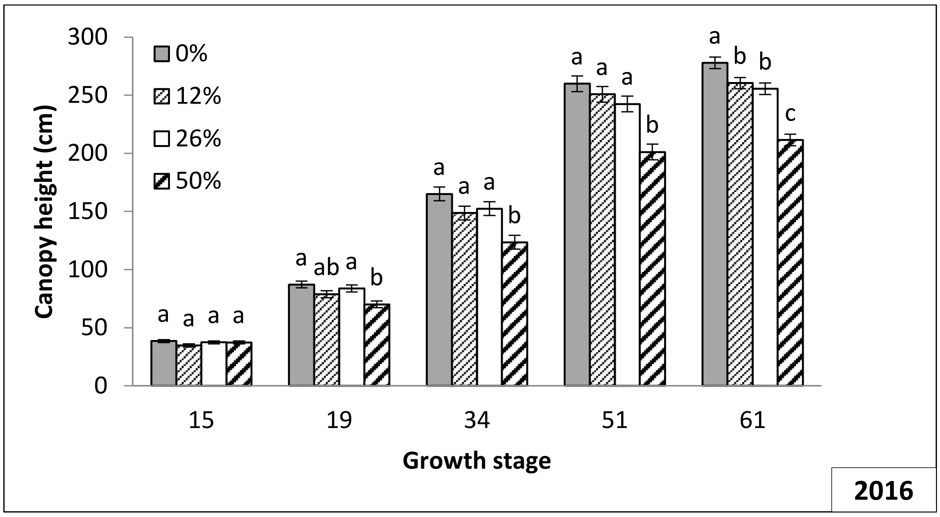
Table 3. Days after sowing (DAS) until the growing stages (GS) emergence (GS 09), tassel initiation (GS 51), silking (GS 61), end of flowering (GS 69), and dough stage (GS 85) under different shading levels (0, 12, 26, and 50%) in 2016 and 2017 and the standard error of means (SEM).

Year	Shade	Emergence [†]		Tassel Initiation		Silking		End of Flowering [†]		Dough Stage	
2016	0%	18	(±0.00)	74 a	(±0.33)	81 a	(±0.71)	97 a	(±0.22)	126 ab	(±1.67)
	12%	18	(±0.00)	74 a	(±0.33)	84 ab	(±0.71)	97 a	(±0.22)	123 a	(±1.67)
	26%	18	(±0.00)	80 b	(±0.33)	85 b	(±0.71)	98 a	(±0.22)	133 b	(±1.67)
	50%	18	(±0.00)	81 b	(±0.33)	90 c	(±0.71)	99 b	(±0.22)	133 b	(±1.67)
2017	0%	23	(±0.00)	67 a	(±0.33)	71 a	(±0.40)	87 a	(±0.00)	110 a	(±2.05)
	12%	23	(±0.00)	68 a	(±0.33)	73 b	(±0.40)	87 a	(±0.00)	112 a	(±2.05)
	26%	23	(±0.00)	73 b	(±0.33)	77 c	(±0.40)	87 a	(±0.00)	117 ab	(±2.05)
	50%	23	(±0.00)	76 c	(±0.33)	82 d	(±0.40)	91 b	(±0.00)	126 b	(±2.05)

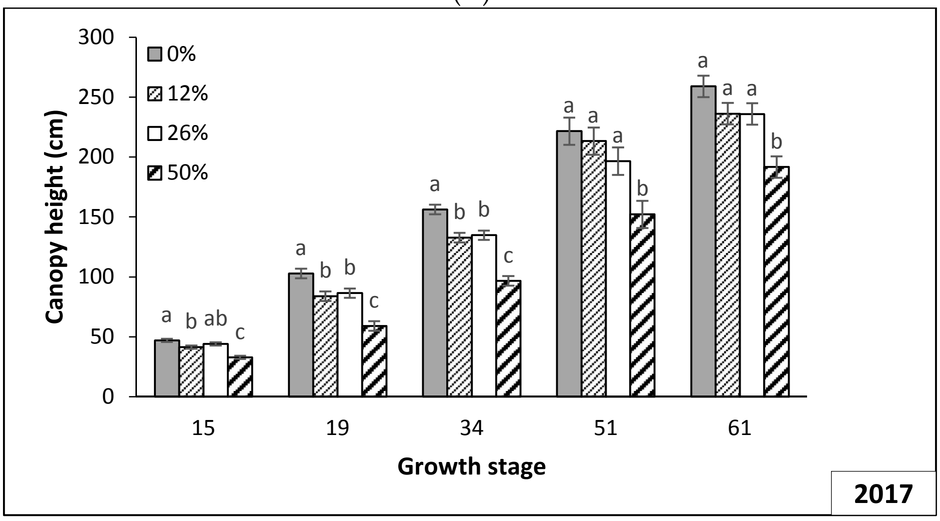
Standard errors of means are given in parentheses. Means with identical letters within each column and year show non-significant differences between the shade levels of the single years (LSD, $p < 0.05$). [†] Note: The SEM was between 0 and 0.005, so rounding to two decimal places resulted in a SEM of zero.

3.2. Plant Growth

Canopy height was reduced by shading, which was especially pronounced at 50% shade during the growing seasons in 2016 and 2017 (Figure 3). In 2016, there was a strong reduction in the maximum canopy height (−24%) under 50% shade with a maximum canopy height of 212 cm compared to the control with 278 cm. Canopy heights under 12% and 26% shade showed only slight differences between each other and were significantly lower than the control with maximum canopy heights of 260 and 256 cm at GS 61, respectively. In 2017, final height under 50% shade was 192 cm, corresponding to a reduction of 26% compared to the control. Under 12 and 26% shade, final canopy heights did not differ significantly from the control.



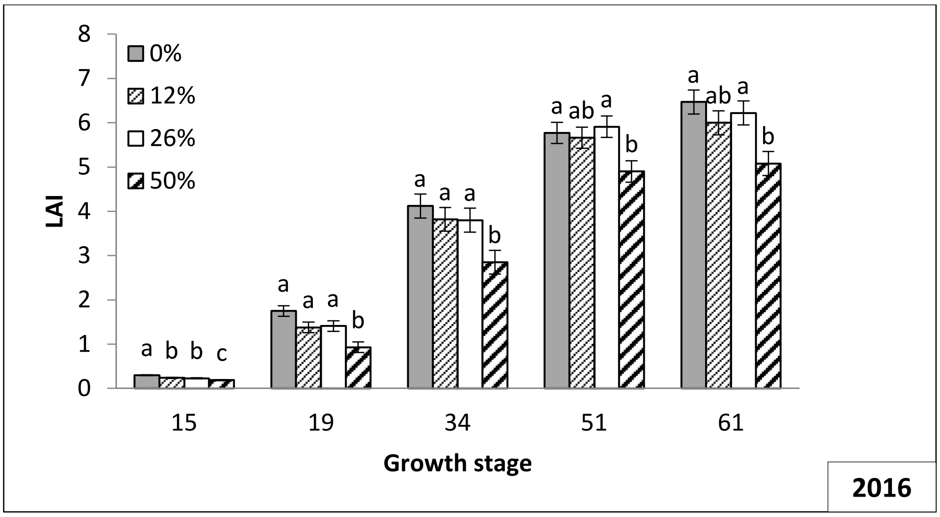
(**A**)



(**B**)

Figure 3. Canopy height (cm) under the different shading levels at the growth stages 15 (5th leaf unfolded), 19 (9th or more leave unfolded), 34 (4 nodes detectable), 51 (tassel initiation), and 61 (silking) of the control in 2016 (**A**) and 2017 (**B**). Black bars represent the standard error of mean. Means with identical letters within a growth stage show non-significant differences between the shade levels (LSD, $p < 0.05$).

In 2016 and 2017, the LAI was significantly reduced under 50% shade at all growth stages until silking compared to the control (Figure 4). The maximum LAI was reduced by 21% and 39% in 2016 and 2017, respectively. Under 12% and 26% shade, the maximum LAI did not differ from each other and was only slightly lower than the control at early growth stages in both years.



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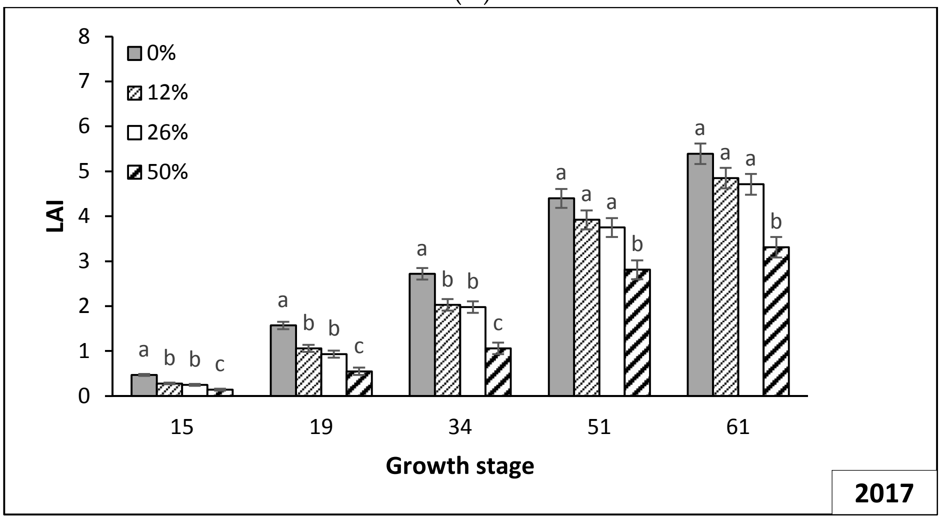
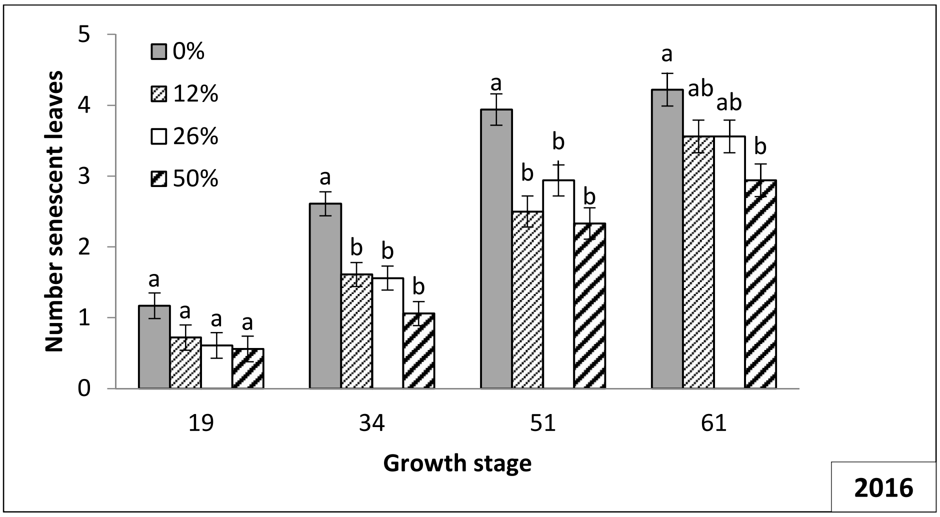


(**B**)

Figure 4. LAI under the different shading levels at the growth stages 15 (5th leaf unfolded), 19 (9th or more leave unfolded), 34 (4 nodes detectable), 51 (tassel initiation), and 61 (silking) of the control in 2016 (**A**) and 2017 (**B**). Black bars represent the standard error of mean. Means with identical letters within a growth stage show non-significant differences between the shade levels (LSD, $p < 0.05$).

Leaf senescence was first observed when the control reached the 8th-leaf stage. In both years, shading delayed senescence from GS 34 onwards (Figure 5). In general, the control had around one senescent leaf more than under 50% shade. In 2017, this effect was less pronounced and high standard errors did not lead to significant differences at GS 51. The high standard errors occurred from the fact, that some of the six observed plants of one plot already did not have any senescent leaf at this time, while other plants in the same plot already had three senescent leaves.



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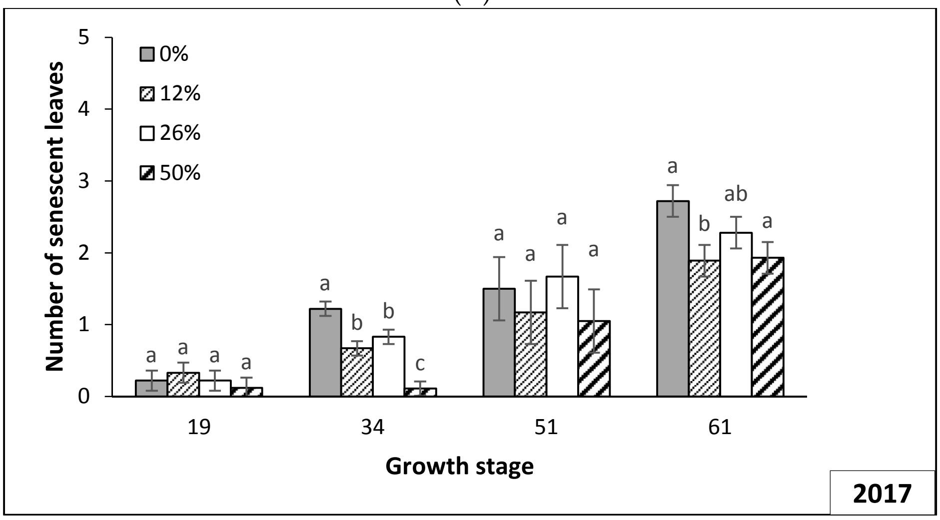

(**B**)

Figure 5. Number of senescent leaves per plant under the different shading levels at the growth stages 15 (5th leaf unfolded), 19 (9th or more leave unfolded), 34 (4 nodes detectable), 51 (tassel initiation), and 61 (silking) of the control in 2016 (**A**) and 2017 (**B**). Black bars represent the standard error of mean. Means with identical letters within a growth stage show non-significant differences between the shade levels (LSD, $p < 0.05$).

3.3. Yield and Quality

There were significant differences between biomass yield as well as the quality parameters and biogas and methane yields at different shade levels (Table 4).

DMY decreased with increasing shade. The mean yield of the control across the three experimental years was 21.05 Mg DM ha^{-1} year^{-1}. The DMY under 12% and 26% was significantly different from the control and the 50% shade, but not from each other. Compared to the control, DMY was 18% and 19% lower under 12% and 26% shade, respectively. The 50% shade resulted in a significantly lower DMY compared to all other treatments, with a reduction of 44% compared to the control.

DS also decreased with increasing light reduction from 39.48% in the control to 32.23% under 50% shade. XP showed a significant increase with increasing shade up to 7.16% in the 50% shade treatment compared to 6.04% of the control. The 12% and 26% shade resulted in an XP content of 6.42% and 6.63%, respectively and did not differ from the control. No significant differences were observed for XF, EE and NfE.

Table 4. Means for DMY (Dry Matter Yield, Mg ha^{-1} $year^{-1}$), DS (Dry Substance, %), biogas, and methane quality parameters (XP, XF, EE, XA and NfE) (% of dry matter), conversion factor C_{biogas} and $C_{methane}$ and theoretical biogas- and methane yield (m^3 ha^{-1}) for the different shade levels (0%, 12%, 26%, and 50%) over the three experimental years (2015–2017).

Constituent	Shade Level								p-Value	LSD
	0%		12%		26%		50%			
DMY	21.05 a	(±0.53)	17.24 b	(±0.53)	17.08 b	(±0.53)	11.73 c	(±0.53)	<0.001	1.54
DS	39.48 a	(±0.71)	36.39 b	(±0.71)	35.98 b	(±0.71)	32.23 c	(±0.71)	<0.001	2.07
XP	6.04 a	(±0.20)	6.42 ab	(±0.20)	6.63 bc	(±0.20)	7.16 c	(±0.20)	0.008	0.58
XF	19.50	(±0.87)	19.52	(±0.87)	19.09	(±0.87)	19.12	(±0.87)	0.973	3.01
EE	2.84	(±0.19)	2.76	(±0.19)	2.99	(±0.19)	3.02	(±0.19)	0.721	0.65
XA	3.01 a	(±0.09)	3.32 ab	(±0.09)	3.45 b	(±0.09)	3.98 c	(±0.09)	0.002	0.31
NfE	68.72	(±1.05)	68.01	(±1.05)	67.87	(±1.05)	66.55	(±1.05)	0.565	3.64
C_{biogas}	54.41	(±0.17)	54.12	(±0.17)	54.10	(±0.17)	53.65	(±0.17)	0.096	0.59
$C_{methane}$	29.39	(±0.09)	29.25	(±0.09)	29.29	(±0.09)	29.12	(±0.09)	0.267	0.30
Biogas	11,455 a	(±280)	9315 b	(±280)	9241 b	(±280)	6321 c	(±280)	<0.001	819
Methane	6188 a	(±150)	5038 b	(±150)	4999 b	(±150)	3437 c	(±150)	<0.001	436

Standard errors of means (SEM) are given in parentheses. Means with identical letters within a shade level show non-significant differences between the shade levels (LSD, $p < 0.05$).

The content of XA increased with increasing shade up to 3.98% under 50% shade corresponding to an increase of 32% compared to the control. Under 26% shade, the XA content differed significantly from the control and 50% shade with an increase of 15% compared to the control.

The conversion factor for biogas C_{biogas} decreased insignificantly, with increasing shade from 54.41% in the control to 53.65% under 50% shade. As a result, together with the decreased DMY, the theoretical biogas yield decreased by 45% from 114,855 m^3 ha^{-1} in the control to 6321 m^3 ha^{-1} at 50% shade. The lower shading levels (12% and 26%) did not differ significantly from each other but were significantly lower than the control with around 9300 m^3 ha^{-1}. The $C_{methane}$ showed no significant differences between the tested shading treatments. The reduction of the value was for all shading treatments under 1%. But in combination with the decreased DMY the theoretical methane yield was reduced about 45% under 50% shade compared to the control, which was similar to the reduction of biogas yield. Both under 12% and 26% shade, the methane yield was 19% lower than the control.

3.4. Macronutrients

The content of the macronutrients P, K, Ca, Mg, and S showed an increase with increasing shade (Table 5). Across the three years, phosphorus and potassium had the largest increase up to 37%. The increase of the other macronutrients was in a range of 10 to 25% from the control to 50% shade. Shading had no significant effect on the calcium, magnesium and sulfur content.

Table 5. Average content of macronutrients (% of dry matter) phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) for the different shade levels (0%, 12%, 26%, and 50%) over the three experimental years (2015–2017).

Constituent	Shade Level								p-Value	LSD
	0%		12%		26%		50%			
P	0.11 a	(±0.01)	0.13 ab	(±0.01)	0.13 b	(±0.01)	0.15 c	(±0.01)	0.003	0.03
K	0.98 a	(±0.04)	0.98 a	(±0.04)	1.12 a	(±0.04)	1.34 b	(±0.04)	0.003	0.15
Ca	0.09	(±0.01)	0.09	(±0.01)	0.10	(±0.01)	0.11	(±0.01)	0.348	0.03
Mg	0.08	(±0.00)	0.09	(±0.00)	0.09	(±0.00)	0.10	(±0.00)	0.105	0.01
S	0.10	(±0.00)	0.10	(±0.00)	0.10	(±0.00)	0.11	(±0.00)	0.349	0.01

Standard errors of means (SEM) are given in brackets. Means with identical letters within a shade level show non-significant differences between the shade levels (LSD, $p < 0.05$).

4. Discussion

In order to counteract the potential negative environmental effects of large maize monocropping areas for biogas production, the cultivation of maize in intercropping systems or AFS is considered as a possible alternative. However, especially in an AFS the maize plant is experiencing competition for light, nutrients and water. It is expected that this competition will impact final biomass and quality of the maize plant and thus overall formation of biogas and methane.

In our study, shading significantly influenced plant development by delaying the occurrence of growing stages as well as senescence. These results are in line with other studies showing delayed development of maize shaded by earlier sown winter wheat in an intercropping system and delayed senescence of winter wheat under 12% of shade [54,55]. Even though the vegetative period of maize was longer under shade, the final plant height and LAI were considerably lower, indicating that plant growth was strongly suppressed by shading.

Finally, changes in height and LAI caused biomass yield to be largely reduced [56–59]. The delayed senescence, in turn, resulted in a lower DS under shade. However, in all treatments, DS of the harvested maize was in the recommended range of 30%–40% for ensiling [60] and, thus, can reach maximum biogas yields [29]. Reynolds et al. [16] observed in an AFS of poplar (*Populus* spp. L.) and maple trees (*Acer* spp. L.) intercropped with maize a significant height reduction of maize plants. The maize height in the poplar intercropping systems was reduced to about 41% in 2 m distance to tree trunk compared to the maize height in 6 m distance. For maple trees, the reduction at the same distances was 37%. Maize genotypes which showed to be intolerant in their growth to low light intensities were intercropped with oil palms and showed a lower plant height under shading levels of 22, 37, and 76% [22].

These reductions were attributed to the changed light conditions and, thus, reduced photosynthesis rates. Maize is known as a shade-intolerant C_4 species. By reducing its photosynthesis under shade, the electron transport and the carboxylation enzymes are reduced in the photosynthetic apparatus [26]. This was shown in a trial with maize/walnut and maize/plum intercropping. In 1 m distance from tree trunk, the plant height was reduced by about 16% and 20%, respectively. The reduction in height was attributed to the reduced photosynthesis rates. As a result, plants had less energy available for growth processes. LAI, as the second major biomass forming parameter, showed the same trend. A shading of 50% resulted in a significantly reduced LAI. Allison and Daynard [61] observed a 20% decrease in leaf area per plant if the time to silking is changed. This is in line with our observed results were a shade-induced prolonged interval of 2 to 9 days from emergence to start of flowering reduced final LAI by 22% and 39%, respectively. Other studies also showed a reduced leaf area under changes in the light environment. Dadashi et al. [62] observed in a weedy plant stand that this reduction was attributed to the reduced plant available light. As a result, the photosynthesis was also reduced, which resulted in lower plant growth.

Dadashi et al. [62] also found that the rates of accumulation of dry matter and leaf area were reduced by stress. Hence, a delayed occurrence of the phenological phases leads to a shorter period of biomass accumulation and, thus, a reduction in the final biomass yield, if harvest is timed on the ripeness of plants in non-shaded field sections. Sinclair and Horie [63] showed that the more LAI is available, the higher the radiation use efficiency is. The potential of a crop to accumulate biomass could be described by a function of the accumulated amount of biomass per unit of solar radiation. Dry matter accumulation rate varied in direct proportion to the amount of intercepted radiation. A shortening of the flowering phase negatively affected the leaf area and, also, on photosynthesis, which causes a reduced biomass growth and dry matter yield.

Our study showed that maize only showed a significant reduction in height and LAI at 50% shade. However, DS was significantly decreased even in the 12% shade treatment. Hence, the combination of growth parameters and DS resulted in a significantly reduced DMY even in the 12% shading treatment.

In addition to the amount of biomass produced, the quality-determining compounds also influence final biogas and methane yield. Results of our study indicated that only XP and XA were significantly influenced by shading. A significant increase in XP occurred at 50% shade, while XA significantly increased in the 12% shading treatment. The other quality determining parameters (XF, EE, NfE) showed no significant change under shading.

XP is an important component for biogas and methane formation in a biogas plant. A high amount of XP leads to a higher concentration of methane in biogas [64,65]. The results of our study were similar to the outcomes of Lin et al. [66] and showed an increasing protein content with increased shade intensity. Under 26% shade there was an increase in XP of 11%. Protein content increased due to an overall reduction in biomass yields. The same amount of nitrogen was available, but less biomass was built. An intercropping trial of loblolly pine trees (*Pinus taeda* L.) with herbages in a silvopastoral AFS in the southeastern USA showed at a shade of 55% that the plants XP of 11.5% was higher, compared to XP of 8.5% at 0% shade [67]. If less yield is formed, available nitrogen is less diluted and accumulates in higher concentrations of XP in these biomasses [33]. As shown by Early et al. [30], an increased protein content of vegetative plant parts grown under shade is associated with a reduction in plant growth and a decrease in kernel production.

XA showed a 10% increase under 12% shade, while under 50% shade there was an increase of 32%. High XA contents can be problematic in the biogas process, as XA is not degradable by the biogas microorganisms. If the XA content is too high, the ash can settle on the bottom of the fermenter and reduce the digestion space. This reduction can disturb and inhibit the biogas process. Maturation of plants is responsible for the number of digestible components. Within the progress of maturity, the proportion of inorganic matter decreases. Since the shaded plants were delayed in maturity, they showed a higher level of inorganic matter or XA [68,69]. We observed a shade-induced prolonged development and, thus, a delayed maturity, which caused higher XA contents.

All tested minerals, except Ca and S, showed a steady increase with shading intensity. However, increases in P, K, Mg were in an acceptable range that would not have a tremendous effect on the biogas formation process [37,38,40,70,71].

If we assume a density of 200 kg m^{-3} for maize silage dry matter, the critical values would be 1.5% for potassium, 0.5% for calcium, 0.3% for magnesium, and 1.9%–4.7% for sulfur [40,70]. If we convert the determined macronutrient contents of the 50% shade treatment on a DS base, values of 0.43% (potassium), 0.04% (calcium), 0.03% (magnesium), and 0.04% (sulfur) would be achieved. Hence, no negative impact on the biogas formation would be expected.

Biogas is a mixture with major proportions of methane (40%–75%) and carbon dioxide (25%–55%), as well as other gases in very low concentrations. Methane has a high energy content, which makes it a valuable compound of the overall biogas composition. In literature, there is no information available on how biogas and methane yield are influenced under different light reductions. Overall, the increase of non-degradable XA and the increase of slowly degradable XP in combination with the reduced biomass yields led to lower biogas and methane yields. Even under 12 % shading, biogas and methane yields were significantly reduced by up to 19%, due to lower biomass yields. A shading of 50% led to almost half of the final biomass and biogas and methane yields.

From yield aspects, silage maize cannot be recommended for the shade conditions in an AFS if compared to monocropping. But even with a shading of 26%, the maize has higher methane yields than cereal monocropping for biogas purpose, which generates 3200 to 4500 m^3 ha^{-1} methane [3]. If the below-ground factors, like nutrient, water and soil fauna are disregarded and the system is only considered for its light competition, silage maize cultivation up to a shading level of 26% can be possible. If political support for AFS in Germany would be granted, as it is already the case in France, silage maize production in an AFS could be profitable for the farmer [13]. However, further studies on the effect of the below-ground interactions would be needed to assess the system as a whole.

5. Conclusions

In all tested shading treatments, final biomass yield was reduced. The results indicated that shading maize at a level of 50% reduced the dry matter biomass yields up to nearly 50%. In addition, contents of XP and XA increased at a shading level of 50% and 12%, respectively. Other biogas parameters were not influenced by shade. Biogas and methane yield were also reduced at 12% shading. Overall, results of our study suggest that shading up to 26% would be tolerable for maize if the positive contribution of the tree strips is also taken into account. However, further investigations on the below-ground interactions are necessary to fully understand and evaluate these systems.

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