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Methane Yield Potential of Miscanthus (*Miscanthus* × *giganteus* (Greef et Deuter)) Established under Maize (*Zea mays* L.)

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Abstract: This study reports on the effects of two rhizome-based establishment procedures 'miscanthus under maize' (MUM) and 'reference' (REF) on the methane yield per hectare (MYH) of miscanthus in a field trial in southwest Germany. The dry matter yield (DMY) of aboveground biomass was determined each year in autumn over four years (2016–2019). A biogas batch experiment and a fiber analysis were conducted using plant samples from 2016–2018. Overall, MUM outperformed REF due to a high MYH of maize in 2016 (7211 $m^3{}_N$ CH_4 ha^{-1}). The MYH of miscanthus in MUM was significantly lower compared to REF in 2016 and 2017 due to a lower DMY. Earlier maturation of miscanthus in MUM caused higher ash and lignin contents compared with REF. However, the mean substrate-specific methane yield of miscanthus was similar across the treatments (281.2 and 276.2 l_N kg^{-1} volatile $solid^{-1}$). Non-significant differences in MYH 2018 (1624 and 1957 $m^3{}_N$ CH_4 ha^{-1}) and in DMY 2019 (15.6 and 21.7 Mg ha^{-1}) between MUM and REF indicate, that MUM recovered from biotic and abiotic stress during 2016. Consequently, MUM could be a promising approach to close the methane yield gap of miscanthus cultivation in the first year of establishment.

Keywords: biogas; biomass; cropping system; establishment; intercropping; low-input; maize; miscanthus; methane yield; perennial crop

1. Introduction

Miscanthus (*Miscanthus* spp.) is a fast growing perennial C4-grass [1], which has the potential to deliver high biomass yields and to grow on marginal agricultural land [2–5]. A wide range of miscanthus genotypes have been screened for different marginality factors such as salinity [6] and erosion [7]. Miscanthus biomass has quality characteristics that allow it to be used to manifold ways: as a combustion fuel, [8–10], bioethanol [11–13], bedding material [10,14,15], building material [16–20] and in biogas production [21,22]. For example, low inorganic constituents and high lignin content is preferred for combustion [23], whereas low lignin is required for efficient biogas [24] as well as ethanol production [25]. Miscanthus can also be a feedstock to processes including pyrolysis that can produce hydrocarbon fuels such as gasoline, diesel and jet fuel [26]. The variety of available genotypes, which have been developed over the years offer the possibility of selecting genotypes with optimal quality characteristics for a specific end use [27]. This study focuses on the use of miscanthus biomass for biogas production as it is considered one of the foremost promising bioenergy pathways [28–33].

Currently, some of the major impeding factors for miscanthus production across Europe are (i) high initial establishment costs, (ii) a lack of harvestable biomass in the first year [34–37] and (iii) a comparatively long crop establishment period [10,38]. Initial establishment costs largely depend on the establishment procedure. Over the years, different establishment procedures such as rhizome plantation [39–41], micro-propagation [42], direct seed sowing [37,39] or the use of plantlets obtained from stems or rhizomes have been tested to optimize the establishment procedure [42]. The adoption of a certain method is largely dependent on initial cost and its compatibility with the existing farming system especially in terms of farm machinery. Currently, direct plantation of rhizomes, which is inexpensive, is the mostly widely practiced establishment procedure [42]. However, it does not fit well with existing agricultural mechanization and requires specific machinery [10]. The adapted plantation method not only influences the initial cost but also impacts crop development especially during crop establishment period [34,37,42–44]. For example, vegetatively (via rhizomes) propagated miscanthus developed better canopies during the establishment period compared to rhizome based plantation [45].

Over time, the development of new machinery and new planting techniques may facilitate miscanthus cultivation and contribute towards reducing the initial establishment cost [42]. However, the absence of harvestable biomass during the first year complemented by rather high initial establishment cost aggravates the issue of economic viability of the crop during establishment period, which is one reason why farmers are reluctant to cultivate miscanthus. Consequently, there is need to identify innovative solutions for an optimized establishment of miscanthus which will make the crop more economically viable especially during the establishment period.

This study explores the potential effects of a recently developed miscanthus establishment procedure 'miscanthus under maize' (*Zea mays* L.; MUM) [34] on both methane yield per hectare (MYH) and fiber composition of miscanthus during the establishment period. It is expected that there will be a trade-off between the achievement of high MYH of the intercropped plant stand (maize and miscanthus) in MUM in the first year and the achievement of high MYH of miscanthus from the second year onwards. This assumption is based on higher biotic (intercropping competition) and abiotic stress (e.g., drought) in the first year of establishment of miscanthus, which can significantly influence its morphological development and thus its suitability as a biogas substrate in the following years [46,47].

2. Materials and Methods

This section reported on where the plant material was collected, how the plant samples were prepared and analyzed and how the results were evaluated. The major focus was on the fiber analyses and the biogas batch-experiments. Here, however, only basic information about the origin of the plant material was presented. For detailed information on the field trial, such as soil type, plant material and cultivation technique, please refer to Von Cossel et al. [34].

2.1. Origin of Plant Material

The plant material was taken from a field trial with randomized block design (three replicates per treatment) located in Hohenheim (southwest Germany). The field trial was established in 2016 (Figure 1) and has run continuously until the present. In this field trial, two miscanthus establishment procedures were tested: sole establishment (REF) and MUM. For miscanthus, rhizome-based plantlets of *Miscanthus* × *giganteus* (Greef et Deuter) were used.

Figure 1. Plant stand of miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) (1) established under maize (*Zea mays* L.) (2) in July 2016.

2.2. Fibre Analyses, Determination of C- and N-Content and Biogas Batch Experiment

The plant material was dried to constant weight at 60 °C to determine the dry matter content (DMC), which was used to calculate the DMY (Equation (1)). Afterwards, the samples were milled using a cutting mill (SM 200, Retsch, Haan, Germany) with a 1 mm sieve for further analysis. No other pre-treatments, e.g., enzymatic hydrolysis, were applied in the conversion process. The contents of ash, lignin, cellulose, hemicellulose, nitrogen (N) and carbon (C) were analyzed for all samples as follows: The ash content was estimated according to Kiesel and Lewandowski [46]. The contents of lignin, cellulose and hemicellulose were analyzed according to VDLUFA Method Book III, methods 6.5.1, 6.5.2 and 6.5.3 [48]. The contents of N and C were measured according to DIN ISO 5725 using the elemental analyzer 'Vario Max CNS' (Elementar Analysensysteme GmbH, Stuttgart, Germany).

Both biogas batchtest and fiber analysis were conducted according to Von Cossel et al. [49] and Kiesel and Lewandowski [46]. For the batchtest, 200 mg of organic dry matter of the plant samples was mixed with 30 g inoculum (4% DMC, origins from a biogas plant) in 100 ml air-tight bottles and kept at 39 °C for 35 days, a standard procedure according to VDI guideline 4630 [46,49,50]. Within this period, all digestible fractions of the plant samples, such as hemicellulose and cellulose, are to a large extend degraded by microorganisms and converted into biogas, which consists predominantly of CH_4 and CO_2 [46,51]. For each sample, there were four replicates within the batchtest. Gas was collected on the third, the 10th, the 22nd and final day of the batchtest (day 35). The gas production was measured via pressure increase using a hand-held pressure measuring devices for external pressure sensors (HND-P pressure meter, Kobold Messring GmbH, Hofheim, Germany). The frequency of these measurements decreased towards the end of the batchtest, because the biogas production also decreased. Therefore, the pressure increase was measured on a daily basis until day 7, every second day until day 17, and every third day until the end of the batchtest. In total, the pressure increase was measured 19 times during the batchtest. For each of these measurements, the surrounding air pressure was also documented to standardize the values (norm conditions: 0 °C and 1013 hPa). The accumulated substrate-specific biogas yield (SBY) was set in relation to the biogas production of the control (inoculum without plant material) and the daily air pressure of the room in which the batchtest was conducted. The methane content (MC) of the collected biogas was determined using a thermal conductivity detector at a detection temperature of 120 °C (GC-2014 gas chromatograph, Shimadzu, Kyoto). The substrate-specific methane yield (SMY) was calculated following Equation (1):

$$SMY = SBY \times MC. \tag{1}$$

2.3. Dry Matter Yield Determination

The agronomic details are presented and discussed in Von Cossel et al. [34]. In addition to the dry matter yield (DMY) presented in [34], in this study the DMY (green harvest) from the vegetation period 2019 was also determined. Therefore, the DMY was calculated using the fresh matter yield (FMY) and the DMC as follows:

$$DMY = FMY \times DMC. \tag{2}$$

Furthermore, the leaf:stem ratio of miscanthus (10 shoots per field replicate) was measured in 2018 and 2019. However, only plant material from the years 2016–2018 was used for the substrate analyses described in the following section.

2.4. Statistical Analyses

The biogas batch experiment was statistically analyzed as described in Von Cossel et al. [49], whereas outliers were omitted given a coefficient of variation of >5%. The F-tests for the effects of establishment under maize on both SMY and MYH were conducted as described in Von Cossel et al. [49]. The model is shown in Equation (3):

$$y_{ijk} = \mu + b_k + (b\varphi)_{jk} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + e_{ijk} \tag{3}$$

where b_k and $(b\varphi)_{jk}$ are the fixed across year and year-specific effect of the kth the pre-treatment, and μ is the intercept. e_{ijk} is the error of observation y_{ijk} with establishment procedure-specific variance. φ_j, τ_i and $(\tau\varphi)_{ij}$ are the fixed effects for the jth year, the ith establishment procedure and their interaction effects. The influence of factors was tested via a global F test. If differences were found, a multiple *t*-test was performed to create a letter display [52]. The assumptions of normality and homogeneous error variance were checked graphically. The best model was selected via the Akaike information criterion (AIC) [53]. All analysis run using the PROC MIXED procedure of the SAS® Proprietary Software 9.4 TS level 1M5 (SAS Institute Inc., Cary, NC, USA). For the correlation matrix and SMY prediction, PROC CORR and PROC REG (SAS ® Proprietary Software 9.4 TS level 1M5, see above) were used (see Appendix A, Table A1). Both degrees of freedom and standard errors were approximated using the Kenward–Roger method [54].

3. Results and Discussion

One of the most important results of the field trial underlying this study was the successful establishment in both establishment procedures REF and MUM. This means that in both REF and MUM all plants survived the winter periods during 2016–2019. Across years and treatments, the morphological and physiological characteristics of all observations (Table A2) were in line with current literature [10,55].

3.1. Dry Matter Yield

In both systems significant increase in dry matter yield were observed between one and four after establishments, whereas the total DMY of MUM (including the proportion of total DMY of maize in 2016) was significantly higher than that of REF (Figure 2).

The proportion of total DMY of miscanthus in MUM was significantly lower than in REF in 2016 and 2017 [34]. In the later years 2018 and 2019, however, there were no significant differences between REF and MUM [34]. This is in line with a finding from a recent study on intercropping miscanthus and legumes, in which similar effects were reported [47]. However, the potentially higher yielding variant REF was water limited in 2018 due to summer drought [34]. Therefore, a significantly higher dry matter yield could have been expected for REF than for MUM under normal precipitation conditions (>700 mm yr^{-1}) in 2018 (Figure 2). The underlying agronomic aspects of this observation are further described and discussed in detail in Von Cossel et al. [34]. Another aspect that could be of great importance in the context of the expansion of miscanthus cultivation in the future is the susceptibility of miscanthus to the Barley Yellow Dwarf Virus (BYDV). BYDV can be transmitted to miscanthus by the corn leaf aphid (*Rhopalosiphum maidis* Fitch) [56]. According to Hugget et al. [56], an expansion of the cultivation of miscanthus could lead to a further spread of the BYDV, which would have to be taken into account in the plant protection management of winter cereals. This has already been observed in France in the course of the spread of maize (also a host crop for the BYDV) [56]. However, BYDV will spread less strongly in miscanthus plant stands harvested in autumn (for biogas production) than in miscanthus plant stands harvested in winter (winter harvest for combustion and other utilization pathways) [56]. This is because miscanthus, which is only harvested in winter, can serve as an intermediate host for the corn leaf aphid before they can infest the winter cereals [56]. However, in the following sections, aspects of DMY formation and the expansion of miscanthus cultivation are not further discussed, as the present study focuses on the effects of establishment procedures on the biogas substrate properties of miscanthus.

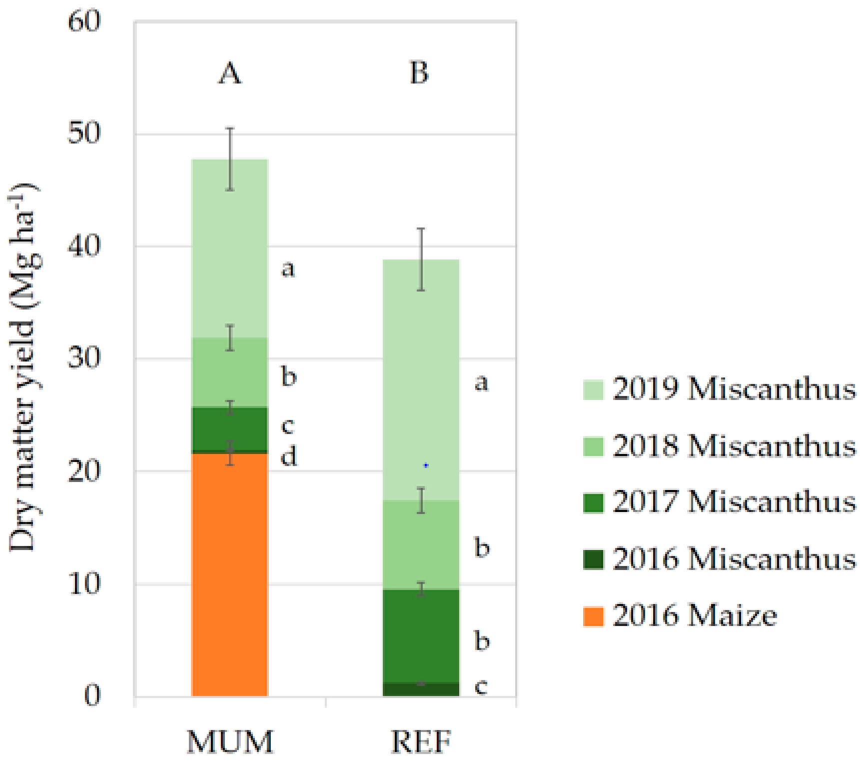

Figure 2. Stacked annual dry matter yields (DMY) of miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) established under maize (*Zea mays* L.; MUM) and alone (REF) during 2016 and 2019. For MUM, the proportion of total annual DMY of maize in 2016 was also included. Different upper case letters denote for significant differences between the four-year accumulated DMY of MUM and REF; different lower case letters denote for significant differences between annual DMY within treatments.

3.2. Methane Yield Potential

It was shown, that the accumulated MYH of a miscanthus biomass production system could be significantly improved during the first years of establishment through establishing miscanthus under maize. This was mainly caused by the high maize MYH in 2016 (Table A3). The MYH of MUM during the years 2016 and 2017 was significantly lower than REF due to an earlier maturation of the miscanthus plants in MUM and better development of miscanthus in REF. This could be explained by a slower development of the miscanthus plantlets in MUM starting from 2016 presumably due to both biotic (intercropping conditions) and abiotic (drought) stress conditions. Von Cossel et al. [34] point out that it must be carefully examined whether MUM starts catching up with REF table onwards. 2018 was a challenging year for both MUM and REF due to low precipitation. For REF, the water limitation was even more severe because the biophysical yield gap (the difference between potentially realizable and actual yield) is higher in REF than in MUM. Abiotic stress, especially drought is critical for defining the biogas substrate quality (BSQ) of miscanthus [46]. Therefore, it can be assumed that the low SMY of both treatments in 2018 (Table 1) was due to changes in biomass composition—especially an increase in lignin content as a response to drought stress. This hypothesis can be supported by the findings of numerous other studies, where lignin content increased under drought stress [57,58]. Furthermore, the results from the stepwise regression analysis show that lignin is the most important regressor for SMY-prediction models (Tables 2 and A1). This is basically in line with the literature [51,59], and a validation of Model 1 (with lignin as sole regressor) based on an external dataset [51] supported the high correlation between lignin content and SMY of miscanthus biomass (Table 2). However, it should still be considered that the plant samples were milled for the biogas batch experiment, which increases the methane yield compared to coarsely chopped material [46]. Therefore, it is generally recommended to evaluate the effects of MUM on the specific methane yield of miscanthus under practical conditions at large scale.

Here it was important to highlight that cellulose content was also highest during the year facing drought stress (2018), whereas hemicellulose content did not change to the same degree. Previous studies showed that (i) the efficiency of bioconversion was significantly influenced by the degree of cellulose crystallinity [60,61] and (ii) hemicellulose content was negatively correlated with cellulose crystallinity [62,63]. In addition, the correlation matrix supports this assumption: both cellulose and hemicellulose significantly correlated with the SMY (Table 3). From this it could be concluded that along with lignin content the ratio of hemicellulose to cellulose was crucial for an efficient bioconversion of miscanthus biomass. The ratio of hemicellulose to cellulose could be optimized to some extent through crop management practices such as adjusting harvesting time. For early green harvest, it was reported that (i) the contents of hemicellulose were higher, and (ii) the contents of cellulose and lignin were lower compared with late green harvest [46,64–66]. Furthermore, at early harvest a high N content is expected in the harvested biomass [8,67], which favors substrate digestion. This was also evident from the correlation matrix, where a highly significant positive correlation between SMY and N content is recorded (Table 3). Due to the earlier maturation, miscanthus biomass of MUM showed significantly lower N contents than REF in 2016. However, starting from 2017 the N contents between both variants were equal. The same applies for the C:N ratio. This indicates that the establishment of miscanthus under maize might not have a lasting effect on N content or the C:N ratio of miscanthus.

It has been reported that a high C:N ratio inhibits the digestion of biomass through production of volatile fatty acids [68–71]. Therefore, early harvest can contribute towards improving the hemicellulose to cellulose and C:N ratio as well, which will subsequently facilitate the bioconversion of biomass. However, it must be considered that the input demand of miscanthus is higher under green harvest regime crop because of poor relocation of nutrients back to rhizomes [67]. This in turn subsequently influences the environmental performance of miscanthus [3,72]. In the case of biogas production, to some extent it could be compensated by recycling nutrients through the application of digested material. Regarding the establishment procedure, this implies that earlier maturation in the first year of establishment increases the C:N ratio to the detriment of BSQ. On one hand, the DM content of miscanthus in the first year of establishment was negligible (Figure 2) and on the other hand earlier maturation had a positive effect on the back-shifting of nutrients (albeit with comparatively low quantitative relevance).

Table 1. Year-specific estimates for qualitative and quantitative traits of the miscanthus biomass in the two establishment systems "miscanthus under maize (MUM)" and "sole establishment of miscanthus (REF)". Different upper case letters denote for significant ($p < 0.05$) differences between establishment procedures within years, lower case letters for significant differences between years within establishment procedures.

Qualitative Parameter	Unit	MUM			REF		
		2016	2017	2018	2016	2017	2018
Substrate-specific methane yield	l_N kg^{-1} volatile $solid^{-1}$	290.8 ± 1.5 Ba	282.8 ± 1.0 Ab	269.9 ± 5.5 Ab	298.6 ± 1.5 Aa	274.3 ± 1.0 Bb	255.7 ± 5.5 Ac
Lignin	% of dry matter	7.4 ± 0.3 Ab	8.2 ± 0.1 Bb	11.1 ± 0.4 Aa	6.4 ± 0.3 Ac	9.0 ± 0.1 Ab	11.2 ± 0.4 Aa
Cellulose	% of dry matter	36.8 ± 0.3 Ac	40.0 ± 0.7 Ab	49.4 ± 0.8 Aa	32.8 ± 0.3 Bc	41.2 ± 0.7 Ab	48.9 ± 0.8 Aa
Hemicellulose	% of dry matter	28.5 ± 0.4 Ba	26.1 ± 0.4 Ab	27.4 ± 1.2 Aab	32.2 ± 0.4 Aa	27.1 ± 0.3 Ab	27.5 ± 1.2 Ab
Ash	% of dry matter	6.8 ± 0.3 Aa	6.1 ± 0.2 Aa	3.1 ± 0.1 Ab	7.3 ± 0.3 Aa	4.0 ± 0.2 Bb	2.1 ± 0.1 Bc
Carbon (C)	% of dry matter	45.7 ± 0.2 Ab	45.7 ± 0.1 Bb	47.3 ± 0.3 Aa	45.8 ± 0.2 Ab	47.1 ± 0.1 Aa	48.2 ± 0.3 Aa
Nitrogen (N)	% of dry matter	0.9 ± 0.0 Ba	0.5 ± 0.0 Ab	0.4 ± 0.1 Ab	1.3 ± 0.0 Aa	0.4 ± 0.0 Ab	0.4 ± 0.1 Ab
C:N ratio	-	2.0 ± 0.1 Ba	1.1 ± 0.1 Ab	0.7 ± 0.2 Ab	2.8 ± 0.1 Aa	0.9 ± 0.1 Ab	0.4 ± 0.2 Ab
Methane yield per hectare	m^3_N CH_4 ha^{-1}	74.1 ± 39.5 Bb	952.5 ± 118.9 Ba	1624.2 ± 285.3 Aa	338.3 ± 39.5 Ab	2256.5 ± 118.9 Aa	1956.5 ± 285.3 Aa

Table 2. Models for predicting the SMY of miscanthus biomass during the years 2016–2018 (* = $p <$ 0.05, ** = $p < 0.01$, *** = $p < 0.001$, n.s. = not significant, n.a. = not added to the model).

Regressor	Model 1	Model 2	Model 3
Intercept	342.35 ***	329.32 ***	299.12 ***
Lignin	−7.18 ***	−3.26 **	n.a.
C:N Ratio	n.a.	1033.88 **	n.a.
Hemicellulose	n.a.	−1.24 n.s.	n.a.
Lignin × Hemicellulose	n.a.	n.a.	−0.13 ***
Coefficient of determination (R^2)	0.8261	0.9752	0.9742
Validation [a] (R^2)	0.7881 *	- [b]	n.s.

[a] Based on miscanthus-specific observations from the supplementary dataset provided by Von Cossel et al. [51]. [b] Not applicable due to missing variables in the supplementary dataset of Von Cossel et al. [51].

Table 3. Correlation matrix (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n.s. = not significant) of qualitative miscanthus biomass traits.

Trait	Ash	Lignin	Cellulose	Hemicellulose	Carbon	Nitrogen
SMY	0.92 ***	−0.91 ***	−0.88 ***	0.59 *	−0.88 ***	0.92 ***
Ash		−0.95 ***	−0.93 ***	0.53 *	−0.95 ***	0.81 ***
Lignin			0.99 ***	−0.65 **	0.87 ***	−0.85 ***
Cellulose				−0.67 **	0.81 ***	−0.83 ***
Hemicellulose					−0.35 n.s.	0.82 ***
Carbon						−0.66 **

Additionally, the morphological development of miscanthus affects the SMY [73]. This was evident from results where cell wall components (lignin, cellulose and hemicellulose) varied between both stands, though the differences for some components were rather small (Tables 1 and 4). Among morphological traits, leaf:stem ratio is important because the composition of biomass varies depending on plant fraction [73]. For example, high hemicellulose, low lignin and cellulose contents were reported in leaves compared with stems [55] and which is why biomass with high leaf share was easily digestible [53,55]. In addition, the better digestibility of miscanthus biomass with high leaf share is also attributed to lignin structural differences such as lower molecular weight of leaf derived lignin compared with stem derived lignin [56]. Therefore, leaf:stem ratio is critical to determine the BSQ and subsequently bioconversion efficiency. In this study, the morphological development of miscanthus plants was also influenced by prevailing stress conditions, whereby the establishment procedure had no significant effect on the leaf:stem ratio on miscanthus from 2018 onwards (Figure A1). However, during 2018, miscanthus leaves became senescent under drought conditions, which reduced their digestibility [74]. Furthermore, leaf:stem ratio varies from species to species [46,74] and also with time of harvesting [53,56,57]. Therefore, miscanthus genotypes with a higher leaf:stem ratio than *Miscanthus × giganteus* (Greef et Deuter) also provide a better BSQ. It remains unclear whether a longer retention time (>35 d) would have significantly increased the specific methane yield of miscanthus (Figure 3), which could be inferred from the research results of Sonwai et al [75]. However, the SMY values of the present study fit well with those of existing literature [46,64,76], which is why it could be assumed that the retention time of 35 d was sufficient to compare the SMYs of miscanthus from MUM and REF. However, it has been shown that there is a clear trade-off between BSQ and biomass yield [46,77].

Table 4. Three-year average qualitative and accumulated quantitative parameters of the miscanthus plant stands established under maize (MUM) and alone (REF). Different letters denote for significant differences between MUM and REF for those parameters without significant interactions between Establishment procedure × vegetation period (Table 5).

Three-Year Average Qualitative Parameter	Unit	Establishment Procedure	
		MUM	REF
Specific methane yield	l_N kg^{-1} volatile solid^{-1}	281.2 ± 9.8	276.2 ± 9.8
Ash	% of dry matter	5.4 ± 1.3	4.4 ± 1.3
Lignin	% of dry matter	8.9 ± 1.3	8.9 ± 1.3
Cellulose	% of dry matter	41.9 ± 4.3	41.1 ± 4.3
Hemicellulose	% of dry matter	27.7 ± 1.2 b	28.8 ± 1.2 a
Carbon (C)	% of dry matter	46.2 ± 0.6 b	47.0 ± 0.6 a
Nitrogen (N)	% of dry matter	0.6 ± 0.3	0.6 ± 0.3
C:N ratio	-	1.2 ± 0.6	1.4 ± 0.6
Three-Year Accumulated Quantitative Parameter	**Unit**	**MUM**	**REF**
Dry matter yield	Mg ha^{-1}	10.2 ± 6.1	17.5 ± 6.1
Methane yield per hectare	m^3_N CH_4 ha^{-1}	2695.8 ± 1565.1	4506.3 ± 1565.1

Table 5. Fixed effects of 'Vegetation period', 'Establishment procedure' and their two-fold interaction on yield and quality parameters of miscanthus as biogas substrate across years (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n.s. = not significant). The fixed effects of 'Pre-crop 2015' and 'Pre-crop 2015 × vegetation period' were non-significant for all parameters and therefore, not added in the table.

Parameter	Effect		
	Vegetation Period	Establishment Procedure	Establishment Procedure × Vegetation Period
SMY	**	n.s.	**
Lignin	**	n.s.	*
Cellulose	**	n.s.	*
Hemicellulose	**	n.s.	n.s.
Ash	***	n.s.	**
Carbon (C)	*	**	n.s.
Nitrogen (N)	**	n.s.	*
C:N ratio	**	n.s.	*
Dry matter yield per hectare	**	*	*
Methane yield per hectare	**	*	*

So far, the differences in yield and quality parameters of MUM have been shown and discussed only regarding the miscanthus biomass. Thus, the share of maize MYH of the total MYH in 2016 must also be considered for MUM to allow for a more holistic comparison of the long-term effects of the two miscanthus establishment procedures MUM and REF. The total three-year (2016–2018) accumulated MYH of MUM—including both miscanthus and maize—accounted for about 9906 m^3_N CH_4 ha^{-} (Table 1, Table A3). This was approximately two and a half times as much as was reached by REF (about 4506 m^3_N CH_4 ha^{-1}; Tables 1 and 4). Consequently, the establishment procedure increased the total MYH of the new establishment procedure MUM compared with REF, even though miscanthus in MUM showed a weaker morphological development of miscanthus (reduced number of shoots, smaller shoots, lower MYH, etc.) compared with REF in the second year after establishment (Tables 1 and 5; Figure 4).

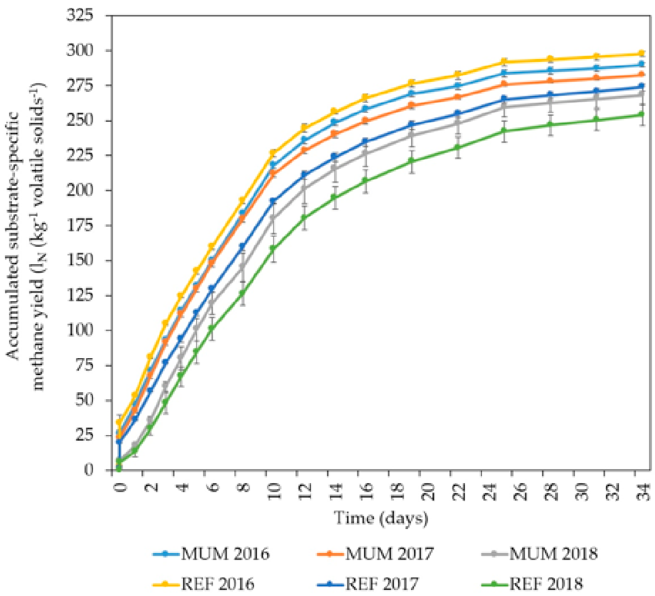

Figure 3. Development of the accumulated substrate-specific methane yields of miscanthus biomass from the two establishment procedures treatments 'miscanthus under maize' (MUM) and 'reference' (REF) during the biogas batch experiments. The error bars show the standard deviation (n = 6).

The establishment procedure MUM therefore provides a clear revenue advantage over the other establishment procedure REF within the first three years of establishment. Furthermore, the costs for soil tillage and herbicide measures should be virtually divided by two (maize cultivation and miscanthus cultivation). This reduces the costs for miscanthus establishment. Since the establishment costs commonly account for about a quarter of the total costs for 20 years of miscanthus cultivation [78] a reduction of the establishment costs may help to foster the implementation of miscanthus into existing farming systems.

However, the effects of both biotic (intercropping competition) and abiotic stress (e.g., drought) need to be further investigated with reference to marginal agricultural land utilization. This is because the cultivation of miscanthus should be promoted on marginal agricultural lands (rather than on favorable sites to avoid land use competition with food crop cultivation) [79–81]. The cultivation conditions on marginal agricultural lands can be challenging for miscanthus, which could worsen the recovery success of miscanthus. On the other hand, intercropping maize and miscanthus could reduce certain marginality constraints, such as wind and water erosion. Furthermore, the overall long-term performance of MUM for different end uses of miscanthus biomass should be evaluated in the future. This is because different end uses require different cultivation practices, which could affect the success of MUM in the long term. For example, a brown harvest (in winter) is usually applied for the end uses 'combustion' or 'isobutanol production'. Brown harvest regimes imply a better nutrient translocation to the rhizomes than a green harvest regimes (in autumn) [46]. A better nutrient translocation may help miscanthus plants to recover much better from the stress during the first year of establishment. Hence, the establishment of miscanthus under maize may be even more suitable for the brown harvest regime of miscanthus, and it should therefore be further investigated in the future.

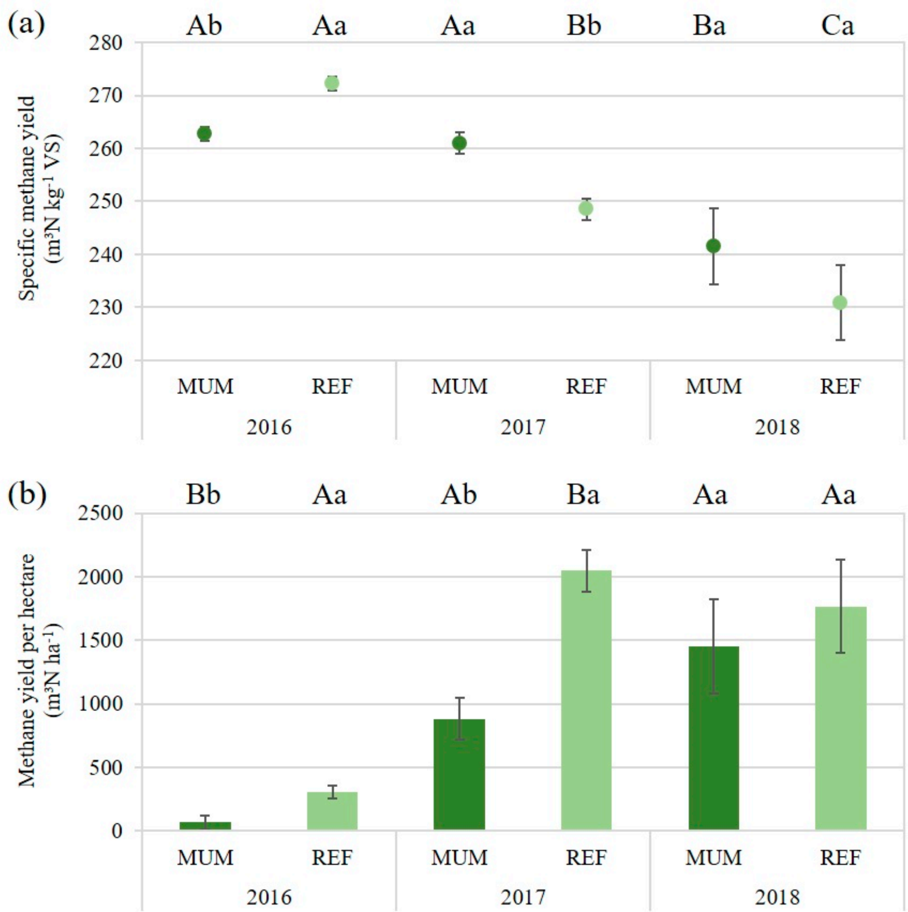

Figure 4. Overview of specific methane yields (**a**) and methane yields per hectare (MYH) of miscanthus biomass from the treatments under maize (MUM) and standard (REF) establishment. In 2016, only the proportion of total MYH of miscanthus is presented for MUM. The proportion of total MYH of maize in MUM 2016 are provided in Table A3. The error bars show the standard errors. Similar capital letters denote for non-significant ($p < 0.05$) differences within treatments between years; similar small letters refer to differences between treatments within years.

4. Conclusions

This study revealed new insights into the effects of a joint establishment of miscanthus (*Miscanthus × giganteus* Greef et Deuter) and maize (MUM) on the overall biogas yield of miscanthus for the establishment period (four years). While intercropping with maize in the first year significantly reduced the biogas yield of miscanthus in the second year after planting, no significant difference between the two establishment variants was observed in the third year after planting. In the fourth year, a non-significant difference in biomass yield indicated that miscanthus recovered from the stress of intercropping in the first year, so that no negative long-term effects on the yield level of miscanthus were to be expected due to the establishment under maize. Moreover, the high biogas yield from the maize proportion in the first year of MUM resulted in a significantly higher total biomass potential within the observation period of four years compared with the conventional establishment variant of miscanthus (REF). From this, it could be concluded that, compared to REF, MUM helped farmers to reduce the costs of miscanthus establishment by providing a first year's revenue from maize biomass.

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Appendix A

Table A1. Setup of Models 1–3 for prediction of substrate-specific methane yield. For selection of regressors, stepwise selection ($p < 0.15$) was chosen.

Model	Input Regressors	Selected Regressors
1	Lignin	Lignin
2	Ash, lignin, cellulose, hemicellulose, C, N, C:N ratio	Lignin, C:N ratio, hemicellulose
3	Ash, lignin, cellulose, hemicellulose, C, N, C:N ratio, ash × ash, ash × lignin, ash × cellulose, ash × hemicellulose, ash × C, ash × N; ash × C:N ratio, lignin × lignin, lignin × cellulose, lignin × hemicellulose, lignin × C, lignin × N, lignin × C:N ratio, cellulose × cellulose, cellulose × hemicellulose, cellulose × C, cellulose × N, cellulose × C:N ratio, hemicellulose × hemicellulose, hemicellulose × C, hemicellulose × N, hemicellulose × C:N ratio, C × C, C × N, C × C:N ratio, N × N, N × C:N ratio, C:N ratio × C:N ratio	Lignin × hemicellulose

Table A2. Simple statistics of both quantitative and qualitative traits across establishment procedures.

Parameter	Unit	Mean	Standard Deviation	Minimum	Maximum	n
Number of shoots per plant	-	21.7	12.1	5.0	41.0	18
Dry matter content	% of fresh matter	0.4	0.1	0.3	0.5	18
Dry matter yield [a]	Mg ha^{-1}	8.1	7.3	0.2	26.4	24 [a]
Specific methane yield	l_N kg^{-1} volatile solid^{-1}	278.7	15.1	248.1	301.0	18
Methane yield per hectare	$m^3{}_N$ ha^{-1}	1200.0	854.6	53.9	2506.0	18
Methane content of biogas produced	%	55.1	0.7	54.0	56.0	18
Ash	% of dry matter	4.9	2.0	1.9	7.7	18
Lignin	% of dry matter	8.9	1.9	6.0	11.9	18
Cellulose	% of dry matter	41.5	6.3	32.7	50.4	18
Hemicellulose	% of dry matter	28.2	2.2	24.7	32.2	18
Carbon	% of dry matter	46.6	1.0	45.3	48.4	18
Nitrogen	% of dry matter	0.7	0.4	0.2	1.3	18

[a] For the dry matter yield, also data from the vegetation period 2019 was available.

Table A3. Yield and quality parameters of maize in MUM 2016.

Parameter	Unit	Value
Dry matter yield	Mg ha^{-1}	21.6 ± 1.0
Dry matter content	% of fresh matter	34.5 ± 1.1
Specific methane yield	l_N kg^{-} volatile $solid^{-1}$	333.2 ± 0.5
Methane yield per hectare	$m^3{}_N$ CH_4 ha^{-1}	7210.5 ± 348.4

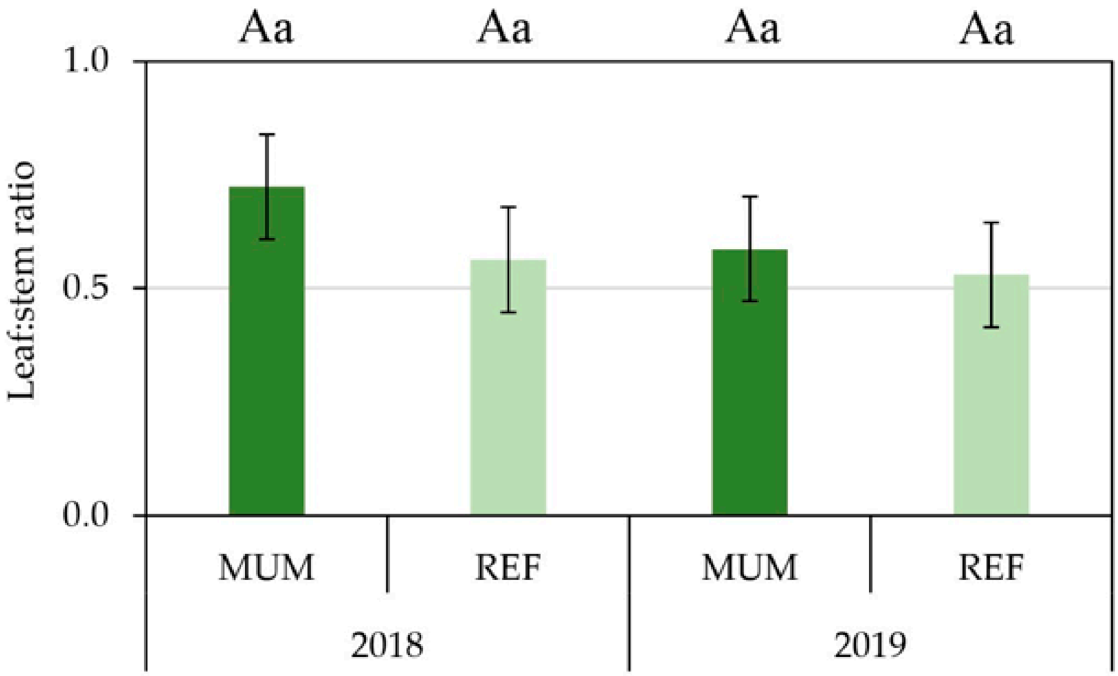

Figure A1. Leaf:stem ratio (and standard error) of miscanthus from different rhizome-based establishment procedures 'under maize' (MUM) and 'alone (REF) in 2018 and 2019 (the planting was conducted in 2016). Similar upper case letters denote for non-significant ($p < 0.15$) differences between treatments within years, lower case letters for non-significant differences between years within treatments.

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