WILEY BIOENERGY

Miscanthus for biogas production: Influence of harvest date and ensiling on digestibility and methane hectare yield

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Abstract

The 8,000 biogas plants currently in operation in Germany are mainly fed with biomass from annual crops. However, feedstock from perennial crops such as miscanthus is expected to be more environmentally benign. If miscanthus is to be used in greater amounts as a substrate for anaerobic digestion, storage will become a relevant topic, as a continuous supply of biomass throughout the year is necessary. The objective of this study was to identify the miscanthus harvest time that best balances the simultaneous achievement of high silage quality, high digestibility and high methane hectare yields. For this purpose, biomass from four miscanthus genotypes with varying senescence characteristics was harvested on three different dates in autumn 2017. Part of the biomass was ensiled, and the methane yield of both ensiled and non-ensiled biomass was analysed in a biogas batch test to assess the effect of ensiling on the methane hectare yield and digestion velocity. The ensiled biomass was found to have an up to 7% higher substrate-specific methane yield and also showed a higher digestion velocity than the non-ensiled biomass. The silage quality was best when miscanthus was harvested in mid-October, due to highest lactic acid content (average: 3.0% of DM) and lowest pH (average: 4.39) compared to the harvests in mid-September and beginning of October. Mass losses during ensiling (as high as 7.6% of fresh matter for the *M. sinensis* genotype *Sin55*) were compensated for by a higher substrate-specific methane yield (up to 353 Nml CH_4 (g oDM)$^{-1}$) in ensiled miscanthus. This resulted in non-significantly different methane hectare yields for non-ensiled (average: 4.635 Nm^3 CH_4/ha) and ensiled miscanthus biomass (4.803 Nm^3 CH_4/ha). A comparison of the four genotypes suggests that *Miscanthus x giganteus* is the most suitable genotype for ensiling as it had the best silage quality.

KEYWORDS
anaerobic digestion, biogas, energy crop, miscanthus genotypes, perennial, silage quality

1 | INTRODUCTION

Currently, there are more than 8,000 biogas plants installed in Germany with an approximate power capacity of 4 GW$_{el}$ (FNR, 2017). The methane produced is most commonly converted into electricity on site. Electricity produced from biogas accounts for approximately 5% of total German electricity generation (FNR, 2017). In future however, the idea is to feed

the methane into the gas grid and use it centrally in larger power stations. The pooling of the produced biomethane via the gas grid has several advantages. If the conversion of methane into electricity and heat takes place at locations with a high heat demand, the overall efficiency and economic viability of biogas plants increases (FNR, 2012; Scholz, Melin, & Wessling, 2013). Moreover, using the gas grid for collection and storage of biomethane facilitates a demand-driven energy supply and is one way in which anaerobic digestion can contribute to balancing out fluctuations in energy supply from wind and photovoltaic (FNR, 2012; Scholz et al., 2013). In addition, biomethane can be used for various other utilization pathways including transportation fuel or chemicals, due to its similarity to natural gas (FNR, 2012; Patrizio, Leduc, Chinese, Dotzauer, & Kraxner, 2015). Today, biogas plants already significantly contribute to the energy mix, but in future are expected to play a crucial role in energy supply systems. This is likely to lead to a stable or even increasing demand for biomass as a substrate for biomethane production.

In Germany, 51% of all biogas plants use biomass crops as feedstock, mostly annual crops (FNR, 2017). Perennial crops such as miscanthus are currently being investigated for their suitability for biogas production (Kiesel, Nunn, & Iqbal, 2017a; Mayer et al., 2014; Ruf, Schmidt, Delfosse, & Emmerling, 2017; Schmidt, Lemaigre, Ruf, Delfosse, & Emmerling, 2018; Wahid et al., 2015). Perennials are expected to be more environmentally benign than annual crops due to their low-input requirements and beneficial environmental profile (Kiesel, Wagner, & Lewandowski, 2017; McCalmont et al., 2017; Wagner et al., 2019). The risk of nutrient leaching and soil erosion, for example, is minimized as a result of undisturbed soil that is covered by vegetation during the whole year (Blanco-Canqui, 2010). In addition, it has been shown that soil organic carbon increases under perennials (Blanco-Canqui, 2010).

If perennials such as miscanthus are to be used in biogas plants with the aim of making biogas production more environmentally benign, several challenges need to be overcome. One of these is to identify the optimal date of a green harvest in autumn. Most studies dealing with green-harvested miscanthus have focused on the question of which harvest date achieves high methane hectare yields, while maintaining the long-term productivity of the crop (Kiesel & Lewandowski, 2017; Schmidt et al., 2018; Wahid et al., 2015). But very few studies have addressed the question of how to store green-harvested miscanthus for anaerobic digestion (Baldini, da Borso, Ferfuia, Zuliani, & Danuso, 2017; Whittaker, Hunt, Misselbrook, & Shield, 2016). Storage, however, is extremely relevant for anaerobic digestion, since a continuous supply of biomass is necessary throughout the year.

Until now, ensiling is the best-known preservation technique for biomass with high water content (Baldini et al., 2017). Whittaker et al. (2016) ensiled *Miscanthus* × *giganteus (M×g)* and *Miscanthus sacchariflorus* harvested in September in Rothamsted (UK) and concluded that additives are necessary. Baldini et al. (2017) demonstrated that *M×g* can be ensiled without additives and showed that, in Italy, the silage quality of miscanthus harvested in autumn was better than that harvested in summer.

Both studies mainly focused on the general feasibility of ensiling miscanthus but did not investigate the extent to which different harvest dates affect the ensiling and subsequent anaerobic digestion. Indeed, the study by Whittaker et al. (2016) found non-ensiled *M×g* to have a non-significant higher biomethane potential than ensiled *M×g*. However, this study only considered one harvest date. Baldini et al. (2017) investigated silage quality from two harvest dates, but did not analyse the effect of ensiled compared to non-ensiled miscanthus on methane hectare yield.

The intention of our study is to investigate both the optimal harvest date of miscanthus and the affects of ensiling on its methane hectare yield. It is known that harvest date of maize effects both processes, ensiling and biogas production (Amon et al., 2007). This is why maize is not harvested when the yield is highest, but when the best silage quality can be expected. However, for miscanthus it is not clear whether the digestibility is affected by the ensiling process. During ensiling, fermentation acids, such as acetic acid, are formed. Acetic acid is an intermediate in the anaerobic digestion process and therefore directly available for methanogen microorganisms. In addition, the fermentation acids may help to reduce the recalcitrance of the lignocellulosic miscanthus biomass and thus positively affect the methane yield of the silage. For this reason, ensiled miscanthus could be expected to be more easily digested than non-ensiled miscanthus. On the other hand, the conversion of sugars into fermentation acids is accompanied by energy losses, which negatively affects methane hectare yields. The question arises whether the two effects compensate each other or whether one is predominant?

The objective of this study was to identify the harvest date that best balances the simultaneous achievement of high silage quality, high digestibility and high methane hectare yield in miscanthus. We hypothesized that a later harvest date would have lower silage quality, lower substrate-specific methane yields and lower digestibility, due to higher dry matter contents. Moreover, we hypothesized that genotypes with earlier senescence would also have lower silage quality and substrate-specific methane yields due to higher dry matter and higher lignin contents. According to Galler (2011), biomass with higher dry matter content builds less lactic acid and is thus more difficult to ensile. In addition, lignin is known to reduce the biodegradability of biomass (von Cossel, Möhring, Kiesel, & Lewandowski, 2018; Fernandes, Bos, Zeeman, Sanders, & van Lier, 2009) and therefore biomass with higher lignin content is expected to have lower methane yields.

	Miscanthus × *giganteus (M×g)*	*GNT1*	*GNT3*	*Sin55*
Type of genotype	Natural hybrid of *M. sinensis* and *M. sacchariflorus*	Artificial hybrids of *M. sinensis* and *M. sacchariflorus*		*M. sinensis* genotype
Origin	South-East Asia	Miscanthus breeding programme of Aberystwyth University		
Propagation characteristics	Vegetative propagation via rhizomes or in vitro culture	Seed propagation		
Senescence characteristics	Early senescence	Later senescence than *M×g*		Stay-green genotype (delayed senescence compared to *M×g, GNT1* and *GNT3*)
Additional information	Currently, single commercially available genotype			High leaf proportion

To test our hypotheses, four miscanthus genotypes with varying senescence characteristics were harvested on three different dates in autumn 2017. Part of the biomass was ensiled, and the methane yield of both ensiled and non-ensiled biomass was then analysed in a biogas batch test to assess the effect of ensiling on the methane hectare yield and digestion velocity.

2 | MATERIALS AND METHODS

The experiment was performed in two phases. The first consisted of a field trial; in the second, samples from the field trial were processed in the laboratory.

2.1 | Field trial

Biomass was harvested in 2017 (third growing season) from a field trial at "Unterer Lindenhof," a research station of the University of Hohenheim. The experimental design was a split-plot design with four replications using genotypes as main plot factor and harvest date as sub-plot factor. Detailed information on the field trial is provided in Mangold et al. (2019). An overview of the weather conditions in 2017 can be found in Supporting Information Table S1.

Four different genotypes, *Miscanthus × giganteus (M×g)*, *GNT1*, *GNT3* and *Sin55,* were established, details of which are provided in Table 1. These genotypes were harvested on three different harvest dates (HD) in 2017: mid-September (18 September; HD 1), beginning of October (4 October; HD 2) and mid-October (17 October; HD 3).

At harvest, the border of each plot was removed and eight plants (approx. 4 m^2) were cut at a height of 20 cm using a field trial harvester "Baural." The chopped plant material was weighed and two subsamples of each plot were taken. The harvested area was measured to determine the fresh matter yield (FMY) per hectare. One subsample (subsample 1) was dried in a cabinet dryer at 60°C to constant weight to determine the dry matter content (DMC). The DMY was calculated based on fresh matter yield (FMY) and DMC. The second subsample (subsample 2) was used for the silage trial. The chopped material was filled into plastic bags and transported to the laboratory.

2.2 | Ensiling miscanthus

In the laboratory, subsample 2 was divided into subsample 2a and 2b. Subsample 2a was used to analyse the buffer capacity and methane yield of the raw, non-ensiled biomass. Subsample 2b was ensiled to analyse the silage quality and methane yield. Analyses of silage quality included silage acids (acetic, lactic, propionic, butyric acid), ethanol, sugars and pH.

Subsample 2a was dried at 60°C to constant weight and milled using a cutting mill (SM 200; Retsch GmbH, Haan, Germany) equipped with a 1-mm sieve.

For the analysis of the buffer capacity, a further subsample of 2a was dried again at 105°C for 4 hr in a drying cabinet and sent to an external laboratory (Center for Agricultural Technology [LTZ] Augustenberg, Karlsruhe, Germany). To estimate the buffer capacity, 100 ml distilled water was added to 1 g of the dry samples (ratio 1:100). After 30–60 min,

lactic acid was titrated until a pH value of 4.0 was reached. The buffer capacity was then calculated by Equation 1:

$$BC = (T - BV) \cdot F \cdot \frac{M_{\text{lactic acid}}}{DM} \qquad (1)$$

where BC is the buffer capacity, T is the titration value (amount of lactic acid); BV = blind value; F = factor of the 0.1 mol/L lactic acid; $M_{\text{lactic acid}}$ = molecular weight of lactic acid 90.08 g/mol; DM = dry matter content in %.

Subsample 2b was used for the ensiling trial. At each harvest date, the biomass was ensiled a few hours after the harvest. This trial was conducted according to the DLG guideline for the assessment of silage additives (2013). Depending on the DMC of each genotype at harvesting, 550–700 g of the chopped biomass was pressed with a wooden pestle into WECK® glass jars of 1.5-L volume. This resulted in different packing densities: *GNT1*, *GNT3* and *Sin55* had a packing density of 465 kg/m³ on all three harvest dates, as 700 g of fresh biomass was pressed into each jar. *M×g* had a packing density of 400 kg/m³ on HD 1 and HD 2 (600 g fresh biomass pressed into jars) and a density of 366 kg/m³ on HD 3 (550 g of fresh biomass). After filling each jar, the rim was cleaned with a paper towel to free it of any biomass particles. The jar was then closed airtight with a rubber ring, a glass lid and two metal clips. This type of sealing ensures that ambient air cannot enter the jar, but that overpressure, originating from gases produced in the ensiling process, is released before critical pressures are reached. Two silage jars were filled from each subsample 2b and thus from each field plot in order to have a "backup" jar should the ensiling of one jar fail. The maximum filling difference was set at 5 g fresh matter over all jars of the same treatment (genotype × harvest date). After all silage jars had been filled, they were stored according to a completely randomized design in a climate chamber (25°C, 60% humidity) for 90 days. The glasses were weighed daily in the first 8 days and then once a week for the remaining storage period to assess the gaseous fresh matter losses of the biomass during the ensiling process.

After the 90-day storage period, the silage jars were removed from the climate chamber and opened. As no fouling or mould was observed in any of the samples, the silage of both jars from the same plot was pooled and a subsample of 50 g taken. This subsample was filled into a plastic bag and stored in a freezer (−20°C) until it was used for silage quality analysis. The remaining silage was dried at 60°C in a drying cabinet to constant weight and then the DMC was calculated. It was then milled following the same protocol as for subsample 2a (cutting mill SM 200 [Retsch GmbH] equipped with a 1-mm sieve). The same procedure was applied for each of the three harvest dates.

Once the ensiling trial from all three harvest dates was complete, the frozen 50-g subsamples of each genotype × harvest date combination were sent to the agricultural centre (LAZBW) Aulendorf for analysis of silage acids (acetic, lactic, propionic and butyric acid), ethanol, sugars and pH.

The silage acids, ethanol and sugars were determined by HPLC analysis. For this, 250 ml distilled water was added to the frozen 50-g subsamples. The water and silage mixture was homogenized twice in a Stomacher 400 circulator on the highest setting, each time for 2 min. An extract was prepared, 10 ml of which was centrifuged for 10 min at 10,000 rpm and then analysed in the HPLC.

2.3 | Biogas batch test

A biogas batch test was conducted according to the VDI guideline 4630 to measure the substrate-specific biogas and methane yield of each "genotype × harvest date combination" for ensiled (subsample 2b after drying and milling as described above) and non-ensiled (subsample 2a after drying and milling) biomass. From each sample, 200 mg oDM (organic dry matter = volatile solids) was filled into a gastight fermentation flask and mixed with 30 g inoculum (4% DM content, 37% ash content). This resulted in an inoculum:substrate ratio of 3.8:1. The inoculum was obtained from the digester of a commercial mesophilic biogas plant that uses maize, grass and cereal whole-crop silage, liquid and solid cattle manure and small quantities of horse manure as substrates. The oDM content was estimated by weight loss during drying of an aliquot of approx. 1 g at 105°C in a cabinet dryer and incineration at 550°C in a muffle kiln to constant weight. The fermentation flasks were placed in a water bath at 39°C in a randomized block design for 35 days. The biogas production was measured via the pressure increase inside the flasks, and the methane content was measured by a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan). The biogas production was calculated as dry gas under standard conditions (0°C, 1,013 hPa). A detailed description of the biogas batch test method is provided in Kiesel and Lewandowski (2017).

Since both ensiled and non-ensiled samples were analysed, it is important to highlight that all samples were dried at 60°C before analysis in the biogas batch test. Drying silage partly removes volatile organic compounds. For this reason, the DM content of such samples is often corrected. However, in our study, we did not make any corrections to the DM content for two reasons. Analysis of ensiled biomass dried at 60°C gives only minor differences between corrected and uncorrected substrate-specific methane yields (SMY; Mukengele & Oechsner, 2007). In addition, it has been shown by Mukengele and Oechsner (2007) that drying at 60°C almost completely removes acetic acid (93%) and ethanol (98%) (lactic acid was

TABLE 2 *p*-Values for *F* tests of fixed effects ($\alpha = 0.05$) of silage quality

	DMC_{silage}	Lactic acid	Acetic acid	Butyric acid	Ethanol	pH	Fructose	Buffer capacity	Mass losses
Genotype	<0.0001	0.0001	0.0533	0.0713	<0.0001	0.0011	<0.0001	0.0216	0.0019
Harvest date	<0.0001	<0.0001	0.0605	0.0007	0.3683	<0.0001	0.0008	0.0391	<0.0001
Genotype × Harvest date	0.4650	0.3813	0.0873	0.8961	0.6702	0.2787	0.0079	0.1931	0.7407

difficult to measure in their study and therefore volatility rate was only estimated). In our study, the amounts of acetic acid and ethanol present in the silage (0.7% of DM and 0.09% of DM, respectively) were negligible.

Additionally, the digestion velocity of the miscanthus biomass was assessed by the volume of biogas produced per hour. The biogas batch test included an internal laboratory maize standard (harvested in 2012) for comparison purposes and to monitor the biological activity of the inoculum.

The biogas production of each substrate and the velocity (biogas produced per hour) presented in the results section are net values, that is, the biogas production of the inoculum has already been deducted.

The methane hectare yield was calculated by multiplication of substrate-specific methane yield and organic dry matter yield. For the silage treatment, the calculation also took the mass losses during ensiling into account.

2.4 | Statistical analysis

Substrate-specific methane yield (SMY) and methane hectare yield (MY) were analysed by a linear mixed model, which considered both field trial and laboratory design (Equation 2).

$$y_{ihjlk} = \mu + g_i + d_h + f_j + (gd)_{ih} + (df)_{hj} + (gf)_{ij} + (gdf)_{ihj} + s_l + (gs)_{il} + r_k + e_{ihjlk} \tag{2}$$

where y_{ihjlk} is the measurement of the *i*-th genotype on the *h*-th harvest date with the *j*-th effect of ensiling in the *l*-th field replication and the *k*-th laboratory replicate. μ is the general effect, g_i is the *i*-th genotype effect *(M×g; GNT1; GNT3; Sin55)*, d_h is the main effect of the *h*-th harvest date (HD 1; HD 2; HD 3), f_j is the main effect of the *j*-th ensiling (non-ensiled; ensiled), $(gd)_{ih}$ is the interaction effect of the *i*-th genotype with the *h*-th harvest date, $(df)_{hj}$ is the interaction effect of the *h*-th harvest date with the *j*-th ensiling, $(gf)_{ij}$ is the interaction effect of the *i*-th genotype with the *j*-th ensiling, $(gdf)_{ihj}$ is the interaction effect of the *i*-th genotype with the *h*-th harvest date and the *j*-th ensiling, s_l is the random effect of the *l*-th replicate in the first phase (field), r_k is the random effect of the *k*-th replication in the second phase (laboratory), and $(gs)_{il}$ is the main plot error associated with the area where genotype *i* in replicate *l* is grown. e_{ihjlk} is the residual error term corresponding to y_{ihjlk}.

As no replicates were performed in the laboratory for the silage quality parameters (silage acids, pH value, sugar content, buffer capacity, mass losses), r_k was removed from the model. In addition, where only ensiled samples were analysed, all effects including ensiling (f_j) were dropped from Equation 2. Thus, the model simplifies to Equation 3.

$$y_{ihl} = \mu + g_i + d_h + (gd)_{ih} + s_l + (gs)_{il} + e_{ihl}, \tag{3}$$

where all effects are denoted as on Equation 2. In all analyses, residuals were graphically checked for normality and homogeneity of variance. Where significant differences were found using an *F* test, a multiple *t* test (LSD) with $\alpha = 0.05$ was performed. A letter display using identical letters for means which are not significant from each other was used. Additionally, simple means were calculated for presentation purpose only. All data analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Dry matter yield

The highest average dry matter yield (DMY) over all three harvest dates was found in genotype *M×g* (20.69 t DM/ha) and the lowest in *Sin55* (13.26 t DM/ha), with genotypes *GNT1* (18.51 t DM/ha) and *GNT3* (16.69 t DM/ha) in between. The differences in DMY between the three harvest dates were only significant for *Sin55*, which had the highest yield at HD 3 (16.59 t DM/ha) and the lowest at HD 1 (9.29 t DM/ha). Genotype *GNT1* had the lowest yield at HD 3, the other three at HD 1.

The average dry matter content (DMC) of all four genotypes and all harvest dates was 33.4%. *M×g* had the highest DMC (39.7%) and *GNT3* the lowest (29.4%). Detailed results for the dry matter yields and contents are shown in Mangold et al. (2019).

3.2 | Silage quality

The silage quality of the genotypes was assessed by analysing the buffer capacity, the content of a number of silage acids, ethanol and sugars, and the pH of the silage.

The test for fixed effects (Table 2) showed highly significant impacts of harvest date on dry matter content (DMC$_{silage}$), lactic acid content, pH value and mass losses within a genotype (level of significance $\alpha = 0.05$). DMC$_{silage}$, lactic acid, ethanol and fructose contents were highly affected by genotype (Table 2). The interactions of genotype × harvest date were only significant for the parameter fructose content. An overview of all results relevant for the silage quality is given in Supporting Information Table S2.

The average dry matter content of the silage was significantly higher for *M×g* (36.7%) compared to 28.6%–30.2% for the other genotypes (see Table 4).

The lactic acid content increased with later harvest date (Table 3). Of all genotypes, *M×g* had the highest lactic acid

TABLE 3 Marginal means of silage quality parameters with their standard error for harvest date. For each parameter, means with identical letters are not significantly different from each other

Quality parameter	HD 1	HD 2	HD 3
DMC$_{silage}$ (%)	29.56[b] ± 0.39	29.41[b] ± 0.39	34.68[a] ± 0.39
Lactic acid (% of DM)	0.66[b] ± 0.27	1.09[b] ± 0.27	3.06[a] ± 0.27
Butyric acid (% of DM)	0.17[a] ± 0.02	0.16[a] ± 0.02	0.06[b] ± 0.02
pH	5.13[a] ± 0.07	4.97[a] ± 0.07	4.39[b] ± 0.07
Buffer capacity	3.67[b] ± 0.22	4.21[ab] ± 0.22	4.50[a] ± 0.22
Mass losses (% of FM)	6.59[a] ± 0.37	6.56[a] ± 0.37	4.28[b] ± 0.37

content (average: 2.97% of DM) and *Sin55* the lowest (average: 0.99% of DM; Table 4). Across genotypes, HD 3 had the significantly highest lactic acid content (Table 3). Acetic acid content was highest in the genotypes *GNT3* and *Sin55* (average content: 0.9% of DM). Butyric acid content was significantly lowest at HD 3 for all genotypes. *M×g* had the lowest average butyric acid content (0.07% of DM) and *Sin55* the highest (0.15% of DM). The propionic acid content was so low in all genotypes that the results are not presented here. The ethanol content of all genotypes was not significantly different between the harvest dates. *M×g*, however, had a significantly higher ethanol content compared to the other genotypes (Table 4).

The ensiling process requires a low pH of max. 4.5 to perform sufficiently and ensure stable preservation of the biomass (Galler, 2011). This pH value was achieved by all genotypes on HD 3, by *M×g* even on HD 2 (Supporting Information Table S2). *M×g* had the lowest average pH value (4.5), the other three genotypes had the same average pH value (4.9; Table 4).

Glucose and sucrose were not detectable in the biomass (data not shown); fructose content was low (Supporting Information Table S2). The average buffer capacity was lowest for *M×g* (3.58) and highest for *GNT1* (4.67; Table 4). The mass losses during ensiling decreased significantly with later harvest date in each genotype (Table 3). *M×g* had the lowest average mass losses (4.3% of FM) and *Sin55* the highest (6.4% of FM; Table 4).

3.3 | Substrate-specific and methane hectare yield

As shown in Table 5, the substrate-specific methane yield (SMY) was affected by interactions of harvest date with genotype and ensiling. Figure 1 shows the mean values of harvest date-by-ensiling combinations for the SMY. Taken as an average across all genotypes, the SMY tends to decrease from HD 1 to HD 3 (Figure 1). Additionally, it can be seen that, as an average across all genotypes, non-ensiled

Quality parameter	$M{\times}g$	GNT1	GNT3	Sin55
DMC$_{silage}$ (%)	36.7^a ± 0.43	30.24^b ± 0.52	29.28^{bc} ± 0.43	28.66^c ± 0.43
Lactic acid (% of DM)	2.97^a ± 0.30	1.24^b ± 0.34	1.21^b ± 0.30	0.99^b ± 0.30
Ethanol (% of DM)	0.12^a ± 0.004	0.08^b ± 0.005	0.09^b ± 0.004	0.09^b ± 0.004
pH	4.51^b ± 0.08	4.97^a ± 0.09	4.93^a ± 0.08	4.91^a ± 0.08
Buffer capacity	3.58^c ± 0.25	4.67^a ± 0.29	4.42^{ab} ± 0.25	3.83^{bc} ± 0.27
Mass losses (% of FM)	4.30^b ± 0.41	6.23^a ± 0.48	6.29^a ± 0.41	6.43^a ± 0.41

biomass had a significantly lower SMY than ensiled biomass at each harvest date (Figure 1). The genotypes $M{\times}g$ and GNT1 had on average 7%, and the genotypes GNT3

TABLE 5 *p*-Values for F tests of fixed effects ($\alpha = 0.05$) of substrate-specific methane yield (SMY) and methane hectare yield (MY)

	SMY	MY
Genotype	<0.0001	<0.0001
Harvest date	0.0005	0.0032
Ensiling	<0.0001	0.5067
Genotype × Harvest date	0.0212	0.0483
Harvest date × Ensiling	0.0315	0.8620
Genotype × Ensiling	0.3865	0.9447
Genotype × Harvest date × Ensiling	0.1097	0.9995
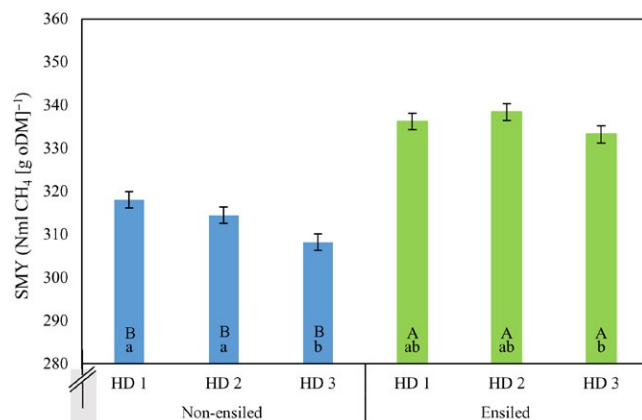
FIGURE 1 Mean substrate-specific methane yield (SMY) [Nml CH$_4$ (g oDM)$^{-1}$] for harvest date × ensiling. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for non-ensiled biomass (blue bars) and ensiled biomass (green bars) are shown by different lower-case letters. Different upper-case letters indicate significant differences between non-ensiled and ensiled biomass for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for SMY

and Sin55 on average over 6% higher SMY for the ensiled than the non-ensiled biomass.

Figure 2 shows the mean values of genotype × harvest date for SMY. Taken as an average of ensiled/non-ensiled biomass, Sin55 had the highest (337 Nml CH$_4$ (g oDM)$^{-1}$) and $M{\times}g$ the lowest (307 Nml CH$_4$ (g oDM)$^{-1}$) average SMY of all genotypes. The SMY of GNT1 remained stable over all three harvest dates, whereas GNT3 and Sin55 had a significantly lower SMY at HD 3 than HD 1. $M{\times}g$ had its significantly lowest SMY at HD 1.

By way of comparison, the inoculum alone produced 42.87 Nml biogas with a methane content of 43.78%; the maize standard had an average SMY of 356 Nml CH$_4$ (g oDM)$^{-1}$) (results not shown).

As can be seen in Table 5, the methane hectare yield (MY) was significantly influenced by genotype × harvest date, but not by the ensiling process. Figure 3 presents the mean methane hectare yield for genotype × harvest date. The highest MY (average of ensiled/non-ensiled biomass) was achieved by $M{\times}g$ at HD 2 (5,978 Nm^3 CH$_4$/ha). The lowest average MY was observed in Sin55 (2,684 Nm^3 CH$_4$/ha) at HD 1. GNT3 and Sin55 had a significantly higher MY at HD 3 than at HD 1. $M{\times}g$ and GNT1 had its highest MY at HD 2 (Figure 3).

3.4 | Velocity of biogas production

The velocity of fermentation of all genotypes, non-ensiled and ensiled, from the three harvest dates is shown in Figure 4. All four genotypes had a considerably lower velocity of biogas production than maize, especially in the first five days of fermentation. On average, $M{\times}g$ biomass produced less biogas per hour than the other three genotypes up to day 11. From this day onwards, a similar or slightly higher velocity was observed for $M{\times}g$ than the other three genotypes.

The digestion velocity was higher in the ensiled than the non-ensiled biomass of all four miscanthus genotypes in the first few days. Non-ensiled biomass of all genotypes had the highest velocity at HD 1, except $M{\times}g$, which had highest velocity at HD 3. By contrast, for the ensiled

FIGURE 2 Mean substrate-specific methane yield (SMY) [Nml CH4 (goDM)−1] for genotype × harvest date. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for each genotype *(Mxg [a], GNT1 [b], GNT3 [c], Sin55 [d])* are shown by different lower-case letters (ns: not significant). Different upper-case letters indicate significant differences between genotypes for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for SMY
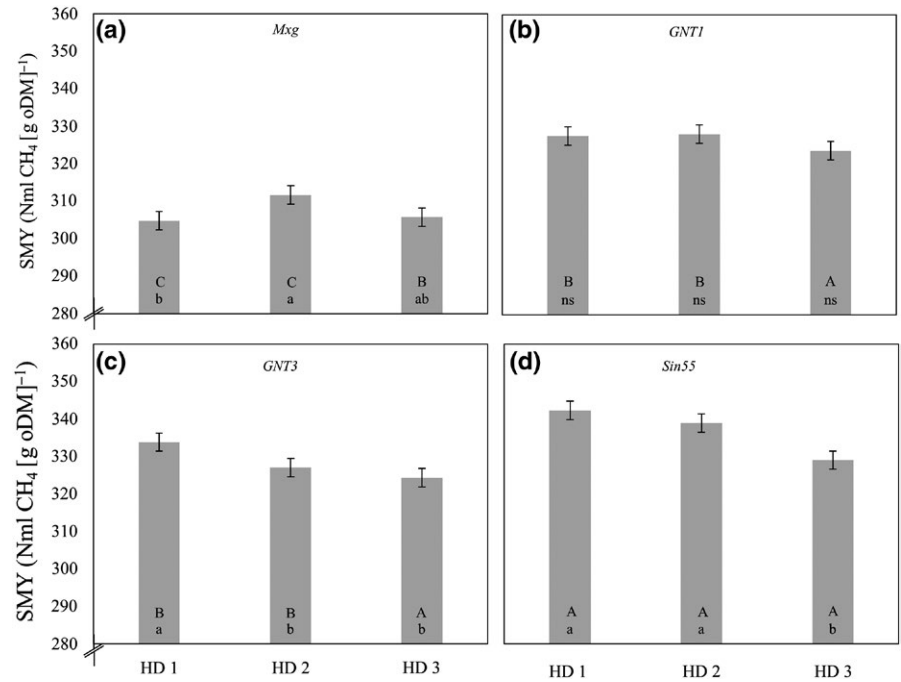
FIGURE 3 Mean methane hectare yield (MY) [Nm3 CH4/ha] for genotype × harvest date. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for each genotype *(Mxg [a], GNT1 [b], GNT3 [c], Sin55 [d])* are shown by different lower-case letters (ns: not significant). Different uppercase letters indicate significant differences between genotypes for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for MY.
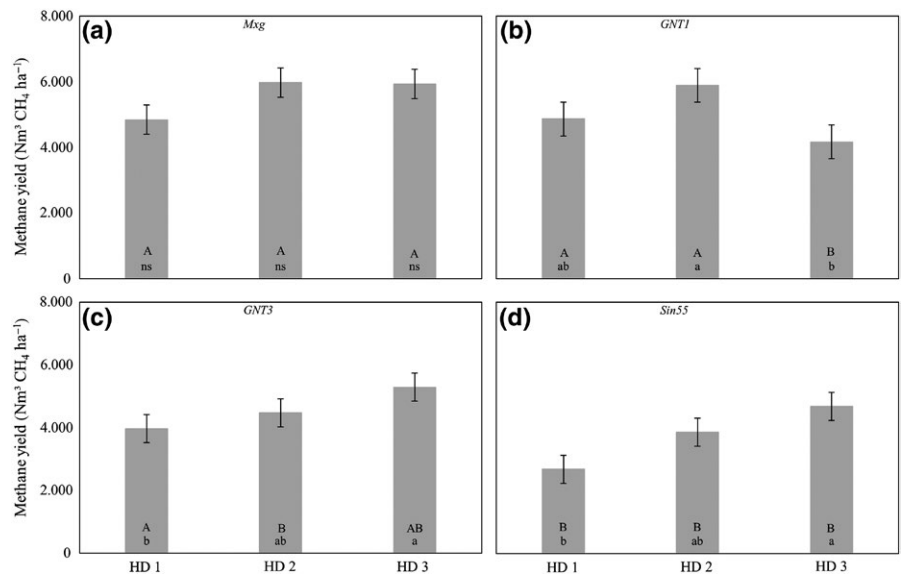
biomass, a later harvest date was more favourable, as the digestion velocity tended to be higher. It is noticeable that the ensiled biomass of all genotypes from HD 3 behaved similarly to maize, that is, the digestion velocity increased within the first day of fermentation and then decreased again.

4 | DISCUSSION

This study demonstrates that the ensiling of miscanthus biomass is possible without additives and that ensiling positively influences the substrate-specific methane yield and digestion velocity. In addition, it was shown that silage quality varies

between genotypes and harvest dates. The following sections discuss the differences in silage quality between the four genotypes and three harvest dates, and also the effect of ensiling on methane yield and digestion velocity. Finally, we give a summary of the results and an outline of what the findings mean for agricultural practice.

4.1 | Ensiling ability of miscanthus biomass

The quality of silage can be measured by various parameters, for example, silage acids and pH value. The two acids, lactic acid and butyric acid, are often used to classify silage quality, as a high level of lactic and low level of butyric acid indicate silage of good quality (Galler, 2011; Liu, Ge, Liu, & Li,
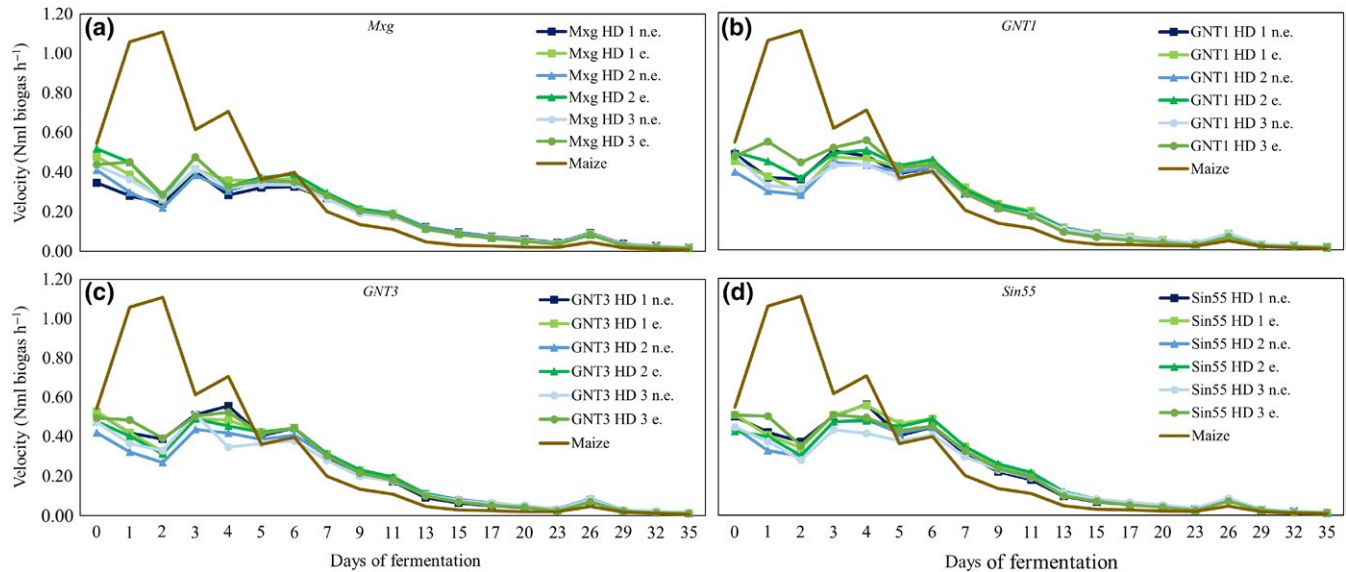
FIGURE 4 Velocity of fermentation [Nml biogas/hr] of the four genotypes ($M{\times}g$ [a], *GNT1* [b], *GNT3* [c] and *Sin55* [d]) from the three harvest dates (HD 1, HD 2 and HD 3), both non-ensiled (n.e.) and ensiled (e.) over the fermentation period of 35 days. Non-ensiled biomass is shown in blue colours, ensiled biomass in green colours. HD 1 is indicated by a square, HD 2 by a triangle and HD 3 by a circle. For comparison purposed, the velocity of maize (brown line) is shown as reference

2016). A number of studies recommend a pH value within the range of 3.7–4.5 to achieve a sufficient silage quality (Galler, 2011; Liu et al., 2016; Teixeira Franco, Buffière, & Bayard, 2016; Vervaeren, Hostyn, Ghekiere, & Willems, 2010).

In our study, all genotypes had the highest lactic acid content, lowest butyric acid content and lowest pH at HD 3 (Table 3). Thus, it could be concluded that, in 2017, HD 3 was the best date to harvest miscanthus for ensiling. A comparison between genotypes shows that $M{\times}g$ seems to be the most suitable for ensiling, as it not only had highest lactic acid contents, but also lowest butyric contents and the lowest pH (Table 4). By contrast, *Sin55* had the least favourable silage quality values, with the lowest lactic acid contents, highest average butyric acid contents and a comparatively high pH (average 4.91). Therefore, our hypotheses that HD 1 and the stay-green genotype *Sin55* are most suitable for ensiling were not confirmed.

According to Teixeira Franco et al. (2016), higher packing density leads to better silage quality, due to higher lactic acid contents, which makes the silage more stable. With increasing DMC, the compaction of biomass becomes more difficult and thus packing density lower (Baldini et al., 2017). This effect was also shown in our study for the genotype $M{\times}g$; at HD 3, only 550 g biomass could be pressed into the jars, as the DMC had increased compared to HD 1 and HD 2 (600 g fresh biomass). This led to a lower packing density of 366 kg/m^3 at HD 3 compared to 400 kg/m^3 at HD 1 and HD 2. In this study, $M{\times}g$ still had the best silage quality at HD 3, despite its lower packing density. However, the higher DMC may lead to compaction problems in agricultural practice.

In our study, the pH value after 3 days of ensiling (indicating speed of pH decrease; results not shown) was significantly lowest for biomass harvested in mid-October (for *GNT1*, it was also lowest but not significantly so). This pH$_{3\ days}$ remained relatively stable until day 90 for each genotype, again showing that mid-October was the most suitable harvest date for miscanthus ensiling in our study.

A possible explanation for the improved silage quality with later harvest dates in our study could be the weather conditions and associated differences in carbohydrate content of the biomass. Purdy et al. (2015) have shown that carbohydrate content in the aboveground biomass of miscanthus fluctuates over the season and can be influenced by weather conditions. In our study, weather conditions at HD 3 did indeed differ considerably from those at HD 1 and HD 2. At and just before HD 3, it was quite sunny and warm, with maximum temperatures around 25°C and minimum temperatures above 8°C, whereas at HD 1 and HD 2, it was cooler with night-time temperatures falling to 4.5 and 5.3°C, respectively. To confirm this hypothesis, further research needs to be performed on the impact of weather conditions on carbohydrate content of aboveground biomass and silage quality.

The differences in silage quality between the genotypes, especially $M{\times}g$ and *Sin55*, can probably be attributed to the differences in potassium content. According to Galler (2011), substances with an alkaline effect, such as potassium, lead to poorer acidification. In general, $M{\times}g$ had lower potassium and higher lactic acid contents than *Sin55* (Mangold et al., 2019). This might also be a possible

explanation for the improved silage quality with later harvest dates, since miscanthus is relocating minerals such as potassium from the aboveground biomass to the rhizomes with ongoing senescence.

The low butyric acid content of the silage, resulting in a good silage quality, can be explained by the high cutting height of the biomass (in our trial about 20 cm). A higher cutting height leads to a lower ash content and less uptake of bacteria, such as *Clostridium*, in turn leading to lower butyric acid contents (Szymańska, Sulewksa, & Selwet, 2014).

Other studies investigating the ensiling of miscanthus had similar results. Baldini et al. (2017) also harvested miscanthus in mid-October and found similar contents of lactic, acetic and butyric acid to those of our study. The pH value in their study was, however, lower (3.9) than in ours. Whittaker et al. (2016) quantified a lower lactic acid content (about 0.5% of DM), but a higher pH value (5.2) of *M×g* harvested in September.

Maize is the most common biogas crop in Germany and known for its good silage quality. Baldini et al. (2017) determined higher contents of silage acids (lactic acid, acetic acid) in maize than in miscanthus. Herrmann, Heiermann, and Idler (2011), however, found similar lactic and butyric acid contents in maize stored for 90 days to those found in our study for *M×g* at HD 3. The lactic and acetic acid contents of maize found by Whittaker et al. (2016) were similar to those of all miscanthus genotypes at HD 3 in our study. Therefore, we conclude in our study that, in 2017, HD 3 was the optimal date to harvest miscanthus to achieve similar silage quality results to those of maize.

4.2 | Effect of ensiling on methane yield and velocity

Our study found significantly higher substrate-specific methane yields (SMY) of ensiled than non-ensiled miscanthus biomass for all four genotypes on all harvest dates (Figure 1). This is in line with the results of Amon et al. (2007) and Herrmann et al. (2011), who also demonstrated a positive effect of ensiling on substrate-specific methane yield. Herrmann et al. (2011) found a positive correlation between ensiling products, such as acetic acid, butyric acid and ethanol, and methane content of various crops, which explains the higher SMY of ensiled than non-ensiled biomass.

When calculating the methane hectare yield (MY), Herrmann et al. (2011) emphasize the importance of considering mass losses during the ensiling process. In our study, we found mass losses of up to 7.43% (Supporting Information Table S2), reducing the dry matter yields (DMY) on a per hectare base. Wahid et al. (2015) demonstrated that dry matter yield correlates positively with methane hectare yield. Therefore, the high mass losses of ensiled biomass in our study, which were significantly higher at HD 1 and HD

2 than HD 3 in all genotypes, reduced the MY from these harvest dates. However, these mass losses were compensated for by a higher SMY, ultimately resulting in similar MY for non-ensiled and ensiled miscanthus biomass.

The average SMY (over all genotypes, HD, ensiling) in our trial was 325 Nml CH_4 (g oDM)$^{-1}$, which is higher than data reported in the literature. For example, Baldini et al. (2017) and Mayer et al. (2014) found a SMY for miscanthus ranging between 160 Nml and 250 Nml CH_4 (g oDM)$^{-1}$. Other studies have reported a SMY of up to 309 Nml CH_4 (g oDM)$^{-1}$ and that SMY generally decreases with later harvest dates (Kiesel & Lewandowski, 2017; Kiesel, Nunn, et al., 2017a).

As maize is the most common biogas crop, it is a good benchmark for alternative biogas crops such as miscanthus. The SMY reported for maize ranges between 285 and 400 Nml CH_4 (g oDM)$^{-1}$ (Baldini et al., 2017; Mast et al., 2014; Mayer et al., 2014), which is higher than that measured for miscanthus in our study. The average miscanthus SMY in our study is also lower than that of the internal laboratory maize standard (356 Nml CH_4 (g oDM)$^{-1}$), which is analysed to monitor the activity of the inoculum in each biogas batch test.

The MYs of miscanthus in our study are in the range of the literature values reported for both maize and miscanthus (Baldini et al., 2017; Kiesel & Lewandowski, 2017; Mayer et al., 2014) with the lowest MY for *Sin55* (average: 3,700 Nm^3 CH_4 ha^{-1} a^{-1}) and the highest MY for *M×g* (average: 5,500 Nm^3 CH_4 ha^{-1} a^{-1}). This reflects the differences in DMY, which is expected to vary with crop stand age, between these genotypes (see Mangold et al., 2019). Mast et al. (2014) found a MY of 6,000 Nm^3 CH_4 ha^{-1} a^{-1} for maize and Kiesel and Lewandowski (2017) even 6,000 Nm^3 CH_4 ha^{-1} a^{-1} for miscanthus (both studies were conducted in similar environmental conditions to our study).

In addition to high methane hectare yields, velocity of digestion is an important parameter in determining the suitability of novel biogas crops. The faster biomass is digested in a biogas plant, the more efficient the process is. Fast digestible biomass requires less electricity in the fermenter, for example, for stirring, until the substrate has been digested. Moreover, fast digestible substrates theoretically require less fermentation volume, which means the digester size could be reduced to save construction costs (Ward, Hobbs, Holliman, & Jones, 2008).

In our study, the ensiling process influenced the digestion velocity of the miscanthus biomass from all harvest dates. For all genotypes, more biogas was produced in the first nine days of fermentation from the ensiled than non-ensiled biomass (Figure 4). In addition, it was found that ensiled miscanthus biomass tended to have better digestion velocity with later harvest date. However, it was still considerably lower than for maize. In this context, Klimiuk, Pokój, Budzyński, and Dubis (2010) attributed this to the higher lignin content of miscanthus than maize. Fernandes et al. (2009) determined

that a higher lignin content decreases the biodegradability of biomass. The higher lignin content of miscanthus renders the breakdown of cellulose and hemicellulose less efficient than in maize and thus lowers the methane productivity (Klimiuk et al., 2010). However, as Zheng, Zhao, Xu, and Li (2014) pointed out, ensiling can have a positive effect on methane yield and can be seen as a pretreatment for miscanthus biomass. Also Liu et al. (2016) found a higher digestibility for ensiled compared to non-ensiled biomass (giant reed). This explains the higher velocity and specific methane yields of ensiled miscanthus compared to non-ensiled miscanthus. Our study confirmed the hypothesis that ensilaging can serve as a pretreatment for miscanthus biomass with the aim of achieving both faster digestion and a higher specific methane yield.

4.3 | Outlook for agricultural practice

The following summary of the findings of this study considers their practical implications for the utilization of miscanthus biomass in biogas plants.

Firstly, we found out that miscanthus biomass ensiles best when harvested in mid-October. Also methane hectare yield was highest at HD 3 in all genotypes, except *GNT1* (which yielded highest at HD 2). This is a further indication for harvest in mid-October. Our expectation that higher DMCs (later harvest date, genotype-specific characteristics) lead to lower silage quality and methane yields, was not confirmed by this study. The best silage quality and methane hectare yields were both found for a later harvest date and the early (in comparison with the other tested genotypes) senescent genotype *M×g*. This leads us to the conclusion that the dry matter content is the most important parameter to consider when determining the optimal harvest date for miscanthus.

Various studies have already recommended harvesting miscanthus in October to give the plant enough time to relocate its nutrients for resprouting in the following year (Kiesel & Lewandowski, 2017; Mangold et al., 2019; Wahid et al., 2015). Thus, in addition to qualitatively better silage, a harvest in mid-October also helps to ensure that enough time is available for relocation of a large fraction of nutrients, which facilitates re-sprouting the following year.

In our study, the miscanthus biomass was milled after ensiling. This generally has a positive effect on the digestibility. However, as the non-ensiled miscanthus biomass was also milled at the same setting in the cutting mill, the observed positive effect can be attributed to the ensiling. Other studies have also found positive effects of ensiling on the methane yield of other crops (Amon et al., 2007; Herrmann et al., 2011). Zheng et al. (2014) suggested ensiling as a pretreatment for miscanthus and various other studies have recommended the pretreatment of miscanthus in general for anaerobic digestion

to achieve higher methane yields (Frydendal-Nielsen et al., 2016; Zheng et al., 2014). Such pretreatment, however, is often energy-intensive and therefore associated with high costs (Zheng et al., 2014). In our study, the ensiling step resulted in a higher substrate-specific methane yield (up to 7% on average) and digestion velocity. Therefore, ensiling may save on, or at least reduce, the pretreatment step for miscanthus.

In addition to the various positive effects of ensiling on methane yield, we also found that the ensiling process led to mass losses of up to 7.6% of fresh matter (*Sin55*). However, these mass losses were compensated for by the higher SMY of ensiled miscanthus, resulting in similar methane hectare yields. Whittaker et al. (2016) demonstrated that silage additives reduce mass losses. Therefore, if additives are added to the biomass and mass losses reduced, the positive effect of ensiling may result in higher methane hectare yields. However, ultimately the increase in methane yield should outweigh the additional costs incurred for additives.

In conclusion, we were able to demonstrate that ensiling is suitable to preserve green-harvested miscanthus and even increases its substrate-specific methane yield and digestion velocity. A harvest in mid-October not only improves silage quality, resulting in high hectare methane yields, but also provides sufficient time for relocation of nutrients for regrowth the following year. It may be possible to reduce dry matter losses and further improve the methane hectare yield through the use of additives. These results can help promote the practical implementation of miscanthus as a biogas crop and thus contribute to making biogas production more environmentally benign.

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