Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion

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Abstract

In the anaerobic digestion and biogas industry in Germany, the step of energy crop production accounts for a high proportion of the greenhouse gas emissions and environmental impacts. Replacing annual energy crops, for example maize, by perennial biomass crops such as miscanthus offers the potential to increase the sustainability of biogas crop production. However, the cutting tolerance of miscanthus and the mechanisms influencing it need to be investigated to assess its potential as a biogas crop. For this purpose, a field trial with different harvest regimes was conducted to identify the potential methane yield and cutting tolerance of Miscanthus x giganteus. Several fertilization regimes were tested under nitrogen-limited conditions in a pot trial to investigate the mechanisms behind the cutting tolerance. The refilling of carbohydrate (starch) stores in the rhizome was identified as a very important factor influencing the cutting tolerance of miscanthus, whereas the nutrient relocation appeared to be of less importance. The field trial revealed that Miscanthus x giganteus offers a very high methane yield potential of approx. 6000 m$^3$ ha$^{-1}$ when harvested in October, which is within the range of the methane hectare yield of energy maize. The substrate-specific methane yield of Miscanthus x giganteus biomass decreased with later harvest dates and reached 247 ml (g oDM)$^{-1}$ in October. This harvest date delivered very high, stable yields of on average 26 t DM ha$^{-1}$ over two years and enabled a good cutting tolerance. Green harvest in October was identified to be suitable for Miscanthus x giganteus and is recommended for biogas utilization. In conclusion, the perennial biomass crop Miscanthus x giganteus is a very promising biogas crop and offers the potential to increase the sustainability of the anaerobic digestion sector in Germany by replacing a substantial area of biogas maize cultivation.

Keywords: anaerobic digestion, biogas, carbohydrates, cutting tolerance, energy crop, green cut, Miscanthus x giganteus perennial, relocation of nutrients

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Introduction

In Germany, almost 8.000 biogas plants with a total installed electric capacity of 3.8 GW were under operation in 2014 (FNR 2014). At the same time, 1.27 Mha or 10.5% of the total arable land in Germany (11.9 Mha) was used for the cultivation of energy crops for biogas production (FNR 2014; Statistisches Bundesamt 2014). Maize is the most important biogas energy crop making an input proportion of 73% of crop-derived biomass (FNR 2014). This is criticized because maize cultivation can be characterized as intensive due to high fertilizer demands often combined with intensive soil cultivation. Also the environmental impact can be high, due to high erosion and nitrate leaching risk and negative impacts on biodiversity caused by pesticide use when monoculture of maize prevails (Altieri, 1999; Svoboda et al., 2013; Vogel et al., 2016).

When considering the entire biogas value chain, the step of energy crop cultivation accounts for a high share of the environmental impact and greenhouse gas emissions (Ljö et al., 2014; Pacetti et al., 2015). Perennial energy crops offer the potential to reduce the environmental impacts of crop production and thereby increase the sustainability of the biogas sector. Various perennial biogas crops are currently being researched as biogas substrates, including cup plant (Silphium perfoliatum L.), szarvasi (Elymus elongatus ssp. ponticus cv. Szarvas-I), energy dock (Rumex schavanat) and giant knotweed (Fallopia sachalensis var. Igniscum). However, Mast et al. (2014) revealed that the overall and substrate-specific methane yield of such novel energy crops is lower than for energy maize. A lower methane yield per hectare means that a larger cultivation area is required which consequently could lead to increased competition with land for food production and biodiversity conservation. To avoid such negative effects, alternative biogas crops should ideally be higher yielding than maize, should have a better environmental profile and be able to be
grown under conditions that are marginal for food production.

Miscanthus is a rhizomatus, perennial C4 grass species, which originates from South-East Asia. The sterile clone Miscanthus x giganteus is a high-yielding genotype, which is currently the standard cultivar in commercial utilization. This high yield potential has led to miscanthus being identified as a promising energy crop in several studies (Lewandowski et al., 2000; Clifton-Brown et al., 2004). As its fertilizer and pesticide requirements are low, miscanthus can be also characterized as a low-input crop (Lewandowski & Schmidt, 2006). Miscanthus x giganteus has a good environmental profile with the potential to increase soil carbon, soil fertility and biodiversity and to reduce nutrient run-off and leaching (McCalmont et al., 2015). Despite these benefits, miscanthus cultivation and the utilization of its biomass are still not widespread in Europe [approx. 38,300 ha in Europe (Elbersen et al., 2012)]. Here, the biomass is mainly used for the low-value application of combustion, mostly for heat generation and therefore harvested in late winter or spring. Water content and concentration of critical elements are the major determinants of the combustion quality of biomass. For miscanthus, both are positively influenced by delaying the harvest to late winter or spring, which is however accompanied by biomass losses (Qabal & Lewandowski, 2014). The low relevance of miscanthus production in Germany, and in Europe in general, has been described by McCalmont et al. (2015) as a ‘chicken-and-egg’ problem. There is no significant market for miscanthus biomass in Europe, and biomass production costs for the low-value application of heat production are still too high. Opening up the biogas sector as a new market for miscanthus biomass could encourage the introduction of this environmentally beneficial crop into European agriculture and thereby help reduce the ecological burden of biogas production.

Miscanthus is currently not used for biogas production on account of the low suitability of the winter-/spring-harvested biomass, which is characterized by high lignin and low water contents. A green cut increases both yield and suitability of the biomass as biogas substrate. However, harvesting miscanthus when it is still green is not recommended by Fritz & Formowitz (2010) as it negatively impacts biomass yields in the following years. Later studies had contradictory findings. Some, such as Mayer et al. (2014) and Wahid et al. (2015), consider green-cut miscanthus to be the most promising future biogas crop. Wahid et al. (2015) identified September to October as the ideal harvest time for miscanthus when its biomass is to be used for biogas production. However, neither of these studies looked into the cutting tolerance of miscanthus. Cutting tolerance has been defined by Kiesel & Lewandowski (2014) in this context as the ability of a crop to recover from an early green harvest without yield reductions in the following year. Miscanthus recycles a large proportion of nutrients from the aboveground biomass to the rhizomes during senescence in autumn and reuses them for the production of new shoots in spring (Lewandowski et al., 2003). The prevention of this nutrient relocation could be one explanation for yield losses in miscanthus in the year following a green cut, but the mechanisms influencing the cutting tolerance are still not clear and need to be explored.

The objectives of this study were to investigate the mechanisms determining the cutting tolerance of miscanthus, to identify green-cut regimes suitable for Miscanthus x giganteus and to quantify the biogas yield derived from these. For this purpose, a field trial with Miscanthus x giganteus and a pot trial with a novel Miscanthus sacchariflorus genotype (OPM 19) were performed. OPM 19 indicates that the Miscanthus sacchariflorus genotype was genotype number 19 of the OPTIMISC (EU FP7 No. 289159) genotype set. The field trial was used to analyse the cutting tolerance of Miscanthus x giganteus with three different green-cut regimes and two nitrogen levels and to measure the effects on dry matter (DM) and specific biogas yield. In the pot trial, the response of miscanthus to different nitrogen levels and application dates was tested under nitrogen-limited conditions. Rhizome weight and starch production were measured to help understand the mechanisms behind cutting tolerance.

Materials and methods

Field trial

The cutting tolerance field trial was performed using a Miscanthus x giganteus stand established in 2008 at the research station ‘Hüniger Hof’ in south-west Germany (48.7° latitude, 8.9° longitude, approx. 480 m a.s.l.). The soil is classified as Haplic Luvisol with a silty clay texture and an overlay of loess loam. The site is characterized by a long-term average annual air temperature and precipitation of 8.3 °C and 689 mm, respectively. The climate data relevant for the field trial (2012–2015) are shown in Table 1 on a monthly basis. As miscanthus is a perennial crop, the 2012 data are included to show that the year preceding the cutting tolerance trial was within the range of average conditions. The original planting density was three rhizomes during senescence in autumn and reuses them for the production of new shoots in spring (Lewandowski et al., 2003). The prevention of this nutrient relocation could be one explanation for yield losses in miscanthus in the year following a green cut, but the mechanisms influencing the cutting tolerance are still not clear and need to be explored.

The cutting tolerance field trial was set up in 2013 in the mature miscanthus crop described above as a randomized...
block design with three replicates. The plot size was 9 m². The central 4 m² were harvested for yield estimation. The variants included three green-harvest regimes, two nitrogen (N) fertilization levels and a winter control (Table 2). The green-harvest regimes comprised one double-cut (July/October) and two single-cut regimes, one early (August) and one late (October). Each green-harvest regime was tested at a lower (80 kg N ha⁻¹) and higher (140 kg N ha⁻¹) nitrogen fertilization level. The winter control was only fertilized with 80 kg N ha⁻¹.

The fertilizer used was also ENTEC® 26. The 2013 N fertilization was split into two applications: 80 kg N ha⁻¹ on 22 April 2013 and 60 kg N ha⁻¹ on 10 June 2013 for the plots with the higher fertilization level. In 2014, the total amount was given in one application on 10 April 2014. During the course of the field trial, the mineral content of the soil (P, K and Mg) was monitored for each plot to avoid negative effects due to nutrient limitation. The plant-available mineral supply was found to be sufficient, and therefore, no mineral fertilizer other than nitrogen was applied. The mineral nitrogen content of the soil was measured after the last green cut each year. As only very low values were detected (on average 4.4 kg N ha⁻¹ in 2013 and 3.6 kg N ha⁻¹ in 2014), these were neglected in the calculation of nitrogen fertilization for the following year.

The crop was harvested using a sickle bar mower at a cutting height of approx. 5 cm. The border of each plot was removed, and the central 4 m² were collected and weighed. In literature, a minimum sampling area of 3 m² is recommended (Knörzer et al., 2013). A subsample of approx. 1 kg was taken and dried at 60 °C in a drying cabinet to constant weight to establish the dry matter (DM) content. The DM yield was calculated based on the fresh matter (FM) yield and the DM content. The dried subsample was milled in a cutting mill SM 200 (Retsch, Haan) using a 1-mm sieve for chemical and biogas analyses. An aliquot of five shoots was used to establish the average dry weight per shoot and the leaf-to-stem ratio. For this purpose, the five shoots were sepa-

| Month  | Average air temperature (°C) | Precipitation (mm) |
|--------|------------------------------|--------------------|
|        | 2012 | 2013 | 2014 | 2015 | 2012 | 2013 | 2014 | 2015 |
| January | 2.0  | 0.5  | 3.2  | 1.9  | 56.1 | 21.6 | 36.5 | 76.5 |
| February | 3.5  | -1.6 | 4.1  | -0.2 | 8.4  | 54.6 | 43.8 | 13.2 |
| March   | 7.3  | 1.4  | 7.2  | 5.4  | 7.7  | 31.0 | 8.4  | 34.3 |
| April   | 8.1  | 8.4  | 10.9 | 9.0  | 42.3 | 60.7 | 49.9 | 31.9 |
| May     | 14.3 | 10.8 | 12.1 | 13.0 | 43.1 | 138.6| 68.2 | 67.7 |
| June    | 16.3 | 15.8 | 16.7 | 16.5 | 116.5| 82.8 | 24.3 | 75.2 |
| July    | 17.3 | 19.8 | 18.4 | 20.8 | 96.0 | 173.4| 162.0| 28.9 |
| August  | 19.2 | 17.5 | 15.7 | 20.0 | 39.3 | 69.5 | 142.4| 75.0 |
| September | 14.0 | 13.7 | 14.6 | 12.6 | 57.2 | 97   | 77.0 | 36.0 |
| October | 8.8  | 10.9 | 12.1 | 8.4  | 58.3 | 87.1 | 50.1 | 16.0 |
| November | 5.5  | 4.0  | 6.7  | 7.2  | 133.7| 60.3 | 59.0 | 69.5 |
| December | 1.8  | 3.0  | 2.8  | NA   | 68.1 | 46.3 | 41.7 | NA   |
| Average | 9.3  | 8.7  | 10.4 | 10.4*| NA   | NA   | NA   | NA   |
| Sum     | NA   | NA   | NA   | NA   | 726.7| 922.9| 763.3| 524.2†|

NA, not assessed.
*Preliminary average, no data from December included.
†Preliminary sum, no data from December included.

Table 2 Experimental treatments of the cutting tolerance field trial

| No. | Harvest regime | Fertilization (kg N ha⁻¹) | Harvest date Year 1 (2013) | Harvest date Year 2 (2014) |
|-----|----------------|---------------------------|---------------------------|----------------------------|
| 1   | Double cut     | 80                        | 1st cut: 18.07.13         | 1st cut: 28.07.14          |
| 2   |                | 140                       | 2nd cut: 24.10.13         | 2nd cut: 23.10.14          |
| 3   | Early single cut | 80                      | 29.08.13                  |                            |
| 4   |                | 140                       | 28.08.14                  |                            |
| 5   | Late single cut | 80                       | 24.10.13                  | 23.10.14                  |
| 6   |                | 140                       |                           |                            |
| 7   | Winter control | 80                        | 20.02.14*                 | 09.03.15†                 |

*Biomass from growing season 2013.
†Biomass from growing season 2014.
The selected plantlets were transferred into pots with a volume of approx. 5 l several months before the beginning of the pot trial. The pots were filled with a soil mixture consisting of fertilized peat, loam and sand in a volumetric ratio of 1 : 2 : 1. The soil mixture was sterilized at 80 °C for 24 h before planting. After successful establishment, the plants were watered with excess water for several weeks to remove the remaining mineral nitrogen in the soil. The excess water was able to run off the saucers, and the water remaining in the saucers was taken up by the plants within few hours to one day. No negative effects on the plants were observed during this period, and viable roots were visible at the bottom of the saucers.

At the start of the pot trial, the plants were approx. 2 years old and the pots were placed in a randomized block design with 4 replications. The treatments of the pot trial are shown in Table 3. Two fertilization levels [100 and 200 mg N (kg soil)⁻¹] were tested in two application regimes (single and split application). For the split applications, half of the fertilizer was applied at the beginning of the trial and the other half directly after the first cut. In addition, a control with no fertilization and a treatment with a reduced fertilizer application [50 mg N (kg soil)⁻¹] after the first cut were conducted. The first fertilizer application was on 23 May 2014. In the following weeks, the plants were watered according to their specific needs without excess water to avoid nitrate leaching. The plants were harvested on 15 July 2014 (after 13 weeks' growth) directly followed by the second fertilizer application. The second harvest was performed on 29 September 2014 (after 11 weeks' growth). Directly after the second harvest, the rhizomes were also harvested by washing off the soil and separating the rhizomes from the roots. The biomass samples from both harvests and the rhizomes were dried at 60 °C to constant weight and milled in a cutting mill SM 200 (Retsch, Haan) using a 1-mm sieve to be used for the chemical analysis.

### Table 3 Overview of the pot trial fertilizer treatments

| Treatment | Description                        | Total fertilization In mg N (kg soil)⁻¹ | 1st application | 2nd application |
|-----------|------------------------------------|---------------------------------------|----------------|----------------|
| 0/0       | Control                            | 0                                     | 0              | 0              |
| 100/0     | Half amount, single application    | 100                                   | 100            | 0              |
| 200/0     | Full amount, single application    | 200                                   | 200            | 0              |
| 0/50      | Reduced single late application    | 50                                    | 0              | 50             |
| 50/50     | Half amount, split application     | 100                                   | 50             | 50             |
| 100/100   | Full amount, split application     | 200                                   | 100            | 100            |

The substrate-specific biogas and methane yields were measured in a biogas batch test under mesophilic conditions at
Formula (2) was used for the subsequent 17 measurements, temperature to 39 °C. Formula (1) was used for the first measurement and takes into account overpressure and the water vapour partial pressure. The fermentation flasks were closed gastight by a butyl rubber stopper and an aluminium cap. The pressure increase in the fermentation flasks was measured by puncturing the rubber stopper and an aluminium cap. The pressure increase due to drying and incineration was recorded before and after drying and incineration. The fermentation flasks were closed gastight by a butyl rubber stopper and an aluminium cap. The pressure increase in the fermentation flasks was measured by puncturing the butyl rubber stopper with a cannula attached to a HND-P pressure meter (Kobold Messring GmbH, Hofheim, Germany). The biogas production was calculated as dry gas (water vapour pressure was considered) from the pressure increase and was standardized to 0 °C and 1013 hPa using Formula (1) and (2).

For the biogas batch analysis, 200 mg organic dry matter (oDM) of milled sample was transferred into a 100 ml fermentation flask, 30 g inoculum was added, and the gas-containing headspace was flushed with nitrogen to attain anaerobic conditions. The oDM content of the milled samples was estimated by drying 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 weight was recorded before and after drying and incineration. The fermentation flasks were closed gastight by a butyl rubber stopper and an aluminium cap. The pressure increase in the fermentation flasks was measured by puncturing the butyl rubber stopper with a cannula attached to a HND-P pressure meter (Kobold Messring GmbH, Hofheim, Germany). The biogas production was calculated as dry gas (water vapour pressure was considered) from the pressure increase and was standardized to 0 °C and 1013 hPa using Formula (1) and (2).

Formula (2) was used for the subsequent 17 measurements, which were taken on a regular basis.

\[
V_{\text{biogas}} = V_{\text{HS}} * T_s / T_F * (P_A0 + P_F1) - (P_A0 * T_s / T_{\text{Lab}}) - P_{\text{WP}} / P_s
\]

where \(V_{\text{biogas}}\) = volume of biogas produced
\(V_{\text{HS}}\) = volume of gas-containing headspace in the fermentation flasks
\(T_s\) = standard temperature (=273.15 K = 0 °C)
\(T_F\) = fermentation temperature (=312.15 K = 39 °C)
\(P_A0\) = ambient pressure at first measurement
\(P_F1\) = overpressure in fermentation flasks at first measurement
\(P_A0\) = ambient pressure at sealing of the fermentation flasks (batch test start)
\(T_{\text{Lab}}\) = laboratory temperature at sealing of the fermentation flasks (batch test start)
\(P_{\text{WP}}\) = water vapour partial pressure at 39 °C
\(P_s\) = standard pressure (1013 hPa)

\[
V_{\text{biogas}} = V_{\text{HS}} * T_s / T_F * ((P_A + P_F) - (P_A0 * T_s / T_{\text{Lab}})) / P_s
\]

where \(P_A\) = ambient pressure at each measurement

\(P_{\text{biogas}} = \) overpressure in fermentation flask at each measurement

\(P_{A(0-1)} = \) ambient pressure at previous measurement

\(P_{F(0-1)} = \) overpressure in the fermentation flasks at previous measurement

During the course of the biogas batch test, it was occasionally necessary to remove the produced biogas from the fermentation flasks. The overpressure in the fermentation flasks was removed using a gastight syringe once it had reached an approximate value of 500 mbar. The biogas was transferred to a gastight evacuated storage flask where it was kept until the end of the batch test. After each gas collection, the remaining overpressure in the fermentation flasks was allowed to level off to ambient pressure by injecting a blank cannula. For the subsequent measurement, \(P_{F(0-1)}\) was then set to zero in formula (2). At the end of the batch test, the remaining biogas in the headspace of the fermentation flasks was removed by active extraction with a gastight syringe and also transferred into the storage flask. An aliquot of the collected biogas was used to analyse the methane content by a GC-2014 gas chromatograph (Shimadzu, Kyoto). The gas chromatograph was equipped with a thermal conductivity detector (TCD), and the detection temperature was set to 120 °C. Two columns (Haye-Sep and Molsieve column) were used (oven temperature 50 °C) with argon as carrier gas. The gas samples were injected using a Combi-xt PAL autosampler (CTC Analytics AG, Zwingen, Switzerland).

Statistical analysis

Statistical analysis was performed using the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The program ‘Procmixed’ was used, and a mixed model was developed for the field trial according to Formula (3). A test on homogeneity of variance and normal probability of residues was performed. The effects were tested at a level of probability of \(\alpha = 0.05\).

\[
y = \mu + \text{rep} + \text{yr} + \text{rep} * \text{yr} + \text{hr} + \text{Nf} + \text{hr} * \text{Nf} + \text{yr} * \text{hr} + \text{yr} * \text{Nf} + \text{yr} * \text{hr} * \text{Nf} + \epsilon
\]

where \(\mu\) = general mean effect
\(\text{rep}\) = effect of field replicate
\(\text{yr}\) = effect of year
\(\text{rep} * \text{yr}\) = effect of interaction of field replicate and year
\(\text{hr}\) = effect of harvest regime
\(\text{Nf}\) = effect of nitrogen fertilization level
\(\text{hr} * \text{Nf}\) = effect of interaction of harvest regime and nitrogen fertilization level

\(\text{yr} * \text{Nf}\) = effect of interaction of year and nitrogen fertilization level
\(\text{yr} * \text{hr} * \text{Nf}\) = effect of interaction of year, harvest regime and nitrogen fertilization
\(\epsilon\) = residual error

The effect of the nitrogen fertilization and the interactions between harvest regime and nitrogen fertilization; year and nitrogen fertilization; and year, harvest regime and nitrogen fertilization were not significant. For this reason, the model shown in Formula (3) was adapted as shown in Formula (4).
Field replicate and year were fixed effects, and the interaction between field replicate and year and the interaction between field replicate, harvest regime and nitrogen fertilization level were random effects.

The data from the pot trial were also analysed by SAS version 9.4, and a mixed model according to Formula (5) was applied. A test on homogeneity of variance and normal probability of residues was performed. The effects were tested at a level of probability of $\alpha = 0.05$.

$$y = \mu + \text{treat} + \text{rep} + \text{treat} \times \text{rep} + e$$  \hspace{1cm} (5)

where $\mu =$ general mean effect
\text{treat} = \text{effect of treatment}
\text{rep} = \text{effect of replicate in the greenhouse}
\text{treat} \times \text{rep} = \text{effect of interaction of treatment and replicate}
$e =$ residual error

The treatment consisted of nitrogen fertilization level and nitrogen application regime. Treatment and replicate were fixed effects, and no random effects were allowed.

Results

Field trial

Plant measurements. In the years 2014 and 2015 – the first and second year after the cutting regimes were first applied – nitrogen fertilization had very little effect on the regrowth in each harvest regime (Figs 1 and 2). Therefore, the following results are discussed based on the harvest regimes and not on fertilization level. The double-cut and the early single-cut regime negatively influenced the regrowth of the following vegetation period (Fig. 1). The lower shoot density and the reduced stem height and diameter revealed that the plants were growing less vigorously and sprouting started later than in the late single-cut regime and the winter control. However, no overwinter plant losses or development of gaps were observed throughout the trial. In the year after the first green cut (2014), the double-cut regime showed a high number of shoots per square metre (Fig. 1a). This could indicate that the crop reacts to this harvest regime by increased shoot numbers in the second season. However, the standard deviation in 2014 was high and this effect was no longer visible in 2015 (Fig. 1b). For this reason, the identified effect may also be caused by chance.

The average dry weight per shoot increased with later harvest in 2013, but the proportion of leaf biomass decreased sharply over winter (Fig. 2). The average dry weight per shoot of the double-cut and the early single-cut regime was lower in 2014 than in 2013. The leaf-to-stem ratio of the early single-cut regime was also lower in 2014 than in 2013. In the single late-cut regime, the average dry weight per shoot was significantly higher in 2014 than 2013, but the leaf-to-stem ratio was stable. However, the weather conditions in 2013 were not ideal for miscanthus growth (cold spring until end of May and drought during June/July), which could explain the higher average dry weight per shoot in 2014 in the late single-cut regime. In the winter control, this effect was not visible. This may be due to losses over winter.

Dry matter yield and methane yield. The double-cut regime showed a low dry matter (DM) yield in both fertilization levels and years, because the low yields of the first cut were not compensated by the second (Fig. 3). It appeared that the first cut was performed before the end of the crop’s main growth phase and therefore the crop was prevented from producing a higher yield. The yield of the early single-cut regime was high in the first year (2013) in both fertilization levels. However, there was a DM yield decrease from 2013 to 2014 in the double-cut and the early single-cut regime of approx. 40% and 60%, respectively. The green cut in the first year (2013) therefore greatly influenced the yield of the second year (2014), whereas the nitrogen fertilization showed almost no effect on the DM yield in the second year. By contrast, the DM yield of the late single-cut regime was even slightly higher in 2014 than in 2013 and showed the significantly highest DM yield in both years and fertilization levels. The DM yield of the late single-cut regime was on average 39% higher than the winter control, as biomass losses occur over winter. The DM content increased with later harvest dates and was ideal for ensiling in the early single-cut regime (on average 35% of fresh matter (FM)).

The substrate-specific methane yield (SMY) decreased with later harvest dates and the significantly highest SMY was measured in the both cuts of the double-cut regime (Fig. 4). The SMY of the late single-cut regime was significantly lower than those of the early single-cut regime, but significantly higher than the winter control. The methane yield per hectare was influenced mainly by the DM yield; the SMY had only of minor influence. The DM and methane yield of the double-cut and early single-cut regime decreased sharply from 2013 to 2014. The late single-cut regime and the winter control delivered stable DM and methane yields. However, the methane yield of the late single-cut regime was about 45% higher than the winter control, due to the higher DM yield and also higher SMY.

Mineral content of and nutrient removal by the biomass. Here, ‘content’ refers to the concentration of the respective nutrient in the biomass (unit % DM). ‘Nutrient removal’ is calculated from the nutrient content and dry matter yield and expresses the amount of nutrients removed from the field (kg ha$^{-1}$).
The nitrogen, phosphorus, potassium, magnesium and calcium contents of the harvested biomass are shown in Table 4. High quantities of potassium and nitrogen in particular were removed by the biomass of the high-yielding green-cut regimes, especially in 2013. In green-cut treatments where the DM yield was low, the nutrient removal was correspondingly low, for example in the double-cut and early single-cut regime in 2014. The removal of both nutrients decreased with later harvest dates, and the lowest nutrient contents were found in the biomass of the winter control. The highest nitrogen and potassium removal by the biomass was found in the late single-cut regime and the first year of the early single-cut regime, where the DM yield was high. The removal in the early and late single-cut regime was considerably lower in 2014 than in 2013. In the early single-cut regime, this was mainly influenced by the reduced DM yield and in the late single-cut regime by a lower nitrogen and potassium content. In the first year, the potassium content of the biomass from the late single-cut regime was much lower than that from the early single-cut regime, indicating that potassium was either actively relocated to the rhizome or lost through leaf fall. In 2013, the leaf-to-stem ratio was lower at the harvest of the late single-cut regime than at the early single-cut regime (Fig. 2), but most of the dead leaves were still attached to the stem. For this reason, potassium seems to be a good indicator of how far the relocation of nutrients and carbohydrates to the rhizomes has proceeded. The nitrogen removal by the biomass in the late single-cut regime is higher than in the winter control, especially in the first year, when the plants had an oversupply of nitrogen. This can be seen from far higher nitrogen fertilization application than removal by the spring-harvested biomass in the previous year, in particular for the higher fertilization level.

**Pot trial**

**Dry matter yield.** Under conditions of limited nitrogen availability, increased nitrogen fertilization led to significantly higher biomass and rhizome production, except the treatment 0/50 (Fig. 5a–c). It is notable that the treatments with single application at the beginning of the trial (100/0 and 200/0) had a significant higher bio-

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mass production at the first cut than the split application treatments. The second nitrogen application after the first harvest stimulated the biomass production and led to nonsignificant differences between both application regimes of the same fertilization level. The rhizome production was not significantly influenced by the application regime, but by the fertilization level. Overall, single application of nitrogen fertilizer was advantageous, due to significantly higher biomass yield at the first cut and similar rhizome production and biomass yield at the second cut.

**Nitrogen and starch content.** Higher nitrogen fertilization resulted in higher nitrogen content in the biomass of both cuts and the rhizomes (Fig. 6). Nitrogen (N) removal was higher in the biomass of the fertilized treatments than in the unfertilized control due to both higher biomass and rhizome yield and higher N content of the biomass and the rhizomes. The N content of the aboveground biomass was higher when N fertilizer was applied in an early single application. These differences were not observed for the N content of rhizomes. Interestingly, the total N removal in the single application
treatments was 792 and 1163 mg N in the treatments 100/0 and 200/0, respectively. This means more N was taken up by the crop than the applied N amount of 500 and 1000 mg N in the treatments 100/0 and 200/0, respectively. This could be due to residual nitrogen and mineralization in the soil or the nitrogen content of the rhizomes at the start of the trial. The split application treatments had a lower total N removal than the single application treatments. However, root biomass and soil were not analysed, so it is possible that a larger proportion of nitrogen was still attached to the roots or in the soil in the split application treatments.

Higher nitrogen fertilization resulted in lower starch content of the rhizomes (Fig. 7), due to the dilution effect of the higher biomass and protein production. The nitrogen fertilization increased growth of above- and belowground biomass production and in particular the amount of starch in the rhizome. The application of fertilizer after the first cut seemed to positively influence the amount of starch in the rhizome, whereas full application at the beginning of the trial seemed to positively influence the aboveground biomass production. However, the quantity of rhizome starch in the 200/0 treatment was not significantly lower than those of the 100/100 treatment.

Discussion

For a perennial crop, such as miscanthus, long-term productivity is the key factor for economically viable crop production. In the literature, the long-term productivity of Miscanthus x giganteus harvested in late winter is reported to be relatively stable with annual fluctuations due to seasonal effects (Christian et al., 2008; Gaulder et al., 2012). It is also reported to be more stable than other perennial energy grasses such as switchgrass (Iqbal et al., 2015). Long-term productivity of green-harvested miscanthus is, however, much more critical on account of the early harvest and very much depends on the cutting tolerance of the crop (Fritz & Formowitz, 2010). The results of this study indicate that Miscanthus x giganteus tolerates harvest in October. However, detailed knowledge of the mechanisms influencing the cutting tolerance is necessary to avoid damaging the crop and to identify optimal harvest regimes. The mechanisms driving cutting tolerance of miscanthus are discussed here, and recommendations for optimal management of miscanthus as a biogas crop are elaborated.

Mechanisms influencing cutting tolerance of miscanthus

The field and pot trial in this study were performed based on the hypothesis that nitrogen fertilization and harvest time interactively affect the cutting tolerance of miscanthus by influencing processes determining cutting tolerance. Under conditions of nitrogen limitation, as here in the pot trial, a positive effect of nitrogen fertilization on the biomass yield, biomass regrowth after the first cut and rhizome weight was indeed recorded. However, under conditions where nitrogen supply is not limited, as in the field trial, only very little effect of nitrogen fertilization on cutting tolerance was observed. The amount of nutrients removed by the harvested bio-

Fig. 4  Mean methane yield per hectare and substrate-specific methane yield (SMY) of the four harvest regimes at two fertilization levels [80 and 140 kg nitrogen (N) ha$^{-1}$a$^{-1}$] in the field trial. Letter display with bold lower-case letters corresponds to total mean methane yield (first and second cut). Letter display with italic lower-case letters corresponds to the SMY of the first cut. The columns with different bold or italic lower-case letters differ significantly from each other according to a multiple t-test $p = 0.05$.
| Harvest regime | Nitrogen fertilizer level kg ha\(^{-1}\) a\(^{-1}\) | Year | Nitrogen Content % DM | Phosphorus Content % DM | Potassium Content % DM | Magnesium Content % DM | Calcium Content % DM |
|----------------|--------------------------------|------|---------------------|----------------------|----------------------|-----------------------|---------------------|
|                |                                 |      | Removal kg ha\(^{-1}\) | Removal kg ha\(^{-1}\) | Removal kg ha\(^{-1}\) | Removal kg ha\(^{-1}\) | Removal kg ha\(^{-1}\) |
| Double cut--first cut 80 | 2013 | 1.0 (0.1) | 103 (8) | 0.2 (0.0) | 19 (1) | 2.4 (0.1) | 243 (12) | 0.1 (0.0) | 12 (1) | 0.3 (0.0) | 27 (2) |
|                  | 2014 | 0.6 (0.1) | 44 (11) | 0.1 (0.0) | 9 (2)  | 1.3 (0.2) | 92 (28)  | 0.1 (0.0) | 6 (1)  | 0.3 (0.1) | 21 (8) |
|                  | 140  | 2013 | 1.1 (0.2) | 103 (20) | 0.2 (0.0) | 18 (3) | 2.3 (0.2) | 219 (45) | 0.1 (0.0) | 12 (2) | 0.3 (0.0) | 25 (3) |
|                  |      | 2014 | 0.7 (0.0) | 51 (5)   | 0.1 (0.0) | 9 (0)  | 1.3 (0.1) | 94 (3)   | 0.1 (0.0) | 7 (1)  | 0.3 (0.1) | 22 (7) |
| Double cut--second cut 80 | 2013 | 1.0 (0.1) | 32 (3)  | 0.2 (0.0) | 6 (1)  | 1.7 (0.2) | 54 (11)  | 0.2 (0.0) | 5 (1)  | 0.4 (0.0) | 14 (3) |
|                  | 2014 | 1.6 (0.0) | 14 (3)  | 0.4 (0.0) | 3 (0)  | 2.9 (0.4) | 24 (4)   | 0.2 (0.0) | 2 (0)  | 0.7 (0.1) | 6 (2)  |
|                  | 140  | 2013 | 1.1 (0.2) | 39 (11) | 0.2 (0.0) | 6 (2)  | 1.8 (0.3) | 64 (14) | 0.2 (0.0) | 6 (2)  | 0.4 (0.1) | 16 (6) |
|                  |      | 2014 | 1.6 (0.1) | 16 (0)   | 0.4 (0.0) | 4 (0)  | 2.9 (0.1) | 29 (2)   | 0.2 (0.0) | 2 (0)  | 0.6 (0.0) | 6 (1)  |
| Early single cut 80 | 2013 | 0.6 (0.0) | 132 (3) | 0.1 (0.0) | 28 (2) | 1.4 (0.1) | 319 (12) | 0.1 (0.0) | 18 (3) | 0.2 (0.0) | 44 (6) |
|                  | 2014 | 0.5 (0.1) | 47 (1)  | 0.1 (0.0) | 12 (0) | 1.0 (0.1) | 96 (5)  | 0.1 (0.0) | 7 (1)  | 0.3 (0.0) | 27 (3) |
|                  | 140  | 2013 | 0.7 (0.2) | 164 (39) | 0.1 (0.0) | 31 (4) | 1.5 (0.2) | 355 (39) | 0.1 (0.0) | 26 (6) | 0.2 (0.1) | 57 (14) |
|                  |      | 2014 | 0.6 (0.0) | 60 (7)   | 0.1 (0.0) | 12 (1) | 1.0 (0.1) | 105 (8) | 0.1 (0.0) | 10 (1) | 0.3 (0.0) | 31 (4) |
| Late single cut 80 | 2013 | 0.6 (0.1) | 135 (22) | 0.1 (0.0) | 21 (4) | 0.8 (0.1) | 192 (44) | 0.1 (0.0) | 19 (3) | 0.2 (0.0) | 52 (6) |
|                  | 2014 | 0.4 (0.1) | 100 (24) | 0.1 (0.0) | 23 (4) | 0.6 (0.1) | 162 (32) | 0.1 (0.0) | 19 (2) | 0.2 (0.0) | 59 (14) |
|                  | 140  | 2013 | 0.7 (0.2) | 184 (46) | 0.1 (0.0) | 22 (3) | 1.0 (0.2) | 237 (46) | 0.1 (0.0) | 23 (3) | 0.2 (0.0) | 58 (8) |
|                  |      | 2014 | 0.5 (0.1) | 121 (37) | 0.1 (0.0) | 20 (3) | 0.6 (0.1) | 171 (32) | 0.1 (0.0) | 24 (4) | 0.2 (0.0) | 66 (10) |
| Winter control 80 | 2013 | 0.3 (0.0) | 52 (12) | 0.1 (0.0) | 12 (3) | 0.5 (0.1) | 103 (34) | 0.1 (0.0) | 10 (3) | 0.1 (0.0) | 26 (8) |
|                  | 2014 | 0.4 (0.1) | 61 (17) | 0.0 (0.0) | 7 (1)  | 0.3 (0.1) | 49 (9)  | 0.0 (0.0) | 7 (1)  | 0.1 (0.0) | 18 (5) |
Nitrogen fertilization also positively affects starch production in the rhizomes, as was measured in the pot trial. This is likely to be the effect of more photosynthetically active biomass present when nitrogen fertilizer is applied. Purdy et al. (2015), and also the observations made in both the pot and the field trial of this study, indicate that carbohydrate – in this case starch – reserves in the rhizomes play an important role in the cutting tolerance of the crop. In the first year, the yield of the late single-cut regime was only slightly higher than that of the early single-cut regime, although the crop had two months more time for yield formation. This leads to the hypothesis that the crop invested a large proportion of the biomass accumulation into belowground biomass to refill the carbohydrate stores in the rhizomes with starch. Purdy et al. (2015) found that starch concentration in miscanthus rhizomes is likely to reach a peak in late autumn (November) and concluded that an earlier harvest could have a negative effect on the crop. The optimal harvest date for a green cut would therefore be at the time of maximum starch content in the rhizomes. Mutoh et al. (1968) revealed that, under Japanese growth conditions, the largest proportion of the net production was used to build up rhizomes, roots and reserve material in August and September. Later studies found that the starch content in the rhizomes increases until the end of the vegetation period and peaks in late autumn (Masuzawa & Hogetsu, 1977; de Souza et al., 2013). Our study found the optimal harvest date for a green cut of Miscanthus x giganteus to be October and indicated that potassium seems to be a good indicator of how far the relocation of nutrients and carbohydrates to the rhizomes has proceeded. Himken et al. (1997) revealed that the content of potassium and nitrogen in the rhizome increased from June to late autumn, which supports this hypothesis. However, senescence and starch storage in the rhizomes are influenced by climatic conditions and accelerated by low daily minimum temperatures (Purdy et al., 2015). In 2013, the first frost occurred at the beginning of October and several cold nights with temperatures around 0 °C were recorded before harvest. Both could have triggered carbohydrate transport to and starch formation in the rhizomes (Purdy et al., 2015). This could explain why Miscanthus x giganteus tolerated cutting as early as October in this study and suggests that not only date, but also daily minimum temperature or first frost should be considered when determining the optimal harvest time for a green cut.
The results of Purdy et al. (2015) indicate that the seasonal fluctuations in rhizome starch content also differ between genotypes and the starch content of the rhizomes increases with ongoing senescence. Therefore, the potential of other genotypes that senesce earlier than Miscanthus x giganteus should be explored for biogas production. These genotypes may be characterized by earlier flowering. The carbohydrate sink in such genotypes may switch earlier from stems to rhizomes, and consequently, the starch stores in the rhizomes are refilled earlier. Such genotypes may tolerate earlier cutting than Miscanthus x giganteus. The advantage of genetic variation in ripening would be the option of broadening the harvest window of miscanthus to enable harvest under ideal climatic and soil conditions.

Cutting tolerance and yield of Miscanthus x giganteus

In the field trial, a significant yield decline was observed in the year after the early single-cut and the double-cut regime with harvests in July and August, indicating that these regimes are not sustainable. Both cutting regimes affected the regrowth of the crop in the second year negatively. This can be seen in a reduced leaf-to-stem ratio and average dry weight per shoot, especially in the early single-cut regime. Negative effects of an early green cut in August on the yield the following year have also been reported by Fritz & Formowitz (2010). The double-cut regime showed noteworthy regrowth after the first cut in 2013, but considerably less than in the late single-cut regime. In the field trial, nitrogen fertilizer was applied in spring and no fertilizer was applied after the first cut of the double-cut regime. In
the pot trial, the split nitrogen application mainly increased the biomass yield of the second cut, but also promoted rhizome starch production. Therefore, we suggest investigating the effect of nitrogen fertilization on the yield of the second cut and the cutting tolerance of the double-cut regime when applied directly after the first cut under field conditions. However, the genotype used in the pot trial was not Miscanthus x giganteus, as in the field trial. Therefore, it cannot be ruled out that the Miscanthus sacchariflorus genotype used starts to relocate carbohydrates to the rhizomes earlier than Miscanthus x giganteus. In addition, the conditions in the greenhouse (e.g. higher temperatures) may have affected the generative development of the plants and thereby promoted the carbohydrate relocation to the rhizome.

In contrast to the early single- and double-cut regime, the late single-cut regime with harvest in October showed a stable or even slightly increased biomass yield from 2013 to 2014. Here, the slightly higher biomass yield and the higher average dry weight per shoot in 2014 may have been influenced by better weather conditions in 2014 than in 2013. Yield stability, with no negative effects on the yield in the following year, has also been reported for October harvest regimes by Mayer et al. (2014) and Yates et al. (2015). Based on these findings, it appears that a green-harvest regime with harvest in October is possible for Miscanthus x giganteus. However, as discussed above, the interactions between the processes of senescence and rhizome starch production with temperature should be further investigated. Such investigations should also include locations characterized by climates different from those for which an October harvest was examined in this study.

It is concluded that, with proper nutrient management and harvest timing, Miscanthus x giganteus tolerates green cutting in October. However, long-term studies are required to assess the cutting tolerance over a longer period and identify the fertilization requirements. As the harvest time is determined here by cutting tolerance, the biomass quality for biogas production may not be ideal. Therefore, the overall suitability of Miscanthus x giganteus for biogas production will be discussed in the following section.

Potential of Miscanthus x giganteus for biogas utilization

For combustion, Miscanthus x giganteus is conventionally harvested in late winter with dry biomass. A major advantage of a green harvest is the higher biomass yield. The average dry matter yield of the winter control (18.7 t DM ha\(^{-1}\)) was about 28% lower than the yield of the late green harvest in October (26.0 t DM ha\(^{-1}\)). Similar biomass losses over winter have been reported in the literature (Lewandowski et al., 2003; Cadoux et al., 2012). Therefore, the utilization of green biomass has the potential to substantially increase the biomass yield per unit area and to exceed that of maize.

The results presented confirm that Miscanthus x giganteus has a high potential for biogas utilization. The methane yield of the October harvest was very high (on average 6153 m\(^3\) ha\(^{-1}\)) and within the range of the methane hectare yield of energy maize (6008 m\(^3\) ha\(^{-1}\)) (Mast et al., 2014). The ability of miscanthus to compete with maize has also been reported by Mayer et al. (2014). Miscanthus x giganteus could thus replace a significant share of maize cultivation for biogas production without increasing the cultivation area required. Schirling et al. (2015) revealed a total potential for Miscanthus x giganteus cultivation in Germany of 4 million ha. This large potential cultivation area indicates that miscanthus could make a significant contribution to substrate production for anaerobic digestion and help increase the sustainability of the biogas sector.

Before using Miscanthus x giganteus biomass in commercial biogas plants, its performance and substrate-specific methane yield (SMY) needs to be further investigated on a larger scale. As its SMY was analysed here in a batch test using milled biomass, the particle size may have positively influenced the SMY and in particular the rate of biogas production. For this reason, further research with commercially chopped biomass is required, also to assess the risk of floating layers forming in wet fermentation plants. As Miscanthus x giganteus biomass has a lower rate of biogas production than maize in anaerobic digestion (data not shown), it may require larger digester volumes or additional pretreatment. For commercial application, the biomass needs to be preserved by ensiling and the high dry matter content in October (on average 44% of fresh matter) may be problematic. However, the successful ensiling of miscanthus biomass has been reported in several studies (Huisman & Kortleve, 1994; Klimiuk et al., 2010; Mayer et al., 2014). On a commercial scale, additional silage additives can be used for efficient ensiling of Miscanthus x giganteus biomass or mixed ensiling with biomass of other crops, such as ryegrass or maize, may be performed.

The SMY decreased with later harvest dates and reached the lowest values at winter harvest [on average 233 ml (g oDM)\(^{-1}\)]. This is due to the effect of progressing lignification, relocation of easily degradable carbohydrates to the rhizomes and losses of faster degradable leaves over winter. As early green cuts are not tolerated by the crop, the SMY of the October harvest [on average 247 ml (g oDM)\(^{-1}\)] is suggested here as a reference SMY of Miscanthus x giganteus biomass. There are diverging findings for SMY of Miscanthus x giganteus biomass in
previous studies. Wahid et al. (2015) and Mayer et al. (2014) found similar values for biomass harvested in autumn as in this study. Menardo et al. (2013) measured the SMY of pretreated miscanthus biomass and reported a very low SMY of 84 ml (g oDM)⁻¹ for the untreated, winter-harvested control. Klimiuk et al. (2010) analysed ensiled Miscanthus x giganteus biomass in a continuously operated digester and measured only 100 ml (g oDM)⁻¹. These lower yields may be an effect of the fermentation technology applied by Klimiuk et al. (2010). It is assumed here that Miscanthus x giganteus biomass requires longer retention times in continuously operated digesters, as the biogas production rate of this lignified biomass is comparatively low.

For anaerobic digestion, a harvest before winter is favourable, but the nutrient removal, especially for nitrogen and potassium, is significantly higher than after winter [see also Cadoux et al. (2012)]. The maximum removal was obtained in August and decreased until winter harvest. As too early harvest resulted in yield decline, only the late single-cut regime (harvest in October) is discussed here. A higher nitrogen fertilization resulted in a slightly higher yield, but also in a higher nitrogen removal. The nitrogen removal in 2013 was higher than in 2014. This can be explained as oversupply, due to lower removal of nitrogen in the year before the trial started. In 2014, the nitrogen removal was slightly higher than the fertilization in the 80 kg ha⁻¹ treatment and slightly lower than that of the 140 kg N ha⁻¹ treatment. Both nitrogen levels delivered high yields, and the yield response to the higher nitrogen level was low. Therefore, the ideal fertilization level for long-term yield stability is considered to be between 80 and 140 kg N ha⁻¹ a⁻¹.

However, deposition from the air and soil fertility should also be taken into account when estimating nutrient requirements. Long-term observations are required to analyse which nitrogen level is sufficient for a steady green harvest of the crop. Low nitrogen fertilization requirements are seen as an important advantage of perennial crops, especially in terms of reducing the environmental impacts of biomass production (McCalmont et al., 2015). Early harvesting of miscanthus decreases this benefit because larger amounts of nutrients are withdrawn and need to be replaced by fertilization. However, in the case of biomass for biogas production, the largest part of these nutrients can be recycled by the application of biogas digestate. This is common practice in commercial biogas production. Direct emissions from nitrogen fertilizer or digestate application increase the global warming potential of crop cultivation, but only low-yield increases of 0.26 to 2.54 t DM ha⁻¹ are required to offset these (Roth et al., 2015).

In conclusion, Miscanthus x giganteus can be used for anaerobic digestion when harvested in October, but long-term effects of the green harvest on the productivity need to be assessed. The removed nutrients need to be replaced to ensure long-term productivity, but recycling of digestates should be sufficient. The methane yield and behaviour of the biomass in large-scale digesters need to be further researched. Due to the slower rate of biogas production, additional pre-treatment or larger digester volumes may be required. Breeding and selection of new miscanthus genotypes for biogas production should focus on development of genotypes with higher substrate-specific methane yield in October (e.g., less lignified) and earlier refilling of rhizome starch stores, to allow a broader harvest window. The replacement of biogas maize by miscanthus offers great potential for reducing the environmental impacts of biogas production without increasing land-use competition.

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