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Effect of silage maize plant density and plant parts on biogas production and composition

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ABSTRACT

Higher maize plant density represents a possible tool for biomass yield improvement but the effect on biogas production and quality has not been intensively investigated so far. The aim of the study was to compare two different plant densities (90 000 and 130 000 plants ha^{-1}) and their effects on yield and biogas production. Experiments were carried out at two sites in Central Bohemia in 2014 and 2015. Specific biogas yield of maize ears and stover was evaluated in batch tests. In both years, there were significantly higher dry matter yield, volatile solids (VS) degradation, and methane content in biogas from ears in comparison with stover. Stover produced on average 90% of biogas per weight unit (625–719 L kg^{-1} VS) compared to ears (721–801 L kg^{-1} VS) depending on locality and year. During the batch tests, ears produced more biogas than stover with the exception of the period from the 4th to 11th day when specific biogas yield of stover was higher. Biogas hectare yield of ears (5039–8962 m^3 ha^{-1}) was 1.3–1.8 times higher than biogas hectare yield of stover (3943–4865 m^3 ha^{-1}). The influence of plant density on dry matter yield and biogas hectare yield was not significant, but higher plant density supported faster dynamics of specific biogas yield of ears in both years and higher volatile solids degradation of ears in 2014.

1. Introduction

Europe is the world's leading producer of biogas [1]. More than 70% of the EU biogas plants operate using agriculture feedstock [2]. Anaerobic digestion in agricultural biogas plants is predominantly based on animal manure and slurries from cattle and pig farming units [3]. However, the total biogas production from manure is limited [4], therefore, biogas plants rely on energy crops with higher specific methane yield [5,6]. Although there is some controversy about the use of arable land for growing energy crops, as they may be in competition with food production, and in some cases may lead to increased soil erosion or negative impacts on the landscape [7–9], energy crops still provide about a half of total EU biogas production [2]. Silage maize is the main crop for biogas production, and it has the highest yield potential among field crops [4].

Maize biomass provides high specific methane yield, i.e. methane yield per unit of biomass (L kg^{-1}) [6], due to high biomass quality which is characterized by high energy content and good degradability [10,11]. Reported specific methane yield for silage maize ranges from 195 to 700 L kg^{-1} of volatile solids (VS) [12], while the methane content in biogas shows a much lower variability from 52 to 62% [6,13]. Large variability in specific methane yield could be partly attributed to different substrate quality, it may depend on a variable composition of the constituent matrix, or on different procedures in the calculations [12].

High dry matter biomass yield (t ha^{-1}) and high specific methane yield are essential for achieving maximal methane hectare yield (m^3 ha^{-1}). Depending on the maize crop dry matter yield and its specific methane yield, the methane hectare yield varies from 3000 up to 12 400 m^3 ha^{-1} [10], but is usually in the range of 6000 to 9000 m^3 ha^{-1} [11,14].

Maize yield per unit area is influenced by individual plant weight and plant density. Plant weight is affected predominantly by fertilization rates [15], type of maize hybrid [16], soil conditions [17], temperature during the vegetation period [18], precipitation and irrigation [19],

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interspecific competition, i.e. crop-weed competition [20], and intra-specific competition [21]. Increasing the plant density in maize stands can lead to a decrease in individual plant weight [17], ear weight [22] and 1000-grain weight [23,24].

Modern maize cropping is based on establishing a relatively high plant density [25], although the actual plant density in the crop depends on the subsequent environmental conditions, the level of growing technologies applied, and the characteristics of the maize hybrids, especially maturity groups. An increase in plant density at establishment enables the yield to increase up to the maximal value, but it may drop consequently due to plant competition [15,26]. Nowadays, the average density in the EU and the USA varies from 60 000 to 80 000 plants ha^{-1} for medium-late maturing hybrids [25]. For Central Europe, plant density of maize is highly variable, and ranges from 40 000 to 135 000 plants ha^{-1} [27,28]. A density of 74 000–95 000 plants ha^{-1} is usual for the Czech Republic [29–31].

Variation in maize plant density has potential to affect biomass quality although this has not yet been fully explained. Results from several studies imply that an increase in the number of plants per unit area causes a decrease in the biomass quality of silage maize when evaluated in terms of feedstuff quality, which means that acid detergent fibre and neutral detergent fibre increase while crude protein and dry matter digestibility decrease [32,33]. These adverse changes in overall biomass quality are usually explained by a drop in ear to stover ratio and leaf to stalk ratio [34]. However, other studies have failed to show that increased plant density impacted on plant morphology [35] and/or biomass quality [15,36]. The ear/stover ratio is an important factor which significantly influences maize biomass quality and usually ranges from 40 to 60% [11,37]. The ear/stover ratio increases during the maturation period where ears provide higher specific methane yield in comparison with leaves and stalks [38]. Biogas production per unit area is related mainly to the total dry matter yield [39] but the ear component can contribute significantly through higher specific methane yield than stover [11]. The importance of ears for biogas fermentation was documented by Rath et al. [13], where the major part of the specific biogas yield, covering about 70% of the total (roughly 500 L kg^{-1} VS) was shown to depend on easily degradable components, with starch contained in the ears representing most of this. The remaining 30% reflects the genotypic differentiation in specific biogas yield, which may be explained by the contents of hemicelluloses, crude fat, acid detergent lignin, and reducing sugars. While hemicelluloses and acid detergent lignin represent the complexity of the carbon binding status, reducing sugars reflects the physiological status of the plant, e.g., the translocation process from vegetative plant parts into developing ears.

It can be summarized that an increase in maize yield and an improvement of biomass quality is a primary goal for researchers and breeders, as well as for maize growers, irrespective of whether it is used as forage for ruminants or as a feedstock for biogas plants. An increase in maize plant density represents an effective tool to improve forage yield; however, less attention has been paid in terms of investigating the interaction between different plant densities and biogas production.

The role of maize in biogas production is controversial because its use leads to competition between food, feed and specific energy crops [9]. However, consideration of the role of maize, and particularly of the different components of the maize crop for biogas production, is necessary to enable an informed analysis of the trade-offs and synergies between bioenergy and food production within a more sustainable, spatially optimized agricultural land use [40]. According to Theuerl et al. [8], the future agricultural biogas plant is likely to be primarily based on residues such as maize stover. Therefore, a field study with maize sown in two different plant densities was conducted at two sites over a two-year period with the following aims: (i) to assess the effect of plant density on maize yield and plant-part proportions; (ii) to evaluate changes in degradation of volatile solids, specific biogas yield and methane content in biogas given by the different plant parts and for different plant densities; and (iii) to determine biogas and methane hectare yield of a maize stand growing in different plant densities. Explanation of these relations may be valuable for optimization of specific or area biogas/methane production in association to maize plant density and the utilization of different maize plant parts.

2. Materials and methods

2.1. Field experiments

Field experiments with silage maize were conducted at two locations in Central Bohemia in the Czech Republic, in the growing seasons of 2014 and 2015.

Experiment A: A plot experiment was carried out in the experimental field of the Czech University of Life Sciences Prague (CZU Prague) in Prague-Suchdol (50°7′39″ N, 14°22′19″ E; 286 m a.s.l.). The soil of the experimental site was clayey-loam Haplic Chernozem [41] with the following characteristics: pH_{KCl} 7.2, phosphorus 156 mg kg^{-1}, potassium 275 mg kg^{-1}, magnesium 177 mg kg^{-1} and calcium 7984 mg kg^{-1} (according to the Mehlich 3 method [42]).

Experiment B: The second experiment was carried out at the experimental field station of the CZU Prague in Červený Újezd (50°04′26″ N, 14°10′25″ E; 405 m a.s.l.). In this area, the soil was clayey-loam Haplic Luvisol [41] which had the characteristics: pH_{KCl} 6.5, phosphorus 100 mg kg^{-1}, potassium 80 mg kg^{-1}, magnesium 110 mg kg^{-1} and calcium 3600 mg kg^{-1} (according to Mehlich 3 method [42]).

The long-term averages and year-specific weather conditions of the experimental locations are shown in Table 1.

The experiments were arranged in a factorial design for both factors in complete randomized blocks with four replications with plot size 17.5 m^2 (3.5 × 5 m). The experiments consisted of 9 treatments combining three plant densities with three spatial arrangements. For biogas evaluation, plant densities of 90 000 and 130 000 plants ha^{-1} at 0.70 m row spacing (with five rows of maize) were considered. Maize (hybrid Kuxxar, FAO 300) was sown (Experiment A: April 24, 2014 and April 21, 2015; Experiment B: April 29, 2014 and April 24, 2015) at a depth of 40 mm. Fertilizers were applied one week before sowing at rates of 120 kg ha^{-1} of nitrogen (ammonium sulphate), 45 kg ha^{-1} of phosphorus (superphosphate) and 120 kg ha^{-1} of potassium (potassium chloride). Weeds were controlled by post-emergent herbicide application with Akris (dimethenamid-P 840 g ha^{-1} and terbuthylazin 750 g ha^{-1}, BASF SE, Germany) in May. There was no irrigation of plots in either of the growing seasons.

At silage maturity, two inner rows (7 m^2) of each plot were manually harvested (Experiment A: September 3, 2014 and August 31, 2015; Experiment B: September 9, 2014 and September 4, 2015) for determination of fresh matter yield (t ha^{-1}). At harvest time, three randomly chosen plants per plot were selected for subsequent analyses. The ears of the sampled plants were separated from the rest (i.e. the stover) and dried at 60 °C to a constant weight in a forced-air dryer (Venticell 404, the Czech Republic) and dry matter content (%) was determined. Dry matter biomass yield (t ha^{-1}) and dry matter weight percentage ratio of the plant parts (ear/stover ratio; %) were calculated. Dried ears and stover were ground separately in a cutting mill (FRITSCH Pulverisette 19, Germany) to pass a sieve with 1 mm mesh size. Milled samples of plant parts from each plot were mixed and average samples of ears and average samples of stover were taken for subsequent anaerobic batch tests.

2.2. Inoculum for batch tests

Inoculum was obtained from the Krásná Hora nad Vltavou biogas plant (49°36′21″ N, 14°17′16″ E) which operates at mesophilic conditions (39 °C). Animal slurry (60% of fresh weight) and maize silage (40% of fresh weight) served as the main substrates for the anaerobic reactor. The inoculum used for batch tests was degassed and subsequently stored for 10 days at 40 °C. Degassing was applied to minimize the effect of

Table 1
Monthly mean air temperature (t; °C) and monthly sums of precipitation (P; mm) in 2014 and 2015 (experimental sites in Prague-Suchdol and Červený Újezd) and long-term averages for the period 1981–2010 for meteorological station Prague-Ruzyně (source: Czech Hydrometeorological Institute).

	Prague-Suchdol				Červený Újezd					
	2014		2015		2014		2015		Long-term averages	
Month	t (°C)	P (mm)	t (°C)	P (mm)	t (°C)	P (mm)	t (°C)	P (mm)	t (°C)	P (mm)
April	11.6	23	9.1	26	11.2	28	9.0	30	8.5	28
May	13.0	137	13.7	32	12.9	92	13.7	45	13.5	70
June	17.3	20	16.8	38	16.7	25	16.2	37	16.2	67
July	20.7	92	21.6	32	20.1	155	20.8	29	18.3	78
August	17.2	43	22.9	60	16.8	57	21.9	55	17.9	65
September	15.7	94	14.5	8	16.1	77	14.6	11	13.5	38
April–September	15.9	409	16.4	196	15.6	434	16.0	207	14.7	346
January–December	10.7	571	10.7	371	10.3	601	10.3	377	8.4	501

biogas production of inoculum on anaerobic batch tests. The average pH of the inoculum was 8.2. The dry matter content reached 6.2%, the volatile solids content was 71.1% of this, and soluble chemical oxygen demand (COD) was 2200 mg L^{-1}.

2.3. Biochemical methane potential tests (batch tests)

Biogas production (L kg^{-1}) was quantified using biochemical methane potential tests (batch tests) according to VDI 4630 [43] and the modified procedure of Mast et al. [44]. Each sample assay was performed in 120 mL glass bottles in five replications where the inoculum was used as a control treatment. The glass bottles were filled with 30 mL of inoculum, maize samples set according to volatile solids amount (0.70 ± 0.002 g), and 80 mL of distilled water to adjust the final volume. Bottles were sealed with butyl rubber stoppers and plastic seals. The inoculum to substrate ratio was 1.9 (on VS basis). The volume of biogas produced during the batch tests was determined by the volumetric method where the produced gas volume was recorded when the levels of the confining liquid in the audiometer tube and in the levelling bottle were the same [43]. The batch tests were carried out under mesophilic conditions at 40 °C and lasted for 40 days. Gas samples were taken through the butyl rubber stoppers by syringe.

During the first two weeks of the tests, biogas production was measured every day. In the following week, measurements were performed every second day and subsequently biogas production was quantified once a week. Gas composition was analysed once a week from the third day of batch test establishment.

At the end of the batch tests, the efficiency of volatile solids degradation (%) was quantified as the difference between a comparison of the amounts of volatile solids at the beginning and at the end of the experiment.

2.4. Analysis and calculations

Characteristics of the samples used for the batch tests (dry matter content, volatile solids content, chemical oxygen demand and pH) were determined according to standard methods [45]. Dry matter content (%) was determined by drying samples at 105 °C according to method SM 2450-B, using a forced-air dryer (Ecocell 55, the Czech Republic). Volatile solids content (%) was determined by incineration of the sample at 550 °C according to method SM 2540-E, using a muffle furnace (ELSKLO MF5, the Czech Republic). Chemical oxygen demand (mg L^{-1}) was measured by the colorimetric method SM 5220-D, using a spectrophotometer (HACH DR/4000, Germany). pH was measured by the electrometric method SM 4500-H + B, using a pH meter IQ 150 equipped with an IS FET PH77-SS electrode (IQ Scientific Instruments, USA).

Methane content in biogas (%) was determined using a gas chromatograph (DANI Master GC, Italy), equipped with a thermal conductivity detector and a 2 m × 1 mm column (Restek ShinCarbon ST, USA). Hydrogen was used as the carrier gas. Injection volume was 0.2 mL, injector temperature was 110 °C, and the detector and oven temperatures were 195 °C.

Specific biogas yield of volatile solids (L kg^{-1} VS) was calculated as biogas cumulative production of each sample after the subtraction of inoculum biogas production. The relative cumulative dynamics of specific biogas yield (%) were determined according to Hakl et al. [46]. Biogas hectare yield (m^3 ha^{-1}) was calculated from the specific biogas yield and dry matter yield of maize. Methane hectare yield (m^3 ha^{-1}) arose from biogas hectare yield and methane content in biogas.

Volatile solids (VS) degradation efficiency was calculated according to Equation (1) where the contribution of volatile solids degradation of inoculum was taken into consideration:

$$VS_{degradation}\ (\%) = \frac{[cVS_{1\ (S+I)} - cVS_{2\ (S+I)}] - [cVS_{1\ (I)} - cVS_{2\ (I)}]}{[cVS_{1\ (S+I)} - cVS_{1\ (I)}]} \cdot 100\% \qquad (1)$$

where: $cVS_{1\ (S+I)}$ – volatile solids content of sample + inoculum at the start

$cVS_{2\ (S+I)}$ – volatile solids content of sample + inoculum at the end
$cVS_{1\ (I)}$ – volatile solids content of inoculum at the start
$cVS_{2\ (I)}$ – volatile solids content of inoculum at the end.

2.5. Statistical analyses

The data of maize yield, plant parts, biogas production and biogas quality were statistically evaluated by using two- and three-way analyses of variance followed by the Tukey post-hoc test (α = 0.05) using data analysis software system Statistica 12 [47].

3. Results

3.1. Maize yield and maize plant part proportions

There were contrasting weather conditions between 2014 and 2015 and these differences were reflected in the observed yield characteristics of silage maize (Tables 2–4). The year 2015 was extremely dry (Table 1), and therefore evaluation of yield and biogas parameters has been made within each year separately.

Table 2 shows a significantly higher plant weight in the treatment with standard plant density of 90 000 plants ha^{-1} in comparison with 130 000 plants ha^{-1}. The ear/stover ratio exceeded 60% in the first evaluated year, but it did not reach this value in the dry year 2015. A higher ear/stover ratio was recorded in the treatment with 90 000 plants ha^{-1} compared with 130 000 plants ha^{-1}, but the difference was not statistically significant.

Plant density influenced neither the dry matter content of maize plants (Table 2) nor dry matter yield per ha of the maize crop (Tables 3 and 4) in any of the evaluated years. A significantly higher dry matter yield of ears (11.8 t ha^{-1} in 2014 and 7.1 t ha^{-1} in 2015) in comparison with dry matter yield of stover (7.3 t ha^{-1} in 2014 and 6.6 t ha^{-1} in 2015) was found regardless of the tested plant density.

Table 2
Plant dry matter weight (PW; g DM plant^{-1}), ear/stover dry matter ratio (E/S R; %), dry matter content of whole silage maize plant (DMC – WP; %), dry matter content of ears (DMC – E; %) and dry matter content of stover (DMC – S; %) at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2014 and 2015.

Year	Experimental site and plant density (plants ha^{-1})	PW (g DM plant^{-1})	E/S R (%)	DMC – WP (%)	DMC – E (%)	DMC – S (%)
2014	Prague-Suchdol	170.8	62.4	33.1^{a}	44.4^{a}	23.3^{a}
	Červený Újezd	180.0	60.9	26.5^{b}	34.2^{b}	19.6^{b}
	P	*0.329*	*0.514*	*< 0.000*	*< 0.000*	*< 0.000*
	PD90	206.1^{a}	63.1	30.1	39.4	21.4
	PD130	144.6^{b}	60.2	29.6	39.1	21.5
	P	*< 0.000*	*0.225*	*0.507*	*0.814*	*0.930*
2015	Prague-Suchdol	153.6^{a}	58.4^{a}	40.8^{a}	52.0^{a}	31.3^{a}
	Červený Újezd	121.9^{b}	44.4^{b}	33.4^{b}	41.3^{b}	29.0^{b}
	P	*0.001*	*0.002*	*< 0.000*	*< 0.000*	*0.036*
	PD90	157.4^{a}	52.3	37.4	48.1	29.8
	PD130	118.1^{b}	50.5	36.8	45.2	30.4
	P	*< 0.000*	*0.626*	*0.681*	*0.122*	*0.536*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

Table 3
Dry matter yield (DMY; t ha^{-1}), specific biogas yield (SBY; L kg^{-1} VS), biogas hectare yield (BHY; m^{3} ha^{-1}), methane hectare yield (MHY; m^{3} ha^{-1}) and efficiency of volatile solids degradation (VS degradation; %) of silage maize at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2014.

Experimental site and plant density (plants ha^{-1})	Plant part	DMY (t ha^{-1})	SBY (L kg^{-1} VS)	BHY (m^{3} ha^{-1})	MHY (m^{3} ha^{-1})	VS degradation (%)
Prague-Suchdol		20.9^{a}	743	15 055^{a}	9599^{a}	80.5^{a}
Červený Újezd		17.2^{b}	749	12 599^{b}	8004^{b}	88.3^{b}
P		*0.006*	*0.704*	*0.009*	*0.008*	*< 0.000*
PD90		18.5	731	13 175	8368	83.0^{a}
PD130		19.6	760	14 480	9234	85.9^{b}
P		*0.319*	*0.067*	*0.127*	*0.113*	*0.001*
	Ears	11.8^{a}	779^{a}	8962^{a}	5833^{a}	92.3^{a}
	Stover	7.3^{b}	713^{b}	4865^{b}	3026^{b}	76.6^{b}
	P	*< 0.000*	*< 0.000*	*< 0.000*	*< 0.000*	*< 0.000*
PD90	Ears	11.7^{a}	756^{ab}	8660^{a}	5639^{a}	90.2^{a}
PD130	Ears	11.8^{a}	801^{a}	9264^{a}	6027^{a}	94.4^{b}
PD90	Stover	6.8^{b}	706^{b}	4514^{b}	2795^{b}	75.7^{c}
PD130	Stover	7.8^{b}	719^{b}	5216^{b}	3256^{b}	77.4^{c}
P		*0.326*	*0.284*	*0.868*	*0.850*	*0.125*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

An effect of experimental site was recorded for dry matter yield in 2014, whilst in 2015 site affected plant weight and ear/stover ratio. The dry matter content of whole plants as well as separated plant parts differed in both years.

3.2. Biogas production and methane content in biogas

Specific biogas yield was influenced by neither the experimental site nor plant density in either of the experiment years (Tables 3 and 4). Specific biogas yield of ears was significantly higher than that of stover. It is evident from evaluation of the plant density × plant part interaction that specific biogas yield of ears and stover were not significantly influenced by plant density.

Table 4
Dry matter yield (DMY; t ha^{-1}), specific biogas yield (SBY; L kg^{-1} VS), biogas hectare yield (BHY; m^{3} ha^{-1}), methane hectare yield (MHY; m^{3} ha^{-1}) and efficiency of volatile solids degradation (VS degradation; %) of silage maize at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2015.

Experimental site and plant density (plants ha^{-1})	Plant part	DMY (t ha^{-1})	SBY (L kg^{-1} VS)	BHY (m^{3} ha^{-1})	MHY (m^{3} ha^{-1})	VS degradation (%)
Prague-Suchdol		14.4	681	9513^{a}	6098^{a}	78.4
Červený Újezd		13.1	677	8450^{b}	5457^{b}	77.8
P		*0.054*	*0.366*	*0.018*	*0.026*	*0.302*
PD90		13.7	679	8965	5707	78.2
PD130		13.8	680	8998	5852	78.1
P		*0.898*	*0.777*	*0.934*	*0.571*	*0.769*
	Ears	7.1^{a}	726^{a}	5039^{a}	3359^{a}	81.9^{a}
	Stover	6.6^{b}	632^{b}	3943^{b}	2447^{b}	74.3^{b}
	P	*0.034*	*< 0.000*	*< 0.000*	*< 0.000*	*< 0.000*
PD90	Ears	7.2	732^{a}	5168^{a}	3387^{a}	82.4^{a}
PD130	Ears	7.0	721^{a}	4916^{a}	3331^{a}	81.5^{a}
PD90	Stover	6.5	625^{b}	3803^{b}	2348^{b}	74.0^{b}
PD130	Stover	6.8	638^{b}	4082^{b}	2546^{b}	74.6^{b}
P		*0.184*	*0.009*	*0.075*	*0.178*	*0.195*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

The dynamics of cumulative specific biogas yield of ears and stover were similar in both years (Fig. 1). The specific biogas yield of ears was higher in the first phase (up to the 4th day) and stover in the second phase (up to the 11th day). After this point, ears again produced more biogas than stover. The effect of plant density on cumulative specific biogas yield of ears is presented in Fig. 2. In 2014, there was a higher specific biogas yield from ears in the treatment with 130 000 plants ha^{-1} in comparison with 90 000 plants ha^{-1} (Fig. 2a) whereas in 2015 only a minimal effect of plant density was found (Fig. 2b). There was no effect of plant density on the dynamics of specific biogas yield of stover in either year of the experiments (data not presented).

The relative cumulative dynamics of specific biogas yield are presented in Tables 5 and 6. Differences between the standard density and high plant density treatments are evident in the initial days of biomass degradation in both years. Evaluation of plant density × plant part interaction shows significantly higher values for ears from the high-density treatment with 130 000 plants ha^{-1} in comparison with 90 000 plants ha^{-1} from the 3rd to the 6th day in 2014 and from the 3rd to the 10th day in 2015. Plant density did not affect the relative cumulative dynamics of specific biogas yield of stover in either year.

Results of methane content in biogas is presented in Tables 7 and 8. The lowest values were determined in all samples on the 3rd day of the batch tests course. Subsequently, methane content increased rapidly and maximum values were observed on the 23rd day. At the beginning of the batch tests, stover produced biogas with a significantly higher content of methane than that from ears. Subsequently, there was a higher methane content determined in samples from ears. In most cases, higher methane contents were found in samples from the treatment with 130 000 plants ha^{-1}.

In 2014, samples originating from the treatment with 130 000 plants ha^{-1} showed a significantly higher efficiency of volatile solids degradation (Tables 3 and 4), but in 2015 no effect of plant density was detected. In both years, higher dry matter degradation occurred in ears in comparison with stover.

Biogas hectare yield (Tables 3 and 4) was calculated using the specific biogas yield and dry matter yield of maize at harvest time. Higher levels of biogas hectare yield were obtained from ears (8962 and 5039 m^{3} ha^{-1}) than from stover (4865 and 3943 m^{3} ha^{-1}) in 2014 and 2015, respectively. Evaluation of the plant density × plant part interaction

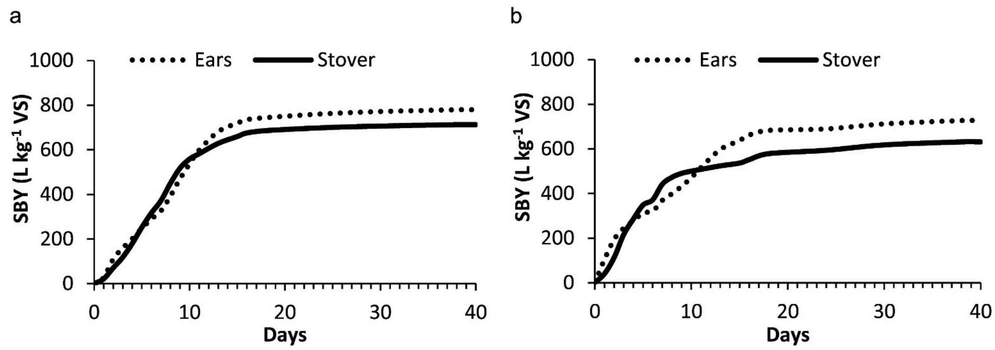

Fig. 1. Cumulative specific biogas yield (SBY; L kg^{-1} VS) of silage maize ears and stover (average of tested plant densities and experimental sites) in 2014 (a) and 2015 (b).

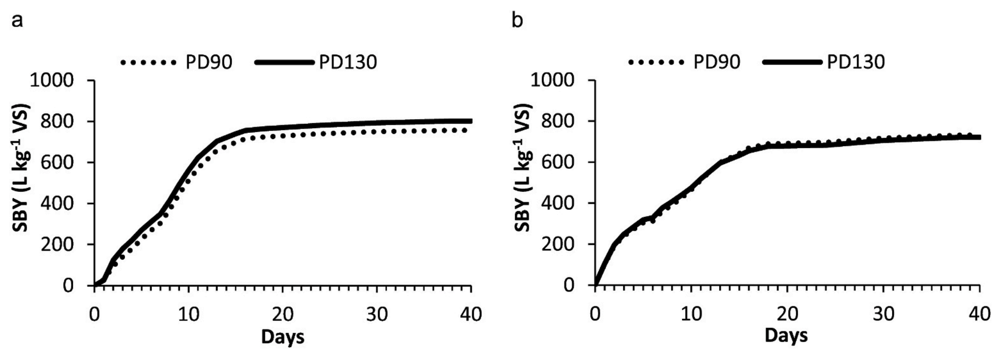

Fig. 2. Cumulative specific biogas yield (SBY; L kg^{-1} VS) of silage maize ears from tested plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively; average of experimental sites) in 2014 (a) and 2015 (b).

Table 5
Relative cumulative dynamics of specific biogas yield (%, 40th day = 100%) of silage maize biomass at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2014.

Experimental site and plant density	Plant part	1st day	3rd day	6th day	10th day	15th day	30th day
(plants ha^{-1})					(%)		
Prague-Suchdol		3.4	17.6	40.2	73.3	92.5	99.1
Červený Újezd		3.1	19.4	41.3	73.8	92.2	99.0
P		*0.100*	*0.052*	*0.156*	*0.500*	*0.309*	*0.100*
PD90		3.4	17.1^{a}	39.5^{a}	72.6^{a}	92.1	99.1
PD130		3.1	19.9^{b}	42.0^{b}	74.5^{b}	92.7	99.0
P		*0.091*	*0.004*	*0.002*	*0.014*	*0.057*	*0.438*
	Ears	3.5^{a}	20.4^{a}	37.1^{a}	68.8^{a}	92.3	99.0
	Stover	3.0^{b}	16.5^{b}	44.4^{b}	78.3^{b}	92.5	99.1
	P	*0.015*	*< 0.000*	*< 0.000*	*< 0.000*	*0.349*	*0.152*
PD90	Ears	3.1^{ab}	18.5^{a}	35.4^{a}	67.6^{a}	92.3^{ab}	99.1
PD130	Ears	2.9^{a}	22.2^{b}	38.7^{b}	69.9^{a}	92.2^{ab}	99.0
PD90	Stover	3.8^{b}	15.6^{a}	43.6^{c}	77.6^{b}	91.9^{a}	99.2
PD130	Stover	3.8^{ab}	17.5^{a}	45.2^{c}	79.1^{b}	93.1^{b}	99.1
P		*0.491*	*0.286*	*0.273*	*0.617*	*0.027*	*0.143*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

showed that the biogas hectare yield of ears and stover, expressed on a per-hectare basis, was not significantly influenced by plant density. Statistical evaluation of methane hectare yield corresponded with the results of biogas hectare yield.

4. Discussion

4.1. Weather conditions

Yield characteristics (plant weight, ear/stover ratio, biomass yield, dry matter content of whole plants and plant parts) were significantly influenced by weather conditions. In 2014, more favourable conditions for maize growth were documented. Mean air temperatures during the vegetation period were 1.2 °C above the long-term average at Prague-Suchdol and 0.9 °C above average at Červený Újezd, and precipitation recorded at both experimental sites (409 mm in Prague-Suchdol and 434 mm in Červený Újezd) was considered to be sufficient. In 2015, however, the vegetation period was unusually hot and dry. The average air temperature during the vegetation period was 1.7 °C above the long-term average at Prague-Suchdol, and 1.3 °C above average at Červený Újezd. Extremely high mean air temperatures occurred in July 2015 (3.3 °C and 2.5 °C above the long-term average) and in August 2015 (5.0 °C and 4.0 °C above the long-term average). At both experimental sites, during May, June and July of 2015, precipitation was approximately 50% below the long-term average and the total precipitation during the vegetation period was only 196 mm in Prague-Suchdol and 207 mm in

Table 6
Relative cumulative dynamics of specific biogas yield (%, 40th day = 100%) of silage maize biomass at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2015.

Experimental site and plant density	Plant part	1st day	3rd day	6th day	10th day	15th day	30th day
(plants ha^{-1})					(%)		
Prague-Suchdol		10.2	34.2	51.5	73.6^{a}	91.0^{a}	98.2^{a}
Červený Újezd		10.5	33.9	51.6	70.4^{b}	87.4^{b}	97.6^{b}
P		*0.319*	*0.319*	*0.724*	*< 0.000*	*< 0.000*	*< 0.000*
PD90		10.2	33.3^{a}	50.7^{a}	71.7	89.7^{a}	98.1^{a}
PD130		10.6	34.7^{b}	52.5^{b}	72.3	88.8^{b}	97.7^{b}
P		*0.161*	*< 0.000*	*< 0.000*	*0.222*	*0.045*	*< 0.000*
	Ears	14.4^{a}	33.7^{a}	44.2^{a}	64.8^{a}	91.1^{a}	98.0
	Stover	6.3^{b}	34.3^{b}	58.9^{b}	79.2^{b}	87.3^{b}	97.9
	P	*< 0.000*	*0.034*	*< 0.000*	*< 0.000*	*< 0.000*	*0.202*
PD90	Ears	14.1^{a}	32.8^{a}	42.8^{a}	63.7^{a}	91.4^{a}	98.1^{ab}
PD130	Ears	14.6^{a}	34.6^{b}	45.7^{b}	65.9^{b}	90.9^{a}	97.8^{ac}
PD90	Stover	6.2^{b}	33.8^{ab}	58.6^{c}	79.8^{c}	87.9^{b}	98.1^{b}
PD130	Stover	6.5^{b}	34.9^{b}	59.3^{c}	78.6^{c}	86.8^{b}	97.6^{c}
P		*0.571*	*0.294*	*0.004*	*< 0.000*	*0.436*	*0.074*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

Table 7
Methane content in biogas (%) from silage maize biomass at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2014.

Experimental site and plant density	Plant part	3rd day	9th day	16th day	23rd day	30th day
(plants ha^{-1})				(%)		
Prague-Suchdol		33.1^{a}	58.0^{a}	65.0^{a}	68.5^{a}	67.3
Červený Újezd		36.2^{b}	64.6^{b}	61.4^{b}	66.6^{b}	67.3
P		*0.001*	*< 0.000*	*0.001*	*0.001*	*0.763*
PD90		34.1	59.9^{a}	64.2^{a}	66.9^{a}	67.1
PD130		35.2	62.6^{b}	62.2^{b}	68.2^{b}	67.5
P		*0.123*	*0.019*	*0.020*	*0.008*	*0.087*
	Ears	27.3^{a}	60.9	67.1^{a}	69.7^{a}	68.9^{a}
	Stover	42.0^{b}	61.6	59.2^{b}	65.3^{b}	65.7^{b}
	P	*< 0.000*	*0.460*	*< 0.000*	*< 0.000*	*< 0.000*
PD90	Ears	26.6^{a}	59.3	68.2^{a}	69.0^{a}	69.0^{a}
PD130	Ears	27.9^{a}	62.5	66.1^{a}	70.4^{a}	68.7^{a}
PD90	Stover	41.5^{b}	60.6	60.1^{b}	64.7^{b}	65.1^{b}
PD130	Stover	42.4^{b}	62.7	58.2^{b}	65.9^{b}	66.3^{c}
P		*0.806*	*0.562*	*0.914*	*0.837*	*0.008*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

Table 8
Methane content in biogas (%) from silage maize biomass at plant densities of 90 000 and 130 000 plants ha^{-1} (PD90 and PD130, respectively) at two experimental sites in 2015.

Experimental site and plant density	Plant part	3rd day	9th day	16th day	23rd day	30th day
(plants ha^{-1})				(%)		
Prague-Suchdol		42.4	59.8^{a}	65.9^{a}	70.5	66.9
Červený Újezd		43.6	61.4^{b}	68.2^{b}	68.0	67.6
P		*< 0.000*	*0.025*	*< 0.000*	*0.363*	*0.782*
PD90		42.5	59.9^{a}	66.3^{a}	67.7	67.1
PD130		43.5	61.3^{b}	67.7^{b}	70.8	67.4
P		*1.000*	*0.034*	*0.007*	*0.266*	*0.916*
	Ears	32.6^{a}	62.5^{a}	73.2^{a}	72.2^{a}	69.5
	Stover	53.4^{b}	58.6^{b}	60.8^{b}	66.2^{b}	65.0
	P	*< 0.000*	*< 0.000*	*< 0.000*	*0.047*	*0.104*
PD90	Ears	30.8^{a}	61.6^{a}	72.1^{a}	71.7	67.6
PD130	Ears	34.4^{a}	63.5^{a}	74.3^{b}	72.8	71.4
PD90	Stover	54.2^{b}	58.1^{b}	60.5^{c}	63.7	66.5
PD130	Stover	52.6^{b}	59.1^{b}	61.2^{c}	68.7	63.4
P		*0.154*	*0.483*	*0.104*	*0.464*	*0.203*

P: probability; different letters indicate significant differences for Tukey HSD (α = 0.05).

Červený Újezd (Table 1). Similar patterns of weather conditions in 2014 and 2015 were described by von Cossel et al. [48] in the context of a methane yield performance study in southwest Germany.

4.2. Effect of maize plant density on biomass production

In both experimental years there were significant plant weight differences between the two tested plant densities (Table 2). The higher plant density of 130 000 plants ha^{-1} reduced the average plant weight relative to the standard plant density of 90 000 plants ha^{-1} (from 206.1 to 144.6 g plant^{-1} in 2014 and from 157.4 to 118.1 g plant^{-1} in 2015, respectively). A similar decrease of plant weight (from 211.9 to 167.6 g plant^{-1}) resulting from a plant density increase (from 102 040 to 142 850 plants ha^{-1}) was reported by Karaşahin [49]. High plant density increases plant stresses due to intensive competition for resources, such as solar radiation, water and nutrients, and it modifies plant morphology and development to the detriment of the single plant yield [50,51].

The effect of plant density on the ear/stover ratio was not significant, but in both years, a higher ear/stover ratio was recorded in treatment with 90 000 plants ha^{-1}. Millner and Villaver [35] reported similar results when the effect of plant density was not significant, but the ear/stover ratio showed a clear tendency to decline at a higher plant density. Studies with a higher range of plant density have usually reported significant changes in the ear/stover ratio [15,49] in relation to site productivity. Morphological adaptation of plants, and particularly of the ears, is clearly influenced by the equidistance space between plants [50]. At high plant density, ear length, the extent of grain-filling and the kernel number are negatively affected by a lower flower primordial formation, poor pollination due to flowering asynchrony, and increased grain abortion after fertilization [52,53].

Dry matter content of whole plants as well as ears and stover was not significantly influenced by plant density. This corresponds with the results of Millner and Villaver [35] and Ma et al. [54] for silage maize densities of 75 000 to 140 000 plants ha^{-1} and 75 000 to 150 000 plants ha^{-1}, respectively. In line with Ma et al. [55], high population density produced biomass with a slightly lower dry matter content of whole plants in both years. A perceptibly lower dry matter content of ears by 2.9% at higher density was manifested in 2015. According to Zhang et al. [53], high plant density may delay the ear development especially under unfavourable weather conditions. Comparison of our experimental sites showed that the dry matter content of ears varied more (by 10% in both years) than the dry matter content of stover (by 3.7% in 2014 and by 2.3% in 2015, respectively), which is related to different weather conditions at the experimental sites. The lack of precipitation was manifested in high dry matter content of ears, especially for the site at Prague-Suchdol in 2015. Increase of whole plant dry matter content varies between years, and the kernel milk line may also not correspond to whole plant dry matter content due to extreme weather conditions [55].

Previous research on the effects of different plant density has shown that the grain or total biomass yield of maize increased linearly [34,56] or quadratically [25,26,57] when plant density was increased. This is also influenced by seasonal weather conditions, and especially in dry years, there was either no significant effect of plant density on yield [58,59] or, in extremely dry years, yield even decreased with an increased density [58]. Many authors [26,51] have pointed out that the optimal plant density depends on several factors, such as water availability, soil fertility, hybrid genotypes and crop management practice. In our experiments, the effect of plant density on dry matter yield was not significant (Tables 3 and 4). In agreement with Testa et al. [50], lower plant weight was fully compensated by higher plant population. Nevertheless, higher dry matter yield (by 1.1 t ha^{-1}) was reached at the plant density of 130 000 plants ha^{-1} compared to that of 90 000 plants ha^{-1} in 2014. A similar increase of dry matter yield (by 0.8 t ha^{-1}) was recorded by Çarpici et al. [15] comparing densities of 100 000 and 140 000 plants ha^{-1}. In the dry and hot year 2015, dry matter yield was the same for both plant densities, which is in accordance with the results of Ren et al. [58]. Water stress at the beginning of the rapid vegetative growth stage negatively affects dry matter production and can be explained by a combination of a reduced plant extension growth rate, delayed leaf tip emergence and a smaller leaf size [60–62]. The negative effect of the combination of low precipitation and high temperature in 2015 caused a reduction in dry matter yield by 6.5 t ha^{-1} (at Prague-Suchdol) and 4.1 t ha^{-1} (at Červený Újezd). Similarly, in southwest Germany, von Cossel et al. [48] recorded a decrease of dry matter yield of silage maize by 9.3 t ha^{-1} in 2015, relative to 2014. Moreover, in our experiment, unfavourable weather conditions in 2015 influenced especially dry matter yield of ears negatively due to the decline in the ear/stover ratio, as described above.

4.3. Effect of maize plant parts and plant density on specific biogas yield, volatile solids degradation and methane content in biogas

Specific biogas yield from maize biomass depends on the proportion of individual parts of maize plants (stalks, leaves and parts of ears, i.e. grains, husks and cobs) [38] and the chemical composition of biomass [6]. The proportion of plant parts, as well as their concurrent chemical composition and biomass degradability, change during maturation. In addition to the time of harvesting, chemical composition is affected by many other factors, such as the maize hybrid, location of cultivation or weather conditions [4,63].

In our experiment, specific biogas yield ranged from 625 to 801 L kg^{-1} VS, depending on plant parts, plant density, locality and year (Tables 3 and 4). The results correspond with many studies [13,14,38]. In 2014, significantly higher levels of specific biogas yield of whole plants could be explained by a higher proportion of ears and lower dry matter content, which was associated with lower maturation in the first year of the evaluations.

In both years, a significantly higher specific biogas yield was obtained from ears in comparison with stover (779 *vs.* 713 L kg^{-1} VS in 2014 and 726 *vs.* 632 L kg^{-1} VS in 2015). According to Menardo et al. [38], the highest specific biogas yield was produced by grains (709 L kg^{-1} VS) in comparison with other parts of maize, such as stalks, leaves, cobs and husks (380–544 L kg^{-1} VS). Similarly, Amon et al. [4] and Seppälä et al. [11] also reported a higher specific methane yield of ears in comparison with specific methane yield of stover.

The high level of specific biogas yield of ears is associated with high volatile solids degradation of grains (about 90%) due to their low fibre and lignin contents [38] and higher content of easily degradable components, such as carbohydrates (mainly represented by starch), fatty acids and proteins [11]. This was confirmed in our experiment (Tables 3 and 4), where ears showed significantly higher volatile solids degradation in comparison with stalks (92.3% *vs.* 76.6% in 2014, and 81.9% *vs.* 74.3% in 2015). Differences in chemical composition between ears and stover are stable and have been proved in many studies [16,63,64]. For biogas production, Grieder et al. [39] recommend hybrids with lower ear proportions but with higher total dry matter yield. Our study shows that stover produced on average 90% of biogas per weight unit in comparison with ears. Therefore, lower ear yield per hectare must be compensated by 1.12 times higher stover yield due to the lower specific biogas yield of stover. It is generally believed that nutritional composition and the C:N ratio are responsible for differences in volatile solids degradation and specific biogas yield [4,6], although Schittenhelm [37] did not find a clear relation between chemical composition and specific methane yield, despite substantially different nutrient content among the maize hybrids.

Higher cumulative (Fig. 1) and relative cumulative dynamics of specific biogas yield (Tables 5 and 6) of ears at the beginning of the batch tests are in line with their high content of easily degradable components, especially water soluble carbohydrates. At the same time, faster biogas production of ears was associated with lower methane content in biogas compared to that of stover (Tables 7 and 8). In the subsequent stages of biomass decomposition, the biogas quality of ears was significantly higher compared to stover in both years. This finding could be explained by a higher starch content and higher volatile solids degradation of ears. Menardo et al. [38] detected significantly higher volatile solids degradation of grains in comparison with stalks and leaves, but they did not record differences in methane content for these different plant parts. Changes of methane content in biogas during anaerobic digestion correspond with the results of Míchal et al. [65]. Production of 80–90% of the maximum of gas production was reached between the 10th and 15th day of the batch tests. Comparable results were found by Hakl et al. [46] for alfalfa and by Míchal et al. [65] for grasses.

In 2014, significantly higher volatile solids degradation of whole plant biomass from the treatment with 130 000 plants ha^{-1} was recorded (Tables 3 and 4). The ear fraction of maize from the treatment with the higher plant density, displayed the best volatile solids degradation (94.4%), although in 2015 this linkage was not confirmed. Intense crowding within the maize stand usually leads to a decrease in biomass feedstuff quality [32,33], but at the present time there is a lack of results about the effect of high plant density on maize biomass for biogas production.

The higher specific biogas yield of ears at the plant density of 130 000 plants ha^{-1} (Fig. 2a) is related to higher volatile solids degradation, which could probably be explained by a higher ratio of grains in 2014. Menardo et al. [38] showed statistical differences of specific biogas yield among particular parts of ears, which means that the ratio of grains, husks and cobs and their chemical compositions affect the specific biogas yield of whole ears. In 2015, specific biogas yield of ears was about 10% lower than in the previous year (Fig. 2b). This can be explained by higher dry matter content of ears in 2015. At the plant density of 130 000 plants ha^{-1}, a higher specific biogas yield of ears was recorded only in the period from the 3rd to 10th day of the batch tests course, which is also evident from the evaluation of relative cumulative dynamics of specific biogas yield (Table 6), but this difference disappeared subsequently. Testa et al. [66] recorded an increase of specific methane yield by 5% at a density of 100 000 plants ha^{-1} in comparison with 75 000 plants ha^{-1}. In 2015, the change of biomass quality was probably caused by the dry and hot weather during the vegetative period. Heat stress has negative effects on flowering dynamics and on grain numbers in ears [67] and has a major adverse effect on grain development [68].

4.4. Effect of maize plant parts and plant density on biogas hectare yield

The total biogas hectare yield and methane hectare yield varied from 8450 to 15 055 m^3 ha^{-1} and from 5457 to 9599 m^3 ha^{-1}, respectively, in the two evaluated years (Tables 3 and 4). These levels of biogas hectare yield are comparable to values reported in other studies [12,14,38,48]. With regard to the greater effect of biogas hectare yield in comparison with the relatively minor effect of methane content, the statistical evaluation of methane hectare yield is in accordance with biogas hectare yield. Our results are consistent with Herrmann and Rath [10] in showing that biogas hectare yield is mainly influenced by the dry matter yield of the maize crop. Nevertheless, the ear/stover ratio should also be considered as a very important factor for biogas and methane production because it represents not only a quantitative, but also, and especially, a qualitative aspect of maize biomass. The results showed that ears were significantly higher in many qualitative characteristics (volatile solids degradation, specific biogas yield and methane content in biogas) in comparison with stover. This was shown in the greater biogas hectare yield of ears compared with that of stover in both years. In the case of the ear/stover ratio above 60% in 2014, the biogas hectare yield of ears was 1.8 times higher (i.e. by 4097 m^3 ha^{-1}) than biogas hectare yield of stover. In 2015, when the ear/stover ratio was about 50%, the biogas hectare yield of ears was 1.3 times higher (i.e. by 1096 m^3 ha^{-1}). A positive effect of ears on biogas production has also been recorded by Seppälä et al. [11]. The authors reported an increase in ear/stover ratio from 32 to 46% which led to an increase in methane hectare yield of ears from 35 to 51%.

The 10% higher total biogas hectare yield was associated with a 6% higher dry matter yield for the treatment with 130 000 plants ha^{-1}, compared with 90 000 plants ha^{-1} in 2014. Testa et al. [66] recorded a 19% higher methane hectare yield from maize grown at a density of 100 000 plants ha^{-1} in comparison with 75 000 plants ha^{-1}. Concurrently in our experiments, the increase in biogas hectare yield was supported by better volatile solids degradation in 2014. In the relatively dry year of 2015, plant density had no effect on volatile solids degradation.

Results of biogas production for the separated plant parts (ears, stover) showed that stover had a potential for biogas hectare yield of about 40% in comparison with the whole plant biomass including ears. Although this is a significantly lower value, it must also be recognized that the ear fraction of maize is of high value in animal nutrition and also as a human foodstuff, in contrast to specific energy crops. Different and complementary utilization of these two parts of maize plants can be considered in the context of improving the efficiency of farming practices, optimization of agricultural land use [40] and seeking to reduce competition between food and energy biomass [9].

5. Conclusions

An increase in maize plant density from a typical 90 000 plants ha^{-1} to 130 000 plants ha^{-1} had no beneficial effect on dry matter yield per hectare and there were no changes in the ear/stover ratio between the two tested plant densities. Some positive effects were detected in biogas production and quality because the higher plant density supported faster dynamics of specific biogas yield, higher volatile solids degradation, methane content in biogas and consequently biogas hectare yield of ears; however, no differences were detected for maize stover. The positive effect observed in 2014 could not be confirmed under conditions of relatively intensive drought stress in 2015. Ears provided significantly higher biogas hectare yield than stover due to a higher hectare yield and higher specific biogas yield of ears. Stover alone produced 40% of biogas hectare yield. The use of higher maize plant density in crops grown for biogas production could be recommended for environments with relatively humid growing conditions, where it can be beneficial not only for higher biomass yield potential, but also for improved specific biogas yield through greater degradability of the ear fraction.

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