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Evaluating Switchgrass (*Panicum virgatum* L.) as a Feedstock for Methane Production in Northern Europe

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Abstract: Interest in using warm-season grasses, including switchgrass (SG) (*Panicum virgatum* L.), as a bioenergy crop has increased in Europe. This study evaluated the effects of harvesting regimes with two cuts per year on the productivity, chemical composition and biochemical methane potential of the SG cultivars 'Dacotah', 'Foresburg' and 'Cave in Rock' in environments with cool and moderate climates in Europe with minimal fertilizer application. The results of two harvest years suggest that the biomass yield, chemical composition and energy potential depend on the grass cultivars and harvesting time. Significant effects ($p < 0.05$) of the harvest date and cultivar were observed for most of the measured parameters for biomass and silage quality. All three SG cultivars harvested on August 8 produced the lowest ($p < 0.05$) volume of methane per kg of biomass (181–202 normal litres (NL) per kg^{-1} volatile solids (VS)) compared to the biomass of the respective cultivar harvested on 14 July (287–308 NL kg^{-1} VS) or on October 3, as regrowth after the first cut made in mid-July (274–307 NL kg^{-1} VS). The stands of all three SG cultivars, when the first harvest was completed in mid-July, achieved a higher annual area-specific methane yield than those harvested first in August (1128–1900 Nm^3 ha^{-1} and 888–1332 Nm^3 ha^{-1}, respectively). Depending on the harvest regime and cultivar, the annual gross energy presented as a lower heating value varied from 31.8 GJ ha^{-1} to 68.0 GJ ha^{-1}. It is concluded that SG growing under the cool temperate climate of Northern Europe could be an interesting alternative crop for methane production. Our study proves that the cultivar choice also plays an important role.

Keywords: switchgrass; biomass yield; biogas production; harvesting regime; low-input farming

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1. Introduction

The development of a sustainable European bio-based economy critically depends on the establishment of a reliable supply of sustainably produced biomass. To this end, policy interventions and technical approaches should focus on the integration of region-specific energy hubs and the implementation of optimized organic waste valorization strategies. In particular, the effective exploitation of residual biomass and livestock waste could significantly enhance local biomass availability while reducing reliance on primary biomass sources [1]. According to de Wit and Faaij [2], the regions that stand out, with respect to their high potential and low costs, are large parts of Poland, the Baltic States, Romania, Bulgaria and Ukraine. A growing interest in the use of agricultural biomass for energy purposes has created demand for novel, high biomass-yielding, specific quality crops for sustainable use [3,4].

Low-input grassland biomass from marginal and other slightly more fertile sites can be used for energy production without competing with food or fodder production [5]. It is predicted that due to global warming, in the next 50 years, in many parts of the world, the main factors slowing plant growth will be higher air temperatures and more frequent droughts [6–8]. Therefore, in areas where these phenomena are most likely to be expected, xerotrophic warm-season C_4 grass species, which are better adapted to changing challenging climate conditions than C_3 species, should become strategic agricultural crops, mainly due to their high light-, water- and nitrogen-use efficiency [9,10]. The C_4 grass switchgrass (*Panicum virgatum* L.) has been identified by the United States Department of Energy as a main herbaceous energy crop because of its potential for high yields, low environmental impact and low input requirements [11]. Liu and Basso [12] found that the majority of Michigan's land could have a high aboveground net primary productivity of switchgrass and a low risk of failure with no more than 60 kg N ha^{-1} of fertilizer input. Switchgrass is a more efficient collector of solar radiation (171.0 GJ of solar energy per ha) and has lower energy input requirements during its production cycle than other agricultural crops—from 37.1 GJ ha^{-1} (rye) to 116.3 GJ ha^{-1} (grain maize) [13]. This efficiency results in a net energy gain per ha of 163.8 GJ ha^{-1}, which is approximately 60% higher than that of grain maize (98.3 GJ ha^{-1}). The species has good adaptive qualities in various climatic and edaphic conditions; the plants are resistant both to drought and waterlogging, as well as to various biotic stressors. Therefore, switchgrass could be cultivated without the use of chemical plant protection products and large amounts of nitrogen fertilizers [14–17]. Studies conducted in Lithuania have shown that switchgrass is well-suited to local climatic conditions, offering high biomass yields with low energy and labour inputs, making it a cost-effective and resource-efficient energy crop [18,19]. Moreover, recent research has indicated that switchgrass cultivation can enhance soil carbon sequestration, thereby contributing to climate change mitigation efforts [20,21]. This benefit is especially relevant in perennial systems, where reduced soil disturbance and robust root systems promote long-term carbon storage in soils.

Switchgrass management in Europe as a bioenergy crop is relatively a new subject matter. Results from various studies have suggested that switchgrass is broadly adaptable to many European countries [22,23]. Currently, the species is being introduced and intensely explored in countries with a Mediterranean climate in the south and the oceanic climate of Western Europe [23]. The switchgrass genotypes originating from North Dakota appear to be adaptable to the European environment, with its cool and moderate climate and short growing season [24]. With reference to calculations based on the 2004 economic situation in Europe, Smeets et al. [22] stated that in the countries of Central Europe, including Lithuania, the costs per tonne of switchgrass dry biomass, counting storage and transportation of about a 100 km radius, are low and equal to EUR 43–64 or EUR 2.4–3.6 per GJ of higher heating value (HHV), and by 2030 the predicted cultivation costs should remain attractive [22].

Biogas production through the anaerobic digestion of feedstocks provides an excellent way to convert the chemical energy accumulated in the biomass of lignocellulosic crops into renewable energy [25,26]. According to Budzianowski [27], in the climates relevant to central EU countries, biogas can be suitably produced from grasses. A Canadian research group reported specific methane yields from switchgrass silage ranging from

0.191 to 0.309 NL gVS^{-1} [28]. The specific methane yields from anaerobically digested switchgrass [28] were the same as from temperate grasses [29], and decreased with advancing stages of crop development; however, the methane concentration in the biogas produced from young biomass was lower at the beginning of the batch assay [29]. Other authors [30,31] have concluded that the specific methane yield is more affected by harvest management than by the genotype. Nevertheless, Oleszek et al. [32] reported substantial differences between varieties of reed canary grass in terms of both their chemical properties and methane yield. Based on a fodder analysis of 41 different energy crops, Dandikas et al. [33] revealed that the specific methane yield was significantly negatively correlated with the acid detergent lignin (ADL). In addition to lignin, the contents of the fibre fractions, namely acid detergent fibre [34] and neutral detergent fibre [35], were moderately negatively correlated with the methane yields from biomass.

Although switchgrass is recognized as a promising energy crop, comprehensive data on its true potential for biogas production are still lacking not only in Northern Europe, including Lithuania, but across the continent. No detailed economic analysis specific to Lithuania is currently available; we acknowledge this as a limitation. Future research should aim to evaluate the costs of harvesting, processing and biogas production to more accurately assess the economic feasibility of utilizing switchgrass regrowth under Northern European conditions.

Life cycle assessment (LCA) studies are crucial for comprehensively evaluating the carbon footprint impact, accounting for emissions and savings across an entire production chain—from cultivation to anaerobic digestion [36]. Biogas produced from switchgrass is generally recognized for its potential to reduce greenhouse gas (GHG) emissions compared to fossil fuels. The carbon stored in soils by perennial grasses, such as switchgrass, can have a net-negative climate effect due to their ability to store carbon [37].

According to Carlsson et al. [38], the GHG emissions from biogas obtained from fertilized biomass were, on average, twice as high as those from unfertilized treatments, as a result of the additional emissions from the mineral fertilizer production and distribution, as well as the nitrogen losses, notably as nitrous oxide, contributing to high climate impacts. Therefore, this study set out to evaluate the potential of biogas production from the biomass of three switchgrass cultivars grown in a low-input farming system in Northen Europe, and to determine the effect of the harvesting regime on the methane yield.

2. Materials and Methods

2.1. Field Experiment Conditions and Switchgrass Management

2.1.1. Field and Weather Characteristics

The field experiments were conducted in the central lowland of Lithuania (55°23′49″ N; 23°51′40″ E), at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, during 2014–2016. The soil of the experimental site was *Endocalcari-Epihypogleyic Cambisol*, a neutral light loam (FAO-UNESCO 1997). Prior to seeding, soil samples from the arable layer (0–30 cm) were analyzed for their chemical and granulometric compositions. The chemical properties were determined using air-dried and sieved ($\leq$2 mm) soil samples. The soil granulometric composition was conducted following the standard method ISO 11277:2020 [39]. The samples were obtained before the beginning of the experiment by forming a composite sample from 8 locations in the field, with 2 replications. The soil pH_{KCl} was determined potentiometrically, the organic carbon (C) content was determined using dichromate oxidation at 160 °C, the contents of available phosphorus (P_2O_5) and potassium (K_2O) were measured using the A-L method and the available sulphur (S) was found by the turbidimetric method using an extracting solution of 1 M potassium (KCl). The soil mineral nitrogen (N) was determined colourimetrically as follows: for N nitrate

(N-NO_3), we used hydrazine sulfate and sulfanilamide, and for N ammonium (N-NH_4), we used sodium phenolate and sodium hypochlorite. The soil was composed of sand (51.1%, 2000-63 µm), silt (27.5%, 63-2 µm) and clay (21.4%, <2 µm). The nutrient contents of the 0–30 cm soil layers in the initial stage of the experiment and at the end of the investigations are presented in Table 1.

Table 1. The chemical properties of the soil arable layer in the experimental plots.

Year	**Soil Property**						
	$C_{org.}$	**N-NO_3**	**N-NH_4**	**K_2O**	**P_2O_5**	**S**	**pH_{KCl}**
	g kg^{-1}	**mg kg^{-1}**					
Before establishment of experiment in 2014	21.3	7.60	1.80	158	196	2.5	6.8
After investigation in 2016	23.1	4.29	1.59	162	154	1.7	6.9

The pre-planting fertilization comprised 15 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1} and 70 kg K_2O ha^{-1}. During the following years after harvesting, additional fertilization was not applied.

According to the environmental stratification of Europe, Lithuania is assigned to the Nemoral zone, with a cool temperate climate and quite a short growing season of 190–195 days [40]. The annual mean precipitation at the experimental site is 550–600 mm and the mean annual temperature is 6.0–6.5 °C. The average temperature in January, the coldest month in Lithuania, is −2.9 °C, and in the warmest month, July, it is 19.7 °C. According to temperature amplitudes, the climatic conditions of Central Lithuania are suitable for plants with a winter hardiness zone 5b.

The daily weather parameters were recorded at an agro-meteorological station situated within 0.2 km of the experimental location. However, during the 2014–2016 experimental period, the weather conditions markedly differed between years. The first season of 2014–2015 was characterized by a late winter with light snow cover; a very early, warm spring; and a changeable summer with heat waves. The winter of 2015 was short, without a permanent snow cover. The weather was uncharacteristically warm and wet. The spring was very early and contrasting—warm days alternated with cold ones. The moisture content for the perennial grasses was critical almost all summer. The precipitation during the 2015 growing season was 55 mm below historic averages for the research site. Moreover, the hydrothermal coefficient (HTK = 0.1) indicated a very severe drought in August. The season of 2016 was characterized by a warm winter and warm, windy and wet spring. The summer season was unusually rainy and changeable in terms of the temperature. The autumn was warm and dry.

2.1.2. Switchgrass Cultivars and Management

The experiment was based on a two-factorial design, consisting of two harvest regimes and three cultivars ('Dacotah', 'Foresburg' and 'Cave in Rock') (Table 2), with three replications. The experimental plots were 11.25 m^2 in size. The preceding crop in the field was wheat. The criteria used for the selection of the cultivars were based on their geographical origin and potential for adaptation to Northern European conditions.

Table 2. Harvest dates and growth stages of switchgrass cultivars.

Harvest Regime	Cut	Harvest Date	Growth Stage		
			'Dacotah'	**'Foresburg'**	**'Cave in Rock'**
I	First	14 July	Heading	Heading	Booting
	Second	3 October		Pre-heading	
II	First	8 August	Flowering	Flowering	Heading
	Second	3 October		Pre-heading	

The plants were not harvested during the establishment year (2014) to enable them to become fully established. In both harvest years (2015 and 2016), all three cultivars were harvested twice per season, on the same dates. The first cut occurred in mid-summer, while the second cut was performed in October, aiming to assess the biomass yield and quality for anaerobic digestion and to ensure good regrowth the following spring (Table 2). The choice of the harvest dates was based on the experiences of other researchers [28,41] and our previous study [42], and took the local climatic conditions into account. Depending on the cultivar, the period from the resumption of vegetative growth to heading (BBCH 52-55) took 68–99 days in 2015 and 51–88 days in 2016, and the duration from the beginning of spring regrowth to flowering (BBCH 61-65) lasted 71–114 days in 2015 and 88–124 days in 2016.

2.2. Sample Preparation and Chemical Analyses

2.2.1. Plant Material and Silage Preparation

The plots were harvested using a self-propelled hay mower at approximately 5 cm above ground level, and the biomass was weighed directly in the mower's bunker. Four replicate samples of 0.5 kg fresh biomass were collected, dried at 105 °C in a well-ventilated oven to a constant weight and used to determine the dry matter yield (DMY). These replicate samples were then pooled for the subsequent chemical analyses and anaerobic digestion procedures. For the chemical analyses, the harvested samples were chopped into 3–5 cm particles, oven-dried at 65 ± 5 °C for 24 h and then ground in a cyclonic mill with a 1 mm screen. The ground samples were stored in plastic vials at room temperature. Before analysis, a small portion (2–3 g) of each sample was dried to a constant mass in a forced-air oven at 105 ± 5 °C so that the data could be expressed per unit dry matter (DM).

After cutting, the chopped switchgrass samples were ensiled for anaerobic digestion tests. The silage was prepared in 3 L glass jars and sealed for at least 120 days. No preservatives for ensiling were used.

2.2.2. Chemical Analyses of Biomass and Silage

Switchgrass plant and silage samples were analyzed according to standard methods as follows: For ash content, dried samples were incinerated at 550 °C. Total carbon (C) and nitrogen (N) contents of switchgrass samples were determined simultaneously by dry combustion using Vario EL III CNS-autoanalyser (Elementar, Langenselbold, Germany). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and ADL were determined using cell wall detergent fractionation method according to Van Soest [43]. NDF and ADF extraction were performed on ANKOM220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) using F57 filter bags (25 µm porosity). Lignin was identified with ADL and it was performed in beakers on remaining material from ADF procedure as insoluble residue in sulfuric acid (72% w/w). Cellulose (Cel) was determined as difference between ADF and ADL, and hemicellulose (HCel) as difference between ADF and NDF. Concentrations of soluble carbohydrates (SCs) in 40% ethanolic extracts of dried samples were measured spectrophotometrically (M107, Camspec, Leeds, UK) using anthrone reagent [44]. Starch was determined in plant material residue after SCs extraction. Remaining plant material was solubilized and hydrolyzed to glucose using enzymes α-amylase and amyloglucosidase, and released glucose was assayed following general procedures described by Zhao et al. [44]. Before analysis, small portion (2–3 g) of each sample was dried to constant mass in forced-air oven at 105 ± 5 °C so that data could be expressed per unit DM or total solids (TS).

Wet samples of ensiled switchgrass were analyzed for TS in forced-air oven at 105 ± 5 °C; pH was determined by pH metre and volatile solids content (VS) was measured as mass loss after dried samples were completely incinerated at 550 °C in muffle furnace.

2.3. Anaerobic Digestion Experiments

The specific methane (CH_4) yield (SMY) of the switchgrass was assessed in batch assays using an automatic methane potential test system (AMPTS, Bioprocess Control AB, Lund, Sweden). Prepared biomass silage samples of 200 g were put into the inoculum (digester volume of 20 L) for acclimation. Anaerobically digested material from a piggery farm biogas plant, with the addition of maize silage, was used as the inoculum. After the acclimation period (50–60 days), a biochemical methane potential (BMP) assay was started. The BMP experiments were performed as previously described by [45–47]. The BMP tests were conducted in triplicate under mesophilic conditions (38 °C ± 1 °C), and the inoculum-to-substrate ratio (based on the VS amount) was set at 2:1 to avoid any inhibition. The total solids and volatile solids of the inoculum were measured every time before test set-up. The volatile solids were adjusted to 40 g kg^{-1} of the inoculum by adding distilled water. An active volume of 400 mL in 500 mL bottles was used in all the tests. The flasks and tubing were purged with pure CH_4 to ensure anaerobic conditions. In the experiments on anaerobic digestion, a control test was included with a sole methanogenic inoculum (without the addition of the switchgrass silage biomass), at the same dilution rate as for the plant samples, to measure its intrinsic CH_4 production. The CH_4 produced by the inoculum was subtracted from the results obtained from the test samples. The substrate was mixed by a stirrer driven by an electric gear with a mixing period of 30 s at a frequency of 1 s^{-1} with 3 min pauses. The volume of CH_4 was calculated to standard temperature and pressure conditions (0 °C and atmospheric pressure of 1 bar). The cumulative SMY was calculated as the sum of the CH_4 produced during the incubation period and expressed as normal litres (NL) per kilogram on the wet mass (WM), TS and VS bases (CH_4Y_{WM}, CH_4Y_{TS} and CH_4Y_{VS}, respectively) of the switchgrass silage added to the test. The BMP tests were stopped according to the specific 1% rule over three consecutive days as outlined in VDI 4630: 2016-11 [48].

Yield of higher heating value (HHVY) and lower heating value (LHVY) per area of switchgrass stand (MJ ha^{-1}) were computed as follows: $HHVY = DMY \times CH_4Y_{TS} \times 39.8$ and $LHVY = DMY \times CH_4Y_{TS} \times 35.8$, where 39.8 is HHV and 35.8 is LHV (MJ) for 1 Nm^{-3} of methane. These values are used by National Renewable Energy Laboratory [49].

2.4. Statistical Analysis

The normality of the data distribution was assessed using the Shapiro–Wilk test, and the data met the assumptions for a parametric analysis. The means were compared by a *t*-test and F-test. Differences were considered to be significant at the 95% level. Only statistically significant effects ($p < 0.05$) are reported in the results. A correlation (Pearson's correlation) and regression analysis of the data sets were completed. An analysis of variance was performed to estimate the differences in the tested parameters among the treatments (year and genotype). Fisher's Protected LSD was used to compare the means at $p = 0.05$ and

$p = 0.01$. Analyses of variances were conducted using the statistical packages SAS Enterprise Guide 7.1 software (SAS Institute Inc., Cary, NC, USA) and MS Excel Analysis ToolPak.

3. Results and Discussion

3.1. Chemical Compositions of Harvested and Ensiled Biomasses

The chemical compositions of the harvested switchgrass biomasses are shown in Table 3. Significant effects ($p < 0.05$) of the harvest date and cultivar were observed for most of the measured biomass quality parameters. The harvest date effected the concentrations of the cell wall-related components (NDF, ADF and ADL); the ash, N, C and C to N ratio (C/N) tended to be more consistent than the cultivar-dependent effect. For the later-harvested (BBCH 61-65, 8 August) biomasses of the three cultivars, the NDF, ADF, ADL and C concentrations and C/N were higher, while the protein and ash concentrations were lower in comparison with the chemical composition of the biomass of the respective cultivars harvested in mid-July and with the switchgrass regrowth harvested in early October. At flowering, the NDF ranged between 734 and 745 g kg^{-1} DM, the ADL measured up to 83.3 g kg^{-1} DM and the ash and N contents declined to 45.7 and 7.18 g kg^{-1} DM, respectively. Due to the higher C and lower N concentrations, the biomass of the flowering plants had higher C/N values (62.4−67.9) than the biomasses of the other two harvests. Although the biomass of the switchgrass regrowth was chemically more similar to the biomass of the heading stage than to that of the flowering, significant ($p < 0.05$) differences were also found between the early first cut and the regrowth: the biomass cut in October had the lowest NDF, starch and C concentrations, but had the highest ash. The concentration of Cel as well as the ADL to Cel and ADL to HCel ratios also differed between the samples of the different cuts, with the later-harvested samples being higher in these biomass quality parameters than the samples cut in mid-July and in early October.

Table 3. Mean values of biomass chemical composition of three switchgrass cultivars at three harvest dates.

Cultivar	Harvest Date	NDF	ADF	ADL	WSC	Starch	Ash	N	C	Cel	HCel	C/N	ADL/Cel	ADL/HCel
		g kg^{-1} DM												
Dacotah	14 July	697 b; x	418 b; x	62.4 b; x	65.9 b; y	91.7 a; x	52.3 b; x	10.30 a; x	481 b; x	356	279	46.7 b; x	0.175	0.223
	8 August	736 a; y	440 a; y	74.3 a; y	95.0 a; x	99.5 a; x	49.3 c; x	7.79 b; x	486 a; y	366	296	62.4 a; y	0.203	0.251
	3 October	666 c; x	405 b; x	58.6 c; x	70.1 b; z	60.6 b; x	66.9 a; x	11.10 a; x	480 b; x	346	261	43.4 b; y	0.169	0.224
Forestburg	14 July	694 b; x	400 b; y	51.4 b; y	61.5 c; y	80.5 a; y	51.9 b; x	10.30 a; x	481 b; x	349	294	46.7 c; x	0.147	0.175
	8 August	745 a; x	457 a; x	79.7 a; x	72.5 b; z	80.7 a; y	49.0 c; x	7.18 c; y	488 a; x	377	288	67.9 a; x	0.211	0.277
	3 October	669 c; x	402 b; x	53.3 b; y	86.5 a; y	58.8 b; x	67.0 a; x	9.60 b; y	478 c; y	349	267	49.8 b; x	0.153	0.200
Cave in Rock	14 July	679 b; y	384 b; z	50.6 c; y	79.7 c; x	90.8 a; x	49.1 b; y	10.20 b; x	482 b; x	334	294	47.2 b; x	0.152	0.172
	8 August	734 a; y	454 a; x	83.3 a; x	89.6 b; y	82.7 b; y	45.7 c; y	7.76 c; x	488 a; x	371	280	62.9 a; y	0.225	0.298
	3 October	654 c; y	385 b; y	57.8 b; x	98.4 a; x	55.4 c; x	61.2 a; y	11.40 a; x	480 b; x	328	269	42.1 c; y	0.176	0.215

	Silage characteristics		
	pH	TS, % WM	VS, % TS
Dacotah	4.2 a; x	32.8 a; x	94.3 a; x
	4.4 a; x	42.4 b; y	94.9 a; x
	4.4 a; x	37.2 b; y	92.6 b; y
Forestburg	4.2 a; x	31.9 a; x	94.1 a; x
	5.1 b; y	37.4 b; z	94.9 a; x
	4.2 a; x	34.6 a; z	92.4 b; y
Cave in Rock	4.0 a; x	32.4 a; x	94.7 a; x
	4.6 a; x	34.5 a; z	94.8 a; x
	4.2 a; x	30.8 a; z	92.3 b; y

The different letters a, b and c in the columns indicate significant differences ($p < 0.05$) in the concentrations of the respective biomass components within the switchgrass cultivars for the harvest date, and the different letters x, y and z indicate significant differences ($p < 0.05$) in the component concentrations among the switchgrass cultivars for the same harvest date.

Among the cultivars, the early-maturing switchgrass 'Dacotah' had the highest NDF, ADF and ADL contents, and the late-maturing 'Cave in Rock' tended to be the lowest in these biomass components. However, such tendencies were evident for the biomass cut in mid-July and partly apparent for the biomass of the regrowth. Conversely, for the biomass of the late first cut (8 August), 'Dacotah' exhibited the least alteration in the concentrations of these components, with a harvest delay from 14 July to 8 August. Among the three cultivars, 'Cave in Rock' had the lowest ($p < 0.05$) ash concentrations in the biomasses of all harvest dates. As regards the concentrations of the other components in the biomasses cut at identical times, the variation among the cultivars was inconsistent, though some significant differences were established.

The silages of the different switchgrass samples also revealed diverse characteristics (Table 3). The average TS content ranged from 30.8% to 42.4%, the pH varied from 4.0 to 5.1 and the VS alternated from 92.3% to 94.9%. The ensiled biomass of the late primary growth exhibited generally higher pH, TS and VS values than the biomass of the other two cuts.

Generally, this study's results addressing the chemical composition of switchgrass biomass agree with the values presented in preceding reports [50–53]. Our study shows an alternating trend in switchgrass quality with a harvest delay, which is consistent with the results of previous studies carried out in the USA and Canada [50,51,54–58]. Similarly as our findings, in their experiments previous researchers observed increases in fibre and its components, and decreases in the nitrogen and ash concentrations of switchgrass with the harvesting date. Aurangzaib et al. [54] and Heaton et al. [59] also found that the C/N increased continuously with advancing maturity. The differences in the chemical composition between harvests might be explained by a decrease in the leaf-to-stem ratios with advancing maturity [60]. Stems have greater fibre and lignin concentrations than leaves [61], resulting in greater NDF, ADF and ADL values for the harvest of 8 August. The higher nitrogen and ash concentrations in the biomass of the leafier heading plants and regrowth than in the flowering plants might be because of the same reason, as leaf components contain more nitrogen and ash than stems [52]. Our results for the NDF and ADF of the switchgrass harvested at the plant heading and flowering stages, as well as at regrowth, are of comparable values to those reported by Richner et al. [51] for switchgrass grown in Missouri State, USA, and harvested at similar stages. In our study, the concentrations of the fibre fractions, both NDF and ADF, of the 'Cave in Rock' biomass from the harvest of 14 July (679 and 384 g kg^{-1} DM, respectively) were very close to those obtained for the biomass of the same switchgrass cultivar cut in late July in eastern Canada (680 and 383 g kg^{-1} DM) [50]. In the biomass of the regrowth, we found more fibre than the Canadian researchers, but our results are in line with the NDF and ADF values reported by Liu et al. [53] from Virginia, USA. However, at the early seed head stage, McIntosh et al. [57] found more NDF and ADF than we determined. On the subject of the ADL content in the biomass, Liu et al. [53] observed less lignified biomass in both harvests in July and the regrowth than we determined. Our ADL values agree with the ones obtained by Richner et al. [51] for the biomass of pre-anthesis harvest. However, concerning the biomass of the regrowth, the ADL varied in different ranges: 40–100 g kg^{-1} DM [51], 38.9–47.0 g kg^{-1} DM [53] and 53.3–58.6 g kg^{-1} DM (the current study). In our research, we found that 'Cave in Rock' had a low ADL at the first harvest date, but was higher or similar to that of Dacotah and Forestburg at the other two harvest dates. According to Casler and Boe [55], most of the variation in the ADL among cultivars can be explained by the cultivar main effect and by differences in the rate of ADL accumulation with later harvest dates. Presumably, 'Cave in Rock' had a higher rate of ADL accumulation with a harvest delay than the other two cultivars. Basically, this is consistent with the observations of Casler and Boe [55] and Aurangzaib et al. [54].

Similarly as in the study of Casler and Boe [55], we also observed that the biomass of 'Cave in Rock' contained less ash than the biomasses of 'Dacotah' and 'Forestburg'. The ash concentration in the biomass of cv. 'Cave in Rock' harvested in mid-July was in line with that determined by [53,62]. As for the N (CP) concentration in switchgrass, the data found in the literature are variable. Sadeghpour et al. [62] determined 5–7.2 g N kg^{-1} DM in biomass harvested in mid-July, McIntosh et al. [57] found 86.8 g CP kg^{-1} DM or 13.9 g N kg^{-1} DM at the early seed head stage and Richner et al. [51] observed 80–100 g N kg^{-1} DM at the boot stage and 80–90 g N kg^{-1} DM at regrowth. The data on the WSCs, starch and carbon concentrations and the carbon-to-nitrogen ratio (C/N) in switchgrass biomass cut in summertime or regrowth are very limited. For the switchgrass 'Cave in Rock', Bélanger et al. [50] reported an increase in the soluble carbohydrates concentration from late July to early September. In our study, we also observed a similar trend from mid-July to the first ten days of August for all three cultivars. We found considerably more starch in the biomasses of all the harvests and all cultivars than Bélanger et al. [50] obtained. Aurangzaib et al. [54] noticed that the changes in the amount of the total non-structural carbohydrates (TNCs) were inconsistent among the varieties throughout the growing season. Contrary, for the WSCs in our study, the authors did not establish significant differences among the varieties for their TNCs concentrations. Sugars are one of the first products of photosynthesis; therefore, their concentration is more dependent on environmental factors than the concentrations of other plant-quality components [63]. Averaged over three locations and two years, the mean C/N of *P. virgatum* was approximately 80 in August [59], i.e., the C/N was higher than what we observed in switchgrass at this time (62.4–67.9). According to the findings of Aurangzaib et al. [54], the C/N in the biomass of five switchgrass cultivars varied within a range of 35–50 on 10 July (190th day of the year) and steadily increased up to 40–70 at the end of July (210th day of the year).

Generally, our findings on the chemical composition are in accordance with the respective literature data. Some perceptible discrepancies among the data from various investigations of biomass composition might be associated with differences in the edapho-climatic conditions, fertilizer applications, cultivars involved and other reasons.

3.2. Cumulative and Area-Specific Methane Yield

The average cumulative and daily methane production of the switchgrass samples as a function of the days of anaerobic digestion over 21 days of incubation at mesophilic temperature are presented in Figures 1 and 2, respectively. A remarkable difference in the methane production curves is observed for the switchgrass biomasses when the plant harvesting dates are compared (Figure 1). During the entire period of observation, the cumulative volumes of methane produced from the switchgrass cut in mid-July and on 3 October were similar but larger than those of the batches of switchgrass sampled on 8 August. The figure also shows that methane formation generally stopped and the VDI criteria were reached after 16 days from the start of the anaerobic digestion of all the samples.

The methane production from the switchgrass samples increased sharply after incubation. The daily methane production reached a peak on the first day for all the samples, followed by a sharp decline during the next 2–4 days and, thereafter, only small volumes of methane were released (Figure 2). The peaks on the first day of anaerobic digestion differed in their values, and the harvesting time showed more obvious differences than the cultivars of the respective harvests. The initial degradation rate was the highest for the samples of the younger plants from the first cut and regrowth. The maximum daily methane production was 71.5–81.8 NL kg^{-1} VS d^{-1} for the switchgrass cut in mid-July, 80.0–89.6 NL kg^{-1} VS d^{-1} for the regrowth biomasses and the switchgrass biomasses sampled on 8 August featured the lowest peaks of 37.3–42.0 NL kg^{-1} VS d^{-1}. This constitutes 24.7–30.4% of the total accumulated SMY (CH_4Y_{VS}) for the biomasses from the early first cut and regrowth, and 18.7–20.8% CH_4Y_{VS} for the biomass cut on 8 August (Table 4). The high initial methane production on the first day was probably due to the easily biodegradable organic compounds of the ensiled biomass: the residual water-soluble carbohydrates and products of biomass fermentation, like volatile acids. For all the batches investigated, it was found that approximately 50% of the CH_4Y_{VS} accumulated during the 16 days of the assay was gained after only 3 days of anaerobic digestion.

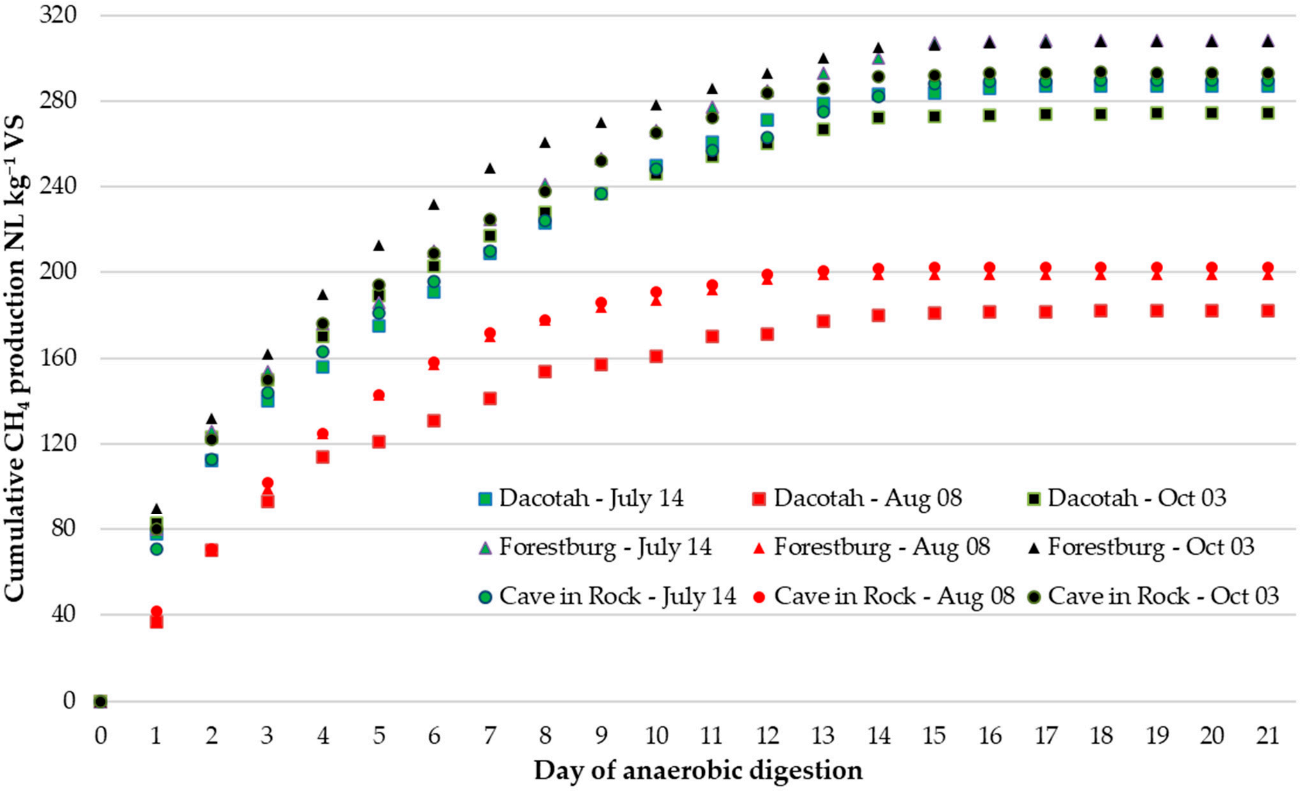


Figure 1. Cumulative methane production from anaerobically digested wet biomass of three switchgrass cultivars harvested on different dates (1st day—start of AD experiment).

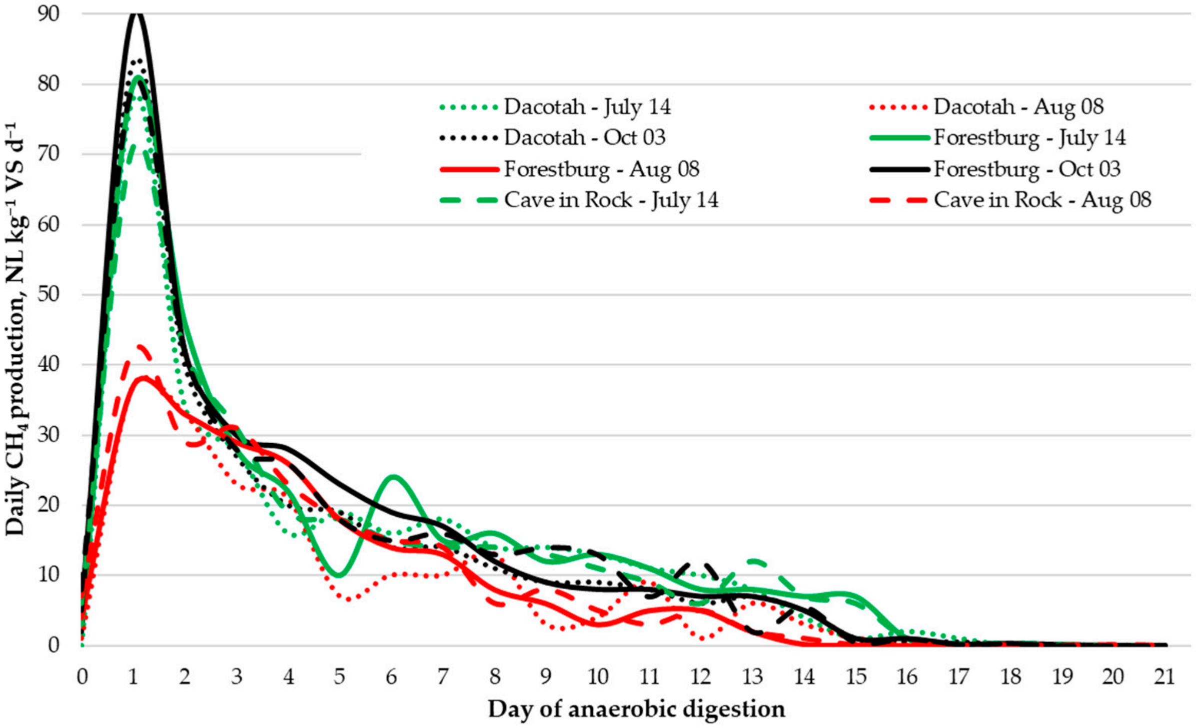


Figure 2. Daily methane production from anaerobically digested wet biomass of three switchgrass cultivars harvested on different dates (1st day—start of AD experiment).

Table 4. The percentage of methane produced during the first four days of the BMP experiment.

Day of BMP Assay	**CH_4, % CH_4Y_{VS}**								
	Dacotah			**Forestburg**			**Cave in Rock**		
	14 July	**8 August**	**3 October**	**14 July**	**8 August**	**3 October**	**14 July**	**8 August**	**3 October**
2nd	27.1	20.8	30.4	26.1	18.7	29.2	24.7	20.8	27.3
3rd	11.9	16.6	14.5	14.7	16.3	14.0	14.4	14.6	14.2
2nd + 3rd	39.0	37.4	44.9	40.8	35.0	43.1	39.1	35.4	41.5
4th	9.77	14.1	10.0	9.11	14.7	9.57	10.8	15.2	10.6
2nd + 3rd + 4th	48.8	51.4	54.8	49.9	49.8	52.7	49.8	50.6	52.2

Ahn et al. [64] also showed rapid biogas production from switchgrass–manure substrates for the first 2 days, followed by a rapid decrease in biogas production between days 2 and 4. Similarly, Dandikas et al. [65] noticed that approximately 80% or more of the total biogas production of energy crops is usually recorded during the first half of an experiment. Luna-delRisco et al. [66] revealed that the methane production from different feedstocks actively started after incubation and for grass silage, the time to reach 80% of the ultimate methane yield was 15 days. Meanwhile, in the experiment carried out by Barbanti et al. [67], switchgrass featured a low peak (7.0 mL CH_4 g^{-1} VS d^{-1}) after ten days of incubation. The CH_4 production assessment was conducted on oven-dried (60 °C) biomass samples harvested on October 5 at the initial senescence of the plants, when their cell walls were highly lignified and their biomass was characterized by a great carbon-to-nitrogen ratio, unfavourable for anaerobic digestion. Ragaglini et al. [68] clearly showed that methane production from frozen giant reed reached its peak during the first days, in accordance with the stage of development of the crop. During the early stage and regrowth, the maximum methane rate was 37–44.4 NL kg VS^{-1} day^{-1}, while during the mid-season stages it ranged between 28.5 and 37.8 NL kg VS^{-1} day^{-1} and at crop maturity it was less than 20 NL CH_4 kg VS^{-1} day^{-1}.

The specific methane yield was primarily related to the harvest timing of the cut: all three switchgrass cultivars harvested on 8 August produced the lowest ($p < 0.05$) volume of methane per kg of biomass (171–191 NL kg^{-1} TS and 181–202 NL kg^{-1} VS) compared with the biomass of the respective cultivar harvested on 14 July or 3 October (254–290 NL kg^{-1} TS and 275–308 NL kg^{-1} VS) (Figure 3). For each corresponding cultivar, statistically significant differences between the specific methane yields produced from the biomass of the first cut on 14 July and that from the regrowth were not found. As regards the cultivar's effect on methane production, the biomass of Forestburg cut on 14 July and its regrowth showed a higher potential ($p < 0.05$) than the other two cultivars. Over 16 days of incubation, the biomasses of Forestburg and Cave in Rock cut on 8 August produced similar CH_4 volumes per kg, which were significantly higher ($p < 0.05$) than that produced from the biomass of the early-maturing Dacotah harvested on the same date. The cultivars Dacotah, Forestburg and Cave in Rock, harvested on 3 October as regrowth after the first cut in mid-July, showed cultivar-dependent ($p < 0.05$) CH_4Y_{VS}, with a total of 275, 307 and 293 NL kg^{-1} VS, respectively.

Massé et al. [28] pointed out that switchgrass remains an interesting renewable alternative energy source under relatively the cool and humid climate of eastern Canada. The biomass of Cave in Rock produced 0.266–0.309 NL CH_4 g^{-1} VS in mid-summer and 0.269–0.276 NL CH_4 g^{-1} VS from the second cut on 01 Oct. These values are comparable to those obtained in our study for the first harvest in late July and the second harvest (regrowth) in October. However, the methane potential of the Cave in Rock biomass harvested on August was lower in our study (202 NL g^{-1} VS) than that obtained by Massé et al. [28] for the same cultivar harvested in late summer (235 NL CH_4 g^{-1} VS).

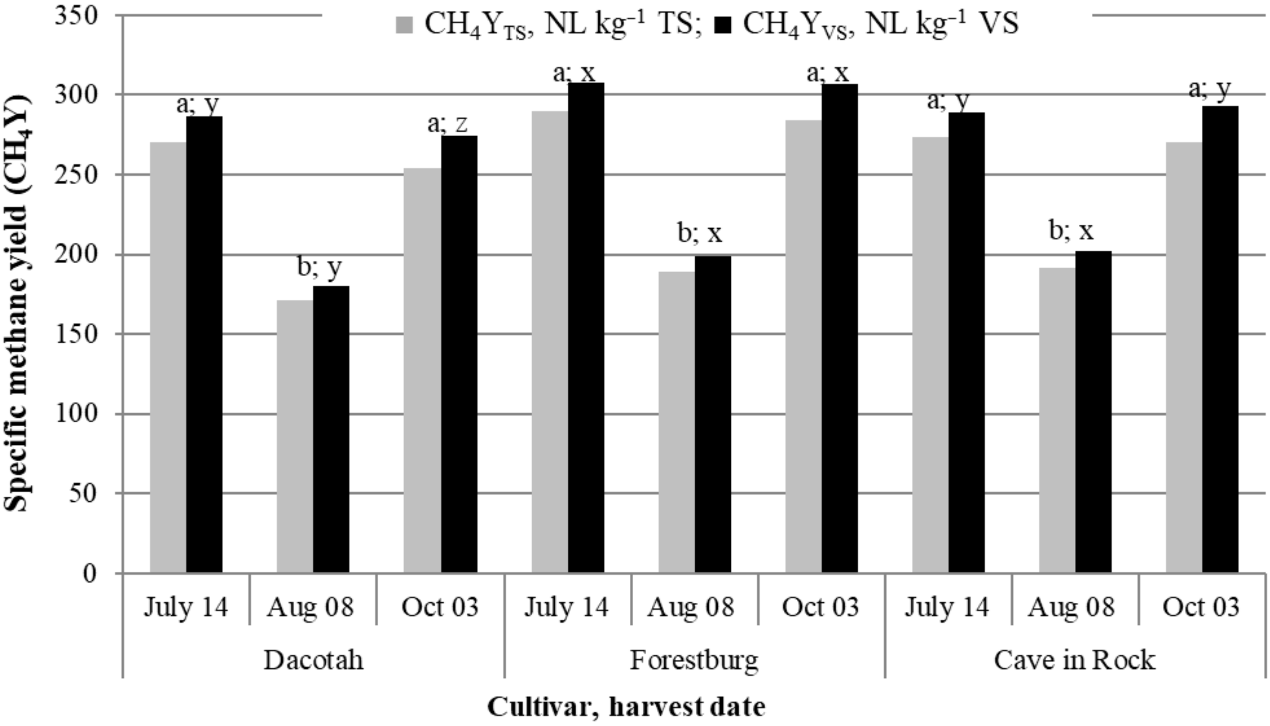


Figure 3. Specific methane yields for three switchgrass cultivars at the end of anaerobic digestion as influenced by different harvest dates. The different letters a and b on the columns indicate significant differences ($p < 0.05$) in the CH_4Y among harvest dates for the same switchgrass cultivar, and the different letters x, y and z indicate significant differences ($p < 0.05$) in the CH_4Y among switchgrass cultivars for the same harvest date.

But it should be noted that wide differences in the potential methane yield of switchgrass have been reported in the literature. The specific methane yield of switchgrass cultivated in other countries has been reported to vary: 127 to 309 NL g^{-1} VS, 127–198 NL g^{-1} VS ([69], USA), 112–298 NL g^{-1} VS ([70], Canada), 184–309 NL g^{-1} VS ([28], Canada), 216 NL g^{-1} VS ([67], Italy), 296 NL g^{-1} VS ([71], France) and 137.5–300.5 NL g^{-1} VS ([72], Italy). These differences might be explained by variations in the switchgrass maturity, cultivar, biomass pretreatment and application of mixtures with additional feedstock. Our results confirm previous observations that crop maturity is an important factor leading to a significant decrease in the specific methane yields of both C_3 and C_4 grasses [28,68,70,73,74]. Switchgrass, as a C_4 perennial grass adapted to more arid conditions, typically has a higher lignin content compared to many C_3 temperate grasses commonly cultivated in Northern Europe [75]. There, El-Mashad [69] reported that the specific methane yields of switchgrass harvested in the post-killing frost stage were only 127 and 198 mL g^{-1} VS at mesophilic and thermophilic temperatures, respectively. Similar methane yields (140–205 mL g^{-1} VS, from winter- and summer-harvested switchgrass biomass, respectively) were obtained by Frigon et al. [70]. The plant maturity effect could be explained by the fact that a lower methane yield during plant senescence is caused by a shortage of nitrogen, resulting in a higher C/N and an increase in the fibre content with higher cell wall lignification. The relationship between the fibre content of switchgrass biomass and the specific methane yield supports this point [50]. Massé et al. [28] noticed that the concentration of non-digestible matter increases with advancing plant maturity.

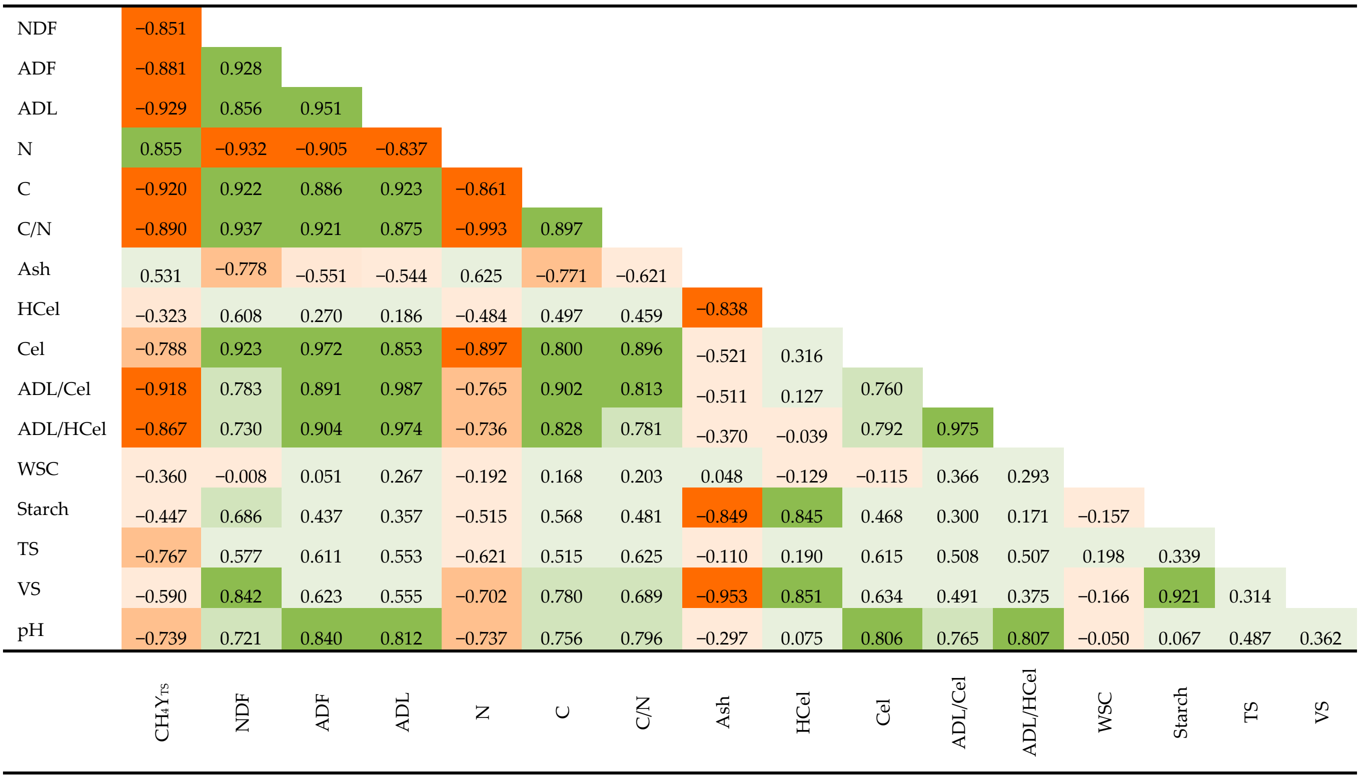
There is a lack of information concerning the cultivar effect on methane production; however, there are such studies that deal with other crops. Oleszek et al. [32] clearly showed that the methane fermentation results revealed a significantly higher ($p < 0.05$) biogas yield from the cultivated variety of canary grass than from the wild one. The BMP of 24 clones of

Arundo donax also varied in a wide range, from 147 mL g^{-1} VS to 243 mL g^{-1} VS [76]. A significant genotypic variation for specific methane yield was revealed in winter rye [31]. Significant differences among *Miscanthus sinensis* genotypes were found both for the quality traits relevant for specific bioenergy conversion routes and for the specific bioenergy yield, including methane [35]. However, according to Dickeduisberg et al. [30], the choice of the wheatgrass germplasm was less important than the cutting frequency and cutting height. As concerns switchgrass, we could not find any information on this subject. The results obtained in our study are logical and may be related to cultivar-dependent variations in chemical composition. The results from the study of Aurangzaib et al. [54] also clearly demonstrated the significance, at $p \leq 0.01$, of the variations between switchgrass ecotypes relating to energy-important quality traits.

Compared to other perennial grasses, *P. virgatum* biomass produced less methane per kg VS than the biomasses of the temperate species *Festuca arundinacea*, *Dactylis glomerata*, *Phalaris arundinacea*, *Phleum pratense* or ×*Festulolium* [77,78]. True, Massé et al. [50] received opposite data: the average specific methane yield from reed canary grass-seeded plots was less than that from switchgrass-seeded plots. On the other hand, there are different ways to improve the methane production potential of switchgrass biomass. Even switchgrass harvested during September, when the plants were mature, could reach 296 L CH_4 kg^{-1} VS, probably because in that experiment, green samples were compressed and stored under anaerobic conditions by N_2 flushing [71]. The storage of the green grass might have affected its biodegradability [69]. Different biomass pretreatments, evocative of the effects of improved accessibility of the cell wall components to microbial attacks, could enhance the value of switchgrass biomass as a feedstock for methane production. These could be physical, chemical or biological pretreatments of lignocellulosic biomass [70–72,79,80], which aim to alter the physical and chemical properties of lignocellulosic biomass to enhance the accessibility of cellulose and hemicellulose, reduce cellulose crystallinity and decrease the degree of polymerization. Despite being generally environmentally friendly and not involving harsh chemicals, physical methods can be energy intensive. For instance, a semi-industrial extrusion pretreatment of grass has shown promising results for improving the biomethane potential (e.g., 11–18% increase for fresh and ensiled grass), and can be economically feasible [81]. An alkaline pretreatment of switchgrass can significantly enhance methane production, with optimal conditions leading to substantial lignin removal while minimizing carbohydrate loss [82]. Biological methods typically require longer treatment times compared to physical or chemical methods [83]. This and other studies have shown a negative correlation between the ADL content and methane yield, highlighting the need for an effective pretreatment to disrupt this structure [75]. In our study, a pretreatment for the switchgrass was not applied prior to anaerobic digestion, as we were focused on evaluating the essential methane production potential of the biomass under standard conditions. Furthermore, to increase the yield of methane production from switchgrass, another feedstock, e.g., *Spirulina platensis* algae, could be used to adjust the carbon-to-nitrogen ratio of switchgrass [69]. A swine manure–switchgrass mixture's anaerobic digestion was also proved to have a high biogas production potential (0.337 L CH_4 g^{-1} VS) [64].

3.3. Association Between Biomass Composition and Specific Methane Yield

Figure 4 presents the matrix of the Pearson correlations, computed to investigate the relationships among the chemical components of the switchgrass biomass and the dependence of the SMY both on the TS and VS based on the chemical composition. The effects of the concentrations of fibres (NDF and ADF), ADL and C, as well as the C/N, on the SMY were significant ($p \leq 0.01$) and negative. The cellulose of the biomass and the pH and TS of the ensiled switchgrass also showed negative but weaker ($p \leq 0.05$) impacts on methane production. The structural carbohydrates of the cell walls had a weak negative impact on methane production, with a significance of $p \leq 0.05$ for cellulose only. However, the cell wall lignification level, i.e., the ratios of lignin to hemicellulose and cellulose, evidenced a negative and significant ($p \leq 0.01$) impact on the accumulated SMY. No or low correlations were found between the CH_4Y_{TS} and non-structural carbohydrates, the ash of plant biomass and the VS of the ensiled samples. The interrelationships among the chemical components in forage crops are well known. Generally, the correlations observed are in agreement with previous studies.



	CH_4Y_{TS}	NDF	ADF	ADL	N	C	C/N	Ash	HCel	Cel	ADL/Cel	ADL/HCel	WSC	Starch	TS	VS
NDF	−0.851															
ADF	−0.881	0.928														
ADL	−0.929	0.856	0.951													
N	0.855	−0.932	−0.905	−0.837												
C	−0.920	0.922	0.886	0.923	−0.861											
C/N	−0.890	0.937	0.921	0.875	−0.993	0.897										
Ash	0.531	−0.778	−0.551	−0.544	0.625	−0.771	−0.621									
HCel	−0.323	0.608	0.270	0.186	−0.484	0.497	0.459	−0.838								
Cel	−0.788	0.923	0.972	0.853	−0.897	0.800	0.896	−0.521	0.316							
ADL/Cel	−0.918	0.783	0.891	0.987	−0.765	0.902	0.813	−0.511	0.127	0.760						
ADL/HCel	−0.867	0.730	0.904	0.974	−0.736	0.828	0.781	−0.370	−0.039	0.792	0.975					
WSC	−0.360	−0.008	0.051	0.267	−0.192	0.168	0.203	0.048	−0.129	−0.115	0.366	0.293				
Starch	−0.447	0.686	0.437	0.357	−0.515	0.568	0.481	−0.849	0.845	0.468	0.300	0.171	−0.157			
TS	−0.767	0.577	0.611	0.553	−0.621	0.515	0.625	−0.110	0.190	0.615	0.508	0.507	0.198	0.339		
VS	−0.590	0.842	0.623	0.555	−0.702	0.780	0.689	−0.953	0.851	0.634	0.491	0.375	−0.166	0.921	0.314	
pH	−0.739	0.721	0.840	0.812	−0.737	0.756	0.796	−0.297	0.075	0.806	0.765	0.807	−0.050	0.067	0.487	0.362

Figure 4. Pearson correlations among specific methane yield (CH_4Y_{TS}) and chemical characteristics of switchgrass biomass and silage. According to statistical significance, reported correlations are colour-coded as follows: dark shades indicate significance at $p \leq 0.01$ (🟧—negative, 🟩—positive), lighter shades indicate significance at $p \leq 0.05$ (🟧—negative, 🟩—positive) and white or neutral colour indicates non-significant correlations (□—negative, □—positive). CH_4Y_{TS}, specific methane yield on a TS basis. Components of plant biomass: NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; N, nitrogen; C, carbon; C/N, C to N ratio; HCel, hemicellulose; CEL, cellulose; WSCs, water-soluble carbohydrates. Characteristics of ensiled switchgrass: TS, total solids; VS, volatile solids.

Thus, the correlation between the methane yields and the main chemical biomasses confirmed that the chemical composition of the fibre fractions of a biomass is essential to estimate the biogas potential [84]. The nitrogen concentration was the sole component which significantly positively impacted the SMY. This was similarly noticed by Herrmann et al. [34]. The significant negative correlations of the SMY with C and the C/N indicate that high values of these parameters in biomass used for biogas production are undesirable traits. Several investigators have clearly shown that the methane potential first increases and then decreases with increases in the C/N [85]. The highest methane potential was observed with a C/N of 25–30. For a set of different agro-industrial biomasses, Dinuccio et al. [84] obtained a negative and statistically significant correlation between the methane yield and ADL. Numerous researchers have investigated the anaerobic digestion of different feedstocks and confirmed the same point about the ADL as a strong negative predictor of methane yield (see [86] for energy crops and animal manures; [87] for reed canary grass leaves and stems, separately; [33] for different plant species; [34] for silages from 43 different crop species; and [35] for miscanthus). A moderate significance to a low negative impact of the concentrations of NDF, ADF and cellulose in a biomass on the SMY_{VS} has also been reported by several research groups [33–35,50,86,87]. These relationships are similar to those revealed in our study.

The strong relationship between the SMY and the ratio of ADL to cellulose established in the current study endorses the previous observation of [80], who stated that the biodegradability of one substrate can be evaluated by the ratio of lignin to cellulose. This is consistent with the fact that biomass pretreatments leading to a decrease in the lignin-to-cellulose ratio improve biodegradability and enhance biogas production [79,80]. In previous studies it has also been observed that the fibre lignification level, expressed as the ratio of ADL to NDF, is statistically significantly correlated with the SMY_{VS} [84]. It has been established that applying both lignin and cellulose increases the probability of the SMY_{VS} prediction compared to that when only lignin or cellulose is used as the independent variable [86,87].

Only weak correlations between the CH_4Y_{TS} and non-structural carbohydrates (both WSCs and starch) were obtained by Dandikas et al. [33]. In the authors' opinion, these parameters are not suitable variables for a linear regression because these carbohydrates exhibit the highest variation among all the parameters. Aurangzaib et al. [54] also noticed that the changes in the amount of total non-structural carbohydrates (TNCs) were inconsistent throughout the growing season. Their concentrations are more affected by environmental factors than the concentrations of other plant-quality components [63].

In general, the Pearson correlations determined in our work are in agreement with those of other researchers; however, most of them are stronger than were established in previous works. This could be due to the fact that most of the cited authors counted correlations for sample sets composed of various feedstocks.

3.4. Area-Specific Methane and Energy Yield

The annual methane yield per hectare displayed evident differences both among cultivars and between harvest regimes, being the highest for the biomass of Cave in Rock or Forestburg, depending on the cutting regime, and the lowest for Dacotah (Table 5). The stands of all three switchgrass cultivars, when the first harvest was completed in mid-July, achieved a higher annual area-specific methane yield than those first harvested in August (1128–1900 $Nm^3\ ha^{-1}$ and 888–1332 $Nm^3\ ha^{-1}$, respectively). Regarding the methane output from the biomass of the second cut, it was lower by 1.53–3.24 folds for the regrowth after an early first cut, and by 4.87–14.7 folds for the regrowth after the first cut was accomplished on 8 August. The primary data from the current study also showed that the regrowth after a later first cut (II regime) was negligible, and it is difficult to conceive the economic benefits of collecting such a low biomass quantity for bioenergy purposes. Therefore, the data on the methane yield from the second cut of the II harvest regime have only theoretical but not practical implications. Assuming that the higher heating value (HHV) and lower heating value for methane are 39.8 $MJ\ m^{-3}$ and 35.8 $MJ\ m^{-3}$, respectively [49], the theoretical energy output per hectare are computed (Table 5). Depending on the harvest regime and cultivar, the annual gross energy, presented as the LHVY, varies from 31.8 $GJ\ ha^{-1}$ (Dacotah of II harvest regime) to 68.0 $GJ\ ha^{-1}$ (Cave in Rock, I harvest regime).

Table 5. Area-specific methane and energy yields.

Cultivar	Harvest Date of the First Cut (Harvest Regime)	Dry Matter Yield (2015–2016), t ha^{-1}			CH_4Y_{TS}, Nm^3 ha^{-1} from Biomass			HHVY GJ ha^{-1}	LHVY GJ ha^{-1}
		First Cut	Second Cut	Annual	First Cut	Second Cut	Annual	Annual	Annual
Dacotah	14 July (I)	5.9	0.7	6.6	862	266	1128	44.9	40.4
	8 August (II)	6.7	0.3	7	828	59 †	888	35.3	31.8
	Average	6.3	0.5	6.8	845	163	1008	40.1	36.1
Forestburg	14 July (I)	5.3	1.5	6.8	1211	522	1732	68.9	62.0
	8 August (II)	9.5	0.3	9.8	1247	85 †	1332	53.0	47.7
	Average	7.4	0.9	8.3	1229	304	1532	61.0	54.9
Cave in Rock	14 July (I)	6.4	2.2	8.6	1148	752	1900	75.6	68.0
	8 August (II)	11.3	0.6	11.9	1004	206 †	1210	48.2	43.3
	Average	8.8	1.4	10.2	1076	479	1555	61.9	55.7
Average for three cultivars	14 July (I)	5.8	1.4	7.3	1074	514	1587	63.2	56.8
	8 August (II)	9.1	0.4	9.5	1026	117	1143	45.5	40.9

† Not investigated for methane production; computed using CH_4Y_{TS} values for regrowth of respective cultivar when first cut was performed during heading stage.

Few studies have evaluated the area-specific methane yield of switchgrass, and researchers' data vary widely. As reported by Massé et al. [28], their preliminary results showed that 2300 to 5400 Nm^3 CH_4 ha^{-1} could be obtained for different varieties of switchgrass grown in Florida. However, the methane yields reported by Canadian researchers, as well as those from our experiment, were noticeably lower than in the study from Florida: 1200–2600 m^3 ha^{-1} [70], 2280–30440 Nm^3 ha^{-1} [28] and 888–1900 Nm^3 ha^{-1}. The LHVY (gross energy) data for switchgrass obtained in the investigation of Barbanti et al. [67] were considerably higher (158 GJ ha^{-1}). One of the crucial factors that enhances the bioenergy output per area is the biomass yield. Barbanti et al. [67] reported a switchgrass yield of 22.4 Mg ha^{-1}, which exceeds what we achieved in our experiment for 2015–2016 (Table 5). A switchgrass biomass yield < 10 t ha^{-1} also determined a low or moderate biogas production in Canada [28,70]. Kandel et al. [78] stated that the biomass yield augmentation is more important than the biomass maturity to achieve a high methane yield per hectare from ×*Festulolium* and tall fescue. Higher biomass yields could be expected with increased fertilization; however, in our study, no additional nitrogen application was performed following the first cut. This low-input management approach reflects typical regional practices and aims to minimize the environmental impacts while assessing the regrowth potential of switchgrass under a limited nutrient supply. Future research could explore the effects of post-cut fertilization on biomass productivity and methane yield to optimize the management strategies for biogas production. However, in our study, we found that although the annual DMY of the first cut harvested on 8 August and its regrowth was 30% higher than that harvested earlier on 14 July (Table 5), the annual methane yields from these biomasses were similar when the same cultivar was compared. Consequently, other factors, including the harvest time and regime, are relevant to methane and energy output via an increase in the biomass convertibility. Approximately 25% more methane was produced per hectare for the two-cut strategy (2900–3440 Nm^3 ha^{-1}) compared to the one-cut strategy with a harvest in late summer (2280–2770 Nm^3 ha^{-1}) [28]. The same rule obtains regarding the harvest time and regime of other grasses [68,88].

If compared with the temperate grasses that have been investigated as feedstocks for biogas production, the results on the yield and methanization of switchgrass biomass from the current study do not seem very promising. Despite potentially lower methane yields, the advantages of switchgrass extend beyond mere energy output, and are critical for its overall viability and sustainability as a bioenergy crop, especially when cultivated on marginal lands [37,89].

The methane yield per hectare (5277−6963 Nm^3 CH_4 ha^{-1}) observed in the study of Kandel et al. [78] represents the high-end values for the temperate perennial grasses ×*Festulolium* and tall fescue in Central and Northern Europe. The energy potential obtained by producing biogas from reed canary grass, cocksfoot and tall fescue grown under Lithuanian climatic conditions ranged from 65 GJ ha^{-1} to 172 GJ ha^{-1} from plots fertilized with 90 kg ha^{-1} or 180 kg ha^{-1} of mineral nitrogen fertilizers [88]. The energy yields from different grasses and harvest years ranged from 1200 to 3600 Nm^3 CH_4 ha^{-1}, corresponding to 43.2 to 129.6 GJ ha^{-1} [77]. Switchgrass, as a biogas feedstock, offers notable environmental and economic advantages that often outweigh its limited methane yield. Its cultivation contributes significantly to greenhouse gas mitigation due to its perennial nature, which reduces the need for frequent tilling and replanting, thereby minimizing soil disturbance, lowering erosion and promoting soil organic carbon sequestration [75]. According to a study focusing on biogas production in northern Germany, perennial cropping systems contribute significantly to greenhouse gas mitigation, with continuous maize (as a comparative feedstock) demonstrating a CO_2 reduction of 55–61% over a fossil fuel-based energy mix [89]. The use of digestate as a fertilizer can further enhance the environmental performance and closed-loop nutrient cycling of switchgrass-based biogas systems [17]. In regions with variable climates, switchgrass is a reliable feedstock source due to its ability to adapt to a wide range of environmental conditions and its drought tolerance.

Greater biomass and methane yields would be expected with fertilization. In areas with sufficient rainfall, sustainable yields of ~15 Mg ha^{-1} yr^{-1} may be achievable by applying ~50 kg N ha^{-1} yr^{-1} at a commercial scale [15]. Hence, N fertilization might also significantly increase the methane and energy yield per hectare [28,88]. In our field experiment, the plots were fertilized with the minimal nitrogen rate (15 kg N ha^{-1}) only once per trial period, i.e., before the trial's establishment. Therefore, in our opinion, growing switchgrass under cool, temperate climate conditions for biogas merits further long-term research for its impacts on environmental conditions, the level of stand fertilization, biomass pretreatments and modelling of the anaerobic digestion process. Our study proves that the cultivar choice also plays an important role.

4. Conclusions

The chemical composition of the switchgrass biomass was significantly influenced by both the harvest date and cultivar. The harvest timing had a greater impact on the cell wall components (NDF, ADF and ADL), ash, nitrogen, carbon and the C/N. Delayed harvesting led to higher fibre and carbon concentrations, as well as a lower protein and ash content, ultimately resulting in an elevated C/N. These findings align with the previous literature highlighting the fluctuating quality of biomass with respect to plants' maturity and genetic variation.

The anaerobic digestion of switchgrass harvested at different times showed significant differences in the methane yield. Comparing the late-harvested (August) biomass to the mid-July and early-October harvests, it was found that the latter two harvests produced comparable or higher cumulative methane volumes. Younger plants, especially after regrowth, produced more methane, likely due to the presence of easily biodegradable compounds. Across all the cultivars, about 50% of the total methane yield was achieved within the first three days of digestion. However, the overall specific methane yield was notably lower for the August harvest. Therefore, the timing of harvesting plays a crucial role in optimizing switchgrass for use as a biogas feedstock.

A correlation analysis revealed a relationship between the biomass composition and methane yield. Specific methane yields were negatively correlated with the fibre compositions (NDF and ADF), lignin composition (ADL), carbon content and C/N. Elevated lignin-to-cellulose and lignin-to-hemicellulose ratios were strongly correlated with reduced methane production, highlighting how lignification hinders the breakdown of the biomass. These findings reinforce the importance of a biomass's chemical composition, particularly the levels of structural carbohydrates and lignin, as key determinants of its potential for anaerobic digestion and subsequent methane production.

The annual area-specific methane yield varied significantly among the different switchgrass cultivars and harvest regimes. Harvesting the first cut in mid-July typically resulted in higher annual methane yields compared to harvesting in early August. The methane yield from the second cut (regrowth) was noticeably lower than that from the first cut, especially when the initial harvest was delayed until August. This indicates limited practical benefits from late regrowth. As a result, the theoretical annual energy output, calculated based on methane's lower heating value, demonstrated that 'Cave in Rock' under the early first-cut regime displayed the highest energy potential. This study emphasizes the importance of cultivar selection and harvest management for maximizing biogas production from switchgrass.

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