

## ORIGINAL RESEARCH

# Species composition of semi-natural mesic grasslands as a factor influencing the methane yield of plant biomass (Central Europe)

Mateusz Meserszmit<sup>1</sup> | Grzegorz Swacha<sup>1</sup> | Lenka Pavlů<sup>2,3</sup> | Vilém Pavlů<sup>2,3</sup> | Adriana Trojanowska-Olichwer<sup>4</sup> | Zygmunt Kački<sup>1</sup>

<sup>1</sup>Botanical Garden, University of Wrocław, Wrocław, Poland

<sup>2</sup>Crop Research Institute, Department of Weeds and Vegetation of Agroecosystems, Grassland Research Station Liberec, Liberec, Czechia

<sup>3</sup>Faculty of Environmental Sciences, Department of Ecology, Czech University of Life Sciences Prague, Praha, Czechia

<sup>4</sup>Institute of Geological Sciences, University of Wrocław, Wrocław, Poland

## Correspondence

Mateusz Meserszmit, Botanical Garden, University of Wrocław, Wrocław, Poland.

Email: mateusz.meserszmit@uwr.edu.pl

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## Abstract

Semi-natural lowland and mountain mesic meadows are grasslands rich in species, and their conservation status depends on treatments such as mowing or grazing livestock. In many countries, the condition of grasslands is deteriorating because of their inappropriate use or abandonment. This study aimed to determine the effects of the species composition of plant communities and functional plant groups on the methane yield from biomass harvested from mesic grasslands in the Sudetes Mountains. Biogas potential analysis was performed based on biomass samples collected from Poland and the Czech Republic. The biogas potential was determined in 40 day-long batch anaerobic digestion tests. The average methane yield obtained from the biomass was  $246 \pm 16$  NL CH<sub>4</sub> kg<sup>-1</sup> VS, whereas the methane yield per hectare was  $870 \pm 203$  m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>. Plant communities comprising different dominant species had no effect on the methane yield but affected the methane yield per hectare. Additionally, the species composition of grasslands with a higher percentage of forbs had lower biomass yield, resulting in lower methane yields per hectare. The continuity of the low-intensity management of mountain grassland, which can be provided by the utilization of their biomass for bioenergy production, sustains high biodiversity and ensures appropriate meadow conservation.

## KEYWORDS

anaerobic digestion, biodiversity, bioenergy, biogas yield, ecosystem services, grassland conservation

## 1 | INTRODUCTION

Seeking solutions to preserve biodiversity is among the important problems of the 21st century. An effective

preservation of biodiversity requires an interdisciplinary approach and the participation of different social groups (Seddon et al., 2016). Among others, human activity has had a significant impact on the declining number of

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species via agricultural intensification (Tollefson, 2019) or the land-use abandonment of low-productive areas (Lindborg et al., 2008). The disappearance of biodiversity worldwide can be slowed down through the active conservation of valuable species, vegetation types and their habitats. Some ecosystems require the human activity to preserve their structure and species richness. This type of communities includes semi-natural meadows, which are valuable from the natural, landscape and cultural viewpoint (Hejcman et al., 2013). Many types of semi-natural grasslands are biodiversity reservoirs as they include a high number of plant species and other organisms inhabiting open landscapes (Raduła et al., 2020). The main conservation measures applied in these ecosystems include mowing, grazing or a combination of these. Otherwise, secondary succession occurs in unused meadows, resulting in the disappearance of communities and closely related organisms (Swacha et al., 2018; Tartally et al., 2019; Van Meerbeek et al., 2016). Extensively used meadows are the most valuable owing to their high level of species richness (Marini et al., 2008). High species richness results from a specific low-intensity management, which can be defined as one or two cuts a year and the application of low-concentration fertilizer. The higher biodiversity index of meadows probably increases the resistance of plants to drought and can be of great importance in extreme weather phenomena caused by climate change (Cole et al., 2019; Isbell et al., 2015).

Semi-natural mountain and lowland hay meadows are valuable ecosystems listed in the Annex I of the EU Habitat Directive (Council Directive, 1992). On average, the status of their preservation in the Sudetes Mountains, both in Poland and the Czech Republic, is assessed as 'unfavourable-inadequate' and 'unfavourable-bad', respectively, suggesting that changes are required in management or policy to restore this habitat to a good conservation status (European Environment Agency, 2013). The plant communities of lowland and mountain hay meadows differ in species composition in response to habitat and management conditions. The species dominating these communities are mostly grasses; however, in certain situations, herbaceous species are equally or even more abundant. The species composition and abundance of individual plant species in grassland vegetation are affected by several soil parameters, including chemical composition, organic matter content, moisture and pH, and also depend on the management and its intensity (Merunková & Chytrý, 2012; Pavlů et al., 2011; Pruchniewicz & Żolnierz, 2014). Soil properties and management practices favour some plants, stimulating their competitive relations. To strengthen the conservation of meadows, hay management methods should be followed, which will encourage farmers to mow them

regularly. This applies to mountain grasslands (Fatyga & Nadolna, 2009) that produce large amounts of biomass, which frequently exceed the demand for animal feed (Nadolna & Żyszkowska, 2011).

The principal role of grasslands is the production of forage for livestock. By contrast, the biomass from grasslands has been found to be a good substrate for biofuel production. Studies have demonstrated that it is possible to produce methane from meadow biomass (Heinsoo et al., 2010; Herrmann et al., 2013; Popp et al., 2017; Prochnow et al., 2009). The use of grassland biomass for energy purposes could contribute to the preservation of this type of habitat, particularly in areas with the problem of grassland biomass surplus (Melts et al., 2014; Meserszmit et al., 2019). If alternative solutions are not applied, the decreased demand for this substrate will result in the abandonment of grasslands or their conversion to other agricultural landforms. The heterogeneous composition of this feedstock causes difficulties in assessing its biogas potential because it comprises many plant species with different chemical properties that can affect the methane yield (Dandikas et al., 2015; McEniry & O'Kiely, 2013; Seppälä et al., 2009). Different plants or plant groups can also exhibit different biomass yields, which have a significant effect on the methane yield per area (Popp et al., 2017).

Recent studies suggest that the areas used for bioenergy crop cultivation can pose a threat to the existence of natural habitats and adversely impact the biodiversity (Dauber & Bolte, 2014; Gevers et al., 2011). A growing number of agricultural biogas plants in Europe could result in increased demand for energy crops, thereby resulting in the conversion of grassland to cropland monoculture (Lüker-Jans et al., 2017). In our opinion, the hay obtained from permanent grasslands could benefit the conservation of natural environment as well as bioenergy production. Studies on biogas production from grassland biomass contribute to the understanding of how grasslands can be effective for renewable energy production. Many types of meadow habitats have not yet been studied with respect to the use of their biomass in biogas production (Theuerl et al., 2019). Mesic grasslands are one of the most common semi-natural plant communities in Central Europe and have not yet been fully studied in terms of methane yield. This study can be useful in predicting the energy potential of grassland habitats, the existence of which is at risk mainly due to abandonment.

This study aimed to determine the effect of the species composition of plant communities and functional plant groups on the methane yield obtained during the anaerobic digestion of biomass harvested from mesic grasslands in the Sudetes Mountains.

## 2 | MATERIALS AND METHODS

### 2.1 | Characteristics of the study areas

This study was conducted in the Sudetes Mountains in Poland and the Czech Republic, with mesic grassland dominance during the peak of the growing season (the second half of June, which is the traditional time for the first meadow mowing in this region) in 2018 and 2019. The mesic grasslands are defined in the Habitat Annex I Directive as lowland hay meadows (habitat code 6510) and mountain hay meadows (habitat code 6520) classified to Arrhenatherion and Trisetum-Polygonion communities respectively (Kački et al., 2020). Habitat type 6510 primarily comprises productive grasses, *Arrhenatherum elatius* and *Dactylis glomerata*, and develops on low to moderately fertilized soils. This habitat contains the following important plant herbaceous species: *Galium mollugo*, *Crepis biennis* and *Heracleum sphondylium*. Habitat type 6520 is majorly dominated by *Trisetum flavescens*, *Agrostis capillaris*, *Festuca rubra* and certain dicots, such as *Hypericum maculatum*, *Cirsium helenioides* and *Crepis mollis* (mostly *C. mollis* subsp. *succisifolia*); these species grow on less fertile soils. The sampling sites were selected based on the assessment of the species composition and management type. Only the extensively mown sites (cut once or twice a year) were considered. Sampling was performed before the first harvest (Table 1).

### 2.2 | Sampling and data collection

Field sampling was performed on 15 different locations in the Sudetes Mountains, seven sites in Poland and eight sites in the Czech Republic. The study area (Sudetes Mountains) was overlaid with a 10 × 10-km grid and we considered only those grid cells with

considerable open-landscape habitats. We used data from Polish Vegetation Database (Kački & Śliwiński, 2012) and Czech National Phytosociological Database (Chytrý & Rafajová, 2003) as reference for mesic grasslands locations. The sampling sites were selected based on the inspection of species composition and management type. Only grasslands with representative species composition for lowland hay meadows (habitat code 6510) and mountain hay meadows (habitat code 6520) were considered as sampling sites. We sampled only extensively mown sites (cut once or two times a year). In each site, 10 × 10-m one focal plot was established at a place homogeneous in terms of topography and vegetation physiognomy. The following operations were performed subsequently within the focal plots.

- a. A 1-m<sup>2</sup> (1 × 1 m) sampling plot was established in the centre of each focal plot using a quadrat metal frame. All vascular plant species within the 1-m<sup>2</sup> plots were recorded, and their percentage cover was estimated to investigate the effect of species composition and functional plant groups on the methane yield and chemical properties of biomass. Then, the standing biomass was cut from these 1-m<sup>2</sup> plots at 3 cm above the ground. The obtained biomass samples were dried at room temperature for at least 10 days and then transported to a laboratory to perform chemical analysis and biogas potential analysis.
- b. From the four 0.4 × 0.4 m (0.64 m<sup>2</sup>) quadrat frames placed evenly in the corners of the focal plots, the biomass was cut at 3 cm above the ground to investigate the biomass yield and methane yield per area. The biomass was divided into the following plant functional groups: grasses (*Poaceae*), forbs (herbaceous, dicotyledonous flowering plants), sedges/rushes (*Cyperaceae/Juncaceae*) and legumes (*Fabaceae*). The collected biomass samples were pre-dried at room

**TABLE 1** Characteristics of plant communities divided by the *k-means* method. The main dominant plant species with the highest cover are highlighted in bold

Groups	Plant community	
	A	B
Dominant species	<b><i>Arrhenatherum elatius</i></b> <b><i>Dactylis glomerata</i></b> <i>Trisetum flavescens</i> <i>Lathyrus pratensis</i> <i>Agrostis capillaris</i> <i>Alchemilla vulgaris</i> agg.	<b><i>Festuca rubra</i></b> <b><i>Agrostis capillaris</i></b> <i>Plantago lanceolata</i> <i>Alchemilla vulgaris</i> agg. <i>Veronica chamaedrys</i> <i>Sanguisorba officinalis</i>
Number of sites	5	10
Altitude (m a.s.l.) min–max	359–696	387–950
Species richness per 1 m <sup>2</sup> : mean (min–max)	26 (16–35)	27 (18–42)

temperature for at least 10 days and then dried in a forced air circulation drying oven at 60°C for 24 h to reach a constant dry matter content, expressed in t DM ha<sup>-1</sup>. For the analysis, the relative percentage proportion of biomass weight by individual functional groups (grasses, forbs, legumes, sedges/rushes) was used.

## 2.3 | Chemical analysis

Using the Kjeldahl method, N (nitrogen) concentration was determined (AOAC, 1990), and it was multiplied by 6.25 to obtain the crude protein content. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were specified according to the protocol described by Goering and Van Soest (1970) and Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Ankom Technology). The acid detergent lignin (ADL) was obtained after 3-h digestion of ADF by 72% H<sub>2</sub>SO<sub>4</sub>. The hemicellulose content was obtained as a difference between NDF and ADF. The amount of cellulose in the samples was determined by subtracting ADL from ADF, and the lignin content was taken as ADL. The fat and ash content were determined by Wenden's method (AOAC, 1984). N, NDF, ADF, ADL, ashes and lipids were analysed in an accredited laboratory of the Crop Research Institute in Chomutov.

## 2.4 | Biogas production

The biomass samples from 1-m<sup>2</sup> plots were mechanically pre-treated by shredding and cutting the plant materials into 2- to 3-cm pieces using electric scissors. Retsch ZM 200 mill, with 0.5-cm trapezoidal mesh screens, was used during the final stage of shredding. The substrate was mixed again to homogenize it. In total, 15 experimental samples from 1-m<sup>2</sup> plots were prepared, each in three replicates. The control fermentation bottles contained only the inoculum. The biogas potential analysis was performed for 40 days using batch anaerobic digestion tests based on the standard VDI 4630 (VDI 4630, 2006). Each test was performed in three replicates in fermentation bottles with a working volume of 0.5 dm<sup>3</sup> and containing 2.9 ± 0.1 g of ground meadow biomass and 494 ± 4.4 g of the inoculum obtained from an agricultural biogas plant. The inoculum was characterized by the following parameters: pH = 7.8, total solid (TS) content = 10 g kg<sup>-1</sup> and volatile solid (VS) content = 565 g kg<sup>-1</sup> TS. Each bottle was purged with nitrogen. Incubation was performed in a water bath at a constant temperature of 37°C. The bottle contents were mixed manually once a day. The volume of emitted biogas was read every day and measured

using a eudiometer connected to a fermenter. The biogas production was standardized under normal conditions (273 K and 1013 hPa), adjusted for the volume of gas produced by the inoculum of the reference sample only, and was expressed in litres per kg (NL kg<sup>-1</sup>) for VS. The biogas composition was tested during the emptying of the eudiometer, that is three times in 40 days. Gas samples were collected to determine their methane content and analysed using a gas chromatograph (Fisons GC8000, Restek Rt-Msieve5A column). The TS and VS contents were tested using a weight-drying method based on the standards PN-EN 12880 (2004) and PN-EN 12879 (2004). The methane yield per area was calculated as the product of the dry matter yield, the percentage of dry organic matter in the biomass and the specific methane yield obtained.

## 2.5 | Statistical analyses

The k-means classification method was used to classify the vegetation compositional data to delimit the types of plant communities. Statistical analyses were performed using Statistica 13.3 software. The data were checked for normal distribution and the homogeneity of variance using the Shapiro–Wilk test and Levene's test respectively. The two groups delimited by the *k*-means were used to compare the chemical parameters, biogas yield, methane yield, area methane yield and biomass yield, and plant groups using the Student's *t*-test. The four functional plant groups (grasses, forbs, sedges/rushes and legumes) were differentiated. Only two groups (grasses and forbs) were included in the analyses as sedges/rushes and legumes had negligible cover in the data set. To investigate the effect of the abundance of functional plant groups on fibre fractions, methane yield, methane yield per area and biomass yield, linear regression analysis was performed at a significance level of 0.05. To investigate the effect of individual chemical parameters on the total methane yield, a correlation matrix (Pearson correlation coefficient) was used.

## 3 | RESULTS

The two groups of plant communities delimited by the *k*-means method differed in both species composition and dominance (Table 1). Group A was primarily dominated by *A. elatius* and *D. glomerata*, whereas group B was mainly dominated by *A. capillaris* and *F. rubra*.

The average biomass TS and VS content in group A was 93.16% and 92.27% TS, respectively, whereas in group B, it was 92.64% and 93.49% TS respectively (Table 2). The average protein and lipid content in group

**TABLE 2** Average values (mean  $\pm$  SD) of chemical composition, methane yield and biogas yield. *F*-ratio = *F*-statistics for the test of a particular analysis; *p*-value = corresponding probability value. For plant community abbreviations (A and B), see Table 1

Characteristics	A	B	<i>F</i> -ratio	<i>p</i> -value	A + B
TS (%)	93.16 $\pm$ 0.52	92.64 $\pm$ 0.44	1.561	0.083	92.81 $\pm$ 0.53
VS (%TS)	92.27 $\pm$ 1.57	93.49 $\pm$ 1.15	2.102	0.136	93.08 $\pm$ 1.43
Protein (%TS)	8.00 $\pm$ 0.97	9.29 $\pm$ 1.60	2.408	0.148	8.86 $\pm$ 1.55
Lipids (%TS)	3.17 $\pm$ 0.32	2.71 $\pm$ 0.63	3.524	0.176	2.86 $\pm$ 0.59
NDF (%TS)	57.30 $\pm$ 3.44	55.73 $\pm$ 6.21	2.909	0.570	56.26 $\pm$ 5.5
Hemicellulose (%TS)	16.59 $\pm$ 1.86	19.46 $\pm$ 4.49	5.179	0.223	18.51 $\pm$ 4.05
Cellulose (%TS)	29.47 $\pm$ 4.38	24.06 $\pm$ 5.16	1.235	0.084	25.86 $\pm$ 5.54
Lignin (%TS)	11.24 $\pm$ 0.79	12.21 $\pm$ 2.16	6.663	0.258	11.89 $\pm$ 1.88
Methane yield (NL CH <sub>4</sub> kg <sup>-1</sup> VS)	248.56 $\pm$ 11.42	244.39 $\pm$ 18.16	2.248	0.669	245.78 $\pm$ 16.34
Biogas yield (NL kg <sup>-1</sup> VS)	452.91 $\pm$ 16.07	470.82 $\pm$ 33.48	3.858	0.311	464.85 $\pm$ 30.08

Abbreviations: TS, total solid; VS, volatile solid.

**TABLE 3** Pearson correlation matrix of chemical compounds and methane yield. Significant *p*-values (*p* < 0.05) are highlighted in bold

	VS (%TS)	Protein (%TS)	Lipids (%TS)	NDF (%TS)	Hemicel. (%TS)	Cellulose (%TS)	Lignin (%TS)	Methane yield (L/kgVS)
TS (%)	0.46	<b>-0.67</b>	-0.12	<b>0.72</b>	0.18	<b>0.64</b>	-0.17	0.12
VS (%TS)		-0.44	-0.19	<b>0.59</b>	0.45	0.32	-0.17	0.10
Protein (%TS)			-0.13	<b>-0.70</b>	-0.07	<b>-0.83</b>	<b>0.57</b>	-0.18
Lipids (%TS)				-0.13	<b>-0.54</b>	0.38	-0.33	0.24
NDF (%TS)					<b>0.56</b>	<b>0.68</b>	-0.30	0.29
Hemicel. (%TS)						-0.16	-0.04	0.25
Cellulose (%TS)							<b>-0.61</b>	0.14
Lignin (%TS)								-0.10

Abbreviations: TS, total solid; VS, volatile solid.

A was 8.00% and 3.17%, respectively, whereas in group B, it was 9.29% and 2.71% respectively. The average NDF content in group A was 57.30% TS, whereas in group B, it was 55.73% TS. The average content of fibre fractions (such as hemicellulose, cellulose and lignin) in group A was 16.59% TS, 29.47% TS and 11.24% TS, respectively, whereas in group B, it was 19.46% TS, 24.06% TS and 12.21% TS respectively. No significant differences were found between the two groups of plant communities (*p* > 0.05).

The relationships between the chemical compounds and methane yield are presented in Table 3. All chemical parameters showed no significant relationship with the methane yield (*p* > 0.05).

Figure 1 shows the relationships between the plant groups (grasses and forbs) and the total percentage content of different fibre fractions. Linear regression showed that the abundance of forbs was positively correlated with the percentage of lignin content in biomass (*p* = 0.021, *r*<sup>2</sup> = 0.346). However, it was the opposite for grasses.

The results of the linear regression demonstrated that the abundance of grasses was negatively correlated with the percentage of lignin content in biomass (*p* = 0.014, *r*<sup>2</sup> = 0.381). No significant correlations were found between the abundance of grasses and forbs and hemicellulose content in sampled biomass (grasses, *p* = 0.197, *r*<sup>2</sup> = 0.125; forbs, *p* = 0.638, *r*<sup>2</sup> = 0.018). Further, no significant correlations were found between the abundance of grasses and forbs and cellulose content in sampled biomass (grasses, *p* = 0.098, *r*<sup>2</sup> = 0.197; forbs, *p* = 0.111, *r*<sup>2</sup> = 0.183).

The average methane and biogas yields in grassland group A were 248.56 NL CH<sub>4</sub> kg<sup>-1</sup> VS and 452.91 NL kg<sup>-1</sup> VS (Table 2), respectively, whereas the area methane yield and biomass yield were 987.40 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup> and 4.29 t DM ha<sup>-1</sup> respectively (Table 4). In group B, the average methane yield and biogas yield were 244.39 NL CH<sub>4</sub> kg<sup>-1</sup> VS and 470.82 NL kg<sup>-1</sup> VS (Table 2), respectively, whereas the area methane yield and biomass yield were 805.04 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup> and 3.53 t DM ha<sup>-1</sup> respectively (Table

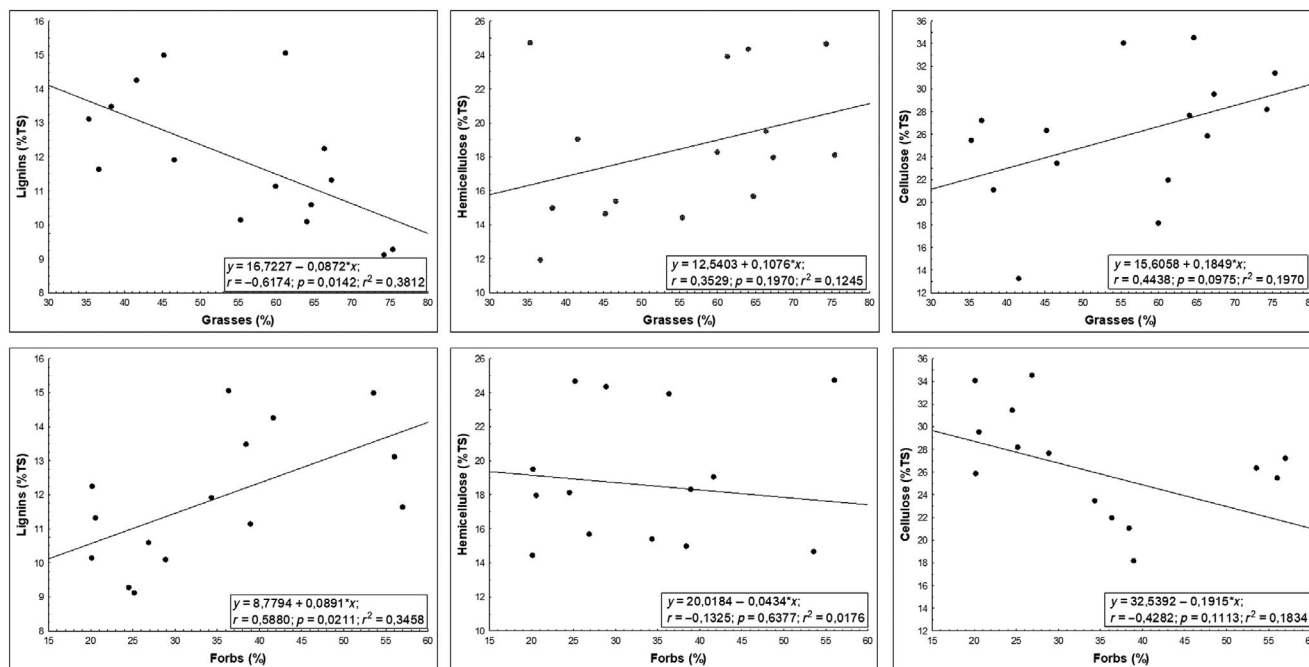


FIGURE 1 Relationship between the lignin, hemicellulose and cellulose contents and the percentage covers of grasses and forbs

TABLE 4 Average percentage contribution of plant groups in the biomass, biomass yield and area methane yield of grasslands. Mean  $\pm$  SD. *F*-ratio = *F*-statistics for the test of a particular analysis; *p*-value = corresponding probability value (no result means an insufficient number of samples for statistical analysis). For plant community abbreviations (A and B), see Table 1

Characteristics	A	B	<i>F</i> -ratio	<i>p</i> -value	A + B
Grasses (%)	68.75 $\pm$ 16.57	58.43 $\pm$ 19.34	1.363	0.336	62.12 $\pm$ 18.46
Forbs (%)	20.27 $\pm$ 9.21	35.08 $\pm$ 15.97	3.009	0.084	29.79 $\pm$ 15.40
Legumes (%)	10.97 $\pm$ 8.08	4.94 $\pm$ 5.54	2.130	0.122	7.09 $\pm$ 6.93
Sedges/Rushes (%)	0.01 $\pm$ 0.02	1.55 $\pm$ 1.93	–	–	1.00 $\pm$ 1.70
Biomass yield (t DM ha <sup>-1</sup> )	4.29 $\pm$ 0.82	3.53 $\pm$ 0.79	1.065	0.115	3.80 $\pm$ 0.86
Area CH <sub>4</sub> yield (m <sup>3</sup> CH <sub>4</sub> ha <sup>-1</sup> )	987.40 $\pm$ 217.28	805.04 $\pm$ 173.83	1.562	0.110	870.17 $\pm$ 203.33

4). Despite a considerable difference in the two groups in terms of species composition, their methane and biogas yields were not significantly different ( $p > 0.05$ ). Grasses have a higher biomass percentage, and its average value in groups A and B was 68.75% and 58.43% respectively. This was followed by forbs, which had a biomass percentage of 20.27% in group A and 35.08% in group B. By contrast, legumes and sedges/rushes had a small biomass fraction (Table 4).

Based on the linear regression analysis, significant negative correlations were found for the proportion of forbs with respect to the biomass yield and area methane yield ( $r^2 = 0.307$ ,  $p = 0.040$ ;  $r^2 = 0.303$ ,  $p = 0.042$  respectively; Table 5). No significant correlations were found between the proportion of grasses and the biomass yield/area methane yield ( $r^2 = 0.255$ ,  $p = 0.066$ ;  $r^2 = 0.252$ ,  $p = 0.068$  respectively).

## 4 | DISCUSSION

### 4.1 | Methane yield

There are many types of extensively used semi-natural communities that have not been studied yet for their methane yield, and in many countries, this type of research has not been conducted at all. The biomass harvested from semi-natural mesic meadows located in the submontane and montane areas in the Sudetes Mountains was characterized; its average methane yield was 245.78 NL CH<sub>4</sub> kg<sup>-1</sup> VS. Some grasslands have been the subject of previous studies, and different results for methane yield have been obtained with respect to their specific types. For instance, the methane yield from wooded, mesic and alluvial meadows in Estonia was 299, 297 and 269 NL CH<sub>4</sub> kg<sup>-1</sup> VS respectively (Melts et al.,

**TABLE 5** Linear regression analysis of methane yield, methane yield per hectare and biomass yield. Linear equation ( $y = a + bx$ ), where 'a' is the intercept and 'b' is the linear coefficient (slope);  $r^2$  = coefficient of determination. Significant  $p$ -values ( $p < 0.05$ ) are highlighted in bold

Effect	Regression equation	$r^2$	Level of significance
Grasses (%)	Methane yield = $253.036 - 0.1196x$	0.010	0.739
	Area CH <sub>4</sub> yield = $33.7236 + 0.3536x$	0.252	0.068
	Biomass yield = $150.031 + 1.4978x$	0.255	0.066
Forbs (%)	Methane yield = $244.4419 + 0.0579x$	0.002	0.880
	Area CH <sub>4</sub> yield = $69.5362 - 0.4647x$	0.303	<b>0.042</b>
	Biomass yield = $301.8382 - 1.9726x$	0.307	<b>0.040</b>
Legumes (%)	Methane yield = $244.4184 + 0.1995x$	0.009	0.745
	Area CH <sub>4</sub> yield = $55.7958 - 0.0148x$	0.000	0.979
	Biomass yield = $241.7455 + 0.1866x$	0.001	0.936

2013). Further, the methane yield from the *Molinia* meadows in Poland ranged from 197 to 221 NL CH<sub>4</sub> kg<sup>-1</sup> VS (Meserszmit et al., 2019). Other studies conducted in Germany showed that the methane yield from meadow foxtail and *Molinia* meadows vegetation ranged from 170 to 200 NL CH<sub>4</sub> kg<sup>-1</sup> VS (Hermann et al., 2013). Two studies conducted on the biomass from mesic (*Arrhenatherion*) grasslands reported a methane yield of 300 NL CH<sub>4</sub> kg<sup>-1</sup> VS (Boob et al., 2019) and a methane yield range from 281 to 297 NL CH<sub>4</sub> kg<sup>-1</sup> VS (Von Cossel et al., 2019). The type of meadow evaluated in these studies is similar in terms of species composition to the meadows investigated in our study. Nonetheless, the information about the species composition of the harvested biomass was not provided in these studies; therefore, it was difficult to make detailed comparisons. However, it should be indicated that biomass, even from similar types of vegetation, comprises of different proportions of individual species and plant functional groups. Different species have different chemical properties and fibre contents, which can affect the fermentation process and methane yield (Dandikas et al., 2015; McEniry & O'Kiely, 2013). The sampled meadows differed in terms of the species composition, number of species in 1-m<sup>2</sup> plots and cover of individual species. Species composition is the basis for differentiating plant communities. Therefore, we analysed how species composition influences the methane potential by evaluating two groups with different species composition delimited by  $k$ -means, which were then interpreted as two different plant communities. Group A corresponds to the mesic meadows of fertile soils dominated by *A. elatius* and *D. glomerata*, whereas group B corresponds to mesic meadows dominated by *A. capillaris* and *F. rubra* on nutrient-poor soils. Although both community groups had different dominant species, their methane yield and biogas yield were not significantly different. This suggests that species composition has no direct effect on the methane yield and that the phenology of the dominant species probably plays a more important role. Other studies

showed that methane yields can vary in different grass species (McEniry & O'Kiely, 2013; Seppälä et al., 2009).

One of the important factors affecting the methane yield is the lignin content. Lignins adversely affect biogas production (Dandikas et al., 2015; Triolo et al., 2012); their amount in biomass increases with increasing plant maturity (McEniry & O'Kiely, 2013; Prochnow et al., 2009; Seppälä et al., 2009). They can be higher in certain plant groups, such as herbs/forbs, in comparison with grasses (Melts & Heinsoo, 2015; Melts et al., 2014). Our study confirmed this relationship: an increase in the percentage of forbs in the biomass contributed to a higher amount of lignins, whereas the lignin content decreased with the increasing percentage of grasses. Moreover, a similar but statistically insignificant trend was observed for the cellulose content. The grass-dominated meadow hay with low fibre fraction could theoretically be easier to degrade during anaerobic digestion. Moreover, it may also have higher methane yield, but this was not confirmed in the present study during the biogas experiment. Based on Melts et al. (2014) study, grasses have higher methane yields than forbs. In our study, the direct impact of lignins on the methane yield was not confirmed despite their varying contents in biomass samples. There could be two reasons. First, the fibre contents in samples were not significantly different. Second, the applied mechanical pre-treatment of the substrate and the high degree of its shredding decreased the effect of lignin content on the methane yield. The type of pre-treatment used in this study reduces the feedstock particle size, increases the digestion area and increases the biogas amount (Rodriguez et al., 2017). This applies to substrates with a complex lignocellulosic structure. The degree of shredding is also important because smaller pieces contribute to an increased biogas production (Menind & Normak, 2008; Tsapekos et al., 2015). Shredding to larger pieces could possibly influence the fibre fraction content (Bridgeman et al., 2007) and significantly affect the fermentation process. Small variations in the protein and lipid contents were found in

the biomass samples. We did not find the direct factor responsible for these changes as well as its possible effects on the methane yield. It can only be presumed that their variable content in the biomass was associated with the different proportions of the different plant groups, species composition or plant maturity (Arzani et al., 2004; Ravetto et al., 2017).

## 4.2 | Methane yield per hectare

The specific methane yield per hectare is an important parameter in studies evaluating the biogas potential of meadow biomass or other feedstock obtained from a particular area. The biomass from semi-natural grasslands is characterized by substantial variation in vegetation and biomass growth, which may affect the methane yield per hectare. In this study, hays were collected before the first cut in the second half of June. Other studies covered this aspect to a small extent, and they were related to the following meadow community types: *Molinia* meadows, from which 482–867 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup> was obtained depending on the harvest time (Meserszmit et al., 2019); wooded meadows (514 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>), mesic meadows (792 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>) and alluvial meadows (1375 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>) in Estonia (Melts et al., 2013); and *Arrhenatherion* meadows in Germany, from which 845–1355 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup> (Boob et al., 2019) or 1903.7–2338.4 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup> (Von Cossel et al., 2019) was obtained on the first day of harvest. The average methane yield per hectare calculated in our study was 870.17 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>, which is similar to those in the above-mentioned studies. In this study, the large variation in the methane yield per hectare was due to the different contents of the individual functional groups in the biomass. Hays with a larger percentage of forbs had less biomass yield, which also resulted in lower methane yield per hectare. By contrast, there was a positive relationship between the increased percentage of grasses and methane yield per hectare. Khalsa et al. (2012) and Popp et al. (2017) also found that area-specific methane yields are greatly affected by different functional plant groups. Moreover, Khalsa et al. (2012) showed a positive correlation between the abundance of legumes and methane yield per area. In our study, this plant group had low percentage in the biomass and thus had no effect on the methane yield per hectare. However, Popp et al. (2017) obtained the highest area-specific methane yields from plots where grasses were sown. In our study, there were no significant differences in area-specific methane yields between the two distinguished communities (Table 4). Despite the lack of a statistically significant difference, we can observe that the average methane yield per hectare and biomass yield were higher in group A than

in group B. Therefore, we can presume that *A. capillaris* and *F. rubra*, which had lower biomass yields than *A. elatius* and *D. glomerata* (Kacorzyk & Kasperczyk, 2016; Łyszczarz et al., 1998), had an effect on the area methane yield. Moreover, group B contained more forbs than group A, which could also result in lower area methane yield.

## 4.3 | Grassland conservation and biogas production

The sustainable management of semi-natural meadows is the most important element in their conservation. From an environmental viewpoint, extensively used meadows are the most valuable because of their high level of species richness (Plantureux et al., 2005). Extensive grassland management involves regular harvest (once or twice a year) or low-intensity animal grazing. Long-term grassland grazing may have less beneficial effects on plant diversity compared with cutting (Pavlů, Pavlů, et al., 2021). The meadows included in this study were cut from 15 June (after grass flowering) once or twice a year. Sampling was performed in accordance with the recommended mowing dates of grasslands and relevant governmental regulation (Regulation of the Minister of Agriculture & Rural Development, 2015). The methane yield obtained in this study confirmed that the biomass from grasslands is the suitable substrate for biogas production. However, this type of substrate may require elaborate pre-treatment due to the high fibre fraction content. A later harvest time results in an increase in the lignocellulosic compound contents in plants (Buxton, 1996; Pavlů, Kassahun, et al., 2021), which may adversely influence the biodegradation process and biogas potential (Triolo et al., 2012). Owing to the intensification of agriculture and decreasing interest to use grassland biomass, the abandonment of large grassland areas has been observed for a long time (Isselstein et al., 2005), particularly in Poland. This is because of the low demand for hay among farmers, making grassland use unprofitable. At present, the active conservation of valuable semi-natural meadow habitats in Europe is financially supported by the European Union grants and the respective agri-environmental schemes. Considering the pace at which semi-natural meadow areas are diminishing, there is an urgent need to establish an appropriate conservation plan (Szymura & Szymura, 2019).

The most important element of grassland conservation is to apply appropriate cutting times and frequencies to preserve the grassland ecosystems from excessive exploitations, making hay harvest still beneficial with respect to the biomass amount. An intensive use of meadows involves several cuts a year, high stocking rate



and a simultaneous application of high fertilizer doses. Several cuts a year increases the quantities of biomass to be obtained, which increases the methane yield per hectare (Von Cossel et al., 2019). This type of meadow use substantially decreases the plant species diversity, and its extensive use results in lower methane yield per hectare (Prochnow et al., 2009). However, it maintains and strengthens the conservation effects on semi-natural meadows. The dynamic development of the biogas sector in Europe may entail future problems in securing meadow habitats and collecting biomass for energy purposes. Certain concerns have been presented by Kallimanis (2018) who suggested that the integration of bioenergy production with nature conservation may cause intensified land use in protected Natura 2000 sites, resulting in reduced biodiversity. As pointed by Van Meerbeek et al. (2018), this type of research was not designed to promote the intensification of the use of protected areas or valuable habitats. In this study, we wished to present an alternative biomass management method and investigate the factors that affect the biogas potential of biomass. This can be useful in predicting the energy potential of grassland habitats, whose existence is at risk owing to abandonment, land-use intensification or conversion. There is a potential in using grassland biomass for energy production under an extensive management.

## 5 | CONCLUSIONS

The plant biomass harvested from species-rich mesic grasslands in the Sudetes Mountains is a suitable substrate in terms of methane yield. The species composition of plant communities and functional plant groups in the investigated meadows had no effect on the methane yield. However, an increase in the lignin content of hay, caused by a higher percentage of forb plants, may theoretically adversely affect the methane yield. Meadows with higher forb percentage had lower biomass yield, resulting in lower methane yield per hectare. The integration of biogas production with the conservation of valuable semi-natural habitats requires an appropriate and well-thought-out approach. An appropriate management of mountain grasslands will allow us to ensure their sustainable use for obtaining their biomass and maintain the proper status of meadow conservation.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Mateusz Meserszmit  <https://orcid.org/0000-0002-1630-9358>

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