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Research article

Effect of mowing versus abandonment of mesic grasslands in Central Europe on biomass use for biogas production: Implications for semi-natural ecosystem conservation

Mateusz Meserszmit [a,*], Grzegorz Swacha [a], Lenka Pavlů [b], Vilém Pavlů [b,c], Jan Titěra [b], Sławomir Jabłoński [d], Marcin Łukaszewicz [d], Zygmunt Kącki [a]

[a] Botanical Garden, Faculty of Biological Sciences, University of Wrocław, Poland
[b] Department of Biology and Ecology, Faculty of Science, Humanities and Education, Technical University of Liberec, Czech Republic
[c] Grassland Research Station Liberec, Crop Research Institute, Praha, Czech Republic
[d] Department of Biotransformation, Faculty of Biotechnology, University of Wrocław, Poland

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ABSTRACT

The economic management of lignocellulosic biomass from semi-natural grasslands is now a challenge across Europe. The abandonment of mowing these grasslands leads to the gradual degradation of these ecosystems. This study investigates how chemical and biological factors affect the suitability of biomass from abandoned grasslands for biogas production. We sampled 30 mown and 30 abandoned grassland sites in the Sudetes Mountains (Poland and Czechia). The cover contribution of short herbs was found to be significantly higher in mown grasslands ($p < 0.001$), while that of tall herbs was more prevalent in abandoned grasslands ($p < 0.01$). The specific biogas yield (SBY, NL kg^{-1} volatile solids) is negatively affected by an increased percentage of herbs in the biomass of mown and abandoned grasslands. This is due to the inhibitory effect of herbs on biodegradation, the increase in lignin content and the decrease in cellulose. This study highlights the importance of individual plant species in assessing grassland biomass for area biogas yield (ABY, m^3 ha^{-1}) and provides new insights into a field that has not yet been extensively investigated. In mown grasslands, ABY was most positively correlated with grass species (*Arrhenatherum elatius*, *Trisetum flavescens* and *Festuca pratensis*). In abandoned grasslands, the ABY was most correlated with herbaceous species (*Galium aparine*, *Urtica dioica* and *Chaerophyllum aromaticum*) and grasses (*A. elatius* and *Elymus repens*). Mown grasslands had significantly higher species richness ($p < 0.001$) compared to abandoned grasslands, but the number of species sampled did not correlate with SBY and ABY. This study contributes to the development of a sustainable bio-economy by highlighting the need for efficient use of grassland biomass. This approach helps protect semi-natural ecosystems and facilitates sustainable management of renewable resources.

1. Introduction

Today, Europe is facing the challenge of transitioning to sustainable energy sources, which is a key element of energy security and socio-economic development. In the framework of the European Green Deal (European Commission, 2019) and global climate protection strategies, such as the United Nations agreement (United Nations, 2016), European Union (EU) countries are investing in low-emission technologies, including renewable energy. Concurrently, the IPCC reports (Intergovernmental Panel on Climate Change IPCC, 2023) provide key scientific information supporting these initiatives. However, not all countries fully utilise their renewable energy potential, especially Central European countries (Igliński et al., 2022). One of the key requirements for the production of renewable energy is that it should be performed in a sustainable manner while considering the protection of the natural environment, which means minimising negative effects on biodiversity and ecosystems.

Specific types of renewable energy sources are being developed in different countries because of their geographical location and specific climatic conditions. According to data published by the Food and Agriculture Organization (2024), the total area of permanent meadows and pastures in the EU in 2021 was 52,119,461 ha. This represents a significant potential source of biomass from mowing that can be used for

* Corresponding author.
E-mail address: mateusz.meserszmit@uwr.edu.pl (M. Meserszmit).

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Abbreviations

SBY	Specific biogas yield (NL kg^{-1} volatile solids)
ABY	Area biogas yield (m^3 ha^{-1})
TS	Total solids (%)
VS	Volatile solids (%)
DM	Dry matter

agricultural and energy purposes. Semi-natural grasslands provide not only fodder for livestock but also various ecosystem services, including carbon sequestration, water retention and purification (Zhao et al., 2020). However, semi-natural grasslands are ecosystem types at high risk of extinction and degradation. More than half of the former grasslands and their associated biodiversity have disappeared from Europe in the last few decades (Habel et al., 2013; Dixon et al., 2014), mainly due to the cessation of their use or conversion to other land use types. The main agricultural activities performed on semi-natural grasslands for their conservation include mowing, grazing or a combination of these. The low-intensity use of meadows also maintains their high multi-functionality (Schils et al., 2022). Meadow hay is generally used as fodder for livestock. However, data indicate that the number of livestock in Europe is declining each year (FAO, 2024), and forage from agricultural crops is increasingly replacing hay as a feed source (Naylor et al., 2005; Erb et al., 2016). This trend has resulted in a lower demand for grassland biomass, leading to the abandonment of grassland use and the disappearance of valuable semi-natural habitats (Janssen et al., 2016). Due to abandonment of agro-ecological activities, secondary succession occurs in semi-natural grasslands (MacDonald et al., 2000; Wehn et al., 2017). The process of secondary succession in grassland communities takes place in several stages and its rate depends on the type of community and the habitat conditions. Usually, a change in vegetation species composition occurs in the first years after the cessation of management. During this time, a greater proportion of tall grasses and herbs in the sward are observed (Pavlů et al., 2011; Pruchniewicz, 2017), and there is a decrease in plant species diversity (Öckinger et al., 2006). Invasive alien and native expansive plant species often appear and disrupt species richness and grassland vegetation structures (Bartha et al., 2014; Czarniecka-Wiera et al., 2019; Swacha et al., 2018). Later stages of succession are followed by the growth of woody plants, i.e. shrubs or trees (Hansson and Fogelfors, 2000). Secondary succession in this type of community typically results in a decline in species diversity, particularly impacting grassland specialist species, which poses a major challenge to their conservation (Gaujour et al., 2012). Depending on the conservation status of the grassland and the advancement of secondary succession, restoration of the habitat through reuse can sometimes be a long and complicated process (Galvánek and Lepš, 2008).

Exploring alternative methods for using hay can facilitate the preservation of existing semi-natural grasslands and restoration of abandoned grasslands, thus significantly enhancing their conservation status. Grassland biomass, which is increasingly considered a potential source of bioenergy, is gaining importance in the context of sustainable use of natural resources. Despite its promising economic potential in the bioenergy sector (Blokhina et al., 2011; Meyer et al., 2015), this type of lignocellulosic biomass is rarely used in agricultural biogas plants. However, in the future, it could play a significant role as a substrate for biogas and biomethane production (Meyer et al., 2018). Grassland biomass is classified as a lignocellulosic substrate from which second-generation, more sustainable and carbon-neutral biofuels are produced (Naik et al., 2010). This type of substrate mainly consists of biomass not associated with human food; hence, it does not compete with food production. The organic matter contained in the biomass, with the participation of microorganisms, is converted to biogas through methane fermentation (Sikora et al., 2017). The biogas potential value from kilogrammes of volatile solids (VS) of hay is often comparable with other substrates commonly used in agricultural biogas plants, such as manure, various types of organic agricultural waste or crops from energy crops (Kougias and Angelidaki, 2018). The analysis of factors related to the biology of grassland vegetation is rare in biogas research, but the most common topics include substrate pre-treatment (Rodriguez et al., 2017), selected grassland plant species in different developmental stages (later stage of plant development increases the amount of lignins in the biomass which results in a reduction of the biogas potential) (Seppälä et al., 2009; McEniry and O'Kiely, 2013; Dandikas et al., 2015), some types of grassland communities and the chemical properties of their biomass (Melts et al., 2013; Herrmann et al., 2014; Meserszmit et al., 2019). However, the effect of early secondary vegetation succession on the biogas potential of grassland biomass, particularly in relation to biomass from regularly and extensively mown grasslands, remains unexplored.

The process of secondary succession not only contributes to changes in grassland plant species composition and species richnes. The change in grassland species composition also affects the chemical composition of the biomass (Archimede et al., 2011; Khalsa et al., 2014; Tonn et al., 2010). Additionally, some chemical changes can affect methane fermentation and biogas yield (Meserszmit et al., 2019; Melts et al., 2014). Some plant functional groups contain higher contents of major fibres such as cellulose, hemicellulose and lignin (Melts et al., 2019; Melts and Heinsoo, 2015). Higher protein contents are associated with dicotyledonous species, which is particularly characteristic of the *Fabaceae* family of plants (Melts et al., 2019). Higher contents of selected macronutrients, e.g., calcium (Ca), nitrogen (N), and magnesium (Mg), are also found in specific plant functional groups (Pirhofer-Walzl et al., 2011; García-Ciudad et al., 1997; Tonn et al., 2010; Melts et al., 2014; Melts and Heinsoo, 2015). Changes in the proportions of the different functional groups of grassland plants, as well as individual grassland plant species, also affect the amount of live biomass produced and the biogas yield per area (Meserszmit et al., 2022; Popp et al., 2017).

This study assessed the biogas potential of grasslands that have been temporarily excluded from use compared with regularly mown grasslands. This study may help to protect the biodiversity of semi-natural grasslands threatened by abandonment. The use of grassland biomass for biogas production is also a key element in the development of a circular bio-economy. In this study, we specifically asked the following questions.

1. Does plant species composition due to different grassland management affect the chemical properties of biomass and its biogas production?
2. Which plant species has the greatest effect on area biogas yield (ABY, m^3 ha^{-1})?
3. Does the species richness in the harvested biomass affect biogas potential? What are the implications of these relationships for the conservation of semi-natural mesic grasslands?

To answer these questions, we conducted a study on Habitats Directive-protected semi-natural mesic grasslands (Council Directive, 1992) with two types of use: (1) mown and (2) abandoned, in Poland and Czechia. This research was conducted in a mountainous area in the Sudetes Mountains, where grassland abandonment problems are increasingly observed.

2. Materials and methods

2.1. Characteristics of the study sites

We sampled 60 sites in the Sudetes Mountains, including sites in Poland and Czechia. The altitude of the sample collection sites ranges from 350 to 950 m above sea level. We selected of 30 regularly mown meadows, hereafter referred to as 'mown', and 30 meadows that had been abandoned for 5–15 years, hereafter referred to as 'abandoned'. All the sites that had been abandoned were in the early stages of secondary succession, with low and infrequent occurrences of late succession (woody) species. The fieldwork was conducted in 2018–2019 during the peak of the growing season, just before the first mowing in late June and early July, which is the standard time for the first mowing of the studied meadow types in Central Europe and the phenological optimum (Preislerová et al., 2024). We focused on a group of mesic meadows comprising two habitat types as defined by the EU Habitats Directive (Council Directive 92/43/EEC): lowland (habitat code 6510) and mountain (habitat code 6520) hay meadows. Habitat type 6510 consists mainly of productive grasses such as *Arrhenatherum elatius* and *Dactylis glomerata*. It also contains important herbaceous species such as *Galium mollugo, Crepis biennis and Heracleum sphondylium*. Habitat type 6520, on the other hand, consists mainly of *Trisetum flavescens, Agrostis capillaris, Festuca rubra*, together with some dicotyledonous species such as *Hypericum maculatum, Cirsium helenioides* and mainly *Crepis mollis* (mainly *Crepis mollis* subsp. *succisifolia*). These habitats phytosociologically correspond to the Arrhenatherion (low- and medium-altitude hay meadow) and Triseto-Polygonion (mountain hay meadow) alliances, as indicated by Chytrý et al. (2020) and Kącki et al. (2020). These meadow types are usually mown once to a maximum of twice a year.

2.2. Sampling and data collection

A focal plot of 10 × 10 m was established at each site, considering the uniformity of topography and vegetation physiognomy. In the central part of each focal plot, a 1-m^2 square was marked out. Additionally, 0.4 × 0.4-m squares were placed in each of the four corners of the focal plot. These measures were aimed at collecting data according to the procedures described in Table 1.

Grass (*Poaceae*) and herb (dicotyledonous flowering plants) species identified in plots were categorised on the basis of their height: grasses – short, 0.18–0.47 m; grasses – medium, 0.48–0.77 m; grasses – tall, 0.78–1.07 m; herbs – short, 0.11–0.35 m; herbs – medium, 0.36–0.63 m; herbs – tall, 0.64–1.43 m. The mean height of each plant species was obtained from the database of Axmanová (2022). We established height thresholds at approximately equal intervals for plant groups. Sedges and rushes (*Cyperaceae/Juncaceae*) were excluded because of their negligible contribution (mean cover = <0.5%). We used the relative coverage for each plant group in the analyses.

Table 1
Description of the data collection process from the 1 m^2 and 0.64 m^2 measurement squares, including aspects such as, sampling methodology and biomass preparation.

	1 m^2	0.64 m^2
Number of sites	30 - mown; 30 - abandoned	28 - mown; 30 - abandoned
Sample size	One square frame (1m × 1m) was placed in the center of the focal plot.	Four square frames (0.4m × 0.4m) were placed evenly in the four corners of the focal plot.
Species recording and biomass collection	Recording of all vascular plant species and estimation of their percentage cover. Fresh biomass was cut at a height of 3 cm above the ground.	Clipping the shoots of individual plant species at ground level to measure their biomass weight. Fresh biomass was manually sorted on site into individual plant species.
Biomass preparation	The samples were air-dried at room temperature, then transported to the laboratory for chemical analysis and biogas potential assessment.	The samples were air-dried at room temperature, then dried in a forced-air circulation drying oven at 60 °C for 24 h. Finally, they were weighed using a laboratory scale with an accuracy of 0.01 g.
Obtained results based on biomass	Total solids (TS), Volatile solids (VS), Protein (%TS), Lipids (%TS) Cellulose (%TS), Hemicellulose (%TS), Lignin (%TS), Specific biogas yield (SBY, NL kg^{-1} VS), Species richness (number of species per 1m^2).	Biomass yield (t DM ha^{-1}), Species richness (number of species per 0.64 m^2).

2.3. Chemical analysis

The concentration of nitrogen was measured using the Kjeldahl method (AOAC, 1990), which was then multiplied by 6.25 to calculate the crude protein content. Neutral (NDF) and acid (ADF) detergent fibres were measured according to the methods of Goering and Van Soest (1970) and Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Ankom Technology). Acid detergent lignin (ADL) was obtained after digestion of ADF with 72% H_2SO_4 for 3 h. The hemicellulose content was measured by the difference between NDF and ADF. The cellulose content of the samples was measured by subtracting the ADL from the ADF, and the lignin content was equal to the ADL. Fat and ash contents were measured using the Wenden method (AOAC, 1984). Analyses of N, NDF, ADF, ADL, ash and lipids were performed in a certified laboratory of the Crop Research Institute in Chomutov.

2.4. Biogas production

Biomass samples collected from 1-m^2 plots underwent mechanical processing, which involved cutting and shredding the plant material into 2- to 3-cm pieces using electric shears. A Retsch ZM 200 Mill equipped with 0.5-cm trapezoidal mesh screens was used for the final shredding stage. The substrate was then thoroughly mixed to ensure homogeneity. A total of 60 test samples were prepared from 1-m^2 plots. Anaerobic digestion tests were performed in 1.1 dm^3 dark glass bioreactors based on the VDI 4630 (VDI 4630, 2006) standard. Each test was conducted in three replicates. Five grammes of air-dried fragmented sample was mixed with 0.05 dm^3 of autoclaved tap water and 0.45 dm^3 of anaerobic sludge. Anaerobic sludge was obtained from an anaerobic digestion bioreactor fed with sugar beet pulp (Süd-zucker Polska S.A., Strzelin, Poland) and maintained in 30-dm^3 laboratory bioreactors fed with 0.5 g VS/dm^3/day of air-dried grass biomass. The parameters of the anaerobic sludge were as follows: total solids (TS) 3.3%, ash 1.0%, VS 2.3% and pH = 7.8. No biomass sample was added to the control bioreactors. Air was removed from the headspace by flushing with nitrogen gas for 60 s. After air removal, the bioreactors were tightly sealed and connected to gas collection plastic bags (1L Tedlar bags with polypropylene fittings). The bioreactors were incubated for 21 days at 37 °C with periodic manual agitation (once a day) in a laboratory incubator (Incubator Shaker 25, New Brenswick). Biogas production was measured on days 1, 2, 4, 7, 11 and 21 using the water displacement method. Biogas measurements were recalculated under the following conditions: t = 25 °C, p = 100,000 Pa according to the ideal gas law. Total biogas production was calculated as the mean of the summed measurements for each replicate minus the average gas production in the control reactors. Specific biogas yield (SBY) was calculated as the quotient of total biogas production, sample mass and VS content. The TS and VS contents were tested using a weight drying method based on the PN-EN 12880 (PN-EN 12880, 2004) and PN-EN 12879 (PN-EN 12879, 2004) standards. ABY was calculated as the product of the dry matter (DM) yield, the percentage of VS in the biomass and the SBY obtained, expressed in cubic metres per hectare (m^3 ha^{-1}).

2.5. Statistical analyses

The mown and abandoned meadows were compared in terms of chemical parameters, SBY, ABY, species richness, biomass yield and functional groups of plants considering their height (the percentage of each functional group was used for the analyses and its effect on the individual parameters was examined). These parameters were analysed using one-way analysis of variance (ANOVA) and the Mann–Whitney *U* test depending on the distribution of the data. The choice of the appropriate statistical test is explained below each figure and table. Differences were considered statistically significant at a level of $p < 0.05$. Statistical analyses were performed using the Statistica software version 13.3. The Shapiro–Wilk test and Levene's test were used to test for normality of data distribution and homogeneity of variances, respectively.

Spearman's rank correlation coefficient was used to investigate the relationships between biomass chemical parameters, species richness and functional plant groups on SBY. Additionally, the relationships of different functional plant groups on ABY and biomass yield were analysed. Results were considered statistically significant at $p < 0.05$.

Principal component analysis (PCA) using Canoco 5 software (Šmilauer and Lepš, 2014) was used to assess the correlation of species composition with supplementary variables, namely, ABY and species richness. The aim of the analysis was to determine how the proportion of each plant species in the biomass correlated with ABY and how this variable was related to the species richness variable. Species that appeared only once or twice in the dataset were excluded from the analysis. The aim of this procedure was to minimise the effect of rare species on the results of the analysis. The analysis was configured to highlight the top 30 species with the strongest associations with the supplementary variables under investigation. The results were visualised using biplots illustrating the correlation of species composition with the two variables. Additionally, the t-value biplot method was used to investigate the effect of plant species composition on ABY. The t-value biplot allows for the relationships between individual plant species and ABY. The t-value biplot is utilized to estimate the t-values of regression coefficients in a multiple regression analysis, with species acting as the response variable and supplemntry factor as predictor. In this biplot, each plant species is represented by an arrow. Around ABY variable, a circle is drawn (referred to as Van Dobben circles), sized to match the length of the arrow representing that variable. If the t-values of the regression coefficients exceed 2 in absolute value, the biplot indicates a statistically significant positive or negative relationship ($p < 0.05$) between species plants and the supplementry variable. Species with arrows terminating within Van Dobben circles show significant relationships with these variables, denoted by red circles for positive relationships and blue for negative ones. The shorter a species' arrow within the circle, the more substantial the influence of the supplementry variable.

3. Results

The study compared two groups of grasslands: mown and abandoned, focusing on the analysis of different parameters (mean values) (Table 2). The TS and VS contents were found to be 92.79% and 93.10% for mown grasslands and 92.92% and 93.41% for abandoned grasslands. For protein (%TS) and lipids (%TS), the values were 9.01% and 2.87% for mown grasslands and 9.67% and 2.89% for abandoned grasslands. For cellulose, hemicellulose and lignin, the results were 25.28%, 19.24% and 12.12% for mown grasslands and 25.61%, 19.56% and 12.32% for abandoned grasslands. The SBY and ABY were 462 NL kg^{-1} VS and 1649 m^3 ha^{-1} for mown grasslands and 470 NL kg^{-1} VS and 1564 m^3 ha^{-1} for abandoned grasslands. The dry biomass yield per hectare was 3.77 t DM ha^{-1} for mown grasslands and 3.55 t DM ha^{-1} for abandoned grasslands. Most of the parameters tested showed no significant statistical differences between the two groups of grasslands, except for species richness, where the mown meadows showed a significantly higher number of species per 1 m^2 (24 species) than the abandoned grasslands (17 species) ($p < 0.001$).

When analysing the percentage cover of each functional plant group (Fig. 1), the following results were observed for mown meadows: grasses – short 13.05%, grasses – medium 32.52%, grasses – tall 10.85%, herbs – short 21.14%, herbs – medium 12.97% and herbs – tall 9.12%. For abandoned grasslands, the results were as follows: grasses – short 14.89%, grasses – medium 29.47%, grasses – tall 16.07%, herbs – short 5.29%, herbs – medium 13.16% and herbs – tall 20.45%. Statistically significant differences between the two meadow groups were observed for the cover of herbs – short ($p < 0.001$) and herbs – tall ($p < 0.01$).

Table 2
Mean values (mean ± SD) of chemical composition, SBY, ABY, biomass yield and species richness. P-values = corresponding probability value. One-way ANOVA[A] test or Mann-Whitney *U* test[M], respectively, was used to compare the two groups. Significant p-values ($p < 0.05$) are highlighted in bold.

Characteristics	Mown	Abandoned	p-value
TS	92.79 ± 0.48	92.92 ± 0.48	0.292[A]
VS (%TS)	93.10 ± 1.33	93.41 ± 1.49	0.387[A]
Protein (%TS)	9.01 ± 1.42	9.67 ± 1.73	0.114[A]
Lipids (%TS)	2.87 ± 0.61	2.89 ± 0.59	0.929[A]
Celulose (%TS)	25.28 ± 5.22	25.61 ± 4.30	0.791[A]
Hemicelulose (%TS)	19.24 ± 4.22	19.56 ± 5.66	0.530[M]
Lignin (%TS)	12.12 ± 2.18	12.32 ± 2.23	0.716[A]
SBY (NL kg^{-1} VS)	462 ± 74	470 ± 83	0.706[A]
ABY (m^3 ha^{-1})	1649 ± 623	1564 ± 443	0.222[M]
Biomass yield (t DM ha^{-1})	3.77 ± 1.27	3.55 ± 0.85	0.091[M]
Species richness (number per 1m^2)	24 ± 6	17 ± 6	< 0.001[A]

Abbreviations: TS, total solid; VS, volatile solid; DM, dry matter; SBY, specific biogas yield; ABY, area biogas yield.

Table 3 presents the relationships between the chemical properties of the biomass and SBY and species richness. The analysis results showed significant relationships between SBY and lipid ($r = 0.390$, $p = 0.033$), cellulose ($r = 0.458$, $p = 0.011$) and lignin ($r = -0.516$, $p = 0.004$) for mown grasslands and protein ($r = -0.387$, $p = 0.035$), lipids ($r = 0.383$, $p = 0.037$), cellulose ($r = 0.383$, $p = 0.037$) and lignin ($r = -0.637$, $p < 0.001$) for abandoned grasslands. For mown grasslands, no significant relationships were found between species richness and biomass parameters. However, for abandoned meadows, significant relationships were found only between species richness and hemicellulose ($r = -0.520$, $p = 0.003$). No significant relationships were found for the other parameters.

Table 4 presents the relationships between the chemical properties of the biomass and the different plant groups. Significant relationships were found for mowed meadows between grasses – medium and hemicellulose ($r = 0.402$, $p < 0.05$), herbs – short and protein ($r = 0.365$, $p < 0.05$) and cellulose ($r = -0.442$, $p < 0.01$), herbs – medium and protein ($r = 0.378$, $p < 0.05$), cellulose ($r = -0.456$, $p < 0.01$) and lignin ($r = 0.529$, $p < 0.01$). Conversely, for abandoned grasslands, significant relationships were found between grasses – short and cellulose ($r = -0.425$, $p < 0.05$), herbs – short and hemicellulose ($r = -0.587$, $p < 0.001$), herbs – medium and cellulose ($r = -0.372$, $p < 0.05$), hemicellulose ($r = -0.519$, $p = 0.01$) and lignin ($r = 0.550$, $p < 0.01$), herbs – tall and hemicellulose ($r = -0.457$, $p < 0.01$).

Table 5 presents the relationships between ABY and biomass yield and the biomass contribution of the different plant functional groups. The analysis results showed significant relationships for mown grasslands between grasses – tall and ABY ($r = 0.501$, $p = 0.007$) and biomass yield ($r = 0.436$, $p = 0.020$). Conversely, for abandoned grasslands, significant relationships were found between grasses – short and ABY ($r = -0.539$, $p = 0.002$) and biomass yield ($r = -0.539$, $p = 0.002$) and between biomass yield and grasses – tall ($r = 0.462$, $p = 0.010$) and herbs – tall ($r = 0.521$, $p = 0.003$).

PCA shows relationships between fractional biomass of species and two supplementary variables (Fig. 2): ABY and species richness. The arrangement of the vectors representing ABY and species richness indicates their independence or near independence. Biplots of t-values showed statistically significant positive and negative correlations between the proportion of individual species in biomass and ABY. In the case of mown meadows (Fig. 2, M1), ABY was positively correlated with

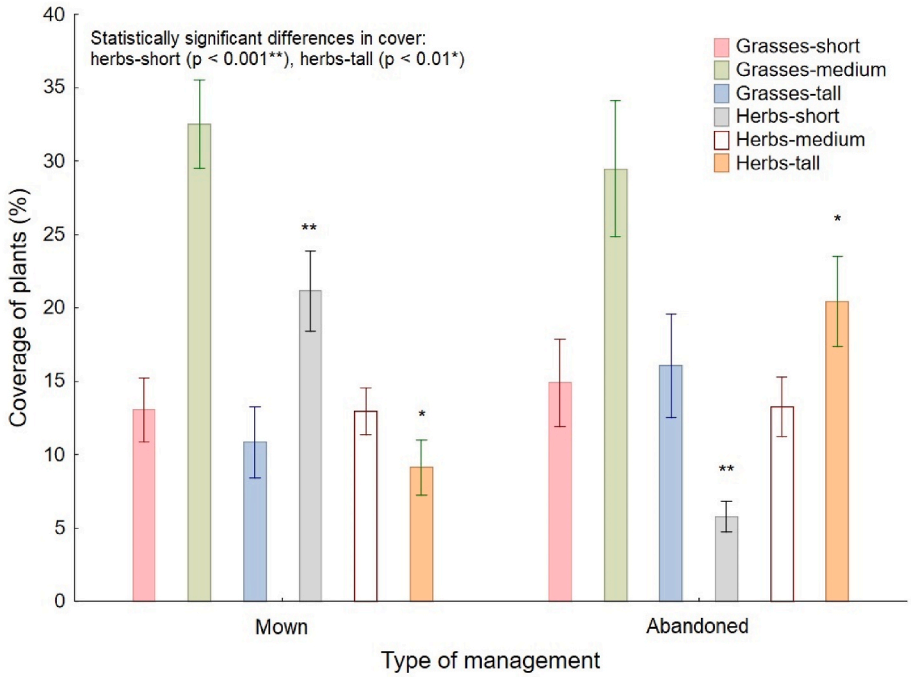

Fig. 1. Coverage by individual plant groups in mown and abandoned meadows. Vertical bars show the standard deviation of the mean value. Mann-Whitney *U* test was used to compare the two types of management. Statistical significance is indicated with asterisks.

Table 3
The relationships between chemical properties, SBY and species richness for two groups (mown and abandoned meadows). Significant p-values ($p < 0.05$) are highlighted in bold. P-values = corresponding probability value, r = correlation coefficient.

	Mown				Abandoned			
	SBY		Species richness		SBY		Species richness	
	r	p-value	r	p-value	r	p-value	r	p-value
Protein	-0.174	0.357	0.100	0.598	**-0.387**	**0.035**	-0.106	0.578
Lipids	**0.390**	**0.033**	0.179	0.345	**0.383**	**0.037**	0.154	0.415
Celulose	**0.458**	**0.011**	-0.210	0.266	**0.383**	**0.037**	-0.113	0.552
Hemicelulose	-0.207	0.273	-0.188	0.320	0.229	0.223	**-0.520**	**0.003**
Lignin	**-0.516**	**0.004**	0.093	0.623	**-0.637**	**<0.001**	0.052	0.786
SBY	-	-	0.062	0.743	-	-	-0.137	0.471

Abbreviations: SBY, specific biogas yield.

seven grass and six herb species, including the most correlated grasses (Fig. 2, M2) *Arrhenatherum elatius* (grasses – tall), *Trisetum flavescens* (grasses – medium) and *Festuca pratensis* (grasses – medium) and negatively correlated with five grass and two herb species including the most correlated grasses *Agrostis capillaris* (grasses – short) and *Festuca rubra* (grasses – medium). Conversely, on abandoned grassland, the increase in ABY was positively correlated with 11 herb and 5 grass species (Fig. 2, A1), including the most correlated grasses (Fig. 2, A2) *A. elatius* (grasses – tall) and *Elymus repens* (grasses – tall) and the herbs *Galium aparine* (herbs – tall), *Urtica diocia* (herbs – tall) and *Chaerophyllum aromaticum* (herbs – tall) and negatively correlated with 5 grass and 6 herb species, including the most correlated grasses *Holcus. mollis* (grasses – short) and *Agrostis capillaris* (grasses – short) and the herb *Hypericum maculatum* (herbs – medium).

4. Discussion

Analysis of the plant cover data revealed marked differences in the species composition of the communities studied. We found that abandoned meadows had a lower proportion of short herbs and a higher proportion of tall herbs than regularly mown meadows. Additionally, abandoned meadows had a lower plant species richness than mown meadows. These differences can be interpreted as progressive processes of secondary succession in semi-natural grassland communities (Pavlů et al., 2011; Pruchniewicz, 2017). Despite the differences in species composition between the two meadow management, we did not observe significant changes in the chemical properties of the biomass. This may be due to the high species diversity of the biomass analysed in this study. The type of meadow communities included in our study can be characterised by various variabilities in the proportions of different plant groups or species (Kącki et al., 2020). This variability can be influenced by many factors, such as the abundance of soil nutrients, pH, moisture content or land use (Pavlů et al., 2011; Merunková and Chytrý, 2012; Swacha et al., 2023).

4.1. Specific biogas yield

Considering only one factor, the type of use of the grasslands studied, we found no significant differences in their SBY. The SBY results were similar to those obtained in other studies reporting the biogas potential of grassland biomass in Europe (Prochnow et al., 2009; Meserszmit et al., 2019). Although SBY did not differ significantly between the two study management, we observed significant results when the biomass from each grassland management was chemically and biologically

Table 4
The correlation coefficient between chemical properties, species richness and cover (%) by grasses and herbs, categorised by mean height for the two groups (mown and abandoned meadows). Statistical significance of the correlations is indicated with asterisks: * indicates significance at $p < 0.05$, ** at $p < 0.01$, and *** at $p < 0.001$; these are highlighted in bold.

	Mown								Abandoned							
	Grasses – short (%)	Grasses – medium (%)	Grasses – tall (%)	Herbs – short (%)	Herbs – medium (%)	Herbs – tall (%)	Grasses (total) (%)	Herbs (total) (%)	Grasses – short (%)	Grasses – medium (%)	Grasses – tall (%)	Herbs – short (%)	Herbs – medium (%)	Herbs – tall (%)	Grasses (total) (%)	Herbs (total) (%)
Protein	−0.236	0.104	−0.261	**0.365***	**0.378***	−0.119	**−0.516****	**0.522****	0.152	0.153	−0.325	0.299	0.307	−0.232	−0.087	0.082
Lipids	−0.273	0.034	0.239	−0.075	−0.018	0.135	0.008	−0.042	−0.277	0.206	−0.138	0.180	−0.171	−0.184	0.139	−0.158
Celulose	−0.032	0.045	0.358	**−0.442****	**−0.456****	0.153	**0.610*****	**−0.618*****	**−0.425***	0.031	0.344	−0.240	**−0.372***	0.147	0.201	−0.158
Hemicellulose	0.310	**0.402***	−0.108	−0.228	0.109	−0.245	**0.493****	**−0.476****	0.137	0.337	0.047	**−0.587*****	**−0.519****	**−0.457****	**0.826*****	**−0.827*****
Lignin	−0.086	−0.249	−0.076	0.302	**0.529****	0.074	**−0.651*****	**0.639*****	0.213	−0.205	−0.178	0.300	**0.550****	0.130	**−0.492****	**0.485****

analysed in detail. One of the most important components of grassland biomass are lignocellulosic fibres, i.e. cellulose, hemicellulose and lignin. For the regularly mown and abandoned meadows, the lignin content of the biomass had a negative effect on SBY. The lignin polymer is difficult to biodegrade under anaerobic conditions because it limits the access of microorganisms to structural carbohydrates. This explains the negative correlation of lignin content with SBY, which is consistent with many studies (Rath et al., 2013; Dandikas et al., 2014, 2015; Thomsen et al., 2014). These results indicate that lignin content tends to be higher in herbs than in grasses (Jarchow et al., 2012; Bovolenta et al., 2008, Melts and Heinsoo, 2015). This observation is consistent with the results of our study, which confirmed that biomass with a higher proportion of herbs had a higher lignin content. Many herbaceous species develop woody stems at later stages of maturity or other structures, which require more lignin for structural support than grasses, e.g. *Chaerophyllum* sp., *Angelica* sp., *Anthriscus* sp., *Aegopodium* sp. Additionally, we investigated the relationship between the functional groups of plants, classified according to their average height and their chemical properties. The results showed high correlations between the lignin content of the biomass and the cover of herbs – medium plants in mown and abandoned meadows. It can be presumed that this is related to the faster attainment of a more advanced stage of maturity and lignification in this group of herbs. Alternatively, it may be the specific chemical characteristics of selected plant species (Bickoff et al., 1972; Dandikas et al., 2015) from this height range. For structural carbohydrates, SBY was expected to correlate positively with hemicellulose because its amorphous structure facilitates the hydrolysis process (Khan and Ahring, 2021). However, similar correlations were not found in our study. Cellulose fibres, in contrast, are composed of glucose, which occurs in both amorphous and highly crystalline forms (Carrere et al., 2016). A higher crystalline cellulose content in the substrate can negatively affect biodegradation and SBY (Triolo et al., 2012). In our study, we found a positive effect of cellulose on SBY in both mown and abandoned meadows. Studies by other authors indicate that grasses have higher cellulose content (Armstrong et al., 1950) and higher biogas potential (Melts et al., 2014) than herbs and that higher cellulose content in biomass typically leads to lower lignin content (Melts et al., 2014; Oleszek et al., 2014). As shown in our study, higher herbaceous cover in grasslands, especially in mown grassland, resulted in lower cellulose content in biomass, which can theoretically have a negative effect on SBY. Our results showed significant positive correlations between the lipid content of biomass and SBY in both mown and abandoned meadows. Compared with other substrates commonly used in agricultural biogas plants (Wang et al., 2014; Yong et al., 2015), lignocellulosic biomass has a low lipid content. Organic compounds such as lipids have a high energy content, are biodegradable under anaerobic conditions and have a higher biogas production potential than proteins and carbohydrates (Raposo et al., 2020). However, we could not explain in detail the reasons for the observed relationships based on the data obtained for the coverage of selected plant groups. Differences in the lipid content of the biomass may be related to the maturity of the plants, as the lipid content usually decreases with increasing developmental stage (Herrmann et al., 2014; Rodriguez et al., 2017) or specific characteristics of the selected species (Dandikas et al., 2015). In our study, we also observed a negative effect of protein on SBY from biomass harvested from abandoned grasslands. Protein-rich substrates usually have a high biogas potential (Moestedt et al., 2016). However, the degradation of amino acids releases ammonia. When present in large quantities, ammonia can be harmful to various microorganisms and lead to reduced biogas production (Chen et al., 2008). Our results do not clearly identify a factor related to vegetation composition and protein content that may have negatively affected SBY. In the abandoned meadow, no significant correlations were observed between the protein content of the biomass and the specific plant group. However, such positive correlations did occur for the biomass of mown meadows for the herbs – short and herbs – medium plant groups. These two herb height ranges included species

Table 5
The relationships between grasses and herbs, categorised by mean height for the two groups (mown and abandoned meadows), ABY (area biogas yield) and biomas yield. Significant p-values ($p < 0.05$) are highlighted in bold. P-values = corresponding probability value, r = correlation coefficient.

	Mown				Abandoned			
	ABY		Biomas yield		ABY		Biomas yield	
	r	p-value	r	p-value	r	p-value	r	p-value
Grasses – short	−0.364	0.057	−0.335	0.081	**−0.538**	**0.002**	**−0.539**	**0.002**
Grasses – medium	0.241	0.217	0.128	0.516	−0.093	0.624	−0.197	0.296
Grasses – tall	**0.501**	**0.007**	**0.436**	**0.020**	0.320	0.085	**0.462**	**0.010**
Herbs – short	−0.288	0.137	−0.204	0.299	−0.118	0.535	0.132	0.486
Herbs – medium	−0.253	0.194	−0.110	0.577	−0.221	0.241	−0.130	0.495
Herbs – tall	0.279	0.151	0.264	0.174	0.322	0.083	**0.521**	**0.003**

from the *Fabaceae* family, which in our study included the genus *Lathyrus* sp., *Lotus* sp., *Trifolium* sp. and *Vicia* sp. This plant family is characterised by a higher protein content in biomass compared with other dicotyledonous plants (Melts et al., 2019) and was found in abandoned grasslands. Therefore, this may have been a factor influencing SBY from the abandoned grassland biomass in our study.

4.2. Area biogas yield

The effect of individual plant functional groups, categorised by height and individual species on ABY was investigated in our analysis. Grassland biomass yield is recognised as a key factor influencing ABY, as reported in several studies that have shown that as biomass yield increases, ABY also increases (Khalsa et al., 2014; Melts et al., 2013; Meserszmit et al., 2019). We found that in mown meadows, ABY was significantly correlated with grasses – tall, which was associated with their higher biomass yield. In abandoned meadows, the grasses – short group of grasses played a key role and was negatively correlated with biomass yield. In the remainder of this study, we focused on investigating how the different plant species found in mesic grasslands, depending on their use, interacted with ABY. To date, there has been a lack of studies with such a detailed approach to ABY assessment, considering the analysis of multi-species plant biomass from grasslands. In our study, we combined two main analytical methods to determine which species are most important for ABY. This approach allowed us to more fully capture the relationship between plant species composition and ABY. In the mown grassland, we identified two groups of species that had a negative or positive effect on ABY. In the first group, a total of seven species were negatively correlated with ABY, of which 43% were short and medium grass species and 43% were short and medium herb species. Most of these species, especially those strongly correlated with ABY (*A. capilaris* and *F. rubra*), are associated with plant communities that prefer oligotrophic to mesotrophic soils (Tichý et al., 2023; Axmanová, 2022) and are often found at higher altitudes (Preislerová et al., 2024). These species tended to be characterised by short to medium height, resulting in lower biomass yield compared with species with higher growth potential. In the second group, 13 species were positively correlated with ABY, of which 46% were medium and tall grass species and 38% were medium and tall herb species. Most of these species, especially those strongly correlated with ABY (*A. elatius*, *F. pratenis* and *T. flavescens*), are mainly associated with plant communities occurring on mesotrophic to eutrophic soils (Tichý et al., 2023; Axmanová, 2022). Most of these species are characterised by medium to tall height, which influences increased biomass yield and ABY values. In contrast, abandoned meadows differed in ABY-related species composition compared with mown meadows. A total of 11 species were negatively correlated with ABY, of which 36% were short and medium grass species and 55% were short and medium herb species. Most of these species, especially those strongly correlated with ABY (*H. mollis*, *A. capillaris* and *H. maculatum*), were plants associated with plant communities that prefer lower nutrient soils ranging from oligotrophic to mesotrophic (Tichý et al., 2023; Axmanová, 2022). Most of these species were characterised by heights classified as 'short' or 'medium', which contributed to lower biomass yield and lower ABY values. Conversely, 16 species were positively correlated with ABY, of which 31% were medium and tall grass species and 56% were medium and tall herb species. In this case, a higher percentage of the species reached heights defined as 'tall' according to the criteria used, resulting in increased biomass yield. Most of these species, especially those strongly correlated with ABY (*G. aparine*, *U. dioica*, *C. aromaticum*, *A. elatius* and *E. repens*), are mainly associated with plant communities occurring on nutrient-rich mesotrophic to eutrophic soils (Tichý et al., 2023; Axmanová, 2022). The change in the species composition of abandoned meadows and the higher proportion of tall herbs compared with mowed meadows is mostly due to the lack of mowing and the deposition of plant biomass, which causes a change in the soil nutrient content. Abandoned grasslands can have high soil nitrogen levels due to the accumulation and mineralisation of organic matter (Pruchniewicz, 2017), which favours the growth of some dicotyledonous plant species that prefer this type of habitat (Pavlů et al., 2011). In abandoned grasslands, where expansive tall grasses are absent, broad-leaved herbs usually dominate over other plants (Huhta et al., 2001).

4.3. Species richness and biogas potential – implications for grassland conservation

Mesic grasslands are among some of the most species-rich semi-natural ecosystems in Europe (Chytrý et al., 2015), and maintaining their high biodiversity is a key element of their conservation. Additionally, research indicates that higher plant species diversity in grasslands may influence their better resilience during extreme weather events (Isbell et al., 2015). Active conservation of semi-natural grasslands involves regular and extensive mowing and hay harvesting practices, up to twice a year after the second half of June in Central Europe. Integrating grassland biodiversity conservation with biogas potential opens new perspectives for sustainable organic waste management and renewable energy production. In our study, most of the analysed parameters did not show a significant correlation between plant species richness and biomass quality or SBY. The exception was the hemicellulose content of the biomass, which showed a negative correlation with both plant species richness and the presence of herbs. We noted that an increase in the proportion of particularly short herbs in the biomass composition had a positive effect on species richness. This increase in species richness was associated with a decrease in the hemicellulose content; however, this did not negatively affect SBY. Herbs growing in the lower vegetation layers are a key element of species diversity in semi-natural grasslands (Swacha et al., 2023), and their presence is an important indicator for assessing the species richness of grassland communities. Studies conducted on grasslands in different European countries have shown that species richness can influence various biomass quality parameters (Hofmann and Isselstein, 2005; French, 2017; Hallikma et al., 2023). Therefore, it appears that the relationship between biomass quality and plant species richness may be complex and dependent on various factors, such as environmental conditions,

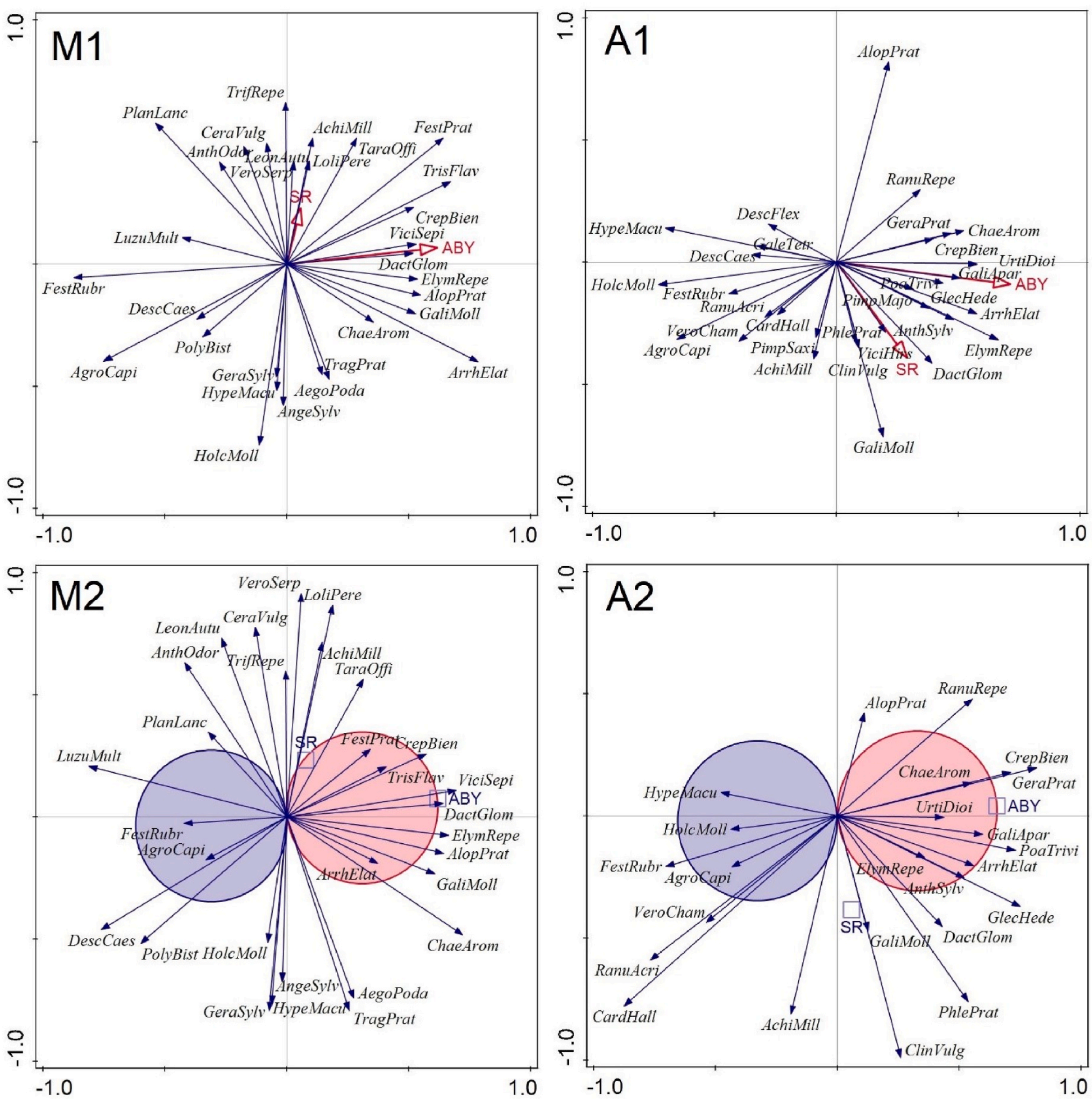

Fig. 2. Principal Component Analysis (PCA) focused on assessing the impact of individual plant species in meadow biomass. It includes two supplementary variables: area biogas yield (ABY) and species richness (SR). The PCA plot showcases the 30 best-fitted species for mown (M1) and abandoned (A1) meadows. For mown meadows, Axis 1 (horizontal) accounts for 24.98% of the total data variation, while Axis 2 (vertical) captures 37.09%. In abandoned meadows, Axis 1 explains 18.48% and Axis 2 explains 31.27% of the variation. Additionally, PCA plots for mown (M2) and abandoned (A2) meadows include t-values and Van Dobben circles, illustrating the relationships between specific plant species in the biomass and the ABY supplementary variable. The supplementary variables are indicated by blue squares in the plot. Red circles indicate a positive correlation, whereas blue circles indicate a negative correlation with ABY.
Species abbreviations: AchiMill – *Achillea millefolium*; AegoPoda – *Aegopodium podagraria*; AgroCapi – *Agrostis capillaris*; AlopPrat – *Alopecurus pratensis*; AngeSylv – *Angelica sylvestris*; AnthOdor – *Anthoxanthum odoratum*; AnthSylv – *Anthriscus sylvestris*; ArrhElat – *Arrhenatherum elatius*; CardHall – *Cardaminopsis halleri*; CeraVulg – *Cerastium vulgatum*; ChaeArom – *Chaerophyllum aromaticum*; ClinoVulg – *Clinopodium vulgare*; CrepBien – *Crepis biennis*; DactGlom – *Dactylis glomerata*; DescCaes – *Deschampsia caespitosa*; DescFlex – *Deschampsia flexuosa*; ElymRepe – *Elymus repens*; FestPrat – *Festuca pratensis*; FestRubr – *Festuca rubra*; GaleTetr – *Galeopis tetrahit*; GaliApar – *Galium aparine*; GaliMoll – *Galium mollugo*; GeraPrat – *Geranium pratense*; GeraSylv – *Geranium sylvaticum*; GlecHede – *Glechoma hederacea*; HolcMoll – *Holcus mollis*; HypeMacu – *Hypericum maculatum*; LeonAutu – *Leontodon autumnalis*; LoliPere – *Lolium perenne*; LuzuMulti – *Luzula multiflora*; PhlePrat – *Phleum pratense*; PimpMajo – *Pimpinella major*; PimpSaxi - *Pimpinella saxifraga*; PlanLanc – *Plantago lanceolata*; PoaTrivi – *Poa trivialis*; PolyBist – *Polygonum bistorta*; RanuAcri – *Ranunculus acris*; RanuRepe – *Ranunculus repens*; TaraOffi – *Taraxacum officinale*; TragPrat – *Tragopogon pratensis*; TrifRepe – *Trifolium repens*; TrisFlav – *Trisetum flavescens*; UrtiDioi – *Urtica dioica*; VeroCham – *Veronica chamaedrys*; VeroSerp – *Veronica serpyllifolia*; ViciHirs – *Vicia hirsuta*; ViciSepi – *Vicia sepium*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grassland management practices or the specificity of the local flora. Another aspect of our study was to test a previously unexplored question: is plant species richness related to ABY? A factor reducing species richness in abandoned meadows may have been the stage of secondary succession. The results of our study did not show that species richness influenced the value of ABY. Despite the significant difference in species richness between mown and abandoned meadows, we found no significant differences in ABY results between them. Changes in species composition in abandoned meadows in the early stages of secondary succession are usually reversible, and after appropriate treatments such as mowing have been implemented for several years, the meadow community can return to a suitable state of conservation (Gaisler et al., 2019). The restoration of grassland use leads to an increase in species richness and diversity, with grasses starting to displace tall herbs in the sward again, becoming an important biomass component. From a methane fermentation perspective, grasses are more biodegradable than herbs, mainly because of their lower lignin content. The incorporation of biomass from semi-natural grasslands can be an important part of a strategy to manage lignocellulosic waste in agriculture through its use in agricultural biogas plants. The use of biomass for biogas production not only contributes to a significant reduction in waste but also provides an opportunity to generate renewable energy sources and can support grassland biodiversity. This, in turn, promotes the protection of semi-natural ecosystems, which is an important step towards achieving the global sustainable development goals and building a circular economy.

5. Conclusions

The range of the quantitative and qualitative characteristics of biomass harvested from both abandoned and regularly mown grassland indicate that it is a suitable substrate for biogas production in agricultural biogas plants. The ability of lignocellulosic biomass, which is rich in plant species, to produce biogas is determined by the complex interactions of different chemical components. On the basis of our study, we can distinguish between lignin and cellulose, which, as chemical factors, had the greatest effect on SBY in mown and abandoned grasslands. An increase in the proportion of herbs in the biomass may hinder biodegradation during methane fermentation and reduce the biogas potential of the biomass due to higher lignin contents. The ABY-increasing and ABY-decreasing species identified in this study are important elements in understanding and assessing the suitability of semi-natural grassland habitats for providing a substrate for biogas production. In mown grasslands, ABY was most positively correlated with grass species (*A. elatius*, *T. flavescens* and *F. pratensis*). In abandoned grasslands, ABY was most positively correlated with herbaceous species (*G. aparine*, *U. dioica* and *C. aromaticum*) and grasses (*A. elatius* and *E. repens*). All plant species that contributed most to higher ABY had medium to tall heights, with the majority being tall. We did not find that species richness had a significant effect on SBY and ABY. However, secondary succession in abandoned grasslands reduces species richness, and changes in species composition and a large increase in the proportion of tall herbs in the biomass of some communities may adversely affect the biogas production process. Restoring the use of abandoned grasslands is crucial for the conservation of semi-natural grasslands, especially those that may be included in the Natura 2000 habitat network in the future. Such grassland management is essential to maintain the biodiversity and ecological functions of these ecosystems, which are important for nature conservation. Additionally, extensive grassland management enables the sustainable production of biomass for biogas production, which supports ecosystem conservation and provides practical solutions for waste management.

CRediT authorship contribution statement

Mateusz Meserszmit: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Grzegorz Swacha:** Writing – review & editing, Methodology, Investigation. **Lenka Pavlů:** Writing – review & editing, Investigation. **Vilém Pavlů:** Writing – review & editing, Investigation. **Jan Titěra:** Writing – review & editing, Investigation. **Sławomir Jabłoński:** Writing – review & editing, Methodology, Investigation. **Marcin Łukaszewicz:** Writing – review & editing, Investigation. **Zygmunt Kącki:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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