GCB Bioenergy

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Global Change Biology Bioenergy
BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY

RESEARCH ARTICLE OPEN ACCESS

Prediction of the Methane Yield From Extensively Managed, Flower-Rich Fen Grassland Based on NIRS Data

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Received: 17 January 2025 | **Revised:** 14 April 2025 | **Accepted:** 19 April 2025

Funding: This work was funded by the German Federal Ministry of Food and Agriculture (BMEL) via the Agency for Renewable Resources (FNR; project number: 22012018).

Keywords: anaerobic digestion | biogas | biomass valorization | ecosystem | flower-rich grassland | methane yield | multiple linear regression | near infrared spectroscopy

ABSTRACT

In many regions of Europe, biogas production is an integral part of farming to generate methane as a sustainable and versatile renewable energy carrier. Besides providing feedstock for ruminants and energy production, grasslands support multiple beneficial ecosystem services, namely diverse flora and habitats that serve as resources for pollinators. The cost-effective utilization of grassland biomass is mainly determined by the biomass quality, which is highly variable and dependent on the management intensities. Besides chemical analyses, biogas models are usually applied to predict the biogas yield of a specific biomass type and quality. However, available models do not apply to mixed grass stands as they primarily refer to individual grass species and/or are just based on single parameters such as lignin. In this work, we evaluated flower-rich extensive fen grassland for its biogas yield using a newly created model based on common chemical parameters. Therefore, flower-rich biomass from a cultivation experiment ($n=48$) was analyzed for its biomass yield (average 9.43 ± 1.26 $t_{VS} \times ha^{-1}$), chemical composition by wet chemical analysis and near-infrared spectroscopy (NIRS), specific methane yield (SMY) potential via batch tests, and methane hectare yield (1505.62 ± 282.86 $m^3_N \times ha^{-1}$). In the results obtained, we found flower-rich grassland biomass characterized by high fiber ($30.1\% \pm 1.7\%$) and high protein content ($11.3\% \pm 1.3\%$) with reliable determinability of chemical composition by NIRS. The most important predictors on SMY assessed by multiple linear regression were crude ash (XA), crude protein (XP), amylase neutral detergent fiber ($aNDF_{vs}$), acid detergent fiber (ADF_{vs}), and enzyme-resistant organic matter (EROM). We conclude that extensive flower-rich grassland biomass composed of diverse species and different growth and ripening stages provides a suitable feedstock for biogas production despite late harvest dates. NIRS proved capable of analyzing the biomass quality of flower-rich grassland and thus contributes to optimizing grassland management strategies and provision of demand-driven feedstock qualities.

Abbreviations: ADF_{vs}, acid detergent fiber (organic matter ash-free); ADL, acid-detergent lignin fraction; $aNDF_{vs}$, amylase neutral detergent fiber (organic matter ash-free, amylase-digested); EROM, enzyme-resistant organic matter; ES, ecosystem services; EU, European Union; FM, fresh matter; Fru, fructose; Gb, gas building (Hohenheimer feed value test); LOOCV, leave-one-out cross-validation; MHY, methane hectare yield; N, nitrogen; NA, not analyzed; Nfe, nitrogen-free extracts; NIRS, near-infrared spectroscopy; PCA, principal component analysis; PLSR, partial least square regression; R, correlation coefficient; R^2, adjusted coefficient of determination; RMSE, the root mean square error; SBY, specific biogas yield determined by batch anaerobic digestion test; SD, standard deviation; SEC, standard errors of calibration; SECV, standard errors of cross-validation; SEP, standard errors of prediction; SMY, specific methane yield determined by batch anaerobic digestion test; TS, total solids; TSSMY, total solids specific methane yield; VS, volatile solids; XA, crude ash; XF, crude fiber; XL, crude lipid; XP, crude protein; XZ, crude sugar; Y_B, predicted biogas yield; Y_M, predicted methane yield.

1 | Introduction

In many regions of Europe, biogas production is an integral part of farming to generate methane as a sustainable and versatile renewable energy carrier. Besides feedstock supply for ruminants and energy production, extensive fen grasslands provide various beneficial ecosystem services (ES). The ES ranges from cultural services (e.g., tourism, education) via supporting (e.g., soil formation) and regulating services (pollination, carbon sequestration) (Fu et al. 2017; Petermann and Buzhdygan 2021). While some ES are not closely linked to land-use intensity, such as groundwater formation (Archer et al. 2016; Behrendt et al. 2013), the provisioning of most ES heavily depends on the magnitude of land-use intensity.

Moderately managed grasslands are global biodiversity hotspots (Feurdean et al. 2018), thus supporting pollination and crop production. Higher management intensities (e.g., fertilization, mowing or grazing) increase the productivity of food and feedstock supply but decrease ES and plant biodiversity. Despite their high value, many grassland systems have suffered from intense management or conversion into arable land over the past few decades. Once a grassland is disturbed, the biodiversity may not be restored within decades (Kaiser and Ahlborn 2021). This degradation and loss of biodiversity impact ecosystems' functioning and capacity to provide essential goods and services to society. Thus, alternatives for the intensive management and use of grasslands need to be evaluated.

Permanent grasslands occupy 47.9 million ha in the European Union (EU) and 4.7 million ha in Germany. This accounts for 30.5% of the utilized agricultural area in the EU and 28.5% in Germany (Eurostat 2020), respectively. The bioeconomic strategy for the energy security of the EU aims to establish a circular economy (European Commission: Directorate-General for Research and Innovation 2018). This circular economy is intended to reduce dependency on fossil raw materials and strive for an economy that conserves resources sustainably. According to estimates, there is still untapped potential for extended utilization of biomass from grasslands in Europe (Meyer et al. 2018). This potential could further increase due to the declining importance of grasslands for food production (Schils et al. 2022). In this context, the increased use of biomass from grasslands for energy production, namely biogas, could be one way of working towards energy security without risking negative impacts on food production.

In parallel, the importance of grasslands and especially the multitude of their ES has been recognized (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) 2018). Some ES, such as the promotion and protection of biodiversity, have been set as objectives at the European level (European Commission: Directorate-General for Research and Innovation 2018). Conservation measures such as late harvesting and reduced cutting are intended to improve the condition of local flora and fauna. A change in utilization also leads to changes in the species composition and the chemical composition of the biomass and thus its quality (Hallikma et al. 2023).

The use of grassland biomass in various value chains is largely determined by the quality of the biomass and established utilization systems (Ding et al. 2024). High-quality biomass, distinguished by high protein and low fiber content, is traditionally used as animal feed in meat and dairy production. Newer value chains include the production of green juice and a further breakdown into individual components such as lactic acid, proteins, or amino acids (Krenz and Pleissner 2024). In contrast, low-quality biomass, characterized by high fiber content and low protein content, can be used as fuel, as feedstock in biogas or paper production, and for other material applications (Ding et al. 2024; Krenz and Pleissner 2024).

To make an informed decision on the valorization of the grass biomass, reliable information on its chemical composition is crucial. Traditionally, the Hohenheim feed value gas test (Gb) (Menke et al. 1979) is used to determine the metabolizable energy for livestock, while wet chemical analytical methods like the Weender and Van Soest analysis (Henneberg and Stohmann 1860; van Soest 1966) have been applied to obtain reliable information on nutrient composition. However, these methodological approaches are comparatively expensive, time-consuming, and produce chemical waste. On the other hand, near-infrared spectroscopy (NIRS) provides a non-destructive alternative method, which requires minimal sample preparation, is time-efficient, inexpensive, and does not produce chemical waste (Roberts et al. 2004). To ensure reliable use, however, calibration procedures and reference values are required (Foley et al. 1998).

Both, the forage quality of relevant grass species (e.g., Oluk et al. 2022; Catunda et al. 2022) as well as the biogas potentials of specific grass biomasses have been determined via regression equations. However, these approaches focus on lignin as the decisive regressor (e.g., Dandikas et al. 2015). So far, the determination of the acid-detergent lignin fraction (ADL) content of grassland biomass by NIRS is still associated with increased inaccuracy (Buonaiuto et al. 2021; Guimarães et al. 2023). In addition, the ADL content is not measured by harvesting machines used in Germany. Currently established land cultivation machinery is often equipped with NIRS sensory systems to determine dedicated crop constituents in real time during harvest, but reliable, certified information on lignin alone is for now not available, especially not for innovative, uncommon harvested crops (DLG TestService GmbH 2019, 2021). This also accounts for flower-rich grassland, which requires extensive modeling of the spectral data with numerous data pairs to determine the nutrient content due to its complex composition. Consequently, a representative number of data pairs from chemical analysis and the corresponding NIRS spectra are essential for trustworthy NIRS calibrations (Norman et al. 2020). The broad species spectrum of flower-rich grassland provides a wide range of resources for pollinating insects and the trophic levels based on them. The phenological diversity can close potential flowering gaps in the agricultural landscape and contribute to its biodiversity preservation. Mixed stands with non-uniform developmental stages can result in a biomass quality with a different composition than grass monocultures. We aimed to answer the following questions in this paper.

What is the nutrient composition of flower-rich grassland and can it be reliably determined using NIRS analysis? We demonstrated an NIRS-based assessment of the nutrient composition of complex flower-rich grassland biomass. In addition, we present a new approach to estimating the quality of grassland biomass.

Secondly, what is the methane yield of flower-rich grassland and can it be modelled using common quality parameters, such as crude fiber (XF), crude protein (XP) or crude ash (XA) as regressors of grassland biomass? To answer these questions, we analyzed the harvested biomass for its chemical composition via both wet chemical and NIRS analysis, determined specific biogas and methane yields (SBY and SMY) in anaerobic digestion batch tests, and predicted the biogas yield (Y_B) and methane yield (Y_M) using a Multiple Linear Regression (MLR). Thereby, we deliberately refrain from using lignin as a regressor, as the value can often not be determined with sufficient quality.

The most relevant regressors of the biogas and methane yield were determined and the performance of the MLR regression was compared with established regression equations proposed by Baserga (1998), Keymer and Schilcher (1999), and Weißbach (2008).

2 | Material and Methods

2.1 | Study Site

The study site (52°41′13.11 "N/12°43′22.14" E) is near Paulinenaue in Brandenburg, Germany, approximately 50 km west of Berlin. The climate is humid with a mean annual precipitation of 545 mm/y and a mean temperature of 9.7°C. The area is strongly influenced by the hydrological regime of the Havel River and its system of channels, with periodical flooding during winter and spring. The surrounding *Havelland* region is characterized by organic soils and its use as grassland.

Fen grassland with dominating species like *Festuca arundinacea, Elymus repens, Poa pratensis, Agrostis stolonifera*, and *Phalaris arundinacea* is typical for the region and was selected as our study site. The grassland is usually cut in May and August, rarely in October. Due to its smooth relief, the site has a moisture gradient (see Figure 1). The soil of the experimental plot is a Hemic Rheic Histosol (WRB). It consists of a 50 cm thick heavily decomposed black upper layer and a less decomposed brown lower layer from 50 to 90 cm above a basic substrate mineral silty mud.

2.2 | Experimental Setup

Seven treatments and one control were established on 96 stripes across 12 blocks on an area with 0.8 ha size in spring 2020: two wildflower mixtures for different moisture levels (see Table 1) were hand-seeded after (a) rotation of the soil and (b) after deep mulching of the vegetation, resulting in the first four treatments. The regional and commercial wildflower mixtures were selected based on the site conditions. Mixtures of different sorts of red clover (*Trifolium pratense*) and white clover (*T. repens*), as well as alsike clover (*T. hybridum*), were slotted with a slotting machine, resulting in three additional treatments. The control completed the experiment. All treatments were fully randomized within each block.

2.3 | Harvesting Details and Sample Preparation

Biomass was harvested in two areas within each stripe ($n = 2$ samples $\times 8$ treatments $\times 12$ blocks $= 192$ per cut) with a plot harvester (Wintersteiger Hege 212, Germany). The first cut was made on August 19, 2020 and 20, to allow the flowering plants to set seeds. The second cut took place on May 31, 2021 and June 1, 2021, and the third cut on August 24, 2021 and 25.

All samples ($n = 192$) were analyzed by wet-chemical feedstuff analysis (see section 2.4) as well as near-infrared spectroscopy

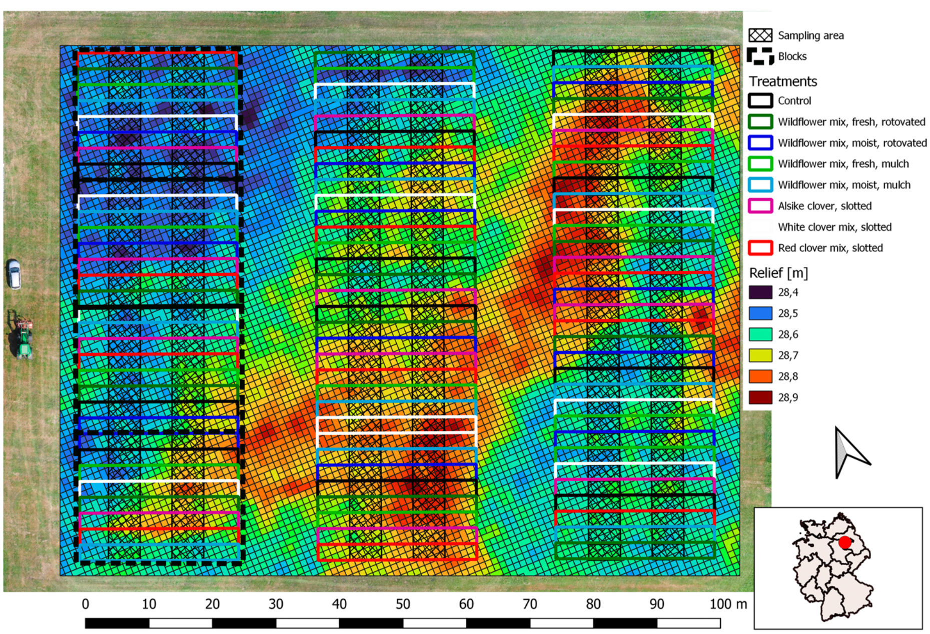

FIGURE 1 | Experimental set-up in Paulinenaue, Brandenburg, Germany. The boxes indicate the treatments and the colors in the background indicate the relief and thus the groundwater level.

TABLE 1 | Seeding mixtures of wild grassland community seeds adapted for two different soil moisture levels.

Fresh	Moist
Achillea millefolium	*Achillea millefolium*
Anthriscus sylvestris	*Achillea ptarmica*
Centaurea cyanus	*Angelica sylvestris*
Centaurea jacea	*Anthriscus sylvestris*
Daucus carota	*Barbarea vulgaris*
Galium album	*Caltha palustris*
Heracleum sphondylium	*Cardamine pratensis*
Knautia arvensis	*Centaurea cyanus*
Leontodon hispidus	*Centaurea jacea*
Leucanthemum vulgare	*Cirsium oleraceum*
Lotus corniculatus	*Filipendula ulmaria*
Lychnis flos-cuculi	*Galium album*
Papaver dubium	*Geum rivale*
Papaver rhoeas	*Heracleum sphondylium*
Plantago lanceolata	*Hypericum tetrapterum*
Prunella vulgaris	*Leucanthemum vulgare*
Rumex acetosa	*Lotus pedunculatus*
Scorzoneroides autumnalis	*Lychnis flos-cuculi*
Silene vulgaris	*Lysimachia vulgaris*
Tragopogon pratensis	*Lythrum salicaria*
Trifolium pratense	*Papaver rhoeas*
	Pimpinella major
	Plantago lanceoloata
	Prunella vulgaris
	Ranunculus acris
	Rumex acetosa
	Scorzoneroides autumnalis
	Succisa pratensis
	Trifolium pratense

(NIRS, Model 5000-M, FOSS Analytical A/S, Denmark, ISI scan) (see section 2.6). For the batch anaerobic digestion test (see section 2.5), a pooled sample of each treatment was taken from all blocks, resulting in a sample size of 12 samples per cut. After harvesting and chopping with a stationary chopper (1–4 cm particle length; rotary mill of Brabender Duisburg, Germany), fresh matter (FM) grass samples were immediately frozen and stored at −18°C until chemical analyses and batch anaerobic digestion tests were conducted. For further analyses of chemical composition, 50 g aliquots were weighed, pre-dried, and then dried at 70°C to a moisture content of 8%–12%. The samples were then ground (< 0.5 mm) and halved to allow wet chemical feedstuff and NIRS analysis. Sample preparation, data acquisition, and analyses are shown in Figure 2.

2.4 | Feedstuff Analysis

Wet chemical feedstuff analyses (Weender and Van Soest analysis) followed European regulations in compliance with the methods of the Association of German Agricultural Analytic and Research Institutes (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (2023), see Table 2). Chemical analyses for listed components are: total solids (TS), crude nutrients, and fiber fractions, namely ash-free, amylase-digested neutral-detergent fiber ($aNDF_{VS}$) and ash-free acid-detergent fiber (ADF_{VS}) were conducted by the *Landeskontrollverband Berlin-Brandenburg* e.V., Germany (see Supporting Information S5; Wendt and Nandke 2025a).

Furthermore, being aware that ADL is the most recalcitrant component in anaerobic digestion (Herrmann et al. 2016) and thus has a relevant effect on biogas potential, we did not gather this parameter for lack of practical application relevance. Even though technical achievements made the on-farm use of NIR sensory technology more practical during harvest, especially the determination of ADL has not reached reliable status for standard purposes (Stubbs et al. 2010) and research is still developing rather specific calibration models to use NIRS as a cost-efficient mass analysis method for animal feedstock (Buonaiuto et al. 2021; Debnath et al. 2022). Partly, the analysis of the ADL content via NIRS in grasses still shows poor reliability (Guimarães et al. 2023). As of date, harvest machine developers only offer a small range of NIRS-based sensory for their fleet with only a little accreditation, mostly for total solids contents (DLG TestService GmbH 2019, 2021).

2.5 | Determination of Specific Biogas and Methane Yields

SBY and SMY of the samples ($n = 48$) were determined at the ATB Potsdam (Germany) in batch anaerobic digestion tests within 3 runs according to German Standard Procedure VDI 4630 (VDI 2016). Tests were performed in a 37°C-tempered water bath using 2 L glass reactors filled with a maximum of 1.5 L inoculum and flower-rich grassland feedstock (31–50 g FM). The inocula were obtained from agricultural biogas fermenters as well as previous laboratory anaerobic digestion experiments (average chemical characteristics and standard deviation: pH 8.1 ± 0.1; TS 3.3% ± 0.1%; VS 65.9% ± 0.3%; TS; N 2.8% ± 0.1 g kg^{-1}; NH_4-N 1.5 ± 0.1 g kg^{-1}; acetic acid 0.2 ± 0.1 g kg^{-1}; propionic acid 0.06 ± 0.04 g kg^{-1}). Reference samples were blank inoculum (control) as well as two microcrystalline cellulose samples (< 1 mm milled crop material as positive reference sample) to monitor the biological activity of the inoculum material. All samples were prepared in triplicate, ensuring a ratio of volatile solids in substrate and inoculum ($VS_{substrate}$: $VS_{inoculum}$) ≤ 0.5. Batch tests were conducted until the daily rate of biogas produced during three consecutive days was < 0.5% of the total biogas obtained up to that time (VDI 2016), which took 35–49 days of incubation.

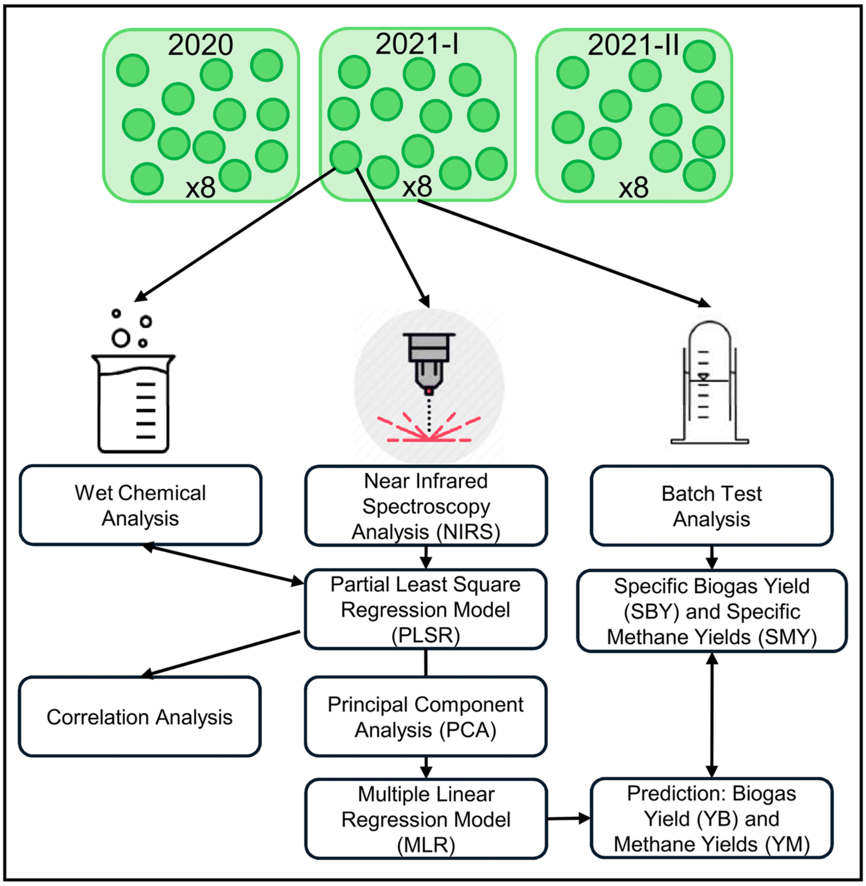

FIGURE 2 | Flow chart of experimental setup and methods applied to determine specific biogas and methane yields from extensive flower-rich fen grassland biomass based on NIRS data. Circles display 12 replications of eight grassland biomass variants; the outer frame displays pooled samples per variant; samples were taken on three dates: one late cut in 2020, one early, and one late cut in 2021. Wet chemical and NIRS analyses were performed for each sample and batch test for pooled samples ($n=48$).

As described by Herrmann et al. (2016), biogas formed during the incubation period was collected in wet gas meters and measured via a liquid displacement approach applying an acidified saturated NaCl barrier solution. The biogas volume was determined daily, corrected for the volume of biogas produced by the inoculum without substrate (blank control) and normalized to standard conditions (dry gas, 0°C, 1013 hPa). The SBY and SMY are given in Normal Liter per kg of fed volatile solids content ($L_N \times kg_{VS}{}^{-1}$).

The biogas composition, namely the content of methane and carbon dioxide, was measured with a portable gas analyzer equipped with infrared sensors (BM5000, Geotechnical Instruments Ltd., Warwickshire, England), considering headspace correction according to VDI 4630 (VDI 2016). Gas analysis required a sufficient volume of biogas, available 9–14 times during a test. SMY of each sample was calculated as the sum of methane generated during the batch test period, in relation to the VS contents.

The methane content in the biogas and the methane hectare yield (MHY) of a biogas feedstock are agronomical key factors. For comparison with maize as a reference, we derived these values from the summed-up biomass volatile solids and SMY of the first and second grassland cuts in 2021.

2.6 | NIRS Analysis & Calibration

The NIRS analysis of the biomass's chemical composition resulted in a high-dimensional data set and expected multicollinearity between the regressors. Partial least squares regression (PLSR) was applied for dimension reduction and to model the chemical components of the feedstuff. PLSR calculations were performed by VDLUFA *Qualitätssicherung* NIRS GmbH, Germany. Components given by NIRS analysis are listed in Table 3.

Enzyme-resistant organic matter (EROM; "organic matter" being volatile solids) has been analyzed as an additional alternative analogue to crude fiber (XF) in the grassland of different intensities and cutting frequencies. Weißbach (2008) found it a more reliable parameter explaining digestibility than crude fiber in extensively managed grasses and grass silages. EROM is determined after hydrolysis by digestion with enzymes and not after hydrolysis by boiling in acids and bases as for XF.

The calibration models were calculated with a partial least squares algorithm (method of least squares) (Haaland and Thomas 1988a, 1988b). The partial least squares factors used in the calibration models cover the wavelength range from

TABLE 2 | Methodology of the components analysis.

Parameter	Abbreviation	Unit	Chapter in VDLUFA Method book volume III
Total solids	TS	% fresh matter (FM)	3.1
Crude ash	XA	% TS	8.1
Crude fats	XL	% TS	5.1.1
Crude fibers	XF	% TS	6.1.1
Crude sugar	XZ	% TS	6.5.1
Neutral-detergent fiber (ash-free, amylase-digested)	$aNDF_{VS}$	% TS	6.5.1
Acid-detergent fiber (ash-free)	ADF_{VS}	% TS	6.5.1

TABLE 3 | Components obtained by NIRS analysis after PLSR modeling.

Components	Abbreviation	Unit
Total solids	TS	% fresh matter (FM)
Crude ash	XA	% TS
Crude fats	XL	% TS
Crude fibers	XF	% TS
Crude sugar	XZ	% TS
Neutral-detergent fiber (ash-free, amylase-digested)	$aNDF_{VS}$	% TS
Acid-detergent fiber (ash-free)	ADF_{VS}	% TS
Gas building (according to Hohenheimer feed value test)	Gb	mL
Fructose	Fru	% TS
Enzyme-resistant organic matter/volatile solids	EROM	% TS

1300 to 2398 nm. At least 100 samples of each substrate were used. The results were evaluated based on the correlation between the reference values of Weender and van Soest analyses and the values calculated using the obtained calibration equations, as well as on the magnitude of the standard errors of calibration (SEC) and prediction (SEP). The fit of the resulting model is also evaluated based on the correlation coefficient (R). The closer the R-value is to 1, the more suitable the model can be considered. Further indicators of the reliability of the model were the values of the standard errors of cross-validation (SECV), 1-VR, and U_e^2 collected (see Supporting Information S2–S4).

2.7 | Statistical Analysis

We performed a principal component analysis (PCA) as an unsupervised dimension reduction method to identify the parameters that explained the most variance in our chemical composition dataset. We have applied a threshold of 60% of the explained variance for our regressors. The PCA was performed using the software R package “factoextra” (Kassambara and Mundt 2020). Then, these parameters were used as regressors in an MLR, and a backward selection was performed based on the corrected Akaike information criterion values. A validation analysis of the model was performed by leave-one-out cross-validation (LOOCV), for which the software R package “caret” was used (Kuhn 2008). The correlation matrix was performed with the R package “ggcorplot” (Kassambara 2023).

The theoretical models according to Baserga (1998), Keymer and Schilcher (1999), and Weißbach (2008) (given in Supporting Information S1) with their specific coefficients were implemented using the package “Rcpp Armadillo” (Eddelbuettel and Sanderson 2014). The regressions were compared based on the adjusted coefficient of determination (R^2), the root mean square error (RMSE) and the mean absolute error (MAE), for which the package “carret” was used (see Wendt and Nandke 2025b).

3 | Results

3.1 | Biogas and Methane Yield and Chemical Composition (NIRS-Analysis)

The mean SBY determined for the green cuttings/flowering plant variants ranges from 490 to 712.7 $L_N \times kg_{VS}$,$^{-1}$ and the mean SMY ranges from 280.3 to 412.3 $L_N \times kg_{VS}{}^{-1}$ and the mean methane contents of the tested samples vary between 55.5 and 60.4 vol% (see Table 4). The results represent typical values for average methane yields (275.1–429.2 $L_N \times kg_{VS}{}^{-1}$) and contents (54.0–62.8 vol-%) of ensiled permanent grassland cuttings from different locations, grassland types, and land use intensities in Germany (Fachagentur Nachwachsende Rohstoffe eV 2016).

The different biomass qualities of the mixed samples tested show a wide range regarding their methane formation potential. Thus, the methane yield plays a major role in the evaluation of individual green cuttings/flowering plant variants concerning their suitability for biogas production beside the yield per unit area achieved. To identify preferred variants, biomass yield,

TABLE 4 | Chemical composition based on the NIRS-analysis, and specific biogas and methane yield based on batch tests of all 48 samples.

Sample ID	SBY (L_N/kg_{VS})	SMY ($L_{N/}kg_{VS}$)	TS in %	XA % TS	XP % TS	XF % TS	XL % TS	XZ % TS	$aNDF_{VS}$ % TS	ADF_{VS} % TS	Gb in ml	EROM % TS	Fru % TS
I 1 20 a	524.00 ± 11.24 SE	298.00 ± 5.51 SE	93.09	5.92	9.51	30.58	2.66	9.47	60.73	36.27	41.55	40.27	9.27
I 2 20 a	506.00 ± 6.66 SE	292.00 ± 1.76 SE	93.18	5.97	9.14	31.44	2.68	9.93	62.14	35.98	42.64	41.36	8.61
I 3 20 a	502.33 ± 3.28 SE	292.00 ± 1.45 SE	93.34	6.61	9.31	30.20	3.16	6.04	48.95	36.22	38.91	40.43	10.11
I 4 20 a	531.00 ± 4.51 SE	301.00 ± 3.79 SE	93.81	8.03	9.73	27.03	2.42	8.44	47.08	34.54	40.32	33.98	9.43
I 5 20 a	555.67 ± 4.33 SE	314.00 ± 1.2 SE	93.68	7.24	10.53	30.52	2.82	7.99	60.40	35.26	42.28	39.70	7.42
I 6 20 a	550.00 ± 7.64 SE	314.00 ± 4.26 SE	93.63	6.47	11.06	30.81	2.80	8.41	61.70	35.13	41.76	42.00	7.36
I 7 20 a	540.33 ± 9.26 SE	309.00 ± 3.46 SE	93.63	6.29	10.01	31.21	2.67	8.52	61.11	35.89	41.85	40.79	7.59
I 8 20 a	527.00 ± 10.82 SE	300.00 ± 6 SE	94.06	6.50	10.94	30.93	2.69	8.14	61.46	35.63	40.12	43.35	7.76
II 1 20 a	549.33 ± 16.15 SE	313.00 ± 7.02 SE	93.87	6.08	8.92	31.01	2.99	8.61	57.38	35.86	41.26	42.59	9.00
II 2 20 a	538.67 ± 7.51 SE	313.00 ± 5.49 SE	93.55	6.61	9.35	31.80	2.69	8.43	62.82	35.81	41.96	43.28	7.50
II 3 20 a	507.00 ± 18.01 SE	297.00 ± 11.26 SE	93.81	6.47	9.24	30.74	3.18	7.06	52.21	35.44	40.34	41.02	9.58
II 4 20 a	547.33 ± 18.19 SE	320.00 ± 11.55 SE	93.48	7.69	9.23	30.04	2.46	7.42	53.47	36.72	39.67	39.86	8.75
II 5 20 a	566.33 ± 4.37 SE	328.00 ± 1.73 SE	93.74	6.97	9.91	31.92	2.76	7.70	63.20	36.21	42.18	41.85	6.65
II 6 20 a	523.33 ± 2.33 SE	311.00 ± 3.51 SE	93.73	6.74	9.47	31.40	2.87	8.48	62.30	34.95	43.09	42.47	7.49
II 7 20 a	561.33 ± 4.84 SE	324.00 ± 2.91 SE	93.57	6.70	9.81	31.55	2.55	8.98	62.23	35.46	43.11	42.23	7.31
II 8 20 a	554.00 ± 4.36 SE	324.00 ± 0.88 SE	93.90	7.06	11.33	31.65	2.80	6.91	63.46	35.86	40.23	43.52	6.06
I 1 21 a	712.70 ± 6.36 SE	407.70 ± 2.4 SE	92.62	7.71	12.99	26.94	2.56	9.82	57.15	31.73	47.60	33.69	7.12
I 2 21 a	640.30 ± 3.93 SE	362.00 ± 4 SE	92.96	7.10	11.86	27.18	2.43	11.12	56.81	31.43	48.74	33.67	7.66
I 3 21 a	694.70 ± 8.41 SE	385.70 ± 6.01 SE	92.68	8.75	12.53	24.05	2.43	9.62	49.29	30.14	46.59	29.36	6.74
I 4 21 a	641.70 ± 10.93 SE	362.00 ± 4.36 SE	92.89	8.68	10.95	24.84	2.14	10.66	48.46	30.85	46.96	30.42	7.36
I 5 21 a	653.00 ± 10.54 SE	369.00 ± 6.11 SE	92.61	7.16	11.87	28.92	2.36	9.70	60.22	33.23	47.90	36.78	6.67
I 6 21 a	727.70 ± 8.67 SE	412.30 ± 4.63 SE	92.21	7.35	11.74	29.75	2.23	8.73	62.21	34.50	46.94	38.37	6.08
I 7 21 a	648.00 ± 13.05 SE	364.70 ± 6.57 SE	92.52	7.61	13.16	27.91	2.51	8.82	59.58	32.55	46.36	36.60	6.38
I 8 21 a	651.00 ± 2.65 SE	370.70 ± 1.33 SE	93.04	7.83	13.24	29.32	2.43	7.57	62.00	33.89	44.50	40.32	5.99
II 1 21 a	649.00 ± 5 SE	364.30 ± 2.19 SE	92.36	7.04	11.10	30.21	2.26	8.60	61.50	34.03	47.77	39.66	6.44
II 2 21 a	660.30 ± 3.38 SE	374.70 ± 3.18 SE	92.46	7.10	11.98	29.00	2.34	8.78	60.41	33.13	47.22	38.23	6.28

(Continues)

TABLE 4 | (Continued)

Sample ID	SBY (L_N/kg_{VS})	SMY ($L_{N/}kg_{VS}$)	TS in %	XA % TS	XP % TS	XF % TS	XL % TS	XZ % TS	aNDF$_{VS}$ % TS	ADF$_{VS}$ % TS	Gb in ml	EROM % TS	Fru % TS
II 3 21 a	655.30 ± 2.4 SE	373.30 ± 1.45 SE	93.51	8.26	11.65	27.33	2.40	9.21	56.11	31.72	47.00	33.79	6.49
II 4 21 a	653.00 ± 12.66 SE	375.70 ± 7.86 SE	92.90	8.62	11.94	26.59	2.37	8.73	53.25	31.73	45.82	33.88	6.43
II 5 21 a	645.70 ± 8.35 SE	368.70 ± 7.33 SE	93.32	7.65	12.54	29.28	2.50	7.89	59.82	32.90	45.30	39.84	5.89
II 6 21 a	659.70 ± 7.06 SE	377.30 ± 3.48 SE	93.11	7.13	11.94	30.01	2.33	8.28	61.24	33.56	46.17	40.24	5.64
II 7 21 a	673.00 ± 8.39 SE	387.30 ± 4.18 SE	93.31	7.88	12.33	29.77	2.37	7.55	61.32	33.73	43.26	40.22	5.87
II 8 21 a	586.30 ± 2.03 SE	354.00 ± 2.08 SE	92.95	7.23	12.34	30.21	2.37	7.47	63.12	33.89	44.43	42.23	5.41
I 1 21 b	509.60 ± 6.8 SE	296.90 ± 0.78 SE	94.44	7.36	13.16	30.07	2.74	7.21	56.99	34.43	38.07	38.74	5.75
I 2 21 b	514.70 ± 3.76 SE	304.00 ± 3.06 SE	94.18	6.77	12.31	29.79	2.65	8.42	58.81	34.40	39.62	38.78	6.29
I 3 21 b	507.30 ± 7.36 SE	296.30 ± 4.84 SE	94.68	7.16	12.95	29.21	2.49	7.58	56.35	34.39	37.96	38.52	6.76
I 4 21 b	490.00 ± 3.79 SE	280.30 ± 3.18 SE	95.58	7.52	11.59	28.41	2.34	7.21	51.41	34.44	35.88	37.54	7.29
I 5 21 b	535.70 ± 3.53 SE	310.70 ± 2.85 SE	95.67	6.47	12.81	29.62	2.81	8.57	58.57	32.37	42.21	37.33	5.48
I 6 21 b	531.00 ± 4.04 SE	315.30 ± 2.33 SE	95.16	6.71	12.31	30.05	2.71	9.09	57.92	32.70	43.01	38.14	5.82
I 7 21 b	521.00 ± 3 SE	309.30 ± 0.33 SE	95.21	6.92	12.92	30.88	2.59	7.74	59.41	34.49	39.15	40.33	4.73
I 8 21 b	556.30 ± 8.45 SE	327.00 ± 6.66SE	94.68	6.96	12.79	29.76	2.65	9.09	58.27	33.14	41.00	38.65	6.09
II 1 21 b	504.30 ± 7.45 SE	301.70 ± 0.33 SE	94.04	6.64	11.01	30.41	2.77	8.82	57.56	34.15	40.81	39.49	6.95
II 2 21 b	590.30 ± 2.96 SE	347.70 ± 1.45 SE	94.16	6.23	10.73	30.63	2.66	9.75	58.32	33.15	43.71	38.92	6.56
II 3 21 b	504.70 ± 7.06 SE	298.30 ± 2.4 SE	95.00	6.46	10.15	31.14	2.91	7.78	54.80	35.01	38.45	41.75	8.77
II 4 21 b	505.00 ± 4.62 SE	294.70 ± 4.37 SE	94.54	7.21	10.36	29.39	2.85	8.49	52.10	33.27	40.95	37.82	8.68
II 5 21 b	527.00 ± 3.46 SE	309.00 ± 3.21 SE	94.49	7.27	10.99	30.52	2.71	9.30	58.71	33.88	40.95	40.47	7.25
II 6 21 b	551.30 ± 2.4 SE	326.30 ± 1.45 SE	94.49	7.10	11.82	29.90	2.69	9.05	58.96	32.87	42.81	38.76	6.32
II 7 21 b	570.21 ± 4.71 SE	321.60 ± 1.22 SE	94.12	6.79	11.26	30.88	2.83	9.08	58.77	33.12	44.17	38.73	5.81
II 8 21 b	546.00 ± 4.62 SE	324.00 ± 1.53 SE	94.51	7.16	11.30	31.52	2.70	8.47	59.95	33.77	42.26	40.65	5.46
Mean	574.99	331.74	93.72	7.11	11.25	29.71	2.62	8.51	58.46	35.71	58.12	34.04	42.81
SD	66.01	34.54	0.85	0.67	1.30	1.73	0.24	0.98	4.00	1.51	4.24	1.56	3.10

Note: The sample ID consists of the I-II = first/s sample, 1–8 = variant; 20–21 = year, a and b = first/second cut.

TABLE 5 | Biomass yield, methane content, specific and hectare-related methane yield of early and late 2121 harvests compared to common maize methane yield.

Biomass yield [$t_{VS} \times ha^{-1}$]	Methane content [Vol.-%]	SMY [$L_N \times kg_{VS}^{-1}$]	MHY [$m^3{}_N \times ha^{-1}$]	MHY relative to maize[a] [%]
9.43 ± 1.26	57.83 ± 1.26	343.60 ± 36.01	3026.26 ± 450.78	53.35

Abbreviations: MHY, methane hectare yield; SMY, specific methane yield.
[a]Maize MHY = 5.672 $m^3{}_N \times ha^{-1}$ (Schmidt et al. 2018).

SMY, and MHY were considered and showed rather large variations (see Table 5).

The value determined for MHY is approximately half of the mean MHY of maize silage due to a lower biomass yield, even though the SMY is slightly higher (333 $L_N \times kg_{VS}^{-1}$, according to Schmidt et al. (2018)). However, it must be taken into account that the SMY of maize is subject to a relatively wide variance and the average SMY of fresh biomass is 375 $L_N \times kg_{VS}^{-1}$ according to KTBL (2021). SMY and MHY of reed canary grass, a typical wet grassland species, which has been grown on two different sites by Schmidt et al. (2018) with nitrogen fertilization rates of up to 160 kg N × ha^{-1}, showed similar SMY (314.92 and 354.92 $L_N \times kg_{VS}^{-1}$, respectively) but in contrast higher biomass yields (12.44 and 15.45 $t_{VS} \times ha^{-1}$) and thus higher MHY. This indicates that extensively managed flower-rich grassland is of the same quality as intensively managed grassland regarding SMY. If the biomass is ensiled, the dry matter content can be expected to decrease by an average of 4% (Weißbach and Strubelt 2008). Consequently, this can also lead to lower nutrient concentrations and lower biogas potential due to the loss of volatile substances. Following Weißbach and Strubelt (2008), an acid correction for ensiled biomass to adjust the dry matter content would be recommended.

The mean TS content of our sampled grassland biomass was 93.72% (±0.85) and ranged between 92.21% and 95.67% (see Table 4). The crude fiber content determined showed a greater range of 7.85% TS, with a mean of 29.71 (±1.73). The mean crude protein content ranged from 8.9% to 13.4% TS with a mean of 11.25% TS (±1.30) which is above the reported content of other mixed grassland biomass (Dandikas et al. 2015; Herrmann et al. 2014; Meserszmit et al. 2022). The crude lipid content ranged from 2.14% to 3.20% TS with a mean of 2.62% TS (±0.24) which is higher than described by Meserszmit et al. (2022) and in the range of the findings of Dandikas et al. (2015) and Herrmann et al. (2014).

3.2 | Partial Least Square Regression

The calibration and validation results for the flower-rich grass substrate (see Supporting Information S3 and S4) suggest that the calibration model for the determination of the chemical composition meets the conditions of a reliable model. The SECV ranged from 0.31 to 2.36, the SEC ranged from 0.30 to 2.19, and the subtracted value of the ratio of unexplained variance from unity (1-VR) ranged from 0.74 to 0.97. The highest estimation quality was found for the parameters crude protein (R^2 = 0.97), fructose (R^2 = 0.97) and EROM (R^2 = 0.95). Total solids achieved the lowest coefficient of determination (R^2 = 0.75), consistent with Oluk et al. 2022, where TS also generated the lowest R^2-values. The standard error of prediction was in good agreement with the standard error of cross-validation.

3.3 | Principal Component Analysis

For the principal component analysis, the parameters XA, XZ, XP, Gb, Fru, TS, XL, $aNDF_{VS}$, ADF_{VS}, EROM, and XF were used. The parameters XZ and TS were below the threshold of 60% explained variance and were excluded as regressors for the MLR model (see Figure 3). The first two principal components explained a total of 66.8% of the total variance.

3.4 | Multiple Linear Regression

An MLR was performed with the parameters XA, XP, XL, Gb, Fru, $aNDF_{VS}$, ADF_{VS}, EROM, and XF as predictor variables and biogas yield (Y_B) as the response variable, as previously determined by PCA (see Figure 3). XL, Fru, XF, and EROM were removed as regressors in the backward selection process.

An analysis of variance revealed a highly significant model (p-value < 0.001). The adjusted R^2-value was 0.88. The absence of a bias in the distribution of the residuals indicates a comprehensive representation of the variability of the data by our model (see Figure 4).

$$Y_B = -1308.85 + 27.95XA + 18.93XP - 2.11aNDF_{vs} + 20.35ADFvs + 21.08Gb$$

The LOOCV results for the biogas model gave an R^2-value of 0.83, an RMSE of 26.75, and an MAE of 19.14.

The same analysis was performed for the calculation of a methane model. In the backward selection process, the regressors XF, Fru, and XL were removed from the model, resulting in a highly significant model (p-value < 0.001).

$$Y_M = -622.62 + 16.61\ XA + 10.22\ XP - 1.52\ aNDF_{vs} + 6.99\ ADF_{vs} + 10.94\ Gb + 2.66\ EROM$$

The adjusted R^2-value for this model was 0.87. The lack of bias in the residuals' distribution suggests that our

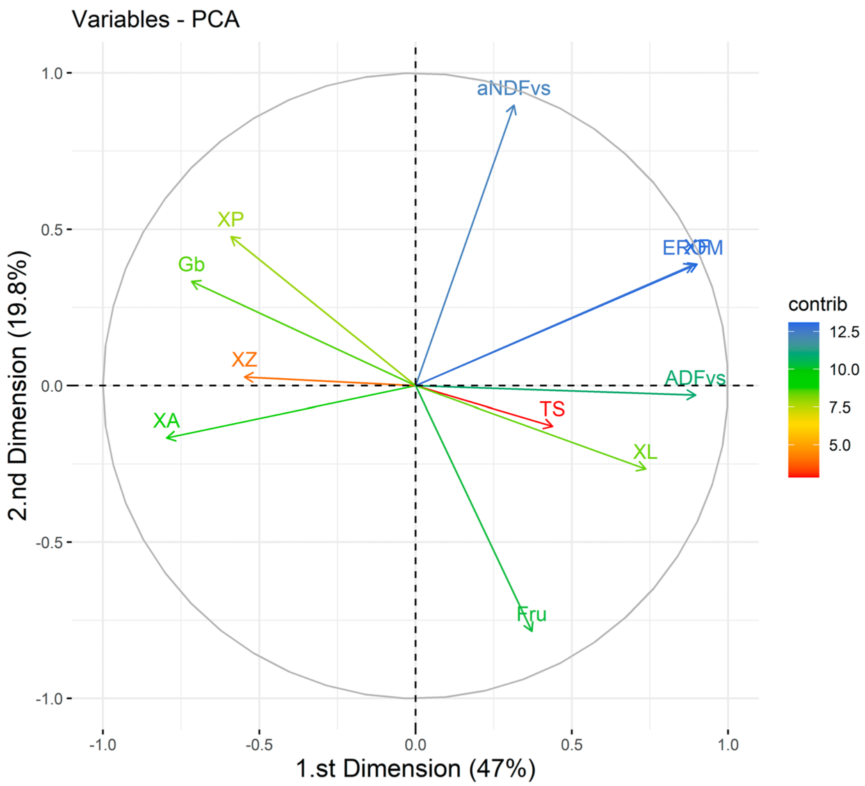

FIGURE 3 | Correlation loading plot for the first and second principal components. The color of the regressors indicates the contribution of explained variance, and the outer circle indicates 100% explained variance.

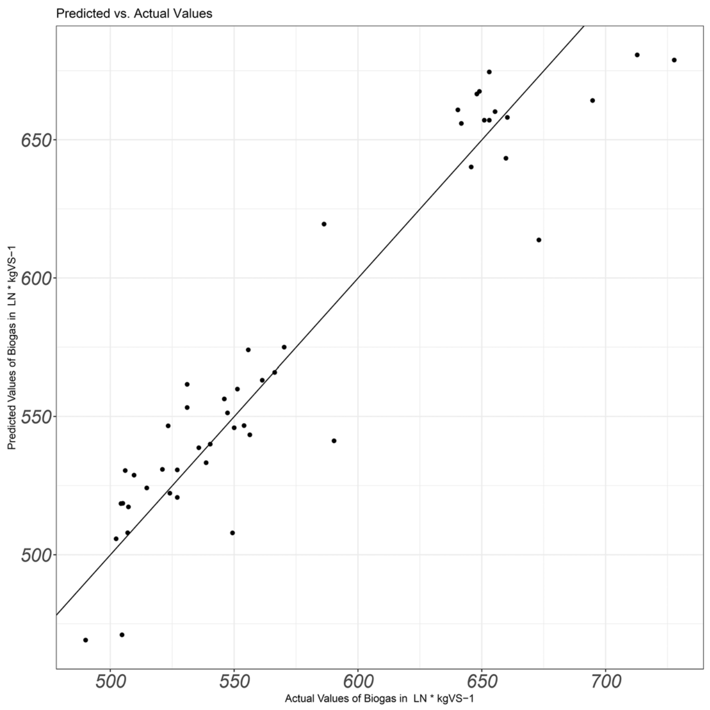

FIGURE 4 | Measured values versus predicted values of the biogas yield (Y_B) based on the MLR model presented here.

methane model accurately captures the variability of the data (see Figure 5). The LOOCV results for the methane model yielded an R^2-value of 0.81, RMSE was 14.78, and the MAE was 10.65.

Additional regression analyses were performed based on the models of Baserga (1998), Keymer and Schilcher (1999), and Weißbach (2008). All three models performed worse than the model presented here (see Table 6).

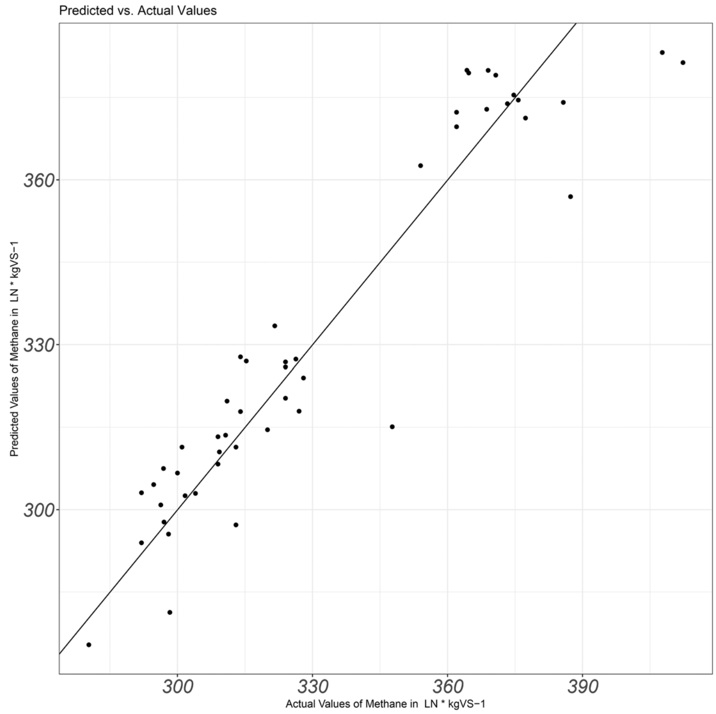

FIGURE 5 | Measured values versus predicted values of the methane yield (Y_M) based on the presented MLR model.

TABLE 6 | Performance of the three regressions based on Baserga, Keymer & Schilcher and Weißbach.

	Biogas model	Methane model	Baserga	Keymer & Schilcher	Weißbach Y_B	Weißbach Y_M
R^2	0.88	0.87	0.48	0.48	0.54	0.58
RMSE	26.75	14.78	45.81	45.72	44.00	22.15
MAE	19.14	10.65	35.91	35.84	32.59	16.74

4 | Discussion

Our newly created model for flower-rich grassland with its regressors ranges in an intermediate position between the Baserga, Keymer & Schilcher and Weißbach models. In the regression models of Baserga and Keymer & Schilcher, the importance of the parameters XF, XP, and XL for the biogas and methane yield was highlighted. Out of these parameters, only XP remained as a regressor in our models after backward selection. XP is positively correlated with the biogas yield Y_B and the methane yield Y_M ($r = 0.44$, $p < 0.001$; $r = 0.48$, $p < 0.001$; see Figure 6), and explains a moderate proportion of the overall variance (see Figure 2). In contrast to the models of Baserga and Keymer & Schilcher, crude lipid content correlates negatively with Y_B and Y_M ($r = -0.67$, $p < 0.001$; $r = -0.66$, $p < 0.001$, see Figure 6). This correlation is also more pronounced than in the case of XP, yet it was discarded as a regressor in the backward selection process in both models. Further differences arise from the coefficients for crude fiber content, which in the Baserga, Keymer & Schilcher models has a positive effect on Y_B and Y_M, but reveals a weak negative correlation ($r = -0.55$, $p < 0.001$; $r = -0.51$, $p < 0.001$) in our model. Gb correlates positively with Y_M and Y_B and also appears as an important positive regressor in the other two models, which seems plausible, as both approaches assess metabolic processes in biomass utilization.

The largest contribution of explained variance in our data set comes from XF and EROM. Like the Weißbach model, EROM is used as a regressor instead of XF. EROM is negatively correlated with the biogas yield ($r = -0.50$, $p < 0.001$) and methane yield ($r = -0.46$, $p = 0.001$). The differences between the type of correlation and the coefficients can be caused by different effects, such as confounding, suppressing, or mediating effects. EROM depicts a stronger correlation with XA, which is also more strongly correlated to Y_M than EROM. By suppressing irrelevant variance in XA, it can lead to a positive coefficient in EROM.

Crude ash content, which appears as a negative regressor in Weißbach's model, showed a positive correlation with Y_B and Y_M ($r = 0.59$, $p < 0.001$; $r = 0.59$, $p < 0.001$, respectively) and contributed to the total variance explained. $aNDF_{vs}$ might be another possible suppressor effect as it is weakly positively correlated ($r = -0.11$, $p < 0.001$; $r = -0.15$, $p < 0.001$) but represented with negative coefficients in our models. $aNDF_{vs}$ is highly positively

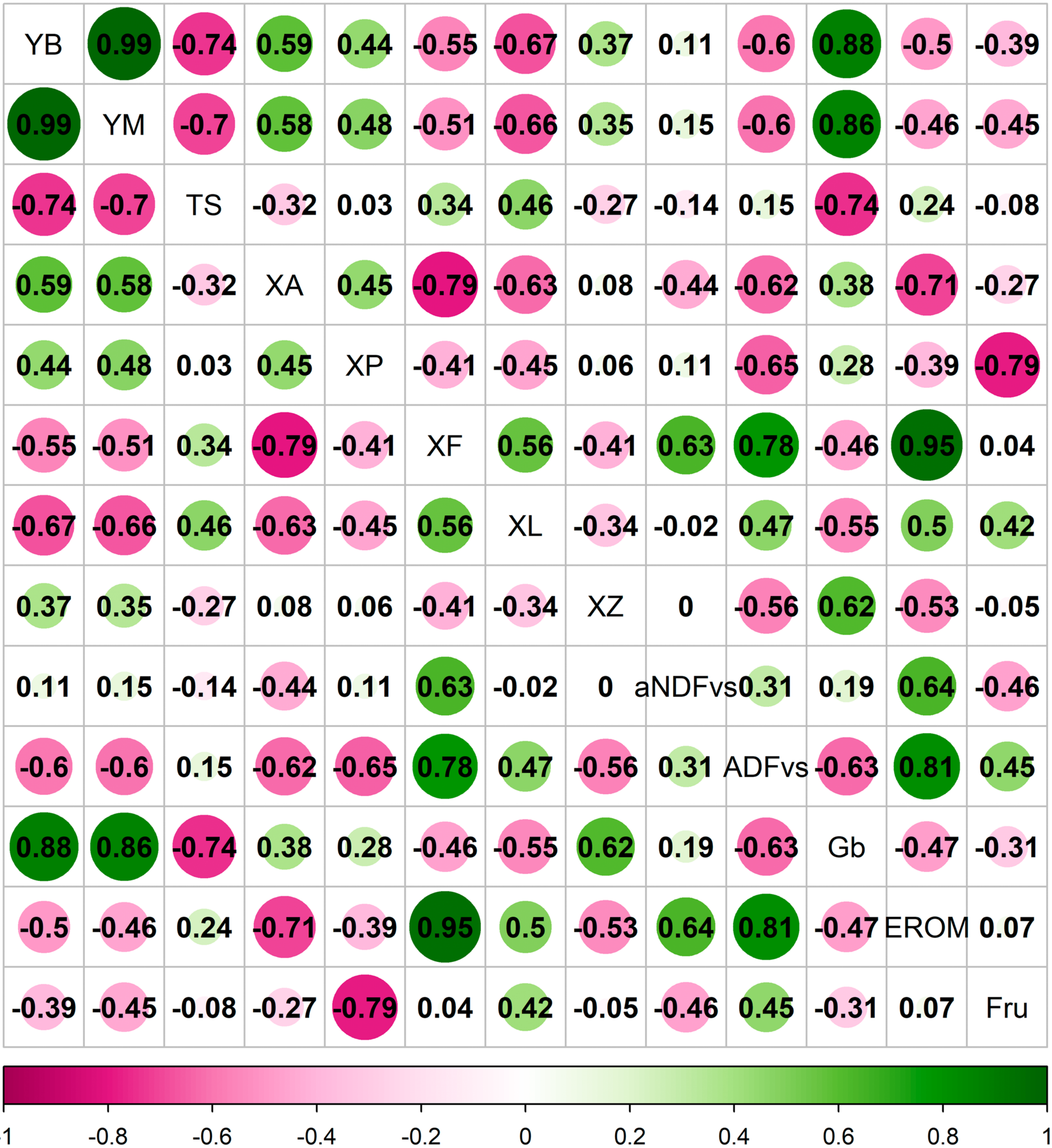

FIGURE 6 | Correlation trimatrix of the biogas yield (Y_B), methane yield (Y_M) and the chemical compounds (NIRS analysis) of all 48 samples.

correlated with EROM and ADF_{vs}, which, in turn, are negatively correlated with Y_B and Y_M.

The development of the models of Baserga and Keymer & Schilcher must be seen in the context of the beginning of the intensive use of food and forage crops for biogas production in Germany in the late 1990s. Thus, the focus in the models is based on prominent parameters such as starch or fat from feedstuff analyses. While these components are highly available in established biogas crops (e.g., maize), they are less present in extensive grassland biomass. Furthermore, the harvest time of biogas crops aims to optimise the chemical composition of the biomass, such as nitrogen concentration, C/N ratio, non-structural carbohydrates, and cell wall components. The hampering influence of increasing crude fiber content with advancing growth stages on the biogas yield is extensively described in the literature (Prochnow et al. 2005; Klimiuk et al. 2010; Kandel et al. 2013; Chiumenti et al. 2018).

The Weißbach model (2008), the most widely used model for estimating the biogas potential of intensive grass (first and second cut) in Germany, includes the crude fiber content as the only used organic parameter. In other established formulas for biogas potential estimation, Weißbach (2009) gradually shifted the focus from crude fiber (linked to a substrate-dependent factor to quantify the usable proportion) to EROM as the single organic and mainly fiber-based parameter for further grass cuts. In this way, these calculation formulas exclude the direct portions of the usable fiber components still included for the first two grass cuts of intensive management.

However, the harvesting time of intensive grassland (as a substrate for Weißbach's model) is similarly determined for other biogas crops. In contrast, the timing of harvesting extensive grassland is guided by factors relevant to nature conservation (e.g., flower availability for pollinators, and breeding birds) and the gross yield of the biomass. The resulting comparatively late harvests are characterized by higher fiber content and lower protein content.

The flowering mixtures investigated in our study were composed of plants with a broad flowering spectrum that extends from early spring to late fall, showing different phenological growth stages when being harvested. As samples from early and late harvests were mixed, a broad spectrum of developmental stages was covered and analyzed. This resulted in a biomass quality characterized by both a high protein and high fiber content (see Melts et al. 2019), which are otherwise nutrients subject to opposing trends (e.g., Krenz and Pleissner 2024). This finding underlines the importance of crude protein and fiber fractions as significant regressors in our models.

Determined values for SMY are well in line with other findings. The SMY and MHY of reed canary grass, a typical wet grassland species also dominantly present in our sites, have been grown on two different sites by Schmidt et al. (2018). Nitrogen fertilization rates of up to $160\,\mathrm{kg\,N} \times \mathrm{ha}^{-1}$ showed similar SMY (314.92 and 354.92 $\mathrm{L_N} \times \mathrm{kg_{VS}}^{-1}$, respectively) to our biomass (343.60 ± 36.01 $\mathrm{L_N} \times \mathrm{kg_{VS}}^{-1}$). Still, most probably due to fertilization rates, it showed higher biomass yields (12.44 and 15.45 $t_{\mathrm{VS}} \times \mathrm{ha}^{-1}$) and thus higher MHY than our extensively managed sites (9.43 ± 1.26 $t_{\mathrm{VS}} \times \mathrm{ha}^{-1}$). This indicates that extensively managed flower-rich grassland is of the same quality as intensively managed grassland, regarding specific methane yields.

5 | Conclusion

Flower-rich fen grassland is composed of a broad spectrum of different species with different stages of growth and maturity, resulting in increased protein and high fiber content. NIRS proved capable of successfully analyzing the nutrient content of this complex biomass. The study confirmed flower-rich fen biomass as an appropriate feedstock for biogas production. Even though the MHY reached only a quarter of that of maize, the SMY was comparable. The additional use of biomass from landscape enhancement measures can contribute to a better economic balance of biodiversity-enhancing measures. Moreover, the wide range of species in flower-rich fen grassland can close flowering gaps and ensure important ecosystem services such as pollination. In addition to promoting insects, important contributions to the biodiversity conservation of higher trophic levels are thus supported, too. Future research should focus on the yields per hectare of extensive flower-rich fen grasslands for a better assessment of its economic significance in the context of demand-driven flexible biogas production.

Author Contributions

M. Wendt: formal analysis, investigation, methodology, software, visualization, writing – original draft, writing – review and editing. **S. Nandke:** investigation, visualization, writing – original draft, writing – review and editing. **M. Heiermann:** data curation, investigation, methodology, resources, validation, writing – original draft, writing – review and editing. **J. Ahlborn:** conceptualization, data curation, methodology, visualization, writing – original draft, writing – review and editing. **M. Thielicke:** conceptualization, formal analysis, writing – original draft. **P. Scharschmidt:** data curation, methodology. **F. Eulenstein:** funding acquisition, project administration, supervision.

Acknowledgements

The authors thank Vincent Plogsties for his valuable support in anaerobic digestion tests and Giovanna Rehde and the staff of her analytical team for conducting the associated chemical analyses at ATB. Also, we would like to thank the staff of the experimental station Paulinenaue, Kerstin Deetz, Dagmar Wacker, Paul Moos, Heiko Biffart, Tilo Henning, as well as Wilfried Bock and Siegfried Kube for their support during the harvest seasons. This research was made possible by funding from the Federal Ministry of Food and Agriculture (FInAL, 22012018). Open Access funding enabled and organized by Projekt DEAL.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Archer, N. A. L., W. Otten, S. Schmidt, A. G. Bengough, N. Shah, and M. Bonell. 2016. "Rainfall Infiltration and Soil Hydrological Characteristics Below Ancient Forest, Planted Forest and Grassland in a Temperate Northern Climate." *Ecohydrology* 9, no. 4: 585–600. https://doi.org/10.1002/eco.1658.

Baserga, U. 1998. "Landwirtschaftliche Co-Vergärungs-Biogasanlagen. Biogas Aus Organischen Reststoffen Und Energiegras." Eidgenössische Forschungsanstalt Für Agrarwirtschaft Und Landtechnik. FAT-Berichte (512, 512).

Behrendt, A., J. Monk, and F. Eulenstein. 2013. "Influence of Cutting Frequency of Grassland on the Hydrological Regime." In Höhere Bundeslehr- Und Forschungsanstalt Für Landwirtschaft Raumberg-Gumpenstein (Ed.): 15. Gumpensteiner Lysimetertagung. Lysimeterforschung als Bestandteil Der Entscheidungsfindung. Lysimeterforschung Als Bestandteil Der Entscheidungsfindung. Raumberg-Gumpenstein, Austria, 16–17 April 2013. Höhere Bundeslehr- Und Forschungsanstalt Für Landwirtschaft Raumberg-Gumpenstein, pp. 165–167.

Buonaiuto, G., D. Cavallini, L. M. E. Mammi, et al. 2021. "The Accuracy of NIRS in Predicting Chemical Composition and Fibre Digestibility of Hay-Based Total Mixed Rations." *Italian Journal of Animal Science* 20, no. 1: 1730–1739. https://doi.org/10.1080/1828051X.2021.1990804.

Catunda, K. L., A. C. Churchill, S. A. Power, and B. D. Moore. 2022. "Near Infrared Spectroscopy Calibration Strategies to Predict Multiple

Nutritional Parameters of Pasture Species From Different Functional Groups." *Journal of Near Infrared Spectroscopy* 30, no. 5: 254–263. https://doi.org/10.1101/2021.07.31.454175.

Chiumenti, A., D. Boscaro, F. Da Borso, L. Sartori, and A. Pezzuolo. 2018. "Biogas From Fresh Spring and Summer Grass: Effect of the Harvesting Period." *Energies* 11, no. 6: 1466. https://doi.org/10.3390/en11061466.

Dandikas, V., H. Heuwinkel, F. Lichti, J. E. Drewes, and K. Koch. 2015. "Correlation Between Biogas Yield and Chemical Composition of Grassland Plant Species." *Energy & Fuels* 29, no. 11: 7221–7229. https://doi.org/10.1021/acs.energyfuels.5b01257.

Debnath, M., A. Sayeed, A. Hannan, and A. Ayele. 2022. "NIR Validation and Calibration of Van Soest Cell Wall Constituents (ADF, NDF, and ADL) of Available Corn Silage in Bangladesh." *Annals of Agricultural & Crop Sciences* 7, no. 3: 1–4. https://doi.org/10.13140/RG.2.2.15179.46887.

Ding, Z., K. T. Hamann, and P. Grundmann. 2024. "Enhancing Circular Bioeconomy in Europe: Sustainable Valorization of Residual Grassland Biomass for Emerging Bio-Based Value Chains." *Sustainable Production and Consumption* 45: 265–280. https://doi.org/10.1016/j.spc.2024.01.008.

DLG TestService GmbH. 2019. "Prüfbericht 7020: CLAAS NIR Sensor. Feuchtemessung in Gras, Groß-Umstadt."

DLG TestService GmbH. 2021. "Prüfbericht 7222: Krone NIR Control Dual. Trockenmassebestimmung in Mais, Groß-Umstadt."

Eddelbuettel, D., and C. Sanderson. 2014. "RcppArmadillo: Accelerating R With High-Performance C++ Linear Algebra." *Computational Statistics & Data Analysis* 71: 1054–1063. https://doi.org/10.1016/j.csda.2013.02.005.

European Commission: Directorate-General for Research and Innovation. 2018. "A Sustainable Bioeconomy for Europe – Strengthening the Connection Between Economy, Society and the Environment – Updated Bioeconomy Strategy Publications Office." https://doi.org/10.2777/792130.

Eurostat. 2020. "Agri-Environmental Indicator - Cropping Patterns." https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agri-environmental_indicator_-_cropping_patterns&oldid=457657.

Fachagentur Nachwachsende Rohstoffe eV. 2016. "Leitfaden Biogas: Von Der Gewinnung Zur Nutzung." Order, (208), 156–157.

Feurdean, A., E. Ruprecht, Z. Molnár, S. M. Hutchinson, and T. Hickler. 2018. "Biodiversity-Rich European Grasslands: Ancient, Forgotten Ecosystems." *Biological Conservation* 228: 224–232. https://doi.org/10.1016/j.biocon.2018.09.022.

Foley, W. J., A. McIlwee, I. Lawler, L. Aragones, A. P. Woolnough, and N. Berding. 1998. "Ecological Applications of Near Infrared Reflectance Spectroscopy–A Tool for Rapid, Cost-Effective Prediction of the Composition of Plant and Animal Tissues and Aspects of Animal Performance." *Oecologia* 116, no. 3: 293–305. https://doi.org/10.1007/s004420050591.

Fu, J., R. Gasche, N. Wang, H. Lu, K. Butterbach-Bahl, and R. Kiese. 2017. "Impacts of Climate and Management on Water Balance and Nitrogen Leaching From Montane Grassland Soils of S-Germany." *Environmental Pollution* Barking, Essex: 1987 229: 119–131. https://doi.org/10.1016/j.envpol.2017.05.071.

Guimarães, I. C. d. S. B., T. H. da Silva, C. C. Picchi, and R. S. Fukushima. 2023. "Assessing the Repeatability and Reliability of NIRS to Predict Nutritional Values and to Evaluate Two Lignin Methods in Urochloa spp. Grasses." *Grasses* 2, no. 2: 112–126. https://doi.org/10.3390/grasses2020010.

Haaland, D. M., and E. V. Thomas. 1988a. "Partial Least-Squares Methods for Spectral Analyses. 1. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information." *Analytical Chemistry* 60, no. 11: 1193–1202. https://doi.org/10.1021/AC00162A020.

Haaland, D. M., and E. V. Thomas. 1988b. "Partial Least-Squares Methods for Spectral Analyses. 2. Application to Simulated and Glass Spectral Data." *Analytical Chemistry* 60, no. 11: 1202–1208. https://doi.org/10.1021/AC00162A021.

Hallikma, T., K. Tali, I. Melts, and K. Heinsoo. 2023. "How Is Plant Biodiversity Inside Grassland Type Related to Economic and Ecosystem Services: An Estonian Case Study." *Agriculture, Ecosystems & Environment* 349: 108429. https://doi.org/10.1016/j.agee.2023.108429.

Henneberg, W., and F. Stohmann. 1860. "Beiträge Zur Begründung Einer Rationellen Fütterung Der Wiederkäuer." Erstes Heft: Das Erhaltungsfutter Volljährigen Rindviehes Und Über Fütterung Mit Rübenmelasse. Praktisch-Landwirtschaftliche Und Chemisch-Physiologische Untersuchungen. Für Landwirte Und Physiologen. Braunschweig: Schwetschke. https://www.digitale-sammlungen.de/de/view/bsb10295869?page=,1.

Herrmann, C., C. Idler, and M. Heiermann. 2016. "Biogas Crops Grown in Energy Crop Rotations: Linking Chemical Composition and Methane Production Characteristics." *Bioresource Technology* 206: 23–35. https://doi.org/10.1016/j.biortech.2016.01.058.

Herrmann, C., A. Prochnow, M. Heiermann, and C. Idler. 2014. "Biomass From Landscape Management of Grassland Used for Biogas Production: Effects of Harvest Date and Silage Additives on Feedstock Quality and Methane Yield." *Grass and Forage Science* 69, no. 4: 549–566. https://doi.org/10.1111/gfs.12086.

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES). 2018. "Summary for Policymakers of the Regional Assessment Report on Biodiversity and Ecosystem Services for Europe and Central Asia of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services." IPBES Secretariat, 22–47.

Kaiser, T., and J. Ahlborn. 2021. "Long-Term Vegetation Monitoring in the Floodplain Grasslands of the Lower Havel Valley (Northeastern Germany) and Conclusions for Sustainable Management Practices." *Journal for Nature Conservation* 63: 126053. https://doi.org/10.1016/j.jnc.2021.126053.

Kandel, T. P., S. Sutaryo, H. B. Møller, U. Jørgensen, and P. E. Lærke. 2013. "Chemical Composition and Methane Yield of Reed Canary Grass as Influenced by Harvesting Time and Harvest Frequency." *Bioresource Technology* 130: 659–666. https://doi.org/10.1016/j.biortech.2012.11.138.

Kassambara, A. 2023. "ggcorrplot: Visualization of a Correlation Matrix Using 'ggplot2'." Version R Package Version 0.1.4.1. https://CRAN.R-project.org/package=ggcorrplot.

Kassambara, A., and F. Mundt. 2020. "factoextra: Extract and Visualize the Results of Multivariate Data Analyses." Version R Package Version 1.0.7. https://CRAN.R-project.org/package=factoextra.

Keymer, U., and A. Schilcher. 1999. "Überlegungen Zur Errechnung Theoretischer Gasausbeuten Vergärbarer Substrate in Biogasanlagen." Landtechnik-Bericht Nr. 32. Landtechnik. Freising.

Klimiuk, E., T. Pokój, W. Budzyński, and B. Dubis. 2010. "Theoretical and Observed Biogas Production From Plant Biomass of Different Fiber Contents." *Bioresource Technology* 101, no. 24: 9527–9535. https://doi.org/10.1016/j.biortech.2010.06.130.

Krenz, L. M. M., and D. Pleissner. 2024. "Valorization of Landscape Management Grass." *Biomass Conversion and Biorefinery* 14, no. 3: 2889–2905. https://doi.org/10.1007/s13399-022-02568-0.

KTBL. 2021. "Gasausbeute in Landwirtschaftlichen Biogasanlagen." Potenziale, Erträge, Einflussfaktoren. Darmstadt, Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL).

Kuhn, M. 2008. "Building Predictive Models in R Using the Caret Package." *Journal of Statistical Software* 28, no. 5: 1–26. https://doi.org/10.18637/jss.v028.i05.

Melts, I., M. Ivask, M. Geetha, K. Takeuchi, and K. Heinsoo. 2019. "Combining Bioenergy and Nature Conservation: An Example in Wetlands." *Renewable and Sustainable Energy Reviews* 111: 293–302. https://doi.org/10.1016/j.rser.2019.05.028.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. "The Estimation of the Digestibility and Metabolizable Energy Content of Ruminant Feedingstuffs From the Gas Production When They Are Incubated With Rumen Liquor In Vitro." *Journal of Agricultural Science* 93, no. 1: 217–222. https://doi.org/10.1017/S0021859600086305.

Meserszmit, M., G. Swacha, L. Pavlů, V. Pavlů, A. Trojanowska-Olichwer, and Z. Kącki. 2022. "Species Composition of Semi-Natural Mesic Grasslands as a Factor Influencing the Methane Yield of Plant Biomass (Central Europe)." *GCB Bioenergy* 14, no. 1: 54–64. https://doi.org/10.1111/gcbb.12902.

Meyer, A. K., E. Paarup, E. A. Ehimen, and J. B. Holm-Nielsen. 2018. "Future European Biogas: Animal Manure, Straw and Grass Potentials for a Sustainable European Biogas Production." *Biomass and Bioenergy* 111: 154–164. https://doi.org/10.1016/j.biombioe.2017.05.013.

Norman, H. C., E. Hulm, A. W. Humphries, S. J. Hughes, and P. E. Vercoe. 2020. "Broad Near-Infrared Spectroscopy Calibrations Can Predict the Nutritional Value of >100 Forage Species Within the Australian Feedbase." *Animal Production Science* 60, no. 8: 1111. https://doi.org/10.1071/AN19310.

Oluk, A. C., H. Yucel, F. D. Bilgin, and U. Serbester. 2022. "Estimation of Forage Quality by Near Infrared Reflectance Spectroscopy in Dallisgrass, *Paspalum dilatatum*, Poir." *Journal of Near Infrared Spectroscopy* 30, no. 4: 189–196. https://doi.org/10.1177/09670335221083070.

Petermann, J. S., and O. Y. Buzhdygan. 2021. "Grassland Biodiversity." *Current Biology: CB* 31, no. 19: R1195–R1201. https://doi.org/10.1016/j.cub.2021.06.060.

Prochnow, A., M. Heiermann, A. Drenckhan, and H. Schelle. 2005. "Seasonal Pattern of Biomethanisation of Grass From Landscape Management." *Agricultural Engineering International: The CIGR Journal* 7: 1–17.

Roberts, C. A., J. Workman, and J. B. Reeves. 2004. *Near-Infrared Spectroscopy in Agriculture*. Vol. 44, 822. American Society of Agronomy, Crop and Soil Science.

Schils, R. L., C. Bufe, C. M. Rhymer, et al. 2022. "Permanent Grasslands in Europe: Land Use Change and Intensification Decrease Their Multifunctionality." *Agriculture, Ecosystems & Environment* 330: 107891. https://doi.org/10.1016/j.agee.2022.107891.

Schmidt, A., S. Lemaigre, P. Delfosse, H. von Francken-Welz, and C. Emmerling. 2018. "Biochemical Methane Potential (BMP) of Six Perennial Energy Crops Cultivated at Three Different Locations in W-Germany." *Biomass Conversion and Biorefinery* 8, no. 4: 1–16. https://doi.org/10.1007/s13399-018-0338-2.

Stubbs, T. L., A. C. Kennedy, and A.-M. Fortuna. 2010. "Using NIRS to Predict Fiber and Nutrient Content of Dryland Cereal Cultivars." *Journal of Agricultural and Food Chemistry* 58, no. 1: 398–403. https://doi.org/10.1021/jf9025844.

van Soest, P. J. 1966. "Nonnutritive Residues: A System of Analysis for the Replacement of Crude Fiber." *Journal of AOAC International* 49, no. 3: 546–551. https://doi.org/10.1093/jaoac/49.3.546.

VDI. 2016. "VDI 4630: Fermentation of Organic Materials: Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests: Beuth Verlag." https://books.google.de/books?id=HmcmygEACAAJ.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. 2023. "Methodenbuch Band III Futtermittel." Darmstadt: VDLUFA-Verl. (VDLUFA-Schriftenreihe, 69).

Weißbach, F. 2008. "Zur Bewertung Des Gasbildungspotenzials Von Nachwachsenden Rohstoffen." *Landtechnik* 63, no. 6: 356–358. https://doi.org/10.15150/LT.2008.880.

Weißbach, F. 2009. "Die Bewertung Des Gasbildungspotenzials Von Nachwachsenden Rohstoffen." In Bayerische Landesanstalt für Landwirtschaft (LfL) (Ed.): Internationale Wissenschaftstagung Biogas Science 2009. Schriftenreihe, Band 3. Band 3. Freising-Weihenstephan (Germany), pp. 517–526.

Weißbach, F., and C. Strubelt. 2008. "Die Korrektur Des Trockensubstanzgehaltes Von Grassilagen Als Substrat Für Biogasanlagen." *Agricultural Engineering* 63, no. 4: 210–211.

Wendt, M., and S. Nandke. 2025a. "Wendt & Nandke et al. 2025 Weender Analysis." Figshare. https://doi.org/10.6084/m9.figshare.28924526.

Wendt, M., and S. Nandke. 2025b. "Wendt & Nandke et al. 2025 GCB R Code." Figshare. https://doi.org/10.6084/m9.figshare.28924532.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.