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Anaerobic Digestion of Cuttings from Grassland in Protected Landscape Areas

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Abstract: Orchard meadows are biodiversity hotspots, as the understory often consists of species-rich lowland hay meadows. Due to the low energy density of the grass, it is not suitable as feed, but the energetic utilisation of cuttings from orchard meadows for biogas production could facilitate the protection of these semi-natural grasslands. Here, lowland hay meadows and extensively used orchards were investigated to assess their potential for anaerobic digestion in biogas plants. Aboveground biomass was harvested weekly from three lowland hay meadows differing in conservation statuses and analysed for cell wall components (aNDF, ADF, and ADL), nutritional values (XF, XL, XP), and methane formation potential by anaerobic digestion. Further, orchard meadows were harvested twice during summer and analysed in the same way. Specific methane yield decreased linearly with cutting dates from 0.325 m^3 $kg^{-1}{}_{(oDM)}$ to 0.237 m^3 $kg^{-1}{}_{(oDM)}$. The cumulated area-related methane yields of the orchards ranged from 818 m^3 ha^{-1} to 1036 m^3 ha^{-1}. Specific methane yields were linearly correlated with XL, aNDF, ADF, and ADL.

Keywords: biomass; biogas; nature conservation; semi-natural grassland; biodiversity; orchard meadow; bioenergy; grassland management; forage quality

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1. Introduction

Orchard meadows are on the Red List of endangered habitat types in Germany and are classified as severely endangered to threatened with complete destruction [1].

The orchard meadows have high biodiversity, provide ecosystem services, and have been part of UNESCO's immaterial cultural heritage since 2021 [2–4]. The high biodiversity of contiguous orchard meadows is based on the combination of the two habitats' open land and forest, creating semi-open and semi-natural tree savannas. This structural richness, as well as high food supply through extensive management of the understory, supports the conservation of 5000 species [5]. The abandonment of meadow orchards is accompanied by a loss of biodiversity, whereas extensive mowing of meadow orchards, for example, promotes the number of herbaceous species [6].

This traditional agroforestry system is widespread in Central, Eastern, and Western Europe, covering about 10,000 km^2 and running like a belt through 11 European countries [7]. One of the largest contiguous stands of traditional orchards is located in the foothills of the Swabian Jura in Baden–Württemberg [8].

A study commissioned by the Baden–Württemberg State Institute for Environmental Protection reveals that the number of extensively managed fruit trees declined from 8.6 million in the year 2008 to 7.1 million in 2021 [9]. The same study projects the possible disappearance of all fruit trees in Baden–Württemberg by the year 2050.

The UNESCO Swabian Alb Biosphere Reserve, also known as the Swabian Jura, is a model region in which attempts are being made to combine economic and nature conservation interests and develop them in a future-oriented and sustainable way [10].

The region is particularly famous for its scattered orchards and diverse flower meadows. The biosphere area covers about 85,000 hectares, of which about a third is designated as Fauna–Flora–Habitat (FFH) areas.

The Natura 2000 network of protected areas includes FFH (habitats) and bird sanctuaries, which serve to protect important natural habitats and species in accordance with European directives. The European Habitats Directive [Annex I; 1992] also designates habitat types of community interest to be protected. This includes habitat type 6510, the lowland hay meadow. The Swabian Jura, in particular, is characterised by well-developed stocks of this habitat type due to the calcareous parent rock [11–15].The meadows are divided into different conservation statuses, the conservation status of which must be recorded as part of the process of mapping the areas.

According to the Habitats Directive, extensively used meadow orchards in Germany do not represent a separate habitat type (HT) to be protected. Only if the orchard meadows are located in designated FFH areas or if the fruit trees are located on protected lowland hay meadows (LRT 6510), in which case the condition of the grassland must not deteriorate, but the tree population does not receive any special protection, and the orchard meadows acquire a protected status.

Traditional hay meadows, where the land was used extensively, are no longer profitable. The reason for this is that the nutritional value of traditional fodder is insufficient for modern livestock production [16,17]. On the other hand, recycling the cuttings in a composting plant is the most expensive recycling method, as fees are incurred for recycling outside the farm's own composting plant.

Currently, a high loss of protected areas can be observed due to intensification, but also extensification measures, and the remaining lowland hay meadows are in poor conservation status, which will further deteriorate over time [18,19]. This has now even led to legal action by the EU Commission against Germany before the European Court of Justice [20].

Restrictions in fertiliser application und extensive mowing with one to two cutting times can be considered optimal for lowland hay meadows [21]. In this context, later cutting dates than envisaged for intensive grassland and the removal of biomass, resulting in nutrient-poor grassland, promote biodiversity. Simply mulching these areas has a detrimental effect on species composition and diversity [22].

While years ago, the construction of biogas plants in Germany was seen as a reason for the decline of species-rich meadows [23,24], the energetic use of the cuttings from orchard meadows (OMs) or species-rich lowland hay meadows (LHMs) in a biogas plant is supposed to be an alternative to sustainably secure this unique cultural landscape. The harvesting of the grass, which typically takes place twice a year, and its utilisation in biogas plants preserves the nutrient-poor grassland and counteracts the destruction of the orchard meadows in the Swabian Jura region [25].

Mowing causes all inflorescences to disappear temporarily [6]. Therefore, large-scale, simultaneous mowing should be avoided, otherwise it can have significant effects on flower-pollinating insects, and there will be no more compensatory areas left where subpopulations of organisms can develop and recolonize the mown areas [6,26,27]. In addition, 10% of the areas should be left standing during each mowing, for example, as overwintering habitats [27–30].

Grass from FFH-mowed meadows does not compete with other cultivated biomass and promises a reduction of greenhouse gas emissions due to its low ecological footprint.

The aim is to demonstrate synergy effects between energy use and landscape conservation, so that the use of the mown grass in a biogas plant is a simple and sensible alternative to the previous use.

Grassland crops differ greatly in their composition, anaerobic degradability, and biogas yield [31]. Therefore, this study addresses the closer examination of these species-rich hay meadows.

The following questions will be addressed in this study: (1) What is the biomass yield in different lowland hay meadows and orchard meadows during a growing season?

(2) What is the influence of harvest timing on biomass chemical composition and specific methane yield, and thus on potential hectare methane yield? (3) Does the energetic use of biomass from orchard meadows offer an alternative utilisation path that also meets the requirements of nature conservation?

2. Materials and Methods

2.1. Experimental Area and Fieldwork

2.1.1. Lowland Hay Meadows

For the first field trial, three meadows were selected, which are classified in the Natura 2000 network as lowland hay meadows (LHM; habitat type 6510), characterised by different conservation statuses (A, B, and C). All meadows are located at the foot of the low mountain range Swabian Jura in Eningen unter Achalm (u. A.), in the southwest of Germany. The entire area is part of the UNESCO Swabian Alb Biosphere Reserve. Meadow A (LHMA) had an excellent conservation status (value A) and is located at 48°29′35.7″ N 9°16′31.1″ E, 560 m above sea level, Meadow B (LHM-B) had a good conservation status (value B) and is located at 48°29′16.9″ N 9°16′03.5″ E, 519 m above sea level, and Meadow C (LHM-C) had a medium conservation status (value C) and is located at 48°29′36.0″ N 9°17′03.3″ E, 583 m above sea level. All meadows are in proximity within an area of 2 km². Slopes of LHM-A and LHM-C face south/southwest, while the slope of LHM-B faces northwest. The vegetation types of LHM-B and LHM-C are *Arrhenatheretum* plant communities, while LHM-A is an *Arrhenatheretum salvietosum* plant community. The exact mapping data can be found in the following references [32–34].

The mean annual temperature in Eningen u. A. was 12.7 °C, and the mean annual precipitation was 647 mm for the year 2020, measured at the research station "Unterer Lindenhof" of the University of Hohenheim.

In each meadow, biomass was harvested on a 1 m^2 area in three replicates on 20 weekly dates between April and September 2020 to determine aboveground biomass. An aluminium cuboid with internal dimensions of 1 × 1 × 1 m^3 was used for harvesting. To avoid contamination of the sample material and to ensure sufficient reserves for regrowth of the meadow, the meadow was cut 7 cm above the ground using a cordless hedge trimmer (Makita DUH751Z, Makita Werkzeug GmbH, Ratingen, Germany). A latinised resolvable row-column design using CycDesigN [35] was applied to randomize sampling points, guaranteeing a buffer zone between sampling points of at least twice the length of the maximum vegetation height. After harvesting, fresh samples were placed in sturdy plastic bags, tightly sealed, and weighed immediately after sampling in the laboratory. For further analysis, replicates were combined into a composite sample.

2.1.2. Extensively Managed Orchard Meadows

For the second trial, 24 orchard meadows (OMs) were selected in the municipalities of Eningen u. A. (OM-E) and Lichtenstein (OM-L). Eningen u. A. is part of the UNESCO Biosphere Reserve Swabian Alb; the municipality Lichtenstein borders this biosphere reserve.

The orchard meadows have an average size of 1673 m^2. Of the 24 orchard meadows, nine were designated lowland hay meadows in the Natura 2000 network (five meadows with conservation status B, four meadows with conservation status C). Another three meadows were in an FFH area. The remaining orchard meadows were not under protection because they were used more intensively before the start of the study. As recommended by the local authorities, the meadows were mowed twice in the summer of 2020, with the first mowing occurring when the stand-forming grasses were in bloom [36] on 5–6 June, and the second in mid-August. Thus, the mowing of the orchard meadows corresponded to a typical hay harvest. The cutting height of the mowers was 7 cm for both hay cuts. To determine yields per hectare, the tractor-drawn loader wagons were weighed with a truck scale before and after unloading the hay at the biogas plant.

The clippings were processed with a crossflow chipper and fed to the research biogas plant. Sub-samples were collected and subjected to further analysis, such as moisture content, chemical components, and specific methane yields, as for the lowland hay meadows samples. The meadows were not fertilised during the experimental period because targeted nutrient removal was to occur on the areas.

2.2. Laboratory Analysis

2.2.1. Sample Preparation and Chemical Composition Analyses

Collected samples were analysed for dry matter and organic dry matter in the laboratory of the State Institute for Agricultural Engineering and Bioenergy (Stuttgart, Germany, Hohenheim), according to the guidelines of the Association of German Agricultural Testing and Research Institutes (VDLUFA, 2012) [37]. For the determination of dry matter, six samples from the composite sample were dried at 105 °C to constant weight. Organic dry matter and crude ash content was then determined by placing the samples in muffle ovens at 550 degrees and then weighing them.

Additional sub-samples were dried in a drying chamber at 60 °C for 48 h to preserve the volatiles. After drying, the substrates were ground with a cutting mill (Pulverisette 19, Fritsch GmbH, Idar–Oberstein, Germany) to a particle size of 1 mm for the HBT batch fermentation test and for the other chemical composition analyses to obtain homogenous samples. After pre-drying the samples at 60 °C, the residual moisture and ash content of these sub-samples were determined by incinerating the samples first at 105 °C and later at 550 °C, noting the weight loss during the process.

The determination of crude ash, crude protein (XP), crude fat (XL), and crude fibre (XF) was performed by the Core Facility Hohenheim (Stuttgart, Germany) in accordance with Commission Regulation (EC) No 152/2009 for Weender analysis [38].

Neutral detergent fibre after amylase treatment (aNDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined by the Core Facility Hohenheim according to the guidelines of the Federation of German Agriculture Investigation and Research Institutes' method 6.5.1, 6.5.2, and 6.5.3 (VDLUFA, 2012) [37].

2.2.2. Biochemical Methane Potential Test

Substrate-specific methane yields of three replications of the samples were determined using the HBT, a standardised method according to VDI 4630 [39]. Specific methane yield refers to the organic dry matter. 100 mL glass syringes were filled with 30 mL of inoculum and 400 mg of the sample material pre-dried at 60 °C. Syringes filled with 50 mL of inoculum were used as blank samples. Syringes filled with 30 mL of inoculum and 400 mg of hay standard or concentrate feed standard were used to control the experimental procedure.

The syringes were rotated in a heating cabinet under mesophilic conditions (37 °C ± 0.5 °C) for 35 days to ensure that the contents remained always in motion. The volume of gas produced was measured at regular intervals using the scale on the syringe. After drying with an absorber (SICAPENT®, Merck, Darmstadt, Germany), the CH_4 concentration of the gas was measured with an infrared spectrometric methane sensor ("Advanced Gasmitter", Pronova Analysetechnik, Berlin, Germany). The methane sensor was calibrated before and after the measurement with a fixed, defined methane concentration (60.0% methane) (GG0007054, Westfalen AG, Münster, Germany). Subsequently, the methane concentrations were corrected to standard conditions (1013.3 hPa, 0 °C).

2.2.3. Inoculum

The inoculum used is from a laboratory-scale 400 L biogas reactor continuously fed with wheat meal, soya meal, corn silage, rapeseed oil, and digestate from a biogas plant. The retention time of the reactor is 200 days, and the organic loading rate (OLR) is 0.3 $kg_{(oDM)}\ m^{-3}\ d^{-1}$. The fermenter temperature is 37 °C [40]. Before starting the experiment, the inoculum was sieved with a mesh size of 0.5 mm.

2.2.4. Kinetics of Methane Formation Process

The kinetics of the methane formation process were simulated using the modified Gompertz function [41], which is used to describe the biogas production in batch experiments based on bacterial growth.

$$M = P \times exp\left\{-exp\left[\frac{R_m \times exp(1)}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

$$R_m(x) = R_m \times e \times exp\left\{-exp\left[\left(\frac{R_m \times exp(\lambda - x)}{P}\right) + 1\right] + \left[\left(\frac{R_m \times exp(\lambda - x)}{P}\right) + 1\right]\right\} \quad (2)$$

M is the accumulated methane yield (m^3 $kg^{-1}{}_{(oDM)}$) as a function of time, P is the methane production potential (m^3 $kg^{-1}{}_{(oDM)}$), R_m is the maximum daily methane formation rate (m^3 $kg^{-1}{}_{(oDM)}$ d^{-1}), λ (d) is the lag phase, i.e., the time required until methane is produced, e is $exp(1)$ or 2.7182818, and x (d) is the trial day. The parameters P, R_m, and λ were determined using nonlinear regression analysis. In addition, the duration (days) during which half of the total methane ½ M (d) was produced was determined. The model parameters of the nonlinear regression equations were determined using the Solver function of Microsoft Excel, and the squared correlation coefficient (r^2) was used to assess the accuracy of the model quality.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS 28.0—IBM (Armonk, NY, USA). For comparison of means, a one-factor analysis of variance (ANOVA) followed by Tukey's post hoc test was performed.

To determine the influence of the factors of meadow and 20th cut timing on the independent variables, a two-factor ANOVA was performed, and a Tukey's post hoc test was used to identify the differences within the two factors. An interaction term was always formed between the two factors. The same approach was true when examining orchards at the two sites (OM-E and OM-L) on the two harvest dates.

For the comparison of the measured values at the respective cutting or harvesting dates, a generalised linear model was applied, and Bonferroni sequential was chosen as the fitting method for multiple comparisons in the case of multiple contrasts. This method reduces alpha error accumulation, so that a reduction of false correctly significant results can be counteracted.

A stepwise selection method was used to determine the coefficients of the multilinear regression equation. To represent the relationship between the variables, the creation of a Pearson correlation coefficient matrix was chosen. Since the orientation of the relationship was not known in advance, a two-sided probability was chosen.

3. Results and Discussion

3.1. Biomass Yields of Lowland Hay and Orchard Meadows during the Growing Season

Aboveground dry biomass (AGB_{dry}) ranged from 0.62 t ha^{-1} on 28 April 2020 (2nd cutting date) in LHM-A to 4.13 t ha^{-1} on 14 July 2020 (13th cutting date) in LHM-B (Figure 1). The AGB_{dry} of the LHM showed a continuous increase from the beginning of sampling until mid-June. On 9 June 2020 (8th cutting date), the AGB_{dry} in LHM-A, LHM-B, and LHM-C was 1.97, 3.29, and 4.04 t ha^{-1}, respectively. At this point, the AGB_{dry} of LHM-A remained constant, while the AGB_{dry} in LHM-B and LHM-C initially remained at a stable level and then tended to decrease. This decline in yield can be explained by the laying down of the grass and the subsequent rotting process in zones close to the ground. LHM-C, in particular, showed a certain heterogeneity of stand density along the slope, which is reflected in high standard deviations.

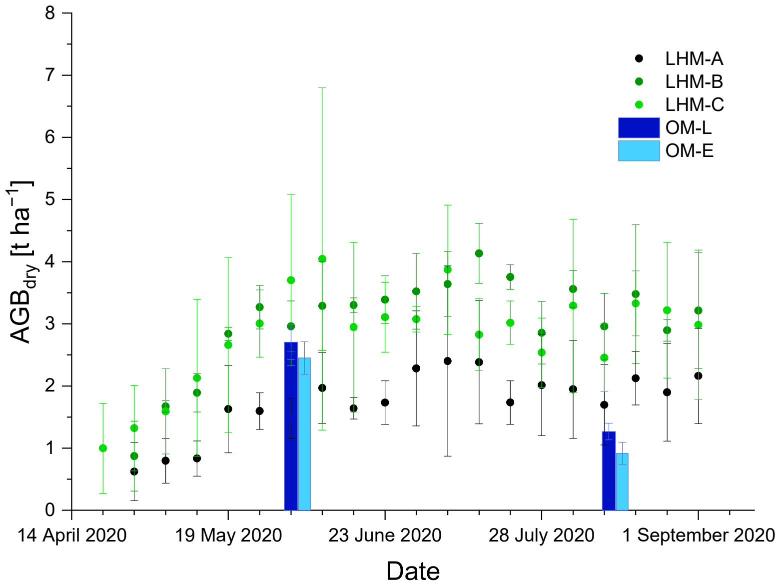

Figure 1. Aboveground dry biomass (AGB_{dry}) (t ha^{-1}) of three lowland hay meadows (LHMs), characterised by different conservation statuses (A = excellent, B = good, and C = average) over the vegetation period and of orchard meadows in two different regions (E = Eningen and L = Lichtenstein) at two harvest dates at Swabian Jura.

On average, across all samplings, AGB_{dry} in LHM-A was 1.65 t ha^{-1}, which was significantly lower than in LHM-B at 2.87 t ha^{-1} and LHM-C at 2.81 t ha^{-1}.

The orchard meadows were harvested on 3 and 6 June 2020 (1st cut) and on 14.08 and 15 August 2020 (2nd cut). In the 1st cut, AGB_{dry} was 2.71 t ha^{-1} in OM-L and 2.45 t ha^{-1} in OM-E, and in the 2nd cut, AGB_{dry} was 1.27 and 0.92 in OM-L and OM-E, respectively. For both cuts, AGB_{dry} was significantly lower at site E than at site L. In comparison, AGB_{dry} of lowland hay meadows at the time of the first and second cuts in the orchard averaged 2.71 and 2.37 t ha^{-1} across conservation status, respectively.

At the first cutting date, only the meadow in medium conservation status had sufficient aboveground biomass for further analyses. Unfertilised semi-natural grassland, unlike agriculturally improved and/or fertilised grassland, can be expected to grow linearly in spring or early summer and reach maximum yield later, in mid-June [42].

LHM-B is the only meadow on the northern slope that is less arid and more nutritious, resulting in the highest biomass yields [33]. LHM-C has a relatively low proportion of herbs in the vegetation, which is why the habitat-typical species inventory is impoverished [32]. In addition, the almost identical biomass yields as LHM-B can be explained by the presence of nitrogen indicator and fat meadow species, despite the predominantly south to southwest exposure [32]. LHM-A has a balanced distribution of grasses and herbs; parts of the meadow are rough grasslands, resulting in lower biomass yields [34].

Lower yields would be obtained for OM-E. The areas are located on the lower slopes of the foothills of the Swabian Jura where the soil types Pararendzina and Rendzina predominate, yielding drier sites due to their soil composition [43,44]. Higher yields were obtained on OM-L. This is because the land at site L is primarily located in the valley bottom and thus closer to the water table. The first cut of the orchard meadows is comparable to the biomass yields of the three lowland hay meadows, because the orchard meadows are predominantly lean lowland meadows. However, the second cut of the orchard meadows is not comparable to the three lowland hay meadows studied because they were not previously mowed and thus the biomass growth rate is stagnant. On the other hand, the biomass of the orchard meadows at the second cut depends mainly on the amount of precipitation in the summer months and the emergence after harvest [45]. Overall, higher yields were obtained in the areas of the orchard meadows. Thus, a second mowing on the same area leads to higher yields.

3.2. Chemical Composition of Harvested Biomass during the Vegetation Period

The ash content of the lowland hay meadows, at 7.58%, was lower than the ash content of the orchard meadows, at 11.28%. The differences are due to contamination of the grass clippings of the orchard meadows with soil from mowing on the slope, and the inclusion of soil piles at a cutting height of 7 cm is reflected in the increased ash contents. This can be counteracted by increasing the cutting height to 10 cm, which also has a positive effect on the mortality rate of insects and amphibians while reducing the rate of introduction of unusable sediments into the fermenter [26].

Crude protein content (XP) ranged from 7.6% on 28 July 2020 (15th cutting date) to 18.1% on 28 April 2020 (2nd cutting date) in the lowland hay meadow, with both values being measured in LHM-B (Figure 2). Crude fat content (XL) ranged from 2.6% on 26 May 2020 (6th cutting date) in LHM-A to 5.2% on 28 April 2020 (2nd cutting date) in LHM-B. The crude fibre content (XF) ranged from 21.2% on 28 April 2020 (2nd cutting date) to 42.7% on 18 August 2020 (18th cutting date), with both values measured in LHM-B. While XP and XL decreased at the beginning of the growing season until early July and mid-June, respectively, and remained constant thereafter, XF increased until almost the end of the growing season.

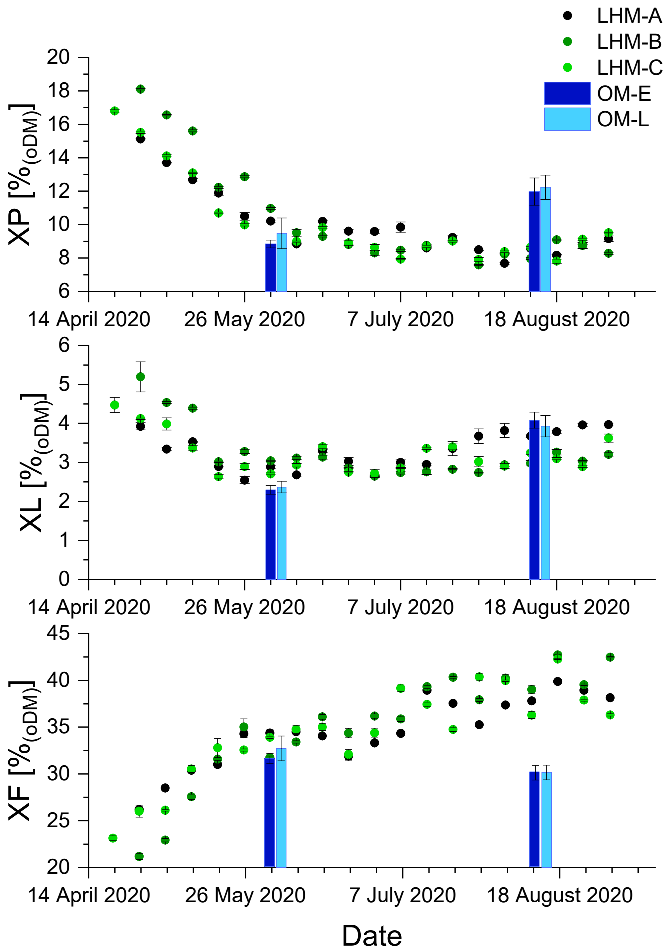

Figure 2. Content of crude protein (XP), crude fat (XL), and crude fibre (XF) ($\%_{(oDM)}$) of three lowland hay meadows (LHMs) characterised by different conservation statuses (A = excellent, B = good, C = average) over the vegetation period and of orchard meadows in two different regions (E = Eningen, L = Lichtenstein) at two harvest dates at Swabian Jura.

On average, over the growing season, the highest XP was found in LHM-B at 10.4%, followed by LHM-C and LHM-A at 10.1 and 10.0%, respectively. XL was highest in LHM-A at 3.3%, followed by LHM-B and LHM-C at 3.3 and 3.2%, respectively. XF was highest in

LHM-B at 35.1%, followed by 34.6 and 34.3% in LHM-A and LHM-C, respectively. Comparison of mean values over the entire growing season revealed no significant differences between the conservation statuses of the meadows in cases of XP, XL, and XF. However, comparison of meadows characterised by different conservation statuses at individual sampling dates revealed significant differences for XP, XL, and XF in most samplings.

XP in OM-L and OM-E were 8.8%$_{(oDM)}$ and 9.5%$_{(oDM)}$ in the 1st cut and 12.0%$_{(oDM)}$ and 12.2%$_{(oDM)}$ in the 2nd cut, respectively. While XP was significantly higher in the 2nd cut than in the 1st cut, no significant differences were found between the two sites. Samplings in the lowland hay meadows conducted at the same time as the 1st and 2nd cut in the orchards revealed XP of 9.9 and 8.4%, respectively, averaged across conservation status.

In the orchards, XL was 2.3%$_{(oDM)}$ and 2.4%$_{(oDM)}$ in OM-L and OM-E, respectively, for the 1st cut, while it was significantly higher for the 2nd cut at 4.18%$_{(oDM)}$ and 3.9%$_{(oDM)}$ in OM-L and OM-E, respectively. Again, no significant differences were found between sites. Sampling in the lowland hay meadows at the same timepoints of the 1st and 2nd cut averaged over conservation status XL of 2.9 and 3.3%, respectively.

At 31.5 and 32.7% for OM-L and OM-E, respectively, XF was significantly higher in the 1st cut than in the 2nd cut at 30.1 and 30.2% for OM-L and OM-E, respectively. No significant differences were found between sites. Samplings of lowland hay meadows during the same periods of the 1st and 2nd cuts revealed XF of 33.4 and 37.7%, respectively, averaged across conservation status.

When temperatures increase during the growing season, the crude protein content decreases, but the content of structural cell wall components increases [46]. In this context, the increase in crude fibre during the growing season is negatively correlated with crude protein content [47] and dependent on growth and maturation. Thus, the increase in structural carbohydrates leads to a decrease in digestibility [48].

The meadows LHM-A and LHM-C are similar in chemical composition; LHM-B starts with a higher crude protein content but drops to the lowest value during vegetation, LHM-B reaches the highest biomass yields. Also, the meadow was more nutrient-rich, causing a steeper drop in protein content, as was also found in another study [48].

Studies on the chemical content of *Arrhenatheretum elatioris typicum* meadows in Poland for the 1st cut, which took place in early June, yielded results in the same range of values [49]. The decrease in crude protein content seems to be steeper during the first growing season (time to first cut) than during the second growing season.

In general, the forage quality of hay is no longer suitable for dairy cattle; at best, it is suitable for cattle and sheep if the crude protein content is at least above 8%, a limit we reached at both cutting dates of the orchard meadows and almost over the entire growing season of the lowland hay meadows [50,51].

aNDF, ADF, and ADL increased during the growing season until mid-August and remained approximately constant thereafter (Figure 3). Averaged across all cutting dates, the highest mean aNDF was measured on LHM-A at 71.0%, while LHM-C and LHM-B reached 70.7 and 68.3%, respectively. The highest mean ADF was found on LHM-B with 51.1%, while LHM-A and LHM-C reached 50.3 and 49.1%, respectively. No significant differences were found in both aNDF and ADF, among meadows characterised by conservation status and cutting dates. However, mean ADL was significantly higher in LHM-A at 8.8% than in LHM-C at 8.1%, while LHM-B was not significantly different from LHM-A and LHM-C, with an ADL of 8.4%. The ADL content ranged from 3.42% on 28 April 2020 (2nd cutting date) in meadow LHM-B to 12.46% on 18 August 2020 (18th cutting date) in the leanest meadow, LHM-A.

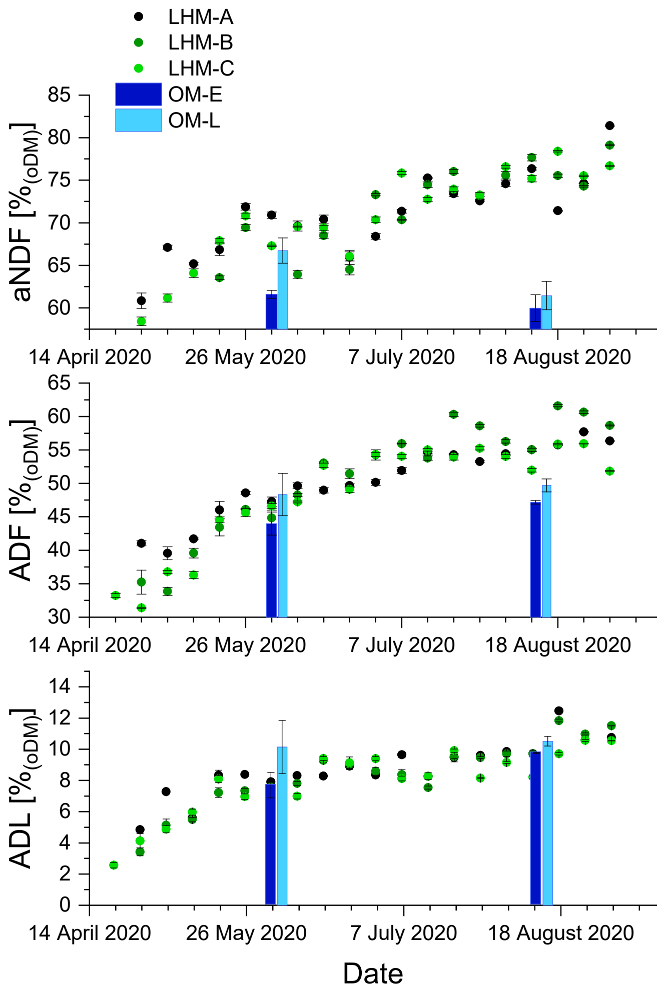

Figure 3. Content of aNDF, ADF, and ADL ($\%_{(oDM)}$) of three lowland hay meadows (LHMs) characterised by different conservation statuses (A = excellent, B = good, C = average) over the vegetation period and of orchard meadows in two different regions (E = Eningen, L = Lichtenstein) at two harvest dates at Swabian Jura.

In the orchards, aNDF was significantly lower in the first cut in OM-L (61.6%) than in OM-E (66.7%), while no significant difference was found between sites in the second cut at 60.0% in OM-L and 61.4% in OM-E. At both cuts, ADF was significantly lower at OM-L, 44.0% at the 1st cut and 47.2% at the 2nd cut, compared to ADF at OM-E, with 48.3% and 49.7% at the 1st and 2nd cuts, respectively. At 7.7%, ADL at the first cut in OM-L was significantly lower than ADL in OM-E at 10.1%, while no significant difference was found between sites at the 2nd cut at 9.8 and 10.5% in OM-L and OM-E, respectively.

Sampling of lowland hay meadows during the same periods of the 1st and 2nd cuts in orchards resulted in aNDF of 66.3 and 76.4%, respectively, on average across conservation status. In addition, in these samplings, the ADF was 46.2 and 53.1%, and the ADL was 7.5 and 9.2% at the time of the 1st and 2nd cuts, respectively.

The change in the content of cell wall components (aNDF, ADF, and ADL) during the season is due to the increasing proportion of cell wall components in the biomass as maturity progresses [52].

The fact that the three meadows were similar in chemical composition is because all three studied meadows belonged to the same plant community and were at a similar elevation above sea level and in the same region. However, it cannot be excluded that biomass from other areas differs from the biomass from the study area in terms of its chemical composition and, in particular, its degree of maturity, e.g., due to differences in altitude.

The higher lignin content in LHM-A may be due to advanced maturity due to lower water availability at this site or to the higher proportion of herbs/forbs resulting in higher lignin content [53]. Lignin in particular has numerous aromatic groups that are capable of replacing aromatics of fossil origin [54]. The degradation of the stable lignin–cellulose structure using suitable pre-treatment methods and the valorisation of lignin are becoming increasingly important [55]. In contrast, lignin is difficult to degrade in anaerobic digestion [56]. However, the complex structure is broken down by pre-treatment processes, and hemicellulose and cellulose can be converted into biogas [57].

In terms of feed digestibility, crude fibre has been largely replaced by the detergent system [58]. aNDF is an estimate of cell wall component content, and ADF is negatively correlated with the digestibility of forages. When considering, for example, alfalfa hay, a value of more than 370 g kg^{-1} of ADF indicates low quality [59]. In addition to ADF, crude protein content also plays an important role in animal nutrition [60]. In biogas production, however, mainly the content of crude fat and lignocellulose (hemicellulose, cellulose, and lignin) is decisive for the expected biogas yield [31,61,62]. The results presented are also consistent with this finding (see Section 3.3). In the study by Mezule et al. (2021), semi-natural grassland habitats were analysed for fermentable sugar for biofuel production [63]. In this study, lowland hay meadows showed the highest productivity in terms of yield per hectare at a harvest date in June and afterwards, decreased over the growing season. In general, the chemical composition of the orchard meadows at the first cutting time was similar to the composition of the FFH meadows that were cut during the same period. It can therefore be assumed that a similar plant community can be found in the orchard meadows, as the orchard meadows are predominantly extensively managed. The second cut of the meadow orchards is not comparable to the weekly samplings in the three lowland hay meadows. When using cuttings as animal feed, the low digestibility and low energy content must be taken into account. Additionally, special attention must be paid to the presence of potentially toxic plants, such as *Colchicum autumnale* and *Jacobaea vulgaris*, which can lead to potential toxicity of the silage [64,65]. Changing the time of cutting and increasing the nutrient supply can suppress these plant species, but this is contrary to the goal of preserving biodiversity [66,67].

In contrast, the substrate can be used in biogas plants after mechanical pre-treatment at both the first and second cutting time without any further restrictions [68–71].

3.3. Methane Yield Potential

Specific methane yield (SMY) ranged from 0.237 $m^3\ kg^{-1}{}_{(oDM)}$, measured for LHM-B on 25 August 2020 (19th cutting date), to 0.325 $m^3\ kg^{-1}{}_{(oDM)}$, measured for LHM-C on 21 April 2020 (1st cutting date) (Figure 4). On the 1st cutting date, only LHM-C had sufficient biomass for harvesting. The specific methane yields of the meadows decreased continuously and linearly over the growing season for the meadows characterised by different conservation statuses. However, regression analysis showed differences in the mean daily decrease in specific methane yield among meadows, with the largest daily decrease of 0.00053 $m^3\ kg^{-1}{}_{(oDM)}$ in LHM-C, followed by LHC-B with 0.00046 $m^3\ kg^{-1}{}_{(oDM)}$, and LHM-A with 0.00033 $m^3\ kg^{-1}{}_{(oDM)}$.

On average, across sampling dates, the highest specific methane yields were recorded in LHM-C with 0.280 $m^3\ kg^{-1}{}_{(oDM)}$, followed by LHM-A with 0.277 $m^3\ kg^{-1}{}_{(oDM)}$ and LHM-B with 0.273 $m^3\ kg^{-1}{}_{(oDM)}$. However, differences in specific methane yields between meadows characterised by different conservation statuses were not significant at $p < 0.05$.

In the orchards, specific methane yields of 0.293 and 0.279 $m^3\ kg^{-1}{}_{(oDM)}$ were measured for hay harvested at the 1st cutting date in OM-L and OM-E, respectively. On the 2nd cutting date, specific methane yields of 0.278 and 0.265 $m^3\ kg^{-1}{}_{(oDM)}$ were measured for OM-L and OM-E, respectively. However, no significant differences were observed between sites or cutting dates.

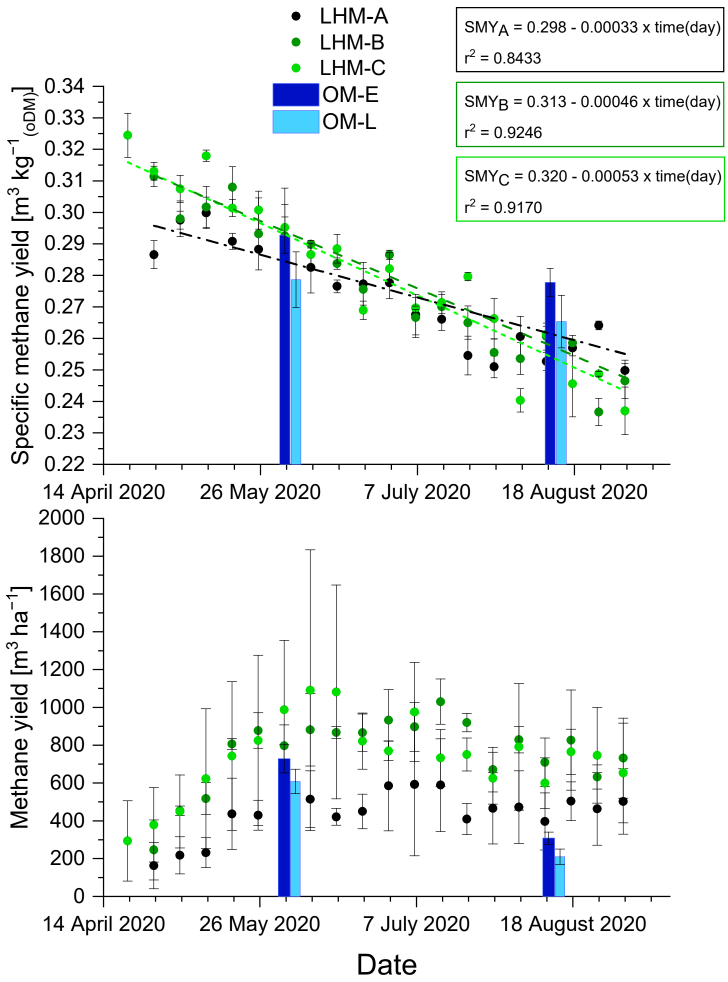

Figure 4. Specific methane yields ($m^3\ kg^{-1}{}_{(oDM)}$) and methane yields per hectare ($m^3\ ha^{-1}$) of three lowland hay meadows (LHMs) characterised by different conservation statuses (A = excellent, B = good, and C = average) over the vegetation period and of orchard meadows in two different regions (E = Eningen and L = Lichtenstein) at two harvest dates at Swabian Jura.

Sampling in the lowland hay meadows at the same 1st and 2nd cutting dates in the orchard meadows averaged specific methane yields of 0.286 and 0.258 $m^3\ kg^{-1}{}_{(oDM)}$, respectively, across conservation status. The differences in the mean specific methane yields between meadows characterised by different conservation statuses and orchard meadows were not significantly different at the first cutting time at $p < 0.05$. The specific methane yields of OM and LHM for the second cut cannot be compared, as the results are based on different harvesting methods. Area-specific methane yields varied from 162.9 $m^3\ ha^{-1}$ for LHM-A on 28 April 2020 (2nd cutting date) and 1090.9 $m^3\ ha^{-1}$ for LHMC on 9 June 2020 (8th cutting date). During the growing season, area yields increased in the early phase, reached their maximum in early mid-June, and remained constant or slightly decreased thereafter. The heterogeneity of the analysed meadows, which led to high standard deviations, is also reflected in the results of the methane yields.

Averaged across all samplings, the highest area-based methane yield was for LHM-B at 762.7 $m^3\ ha^{-1}$, followed closely by LHM-C at 732.8 $m^3\ ha^{-1}$. At 433.0 $m^3\ ha^{-1}$ across all samplings, the methane yield of LHM-A was significantly lower than at the other sites.

In the orchards, area methane yields were significantly higher at the 1st cutting date, 729.1 and 608.0 $m^3\ ha^{-1}$ in OM-L and OM-E, respectively, than at the 2nd cutting date, 308.6 and 209.7 $m^3\ ha^{-1}$ in OM-L and OM-E, respectively. In addition, area methane yields were significantly higher in OM-L than in OM-E.

Sampling in the lowland hay meadows during the same periods of the 1st and 2nd cutting in orchards resulted in area methane yields of 722.4 and 568.5 m^3 ha^{-1}, respectively, averaged across conservation status.

Biomass yields increase until mid-June, while specific methane yields decrease. Despite advancing maturity, comparable area methane yields are achieved. Adjusting the mowing time after the optimum phenological time does not affect the possible area methane yield. In studies on Molinia meadows in Poland, where *Arrhenatherum elatius* was also found as the dominant species, low methane yields of 867 m^3 ha^{-1} were determined at the beginning of July (July 1). In this study, too, specific methane yields decreased over the course of vegetation from 0.221 m^3 $kg^{-1}{}_{(oDM)}$ in May to 0.197 m^3 $kg^{-1}{}_{(oDM)}$ in September [72]. In contrast, yields of between 5300 and 9400 m^3 ha^{-1} can be expected from energy maize cultivation, and the SMY ranges between 0.251 und 0.366 m^3 $kg^{-1}{}_{(oDM)}$, depending on the variety and the harvest time [73,74].

The higher proportion of forbs in the LHM-A meadow results in a lower specific methane yield than in the meadows dominated by grass species [75].

Herrmann et al. (2013) found a correlation between increasing crude fibre content and decreasing methane yield in grass from landscape maintenance materials [47]. However, there was no clear trend in the orchards, because although the content of crude protein and crude fat increased significantly in the second cut, no significant increase in specific methane yield was observed. However, for cell wall components (aNDF, ADF, and ADL), there was also no clear indication between the cutting dates; the differences were site-dependent.

In summary, there is also sufficient methane yield potential in the second cut of the orchards, with yields per hectare depending mainly on the amount harvested. Both cuts of the orchards are therefore well-suited for biogas production. However, the amount of biomass produced is also crucial for economic feasibility, so the second cut is less useful due to the small amount harvested with almost the same amount of labour required for harvesting.

In our trial, which took place under laboratory conditions, the lignocellulose-rich substrate samples were first finely ground to 1 mm. As a result, the accessible surface area for enzymes and microbes increased, thus increasing the digestibility of the biomass. Higher specific methane yields can be achieved using this process. Zheng et al. (2014) provide a good overview of the various methods of pre-treatment, the effect of the pre-treatment and the resulting increase in methane yield from lignocellulosic material [76]. For the substrate delivered directly from the field to the biogas plant, the actual biogas yields to be obtained depend on the harvesting technique and also on the mechanical pre-treatment method to increase the conversion of the cell wall structural substances to biogas [68,77,78].

Specific methane yields were significantly correlated with the parameters describing the chemical composition of the harvested biomass (Table 1). While positive correlations were found with XP and XL, XF was strongly but negatively correlated with specific methane yield. Furthermore, strong and negative correlation with specific methane yields was found for aNDF, ADF, and ADL.

Table 1. Pearson correlation of chemical composition and specific methane yields (SMYs) of three lowland hay meadows characterised by different conservation statuses. Sampling on 20 cutting dates over one growing season, with three replicates per sample. The first cutting date did not provide material for studies in two meadows.

		XP	XL	XF	aNDF	ADF	ADL	SMY
SMY	Pearson Correlation	0.724 **	0.252 **	−0.795 **	−0.795 **	−0.841 **	−0.804 **	1

XP, XL, aNDF, ADF, ADL ($\%_{(oDM)}$); SMY (m^3 $kg^{-1}{}_{(oDM)}$); n = 174; ** significant at $p < 0.001$.

The performance of a multilinear regression model using chemical parameters to predict specific methane yield showed that the highest model quality could be achieved by including XL, aNDF, ADF, and ADL (adjusted $r^2 = 0.815$) (Figure 5).

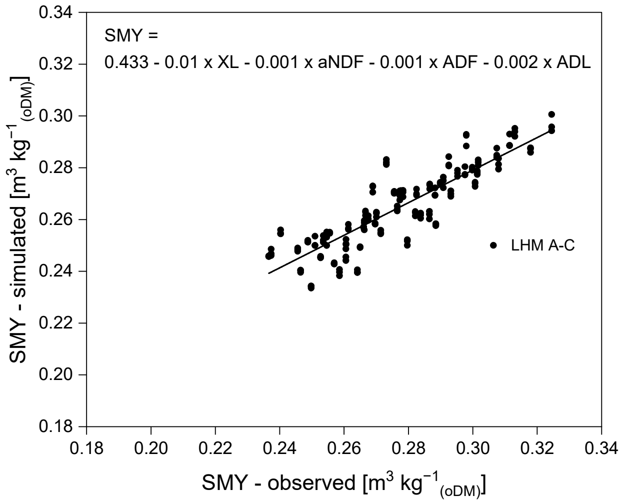

Figure 5. Simulated versus observed specific methane yield (SMY) of three lowland hay meadows after multiple regression analysis for specific methane yields and the corresponding parameters describing the chemical composition of the substrate. XL, aNDF, ADF, ADL ($\%_{(oDM)}$); SMY ($m^3\ kg^{-1}{}_{(oDM)}$); n = 174; LHM = lowland hay meadow.

3.4. Kinetics Analysis of Methane Formation (Gompertz)

The daily methane formation rate R_m varied from 0.0169 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ on 1 September 2020 (20th cutting date) to 0.0380 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ on 28 April 2020 (2nd cutting date), both on LHM-B (Figure 6). R_m decreased steadily during the growing season at all sites. Averaged over the sampling dates, the highest R_m on LHM-B, 0.0281 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$, was significantly higher than on LHM-A, 0.0252 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$, while LHM-C 0.0271 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ did not differ from the other meadows.

In the orchard meadows, the R_m of 1st cut hay was significantly lower at 0.0261 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ on OM-E than on OM-L at 0.0301 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ and also lower than the R_m of 2nd cut hay at 0.0317 and 0.0306 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$ on OM-L and OM-E, respectively.

Samplings in the lowland hay meadows, which occurred at the same dates of 1st and 2nd cutting in the orchards, averaged R_m of 0.0310 and 0.0216 $m^3\ kg^{-1}{}_{(oDM)}\ d^{-1}$, respectively, across conservation status.

The lag phase λ ranged from undetectable in LHM-B (20th sampling date) and LHM-C (18th and 19th sampling dates) to 0.863 days in LHM-B (3rd cutting date). λ decreased from the beginning of the growing season to early June and fluctuated widely thereafter. On average, across sampling dates, λ ranged from 0.467 days in LHM-B, closely followed by 0.463 days in LHM-A and 0.383 days in LHM-C. However, no significant difference in λ was observed among meadows characterised by different conservation statuses.

In the orchard meadows, λ was significantly lower in the 1st cutting period at 0.352 and 0.167 days for OM-L and OM-E, respectively, than in the 2nd cutting period at 0.701 and 0.676 days at OM-L and OM-E, respectively. Sampling in the lowland hay meadows during the same periods of 1st and 2nd cutting in the orchards yielded λ of 0.537 and 0.395 days, respectively, on average across conservation status.

Half production time ranged from 4.708 days at the 2nd cut to 6.825 days on the last cut, both at LHM-B. ½ M constantly increased steadily during the growing season. Averaged across all sampling dates, ½ M at LHM-A was significantly higher at 5.559 days than at LHM-B at 5.327 days, while LHM-C was not significantly different from the other sites at 5.487 days.

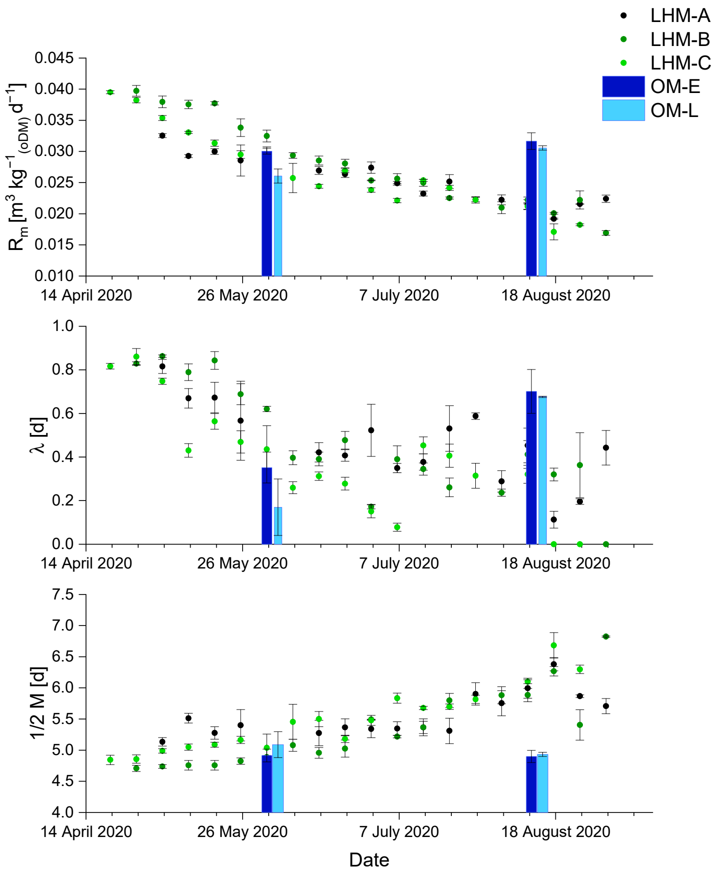

Figure 6. Kinetic parameters R_m (m^3 kg^{-1}$_{(oDM)}$ d^{-1}), λ (d), ½ M (d), using the modified Gompertz function of three lowland hay meadows (LHMs) characterised by different conservation statuses (A = excellent, B = good, and C = average) over the vegetation period and of orchard meadows in two different regions (E = Eningen and L = Lichtenstein) at two harvest dates at Swabian Jura.

In orchards, ½ M was significantly higher at the 1st cut in OM-E at 5.089 days than in OM-L at 4.915 days and higher than at the 2nd cut at 4.900 and 4.933 days in OM-L and OM-E, respectively. Sampling in the lowland hay meadows at the same timepoints of the 1st and 2nd cut in the orchards yielded a mean over conservation status in ½ M of 4.964 and 5.994 days, respectively.

No kinetics data could be obtained for meadow LHM-A at the 2nd, 7th, and 8th cutting dates, for meadow LHM-B at the 15th cutting date, and for meadow LHM-C at the 16th and 20th cutting dates.

The Pearson correlation matrix (Table 2) shows the significant correlations between the modelled kinetic parameters and the measured specific methane yields. The daily maximum methane formation rate R_m and the lag phase are positively correlated with the specific methane yield; ½ M is inversely correlated.

Table 2. Pearson correlation of kinetic parameters of modified Gompertz function and specific methane yields (SMYs) of three lowland hay meadows (LHMs) characterised by different conservation statuses. Three replicates per sample. Data are not available for eight samples.

		R_m	λ	½ M	SMY
R_m	Pearson Correlation	1			
λ	Pearson Correlation	0.876 **	1		
½ M	Pearson Correlation	−0.897 **	−0.766 **	1	
SMY	Pearson Correlation	0.901 **	0.693 **	−0.777 **	1

R_m (m³ kg^{-1}$_{(oDM)}$ d^{-1}); λ (d); ½ M (d), ADF, ADL (%$_{(oDM)}$); n = 156; ** significant at $p < 0.001$.

All samples had a lag phase value of less than one day. This indicates a well-run biogas process with a well-adapted reactive inoculum and an existing substrate that does not have high levels of crude fat or other inhibitors [79]. When looking at the crude fat content and lag phase values of the lowland hay meadows, it is noticeable that both values decrease until the first half of vegetation period, and no clear trend can be seen in the second half of the vegetation period.

Pretreatment of the material has a positive influence on the kinetics of the biogas process [68,80]. However, the parameters for R_m and ½ M are in ranges of values that would generally be too inert for demand-oriented biogas production, as currently promoted by policy in Germany [81]. Seasonal substrate of lignocellulose is often used as a co-substrate [82,83]. Also, the base gas production of seasonally flexible biogas plants is greatly reduced in the summer months, and demand peaks are then covered by gas storage [84,85].

4. Conclusions

The use of cuttings from species-rich hay meadows and orchard meadows for biogas production as seasonal biomass can be an important component in the protection and conservation of these habitats.

Later cutting dates, which make sense from a nature conservation perspective, have no influence on the potential area methane yield. The newly accumulated AGB compensates for the simultaneous decrease in specific methane yield. Mowing before the flowering of main grasses and fertilisation of the areas increase the specific methane yield and promote biomass production but deteriorate the conservation status of the meadows and significantly change the species composition [86,87].

The hectare methane yields depend primarily on the AGB, with the amount of precipitation or water availability playing a decisive role, especially for the amount of AGB that can still be harvested during the second cut. The cuttings are well-suited for biogas production due to the good specific methane yields that can be achieved, but the harvest quantities are low in contrast to other substrates.

Cost-effective pre-treatment of the lignocellulosic material is essential, as otherwise, the material cannot be economically fed into a biogas plant. Further, pre-treatment prevents undesirable process disruptions such as the formation of floating layers or clogging of plant components [88,89].

However, mowing orchard meadows is a labour-intensive task that cannot be compensated for by the expected biogas yields alone [68]. Nevertheless, the maintenance of these meadows represents a cultural landscape and nature conservation measure that has a high ecological value, which must be taken into account in monetary terms.

The treatment of the cuttings in practice, e.g., handling, storage, pre-treatment, should be the subject of further research so that the energetic use of extensive grassland systems can find its way into modern agricultural systems in which biodiversity is also no longer neglected.

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Conflicts of Interest: The manuscript is the original work of the authors and was not previously submitted to Inventions. 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.

References

1. Finck, P.; Heinze, S.; Raths, U.; Riecken, U.; Ssymank, A. *Rote Liste der Gefährdeten Biotoptypen Deutschlands: Dritte Fortgeschriebene Fassung 2017*; Naturschutz und Biologische Vielfalt 156; Bundesamt für Naturschutz: Bonn, Germany, 2017. [CrossRef]
2. Deutsche UNESCO-Kommission e.V. Bundesweites Verzeichnis Immaterielles Kulturerbe Nationwide Inventory of Intangible Cultural Heritage in Germany: Jubiläum 20 Jahre Konvention 10 Jahre Verzeichnis. Immaterielles Kulturerbe Wissen. Können. Weitergeben. Available online: https://www.unesco.de/publikationen?page=2#row-9911 (accessed on 24 November 2023).
3. Plieninger, T.; Levers, C.; Mantel, M.; Costa, A.; Schaich, H.; Kuemmerle, T. Patterns and drivers of scattered tree loss in agricultural landscapes: Orchard meadows in Germany (1968–2009). *PLoS ONE* **2015**, *10*, e0126178. [CrossRef]
4. Forejt, M.; Syrbe, R.-U. The current status of orchard meadows in Central Europe: Multi-source area estimation in Saxony (Germany) and the Czech Republic. *Morav. Geogr. Rep.* **2019**, *27*, 217–228. [CrossRef]
5. Kilian, S. Streuobst-unverzichtbar für unsere Kulturlandschaft. In *Wildtiere in der Agrarlandschaft 14. Kulturlandschaftstag*; Bayrische Landesanstalt für Landwirtschaft: Freising, Germany, 2016; pp. 29–39.
6. Steffan-Dewenter, I.; Leschke, K. Effects of habitat management on vegetation and above-ground nesting bees and wasps of orchard meadows in Central Europe. *Biodivers. Conserv.* **2003**, *12*, 1953–1968. [CrossRef]
7. Herzog, F. Streuobst: A traditional agroforestry system as a model for agroforestry development in temperate Europe. *Agrofor. Syst.* **1998**, *42*, 61–80. [CrossRef]
8. Ministerium für Ländlichen Raum und Verbraucherschutz. Streuobstkonzeption Baden-Württemberg: Aktiv für Reichtum und Viefalt Unserer Streuobstlandschaften. 2015. Available online: https://mlr.baden-wuerttemberg.de/fileadmin/redaktion/m-mlr/intern/dateien/publikationen/Streuobstkonzeption.pdf (accessed on 9 December 2022).
9. Borngraeber, S.; Krismann, A.; Schmieder, A. Ermittlung der Streuobstbestände Baden-Württembergs durch Automatisierte Fernerkundungsverfahren; Naturschutz und Landschaftspflege Baden-Württemberg No. 81. 2023. Available online: https://pudi.lubw.de/detailseite/-/publication/10128 (accessed on 16 November 2023).
10. Ministerium für Ländlichen Raum und Verbraucherschutz. Das Rahmenkonzept Band 2: Unsere Strategie: Leitbilder, Entwicklungsziele, Leitprojekte (Kurzfassung). 2012. Available online: https://www.biosphaerengebiet-alb.de/fileadmin/page_images/biosphaerengebiet/rahmenkonzept/Band_2_Unsere_Strategie_24.08.2012.pdf (accessed on 21 November 2023).
11. Ashwood, F. *Lowland Calcareous Grassland: Creation and Management in Land Regeneration*; The Land Regeneration and Urban Greenspace Research Group: Farnham, UK, 2014.
12. Karlik, P.; Poschlod, P. History or abiotic filter: Which is more important in determining the species composition of calcareous grasslands? *Preslia* **2009**, *81*, 321–340.
13. LUBW. FFH-Lebensraumtyp 6510: Magere Flachland-Mähwiese. Available online: https://www.lubw.baden-wuerttemberg.de/natur-und-landschaft/6510-magere-flachland-mahwiesen (accessed on 25 November 2023).
14. Fischer, M.; Stöcklin, J. Local Extinctions of Plants in Remnants of Extensively Used Calcareous Grasslands 1950–1985. *Conserv. Biol.* **1997**, *11*, 727–737. [CrossRef]
15. Willems, J.H. Phytosociological and geographical survey of Mesohromion communities in Western Europe. *Vegetatio* **1982**, *48*, 227–240. [CrossRef]
16. LfL. Gruber Tabelle zur Fütterung der Milchkühe, Zuchtrinder, Schafe, Ziegen. 2023. Available online: https://www.lfl.bayern.de/publikationen/informationen/040183/index.php (accessed on 18 November 2023).
17. Jilg, T.; Aulendorf, L.A.Z.B.W. Fütterung von Hochleistungskühen mit FFH-Grünland–Geht das? *Landinfo* **2011**, *6*, 33–36.
18. Bundesministerium für Umwelt, Naturschutz und nukleare Sicherheit; Bundesamt für Naturschutz Die Lage der Natur in Deutschland: Ergebnisse von EU-Vogelschutz- und FFH-Bericht, Berlin, Bonn. 2020. Available online: https://www.bfn.de/sites/default/files/BfN/natura2000/Dokumente/bericht_lage_natur_2020.pdf (accessed on 14 February 2024).
19. Scheibe, U. FFH-Mähwiesen (Flachland- und Bergmähwiesen). *Landinfo* **2015**, *5*, 20–23.
20. NABU. *Vollzugsdefizite und Verstöße Gegen das Verschlechterungsverbot bei FFH-Lebensraumtypen auf Grünlandstandorten in Deutschland: Beschwerde des NABU an die Europäische Kommission Wegen Nichtbeachtung des Gemeinschaftsrechts*; NABU: Berlin, Germany, 2014.
21. LAZBW. FFH—Mähwiesen: Grundlagen—Bewirtschaftung—Wiederherstellung, Aulendorf. 2018. Available online: https://pudi.lubw.de/detailseite/-/publication/86574 (accessed on 24 November 2023).
22. Dalle Fratte, M.; Montagnoli, A.; Anelli, S.; Armiraglio, S.; Beatrice, P.; Ceriani, A.; Lipreri, E.; Miali, A.; Nastasio, P.; Cerabolini, B.E.L. Mulching in lowland hay meadows drives an adaptive convergence of above- and below-ground traits reducing plasticity and improving biomass: A possible tool for enhancing phytoremediation. *Front. Plant Sci.* **2022**, *13*, 1062911. [CrossRef]

23. Baumann, A.; Bergmann, F.; Neub, M.; Opitz, H.; Rupp, J.; Westermann, K. Naturschutzfachliche Bedeutung, Gefährdungen und Schutzmöglichkeiten der Elzniederung östlich der A5 bei Kenzingen und Herbolzheim. *Naturschutz SüDlichen Oberrh.* **2014**, *7*, 249–254.
24. Lüker-Jans, N.; Simmering, D.; Otte, A. The impact of biogas plants on regional dynamics of permanent grassland and maize area—The example of Hesse, Germany (2005–2010). *Agric. Ecosyst. Environ.* **2017**, *241*, 24–38. [CrossRef]
25. Plieninger, T.; Bieling, C.; Ohnesorge, B.; Schaich, H.; Schleyer, C.; Wolff, F. Exploring Futures of Ecosystem Services in Cultural Landscapes through Participatory Scenario Development in the Swabian Alb, Germany. *Ecol. Soc.* **2013**, *18*, 39. [CrossRef]
26. Humbert, J.-Y.; Ghazoul, J.; Walter, T. Meadow harvesting techniques and their impacts on field fauna. *Agric. Ecosyst. Environ.* **2009**, *130*, 1–8. [CrossRef]
27. Van de Poel, D.; Zehm, A. Die Wirkung des Mähens auf die Fauna der Wiesen—Eine Literaturauswertung für den Naturschutz. In *Handbuch Naturschutz und Landschaftspflege*; Konold, W., Böcker, R., Hampicke, U., Eds.; Wiley: Weinheim, Germany, 2014; pp. 1–19, ISBN 9783527678471.
28. Unterweger, P.A.; Klammer, J.; Unger, M.; Betz, O. Insect hibernation on urban green land: A winter-adapted mowing regime as a management tool for insect conservation. *BioRisk* **2018**, *13*, 1–29. [CrossRef]
29. Diacon, J.; Bürgi, M.; Dalang, T. Systematisches Review zu Bewirtschaftungseinflüssen auf Trockenwiesen und –Weiden (SR-TWW). Schlussbericht, Birmensdorf. 2011. Available online: https://infohabitat.ch/wp-content/uploads/2019/01/Tw_SystemReview_TwwBewirtschaftungseinfluesse_15022011_de.pdf (accessed on 26 November 2023).
30. Dieterich, M.; Dorsch, H.; Bahrs, E. Übersicht Möglicher Biodiversitätsmaßnahmen, Kirchheim unter Teck. 2021. Available online: https://mlr.baden-wuerttemberg.de/fileadmin/redaktion/m-mlr/intern/dateien/PDFs/Biodiv/biodiv-ma%C3%9Fnahmenkatalog_demobetriebe.pdf (accessed on 26 November 2023).
31. Dandikas, V.; Heuwinkel, H.; Lichti, F.; Drewes, J.E.; Koch, K. Correlation between Biogas Yield and Chemical Composition of Grassland Plant Species. *Energy Fuels* **2015**, *29*, 7221–7229. [CrossRef]
32. Data and Map Service of the LUBW. Erfassungseinheit Nr.: 6510800046030080; Flurstücksnummer: 7429; Gemarkung Eningen unter Achalm (Landkreis Reutlingen, Germany). Available online: https://udo.lubw.baden-wuerttemberg.de/public/api/processingChain?conditionValuesSetHash=C10A56A&selector=ROOT.Natur%20und%20Landschaft.Natura%202000%20-%20FFH-M%C3%A4hwiesen.nais_map:nais_map_z_mw_udo_permalink.sel&processings=nais_map:nais_map_mw_erhebungsbogen/nais_map_mw_erhebungsbogen_udo.rpt&sourceOrderAsc=false&columns=020789b6-1f17-4820-a4a7-a5823f7e35b8&offset=0&limit=2147483647&executionConfirmed=false (accessed on 28 November 2023).
33. Data and Map Service of the LUBW. Erfassungseinheit Nr.: 6510800046029669; Flurstücksnummer: 4931; Gemarkung Eningen unter Achalm (Landkreis Reutlingen, Germany). Available online: https://udo.lubw.baden-wuerttemberg.de/public/api/processingChain?conditionValuesSetHash=C4950AD&selector=ROOT.Natur%20und%20Landschaft.Natura%202000%20-%20FFH-M%C3%A4hwiesen.nais_map:nais_map_z_mw_udo_permalink.sel&processings=nais_map:nais_map_mw_erhebungsbogen/nais_map_mw_erhebungsbogen_udo.rpt&sourceOrderAsc=false&columns=0bc54797-738d-4545-aea6-5c88c242b380&offset=0&limit=2147483647&executionConfirmed=false (accessed on 28 November 2023).
34. Data and Map Service of the LUBW. Erfassungseinheit Nr.: 6510800046030070; Flurstücksnummer: 7430; Gemarkung Eningen unter Achalm (Landkreis Reutlingen, Germany). Available online: https://udo.lubw.baden-wuerttemberg.de/public/api/processingChain?conditionValuesSetHash=50FC495&selector=ROOT.Natur%20und%20Landschaft.Natura%202000%20-%20FFH-M%C3%A4hwiesen.nais_map:nais_map_z_mw_udo_permalink.sel&processings=nais_map:nais_map_mw_erhebungsbogen/nais_map_mw_erhebungsbogen_udo.rpt&sourceOrderAsc=false&columns=4e79a835-0123-42aa-8d88-775ba81bddfc&offset=0&limit=2147483647&executionConfirmed=false (accessed on 28 November 2023).
35. Piepho, H.-P.; Michel, V.; Williams, E. Neighbor balance and evenness of distribution of treatment replications in row-column designs. *Biom. J.* **2018**, *60*, 1172–1189. [CrossRef]
36. Seither, M.; Engel, S.; King, K.; Martin, E. FFH—Mähwiesen: Grundlagen—Bewirtschaftung—Wiederherstellung, Aulendorf. 2018. Available online: https://fortbildung-lazbw.lgl-bw.de/lazbw/webbasys/download/Shop/2018_GL_lazbw_FFH_Maehwiesen_Grundlagen.pdf (accessed on 28 November 2023).
37. VDLUFA. *Method Book III—The Chemical Analysis for Feedstuffs*; VDLUFA: Darmstadt, Germany, 2012.
38. European Commission. *Commission Regulation 2009/152/EC: Laying Down the Methods of Sampling and Analysis for the Official Control of Feed*; Official Journal of the European Union: Luxembourg, 2009.
39. Energie und Umwelttechnik. *Fermentation of Organic Materials—Characterization of the Substrate, Sampling, Collection of Material Data, Fermentation Tests: VDI 4630*; Beuth Verlag GmbH: Berlin, Germany, 2016.
40. Hülsemann, B.; Zhou, L.; Merkle, W.; Hassa, J.; Müller, J.; Oechsner, H. Biomethane Potential Test: Influence of Inoculum and the Digestion System. *Appl. Sci.* **2020**, *10*, 2589. [CrossRef]
41. Lindner, J.; Zielonka, S.; Oechsner, H.; Lemmer, A. Effects of mechanical treatment of digestate after anaerobic digestion on the degree of degradation. *Bioresour. Technol.* **2015**, *178*, 194–200. [CrossRef]
42. Tallowin; Jefferson. Hay production from lowland semi-natural grasslands: A review of implications for ruminant livestock systems. *Grass Forage Sci.* **1999**, *54*, 99–115. [CrossRef]
43. Landesamt für Geologie, Rohstoffe und Bergbau. Bodenkunde; GeoLa Boden; Bodenkundliche Einheiten; Pararendzina und Rendzina aus schuttreichen Fließerden und Hangschutt, Kartiereinheit q21. Available online: https://media.lgrb-bw.de/link/bod3200/q21.pdf (accessed on 28 November 2023).

44. Landesamt für Geologie, Rohstoffe und Bergbau (LGRB) Wissen. Böden der Trauf- und Talhänge. LGRBwissen. Available online: https://lgrbwissen.lgrb-bw.de/bodenkunde/mittlere-westliche-alb/bodenlandschaften/boeden-trauf-talhaenge (accessed on 29 November 2023).
45. Ludewig, K.; Donath, T.W.; Zelle, B.; Eckstein, R.L.; Mosner, E.; Otte, A.; Jensen, K. Effects of reduced summer precipitation on productivity and forage quality of floodplain meadows at the Elbe and the Rhine River. *PLoS ONE* **2015**, *10*, e0124140. [CrossRef]
46. Zhao, X.-Q.; Zhou, X.-M. Ecological Basis of Alpine Meadow Ecosystem Management in Tibet: Haibei Alpine Meadow Ecosystem Research Station. *Ambio* **1999**, *28*, 642–647.
47. Herrmann, C.; Prochnow, A.; Heiermann, M.; Idler, C. Biomass from landscape management of grassland used for biogas production: Effects of harvest date and silage additives on feedstock quality and methane yield. *Grass Forage Sci.* **2014**, *69*, 549–566. [CrossRef]
48. Michaud, A.; Andueza, D.; Picard, F.; Plantureux, S.; Baumont, R. Seasonal dynamics of biomass production and herbage quality of three grasslands with contrasting functional compositions. *Grass Forage Sci.* **2012**, *67*, 64–76. [CrossRef]
49. Grygierzec, B. Fodder value of hay from extensively used Arrhenatheretum elatioris typicum community. *Fragm. Agron.* **2012**, *29*, 37–44.
50. Thumm, U.; Tonn, B.; Schreiber, K.F.; Brauckmann, H.J.; Broll, G.; Krebs, S.; Poschlod, P. *Grünlandaufwüchse—Futter oder Bioenergie*; Regionalkultur: Ubstadt-Weiher, Germany, 2009.
51. Dullau, S.; Rydgren, K.; Kirmer, A.; Jäger, U.G.; Meyer, M.H.; Tischew, S. The Dessau Grassland Experiment—Impact of Fertilization on Forage Quality and Species Assembly in a Species-Rich Alluvial Meadow. *Agriculture* **2021**, *11*, 339. [CrossRef]
52. Bruinenberg, M.H.; Valk, H.; Korevaar, H.; Struik, P.C. Factors affecting digestibility of temperate forages from seminatural grasslands: A review. *Grass Forage Sci.* **2002**, *57*, 292–301. [CrossRef]
53. Meserszmit, M.; Swacha, G.; Pavlů, L.; Pavlů, V.; Trojanowska-Olichwer, A.; Kącki, Z. Species composition of semi-natural mesic grasslands as a factor influencing the methane yield of plant biomass (Central Europe). *GCB Bioenergy* **2022**, *14*, 54–64. [CrossRef]
54. Khan, R.J.; Lau, C.Y.; Guan, J.; Lam, C.H.; Zhao, J.; Ji, Y.; Wang, H.; Xu, J.; Lee, D.-J.; Leu, S.-Y. Recent advances of lignin valorization techniques toward sustainable aromatics and potential benchmarks to fossil refinery products. *Bioresour. Technol.* **2022**, *346*, 126419. [CrossRef] [PubMed]
55. Mankar, A.R.; Modak, A.; Pant, K.K. Recent Advances in the Valorization of Lignin: A Key Focus on Pretreatment, Characterization, and Catalytic Depolymerization Strategies for Future Biorefineries. *Adv. Sustain. Syst.* **2022**, *6*, 2100299. [CrossRef]
56. Agregán, R.; Lorenzo, J.M.; Kumar, M.; Shariati, M.A.; Khan, M.U.; Sarwar, A.; Sultan, M.; Rebezov, M.; Usman, M. Anaerobic Digestion of Lignocellulose Components: Challenges and Novel Approaches. *Energies* **2022**, *15*, 8413. [CrossRef]
57. Li, W.; Khalid, H.; Zhu, Z.; Zhang, R.; Liu, G.; Chen, C.; Thorin, E. Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. *Appl. Energy* **2018**, *226*, 1219–1228. [CrossRef]
58. Van Soest, P.J. *Nutritional Ecology of the Ruminant*, 2nd ed.; Cornell University Press: Ithaca, NY, USA, 1994; ISBN 978-1-5017-3235-5.
59. Buxton, D.R. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Anim. Feed Sci. Technol.* **1996**, *59*, 37–49. [CrossRef]
60. Dal Prà, A.; Davolio, R.; Immovilli, A.; Burato, A.; Ronga, D. Plant Composition and Feed Value of First Cut Permanent Meadows. *Agronomy* **2023**, *13*, 681. [CrossRef]
61. Rath, J.; Heuwinkel, H.; Herrmann, A. Specific Biogas Yield of Maize Can Be Predicted by the Interaction of Four Biochemical Constituents. *BioEnergy Res.* **2013**, *6*, 939–952. [CrossRef]
62. Lymperatou, A.; Engelsen, T.K.; Skiadas, I.V.; Gavala, H.N. Prediction of methane yield and pretreatment efficiency of lignocellulosic biomass based on composition. *Waste Manag.* **2023**, *155*, 302–310. [CrossRef]
63. Mezule, L.; Strazdina, B.; Dalecka, B.; Skripsts, E.; Juhna, T. Natural Grasslands as Lignocellulosic Biofuel Resources: Factors Affecting Fermentable Sugar Production. *Energies* **2021**, *14*, 1312. [CrossRef]
64. Hoiß, B.; Berg, M.; Krämer, M. Die Herbstzeitlose im extensiven Grünland. *Anliegen Nat.* **2022**, *3*, 123–126.
65. Chizzola, R.; Bassler-Binder, G.; Winter, S.; Zebeli, Q.; Kriechbaum, M. Persistence of alkaloids of typical poisonous plants autumn crocus and marsh ragwort in grass silage. *Vet. Med. Austria* **2015**, *102*, 285–292.
66. Winter, S.; Penker, M.; Kriechbaum, M. Integrating farmers' knowledge on toxic plants and grassland management: A case study on Colchicum autumnale in Austria. *Biodivers. Conserv.* **2011**, *20*, 1763–1787. [CrossRef]
67. Wiggering, H.; Diekötter, T.; Donath, T.W. Regulation of Jacobaea vulgaris by varied cutting and restoration measures. *PLoS ONE* **2022**, *17*, e0248094. [CrossRef]
68. Heller, R.; Brandhorst, C.; Hülsemann, B.; Lemmer, A.; Oechsner, H. Comparison of Different Mechanical Pretreatment Methods for the Anaerobic Digestion of Landscape Management Grass. *Energies* **2023**, *16*, 8091. [CrossRef]
69. Khan, M.U.; Usman, M.; Ashraf, M.A.; Dutta, N.; Luo, G.; Zhang, S. A review of recent advancements in pretreatment techniques of lignocellulosic materials for biogas production: Opportunities and Limitations. *Chem. Eng. J. Adv.* **2022**, *10*, 100263. [CrossRef]
70. Rodriguez, C.; Alaswad, A.; Benyounis, K.Y.; Olabi, A.G. Pretreatment techniques used in biogas production from grass. *Renew. Sustain. Energy Rev.* **2017**, *68*, 1193–1204. [CrossRef]
71. Meyer, A.; Raju, C.S.; Kucheryavskiy, S.; Holm-Nielsen, J.B. The energy balance of utilising meadow grass in Danish biogas production. *Resour. Conserv. Recycl.* **2015**, *104*, 265–275. [CrossRef]

72. Meserszmit, M.; Chrabąszcz, M.; Chylińska, M.; Szymańska-Chargot, M.; Trojanowska-Olichwer, A.; Kącki, Z. The effect of harvest date and the chemical characteristics of biomass from Molinia meadows on methane yield. *Biomass Bioenergy* **2019**, *130*, 105391. [CrossRef]
73. Amon, T.; Amon, B.; Kryvoruchko, V.; Zollitsch, W.; Mayer, K.; Gruber, L. Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agric. Ecosyst. Environ.* **2007**, *118*, 173–182. [CrossRef]
74. Oslaj, M.; Mursec, B.; Vindis, P. Biogas production from maize hybrids. *Biomass Bioenergy* **2010**, *34*, 1538–1545. [CrossRef]
75. Melts, I.; Normak, A.; Nurk, L.; Heinsoo, K. Chemical characteristics of biomass from nature conservation management for methane production. *Bioresour. Technol.* **2014**, *167*, 226–231. [CrossRef]
76. Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. *Prog. Energy Combust. Sci.* **2014**, *42*, 35–53. [CrossRef]
77. Tsapekos, P.; Kougias, P.G.; Egelund, H.; Larsen, U.; Pedersen, J.; Trénel, P.; Angelidaki, I. Mechanical pretreatment at harvesting increases the bioenergy output from marginal land grasses. *Renew. Energy* **2017**, *111*, 914–921. [CrossRef]
78. Dahunsi, S.O. Mechanical pretreatment of lignocelluloses for enhanced biogas production: Methane yield prediction from biomass structural components. *Bioresour. Technol.* **2019**, *280*, 18–26. [CrossRef]
79. Herrmann, C.; Idler, C.; Heiermann, M. Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. *Bioresour. Technol.* **2016**, *206*, 23–35. [CrossRef]
80. Rani, P.; Bansal, M.; Pathak, V.V. Experimental and kinetic studies for improvement of biogas production from KOH pretreated wheat straw. *Curr. Res. Green Sustain. Chem.* **2022**, *5*, 100283. [CrossRef]
81. Maurus, K.; Ahmed, S.; Getz, W.; Kazda, M. Sugar beet silage as highly flexible feedstock for on demand biogas production. *Sugar Ind.* **2018**, *143*, 691–698. [CrossRef]
82. De Moor, S.; Velghe, F.; Wierinck, I.; Michels, E.; Ryckaert, B.; de Vocht, A.; Verbeke, W.; Meers, E. Feasibility of grass co-digestion in an agricultural digester, influence on process parameters and residue composition. *Bioresour. Technol.* **2013**, *150*, 187–194. [CrossRef]
83. Hidaka, T.; Arai, S.; Okamoto, S.; Uchida, T. Anaerobic co-digestion of sewage sludge with shredded grass from public green spaces. *Bioresour. Technol.* **2013**, *130*, 667–672. [CrossRef]
84. Güsewell, J.; Eltrop, L.; Hufendiek, K. Seasonal flexibilisation: A solution for biogas plants to improve profitability. *Adv. Appl. Energy* **2021**, *2*, 100034. [CrossRef]
85. Thrän, D.; Dotzauer, M.; Lenz, V.; Liebetrau, J.; Ortwein, A. Flexible bioenergy supply for balancing fluctuating renewables in the heat and power sector—A review of technologies and concepts. *Energy Sustain. Soc.* **2015**, *5*, 35. [CrossRef]
86. Boob, M.; Elsaesser, M.; Thumm, U.; Hartung, J.; Lewandowski, I. Different management practices influence growth of small plants in species-rich hay meadows through shading. *Appl. Veg. Sci.* **2021**, *24*, e12625. [CrossRef]
87. Boob, M.; Truckses, B.; Seither, M.; Elsäßer, M.; Thumm, U.; Lewandowski, I. Management effects on botanical composition of species-rich meadows within the Natura 2000 network. *Biodivers. Conserv.* **2019**, *28*, 729–750. [CrossRef]
88. Mönch-Tegeder, M.; Lemmer, A.; Oechsner, H. Enhancement of methane production with horse manure supplement and pretreatment in a full-scale biogas process. *Energy* **2014**, *73*, 523–530. [CrossRef]
89. Al Seadi, T.; Rutz, D.; Prassl, H.; Köttner, M.; Finsterwalder, T.; Volk, S.; Janssen, R. Biogas: Handbook. 2008. Available online: https://www.wip-munich.de/pv-policy-group-european-best-practice-report/annex2-39_wp4_d4.1_master-handbook.pdf (accessed on 23 January 2024).