agriculture

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Harvest Time Determines Quality and Usability of Biomass from Lowland Hay Meadows

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Received: 31 July 2019; Accepted: 6 September 2019; Published: 10 September 2019

Abstract: Species-rich hay meadows are usually managed extensively to maintain their biodiversity, with the harvested biomass traditionally being fed to ruminants for milk or meat production. The quality of the biomass is, however, variable, difficult to predict and often does not fulfil today's requirements. This study established a field trial at two species-rich hay meadows to investigate the combined effect of fertilisation (none, phosphorus and potassium (PK), nitrogen, phosphorus and potassium (NPK)) and date of first cut (at different phenological stages) on biomass quality and quantity. In addition, the most suitable uses of the biomass were explored, including the alternatives biogas and combustion. After four years of the field trial, the stage of maturity at the time of first cut had a greater influence than extensive fertilisation on biomass quality. Dry matter yield (DMY) of the first cut was about 40%–60% of annual DMY (53.99 ± 12.51 dt ha^{-1} a^{-1}) depending on site, fertilisation and harvest time. Fertilisation had a stronger effect than harvest time on DMY and annual methane yield. In most cases, there was no significant difference in chemical composition between biomass harvested at the end of the grass-flowering stage and at the seed-ripening stage. Thus, a late cut for hay proved to be the most flexible option.

Keywords: grassland management; Natura 2000; forage quality; bioenergy; biorefinery

1. Introduction

Approximately 10% of the utilised agricultural area in the European Union (EU) belongs to the Natura 2000 network [1]. This network includes lowland hay meadows, which are extensively managed meadows protected as 'Habitat Types of Community Interest' by the EU Habitats Directive [2]. The directive stipulates that the typical botanical composition of these lowland hay meadows is to be maintained. This can only be achieved through extensive management, usually with two cuts per year and fertilisation without mineral nitrogen (N) [3]. The biomass harvested is different to that of intensively used grassland in that it contains, depending on site conditions, a diverse range of plant species at different stages of maturity. Therefore, the quality of biomass harvested from lowland hay meadows is variable, difficult to predict and often unknown.

One factor influencing the chemical content of herbage is its botanical composition. This is in turn significantly influenced by management practices and other site-specific factors such as soil. For example, a study examining the nutritive value of various grass and legume species found that legumes always had the highest N content [4]. Another found forbs to be rich in mineral nutrients [5].

It is known that the stage of maturity at the time of harvest has a great influence on forage quality, because cell wall components increase during plant development [6]. Thus, grassland management can influence the quality of cut herbage both directly and indirectly.

To integrate grassland biomass into modern agricultural systems and to determine which value chain is most suitable for its use, information on its quality, in particular chemical composition, is invaluable. Traditionally, grassland biomass has been used as forage. Freshly cut forage is conserved as hay or silage and fed to ruminants for the production of milk or meat. In modern livestock systems, however, this traditional usage is restricted because highly productive dairy cows have high nutritional demands, which cannot be met by extensively managed grassland [7,8]. The quality of ruminant forage is often estimated based on content of usable energy (measured as net energy for lactation, NEL) and protein content. Both are reduced with advancing maturity of grassland biomass as fibre content increases. Thus, the question arises on the extent to which biomass from species-rich hay meadows can be integrated into modern livestock systems.

There are alternative energetic uses of grassland biomass as solid fuel or as substrate for biogas production. Although the feedstock-specific methane yield (SMY) of biomass from extensively managed hay meadows has not yet been sufficiently established [9], it is known that the higher lignin content of grassland biomass harvested at a later maturity stage reduces its digestibility and biogas yield. The same is true for its use as forage. On the other hand, the management required for use in biogas production can be less intensive than for dairy use, especially with regard to nitrogen (N) content [10]. Biomass rich in fibre is more suitable for combustion than either fermentation or forage use [11]. Here, low amounts of ash-forming components and N content are preferable, as these can cause problems such as sintering in the combustion chamber and harmful emissions [12].

Finally, there is also increasing interest in the material use of grassland biomass. One example is the green biorefinery, which combines the production of materials and energy, and commonly uses fresh green herbage or silage as substrate. Other examples are the thermo-chemical splitting of grassland biomass through pyrolysis into gaseous, liquid and solid components [13], and hydrolysis, which is the enzymatic production of sugars for bioethanol [14]. For use in biorefineries, grassland biomass needs to be stored in the form of silage to ensure year-round availability. However, biomass from extensively used meadows often does not contain the necessary levels of components such as sugars and amino acids [10].

The economic feasibility of the use of grassland is mainly determined by the yield and quality of the biomass. The DMY of extensively used grassland depends on water availability [15]. Where water supply is sufficient, management practices such as fertilisation and cutting regime are the key factors regulating DMY. Long-term fertilisation experiments have shown that nitrogen, phosphorus and potassium (NPK) fertilisation increases DMY in both intensively (e.g., [16]) and extensively used permanent grassland (e.g., [17]). However, increased DMY has also been reported with PK fertilisation only [18] in an alluvial meadow fertilised for 25 years. Thus, the grassland productivity in this fertilisation experiment was not N-limited [19]. Extensively used meadows often exhibit low soil P (phosphorus) and K (potassium) contents. The fertilisation effect depends on the soil nutrient status and pH value. For example, a long-term fertilisation experiment by [20] found that N application to a hay meadow led to P limitation.

Kirkham and Tallowin [21] found a poorer forage quality of species-rich hay meadows at late dates of first cut. They compared unfertilised plots and plots previously fertilised with NPK and found no significant effects of previous fertilisation or fertilisation x date of first cut interactions. By contrast, [22] reported highest DMY for NPK-fertilised plots combined with a late date of first cut in September, even though in their experiment only low doses of NPK were used to restore former agriculturally improved meadows. Thus, when deciding on the optimal date of first cut of hay meadows, there is a trade-off between high forage quality and high DMY.

The aim of our study is to assess the influence of harvest date and fertilisation on biomass quality and quantity from species-rich meadows. This is discussed in the context of the usability of biomass provided by lowland hay meadows.

We hypothesized that the stage of maturity has a stronger effect than fertilisation on biomass quality, especially on NEL, protein and fibre content. Further, we expected a better suitability of an early date of first cut for forage use and a late date of first cut for combustion. We anticipated site-specific effects of the treatments and an influence of botanical composition on biomass quality and quantity. To test these hypotheses, a 5-year field trial with three replicates was established at two lowland hay meadows in south-west Germany. The influence of the two factors fertilisation (none, PK and NPK) and date of harvest (before, at beginning of, at end of and after flowering of main grasses) was investigated.

2. Materials and Methods

2.1. Field Trial Location

The field trial was established in 2013 on two species-rich hay meadows (Habitat Type 6510) in a special area of conservation, approximately 30 and 40 km respectively from Stuttgart, south Germany. The first site, "Swabian Jura", is located on this low mountain range at 774 m above sea level. The second site "Foothills" (470 m a.s.l.) is located approximately ca.10 km away at the base of the Swabian Jura (Table 1). Both meadows belong to the *Arrhenatherion* alliance, with typical species of the *Geranio-Trisetetum* association at Swabian Jura (mean ± standard deviation, n = 36) and a typical *Arrhenatheretum elatius* community at Foothills [23]. In 2013, average soil N and C content (in % of dry matter (DM)) were 0.63 ± 0.07 N and 7.87 ± 1.09 C at Swabian Jura, and slightly higher at Foothills (0.79 ± 0.05 N, 8.42 ± 0.55 C). The soil is alkalescent at both sites (mean pH 7.4). There were no significant differences in pH values between sites and treatments. Meteorological data are shown in Figure A1 (Appendix A).

Table 1. Site characteristics at the beginning of the field trial (in 2013).

	Swabian Jura	Foothills
Coordinates	48°34′27.8′′ N, 9°26′29.7′′ E	48°31′38.5′′ N, 9°31′53.9′′ E
Mean Annual Temperature	7.4 °C	9.6 °C
Mean Annual Precipitation	1040 mm	970 mm
Altitude	774 m a.s.l.	470 m a.s.l.
Soil N content	0.63 ± 0.07% DM	0.79 ± 0.05% DM
Soil C_{total} content	7.87 ± 1.09% DM	8.42 ± 0.55% DM
pH	7.2 ± 0.1	7.2 ± 0.1
Soil K_2O content	13.38 ± 1.43 mg $100g^{-1}$	8.97 ± 1.42 mg $100g^{-1}$
Soil P_2O_5 content	4.10 ± 0.71 mg $100g^{-1}$	2.70 ± 0.68 mg $100g^{-1}$

2.2. Design and Management

A randomised block-design field trial with three replications was set up at both sites. Each block was divided into 12 plots of 25 m^2, one for each treatment. Treatments were a combination of three fertilisation variants (none, PK and NPK) and four cutting variants (date of first cut).

The three fertilisation variants were none (unfertilised), PK (35 kg P_2O_5 and 120 kg K_2O ha^{-1} a^{-1}) and NPK (35 kg N, 35 kg P_2O_5 and 120 kg K_2O $ha^{-1}a^{-1}$). Fertiliser amounts were chosen to simulate traditional manure application according to recommendations by the governmental institute LAZBW [3] and applied every year in March. The meadows at both sites were cut twice per year, with the first cut being performed on four different dates and the second cut on the same day in September. The dates of first cut were based on growth stages. The earliest date of first cut (D1) was timed to represent an early cut before the main flowering period, the second (D2) and third (D3) dates were in the main flowering period and the latest date of first cut (D4) was chosen to represent an extensive cut for hay at seed-ripening stage (Table 2). Data were taken in four subsequent years (2013 until 2016).

Table 2. Cutting dates of first (variants D1 = early date of first cut to D4 = late date of first cut) and second cut 2013–2016.

Year	1st Cut				2nd Cut
	D1	D2	D3	D4	
	Before Flowering	Start of Flowering	Flowering Period	Seeds Ripening	
2013	May 28 and June 4	June 14	July 3	July 18	September 23
2014	May 8	May 26	June 12	July 1	September 16
2015	May 13	June 2	June 16	June 30	September 15
2016	May 19	June 2	June 17	June 29	September 14

2.3. Sampling and Laboratory Analysis

Sampling and laboratory analyses were performed each year from 2013 to 2016. All plots were mown with a sickle bar mower to a sward height of 5 cm. The fresh weight of the harvested biomass was determined directly on the field. A subsample (about 0.5 kg) was selected randomly for the determination of DM content (60 °C for at least 48 h), and then ground in a cutting mill.

The specific methane yield (SMY) in Nm^3kg^{-1} organic DM (ODM) was determined using the Hohenheim biogas yield test [24]. Gas production potential (GP) was determined [25] by means of the Hohenheim feed value test. Net energy for lactation content (NEL in MJ kg^{-1}DM) was calculated using the following equation [26]:

$$NEL = 0.54 + 0.096 \times GP + 0.038 \times CP + 0.00173 \times CL^2$$

where CP is the crude protein content (g/kg) and CL the crude fat content (g/kg). Additionally, the dried samples were analysed for contents of ash (XA), fibre, and the mineral nutrients potassium (K), phosphorus (P), magnesium (Mg), and calcium (Ca) [25]. The dry matter fibre content (all cell wall components (NDF), consisting of lignin (ADL), cellulose and hemicellulose) was determined by the Van Soest method.

Soil samples were taken after the second cut in 2013 using a soil corer at a depth of 0–10 cm. For each plot, at least 10 soil samples were mixed together. Mixed samples were air-dried, sieved and the pH value, K_2O and P_2O_5 (extracted by calcium acetate lactate) contents determined [27]. C and N contents were determined in an elemental analyser (Vario Max CNS, Elementar, Langenselbold, Germany).

2.4. Statistical Analyses

A linear mixed-effect model was fitted to each response variable and each site and residuals were checked graphically for homogeneity of variance and normal distribution. Where the residual distribution was not normal, data were square root-transformed. In this case, means were back transformed for presentation purpose only. The single-site statistical model used for each response is as follows:

$$y_{ijkl} = \mu + a_l + b_{kl} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + (a\tau)_{il} + (a\varphi)_{jl} + (\tau\varphi a)_{ijl} + e_{ijkl},$$

where μ is the intercept, a_l is the fixed effect of the lth year, b_{kl} is the random effect of the kth replicate in year l, τ_i, φ_j and $(\tau\varphi)_{ij}$ are the fixed effects of the ith cutting regime, the jth fertiliser level and their interactions, respectively. $(a\tau)_{il}$, $(a\varphi)_{jl}$ and $(\tau\varphi a)_{ijl}$ are the corresponding fixed interaction effects with year l. e_{ijkl} is the error of observation y_{ijkl}. For b_{kl} and e_{ijkl} homogeneous or heterogeneous variances with independence or a first order autocorrelation were assumed. The best variance-covariance structure was selected via AIC (Akaike information criterion) [28]. In case of significant F tests, a multiple comparison of means was performed and presented via letter display [29]. Statistical analyses were performed using the PROC MIXED procedure of the SAS system (version 9.4, SAS Institute, Cary, USA).

3. Results

Most results showed significant influences of treatments x year interactions. While for most variables both two-way interactions (date of first cut × year and fertilisation × year) were significant for both sites, the three-way interaction was significant only for K. We found no common effects across the years 2013–2016 (except for fertilisation for CP at Foothills) or fertilisation × date of first cut interactions across these years except for P at Foothills. Thus, year-specific means were calculated throughout the paper. As year four (2016) is the most recent year, means for 2016 are shown in this study.

3.1. Dry Matter Yield

There were significant year x fertilisation and year x date of first cut interactions ($p < 0.05$) at both sites. Regardless of date of first cut, annual DMY increased from 48.2 ± 3.6 or 48.3 ± 2.5 to 69.7 ± 3.6 or 77.3 ± 2.5 dt ha^{-1}a^{-1} (1 dt = 100 kg, ha = hectare, a = year), respectively, with increasing DMY with increasing amount of fertiliser used (Figure 1).

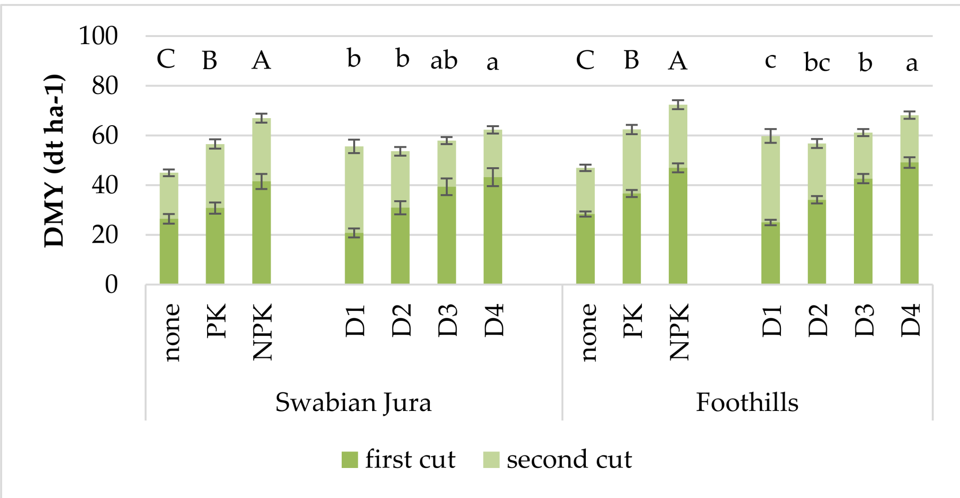

Figure 1. Means of dry matter yield (DMY) (dt ha^{-1}) at Swabian Jura and Foothills sites in 2016 with standard deviation. Data are shown for different fertilisation levels (none, PK, NPK) and cutting regimes (D1 = early date of first cut, D4 = late date of first cut). Identical lower-case letters between cutting variants ($p < 0.05$, $n = 9$) and identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) for each site.

3.2. Organic Components, Net Energy Content and Specific Methane Yield (SMY)

Fertilisation had no significant effect on ADL or SMY. NDF content was significantly influenced by fertilisation × year interactions and date of first cut x year interactions at both sites (Table 3).

ADL and SMY were influenced by date of first cut x year interactions only in both sites. For CP and NEL, significant terms vary between sites. For CP at Foothills, fertilisation and year x cutting interactions were significant, for Swabian Jura, the three-way interactions are significant. For NEL, year x cutting interactions were significant at both sites while year x fertilisation interactions were significant at Foothills only. In contrast, in 2016 date of first cut always had a significant effect on organic components, energy content and SMY of the first growth (harvested biomass of first cut), except for CP at Swabian Jura. There were significant year x date of first cut interactions ($p < 0.05$) (Table 4).

Table 3. Least square means of organic components, net energy for lactation (NEL) and specific methane yield (SMY) of first cut in 2016 with standard error (±SE).

Site	Treatment	CP (Protein)		NDF (Fibre) g kg^{-1} DM		ADL (Lignin)		NEL MJ kg^{-1}		SMY Nm^3 kg^{-1} ODM	
Swabian Jura	D1	135.2^a	±2.2	498.5^c	±0.8	58.63^a	±0.19	5.81^a	±0.10	0.320^c	±0.003
	D2	104.6^b	±2.2	527.3^b	±0.8	66.00^b	±0.19	5.39^b	±0.10	0.300^b	±0.0003
	D3	90.0^c	±2.2	550.8^a	±0.8	69.71^b	±0.19	4.54^c	±0.10	0.262^a	±0.003
	D4	83.9^d	±2.2	537.6^{ab}	±0.8	69.81^b	±0.19	4.64^c	±0.10	0.269^a	±0.003
	none	103.0	±1.98	511.9^A	±0.7	68.52	±0.17	5.10	±0.10	0.290	±0.003
	PK	104.2	±1.98	515.5^A	±0.7	64.70	±0.17	5.12	±0.01	0.287	±0.003
	NPK	103.2	±1.98	558.2^B	±0.7	64.89	±0.17	5.07	±0.01	0.283	±0.003
Foothills	D1	112.5^a	±3.26	500.0^c	±0.8	53.66^a	±0.15	5.56^a	±0.08	0.312^a	±0.004
	D2	86.2^b	±3.26	528.6^b	±0.8	60.46^b	±0.15	5.22^b	±0.08	0.288^b	±0.004
	D3	78.8^c	±3.26	557.7^a	±0.8	63.03^{bc}	±0.15	4.52^c	±0.08	0.259^c	±0.004
	D4	68.0^d	±3.26	572.9^a	±0.8	66.30^c	±0.15	4.46^c	±0.08	0.262^c	±0.004
	none	81.3	±3.04	497.0^C	±0.8	59.65	±0.13	4.94^A	±0.07	0.283	±0.004
	PK	88.1	±3.04	550.2^B	±0.8	61.83	±0.13	4.88^B	±0.07	0.280	±0.004
	NPK	89.8	±3.04	572.2^A	±0.8	61.10	±0.13	5.00^A	±0.07	0.278	±0.004

Identical lower-case letters between cutting variants ($p < 0.05$, $n = 9$) and identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) for each site.

Table 4. F statistics of three-way analyses of variance (ANOVAs) (factor year (Y), date of first cut (D) and fertilisation (F)) and significant interactions for organic components at Swabian Jura and Foothills site.

Site	Factor		CP	NDF	ADL	NEL	SMY
Swabian Jura	date of first cut	*F* value	373.81 ***	142.16 ***	75.29 ***	544.3 ***	288.1 ***
	Fertilisation	*F* value	0.67	27.37 ***	0.94	1.15	2.34
	Year	*F* value	1.75	11.70 **	182.6 ***	63.1 ***	204.27 ***
	Significant interactions		DxY	DxY, FxY	DxY	DxY	DxY
Foothills	date of first cut	*F* value	478.59 ***	117.59 ***	82.11 ***	652.97 ***	269.67 ***
	Fertilisation	*F* value	5.15 *	52.97 ***	1.60	4.91 **	1.59
	Year	*F* value	0.79	54.53 **	70.48 ***	11.23 **	32.11 ***
	Significant interactions		DxY	DxY, FxY	DxY	DxY, FxY	DxY

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. *F* and *p* values are results of data for years 2013–2016.

The first growth was rich in fibre, with cell wall components (NDF) constituting about 50% of DM. In 2016, the NDF and ADL content significantly increased with later date of first cut, while CP, NEL and SMY decreased at both sites. Furthermore, NDF increased with NPK fertilisation (Table 3).

Content of usable energy (measured as NEL) is often considered when estimating forage quality for ruminant feed. The NEL content was above 5 MJ kg^{-1} DM at both sites each year at early date of first cut, but declined significantly at later date of first cut (Table 3).

The mean specific methane yield (SMY) was about 0.30 ± 0.003 Nm^3 CH_4 kg^{-1} DM at both sites. Fertilisation had no significant influence on SMY. As with NEL content, SMY decreased with later date of first cut at both sites (to 0.28 ± 0.02 Nm^3 CH_4 kg^{-1} DM).

Due to its better suitability for biogas production, the biomass of the first growth only was analysed for its biogas potential and taken into account in the assessment of methane yield per ha and year. Methane yield is calculated by multiplying DM yield by SMY. For this reason DM and methane yields showed similar results. However, calculated methane yields of the first growth (Nm^3 CH_4 ha^{-1}) were comparable between sites, years and treatments. At Swabian Jura, the methane yields of the first growth cycle increased continuously during the four years of the field trial from 845 (2013) to 983 Nm^3 CH_4 ha^{-1} (2016). At Foothills, methane yields were generally higher than at Swabian Jura and in 2016 highest on NPK-fertilised plots. At this site, the highest methane yield was reached on the latest date of first cut in 2016 (1396 m^3 CH_4 ha^{-1}). Additionally, annual methane yields (AMY) in Nm^3 CH_4 ha^{-1} a^{-1} were calculated based on the SMY of the first growth cycle and the annual DMY to give a rough estimate for each site and treatment. In 2016, estimated AMY of D1 was 1355 (none), 1606 (PK) and 2177 Nm^3 CH_4 ha^{-1} a^{-1} (NPK) at Foothills and 1565 (none), 1790 (PK) and 2087 (NPK) at Swabian Jura. At both sites, it was significantly increased by NPK fertilisation and there were significant differences between cutting variants in 2016 (Figure 2).

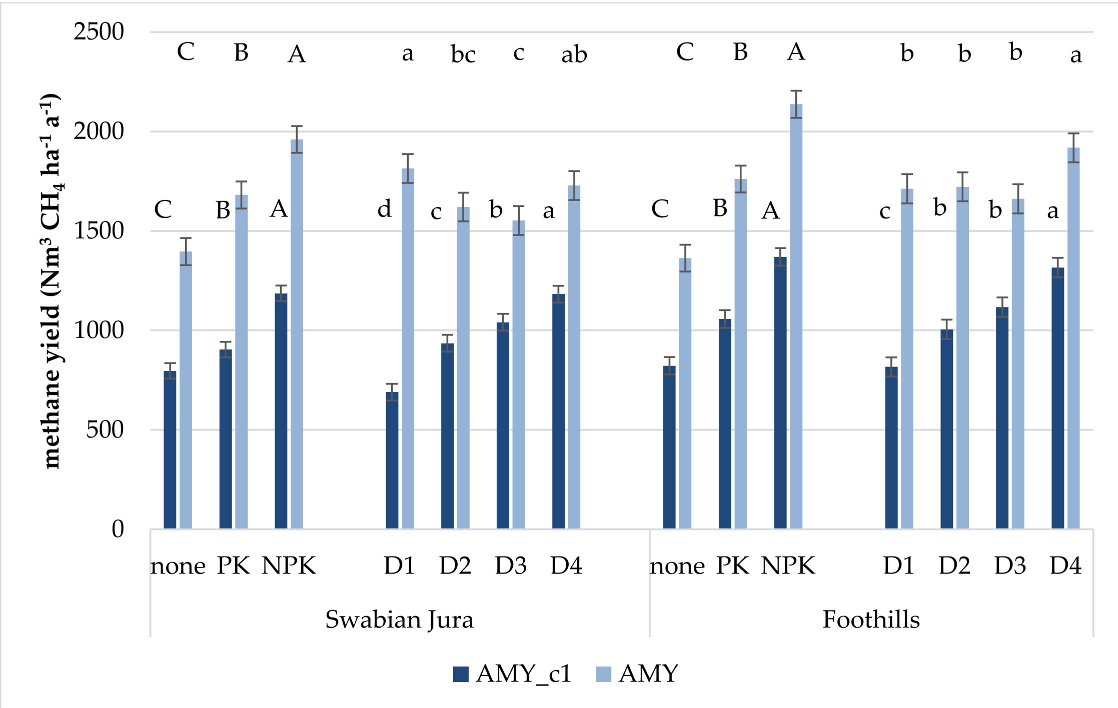

Figure 2. Mean methane yield from first growth (AMY_c1) and estimated annual methane yields (AMY) in Nm^3 CH_4 ha^{-1} a^{-1} at Swabian Jura and Foothills sites in 2016. Data are shown for different fertiliser levels (none, PK, NPK) and cutting variants (D1 = early date of first cut, D4 = late date of first cut). Error bars indicate standard deviation. Identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) and lower-case letters between cutting variants ($p < 0.05$, $n = 9$) for each site.

3.3. Mineral Nutrients and Ash Content

There were significant differences between treatments in crude ash content and ash components of biomass harvested from the first growth and significant interactions with the factor 'year'. Mean contents of mineral nutrients and ash are shown in Table 5.

Table 5. Least square mean content of mineral nutrients potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and ash (g kg^{-1} DM) of first cut in 2016 with standard error (±SE).

Site	Treatment	K		P		Ca		Mg		Ash Content	
		g kg^{-1} DM									
Swabian Jura	D1	25.9	±1.4	3.41^{a}	±0.31	10.4	±1.0	1.50^{b}	±0.07	108.8^{a}	±4.5
	D2	23.3	±1.4	3.12^{b}	±0.31	10.2	±1.0	1.69^{a}	±0.07	103.7^{ab}	±4.4
	D3	19.1	±1.4	2.72^{c}	±0.31	11.1	±1.0	1.50^{b}	±0.07	105.3^{ab}	±4.4
	D4	19.5	±1.4	2.57^{c}	±0.31	10.7	±1.0	1.53^{b}	±0.07	93.9^{b}	±4.2
	none	22.2	±1.4	2.90	±0.31	12.0	±0.9	1.61	±0.06	115.2^{A}	±4.0
	PK	22.2	±1.4	2.99	±0.31	10.0	±0.8	1.53	±0.06	97.1^{B}	±3.7
	NPK	21.5	±1.4	2.98	±0.31	9.8	±0.8	1.53	±0.06	96.8^{B}	±3.7
Foothills	D1	24.9	±1.9	2.88^{a}	±0.35	10.0	±0.5	1.97^{a}	±0.15	112.5^{a}	±3.7
	D2	20.4	±1.9	2.33^{b}	±0.35	9.8	±0.5	1.87^{ab}	±0.15	95.0^{b}	±3.4
	D3	16.8	±1.9	2.16^{c}	±0.35	9.7	±0.4	1.64^{c}	±0.15	89.2^{bc}	±3.3
	D4	16.6	±1.9	1.92^{d}	±0.35	9.5	±0.4	1.72^{bc}	±0.15	84.7^{c}	±3.2
	none	19.0	±1.9	2.24^{A}	±0.35	9.0^{B}	±0.4	1.64^{B}	±0.14	92.0	±3.0
	PK	20.1	±1.9	2.43^{B}	±0.35	10.0^{A}	±0.4	1.83^{A}	±0.14	97.5	±3.1
	NPK	19.9	±1.9	2.29^{AB}	±0.35	10.4^{A}	±0.4	1.93^{A}	±0.14	95.8	±3.9

Identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) and lower-case letters between cutting variants ($p < 0.05$, $n = 9$) for each site.

At both sites, date of first cut and fertilisation significantly influenced P, Mg, Ca content in 2016. For K, date of first cut and fertilisation interact, thus means for each combination should be compared in 2016. The P content of the harvested biomass was significantly decreased by later date of first cut (Table 5).

4. Discussion

4.1. Early Date of First Cut before Flowering Stage (D1)

As biomass cut early had the highest NEL and protein content, its use as forage would seem appropriate. However, although NDF and ADL values were lowest at early date of first cut, the fibre contents were comparable to those found for hay meadows in previous studies (e.g., [30]). For this reason, the biomass needs to be chopped if it is conserved as silage. Herrmann et al. [31] recommend silage additives for extensively used *Alopecurus pratensis* wetland meadows because the compactibility of fibrous material is poor.

In 2016, the biomass harvested at the earliest date of first cut had a CP content of 135.2 ± 2.2g kg^{-1} DM at Swabian Jura, but only 112.5 ± 3.3 g kg^{-1} at Foothills. This was because the plants were at different stages of maturity. They were younger at Swabian Jura due to delayed development at this site (Figure A1). Roughage with a CP content of 160–220 g kg^{-1} and below 22% NDF is suitable for dairy cows [32]. Forage of lower nutritional quality is not adequate. However, roughage with a CP content of at least 80 g kg^{-1} DM can be fed to beef cattle and non-lactating sheep [32].

The net energy for lactation (NEL) of the biomass was calculated to estimate its forage quality. Lactating dairy cows require energy contents of at least 6.0 MJ NEL kg^{-1} [7]. This level was not achieved at either site. In addition, protein and energy content losses can be expected during haymaking, especially with field-dried hay (e.g., [33]). Consequently, early-cut hays are not suitable as exclusive forage for dairy cows. In terms of CP and NEL content, they would be preferable for more extensively kept animals such as suckler cows and sheep.

Due to the decreasing demand for grassland biomass as forage [34], there is also the option of using it for renewable energy production in biogas plants. Germany has the highest number of biogas plants in Europe. Of these, 50% use grass silage as a co-substrate [35]. At both sites of our field trial, the feedstock-specific methane yield (SMY) of the early first cut was 15%–18% higher than that of the later first cuts. The SMY of the early cut at both Swabian Jura (0.32 ± 0.003 Nm^3 kg^{-1}) and Foothills (0.31 ± 0.004 Nm^3 kg^{-1}) was similar to that of maize (0.30–0.38 Nm^3 kg^{-1}), the most commonly used feedstock, but maize can yield more biomass (15–30 t ha^{-1}) [35]. DMY is the main determinant of high methane yields per hectare.

The AMY was significantly increased by NPK fertilisation at both sites. Similar to our field trial, [36] found an increased area-specific methane yield through an increase in biomass. In their experiment, different sown species mixtures yielded on average 1674±487 m^3 CH_4 $ha^{-1}a^{-1}$ when cut twice a year. Compared to other long-term grassland experiments e.g., [37] the AMY in our study was relatively low. Only that of the NPK-fertilised plot at Foothills was comparable to levels, where 2157.3 m^3 $ha^{-1}a^{-1}$ were calculated for a two-cut system fertilised with 30 kg N per cut [37]. For intensive grassland, even higher methane yields of 2700–3500 m^3 ha^{-1} have been reported [38]. By contrast, extensive mountain grassland (890 m a.s.l.) yielded only about 910 m^3 CH_4 $ha^{-1}a^{-1}$ when cut twice [38].

4.2. Cut at Beginning of Grass Flowering Stage (D2)

The biomass of cutting variant D2 showed a significant reduction in many of the quality parameters examined (NEL, SMY and Mg) compared to D1. The P, Ca and K contents were sufficient for forage use [6], but the Mg content was low (1.72 ± 0.72 g kg^{-1}, average of both sites). In agricultural practice, mineral nutrient contents are of minor importance for livestock, because they can be supplemented by mineral feed.

The feed energy content of the biomass from D2 was low, but could be included into feed rations if supplemented with concentrates or energy-rich silage [7]. Hay of about 5 MJ NEL kg^{-1} can be used for up to 80% of feed rations for pregnant suckler cows [39]. If forage quality is not sufficient, upgrading treatments can be applied. For example, adding ammonium salts to hay from species-rich meadows can improve its forage quality [6]. As the methane yield was similar to that of D1, the biomass from D2 can also be used for the production of biogas.

4.3. Late First Cut at Flowering (D3) or Seed-Ripening Stage (D4)

The first cut of species-rich hay meadows is often delayed for ecological reasons: the plants have time to develop, some species already produce seeds and there is a broader range of pollinators. This is why these meadows are protected as habitats. The main flowering period of grasses is in June, corresponding to our cutting variant D3. At the end of June, most grass species in our field trial had produced seed and many seeds were already ripening. At the same time, the NEL and SMY, but also N and K content, were continuing to decrease compared to D2. In 2016, there were no significant differences in chemical composition between D3 and D4, except for P at Foothills and CP. Thus, the first cut in the main flowering period can be delayed up to 14 days without substantially impairing forage quality. This is due to an increased proportion of forbs with later date of first cut as these do not mature as fast as most grass species [5].

At the latest date of first cut (D4), CP was reduced by 34%–41% and NEL by 20% compared to D1 (average of both sites). The fibre content increased, depending on fertilisation level. NDF content was significantly increased by NPK fertilisation due to the high percentage of grasses. Similar results were reported by [40]. Due to the NEL values below 5 MJ, the biomass from the D4 date of first cut cannot be recommended as forage for most types of livestock; biomass of this quality can only serve as exclusive feed for horses with low performance [7]. However, it should be kept in mind that hay meadows cut late for several years in succession may contain plant species that are toxic to animals if eaten in large quantities. In European hay meadows, these include in particular *Colchicum autumnale* and *Senecio* species [41].

The mean AMY of D4 was significantly higher than D1 at Foothills. This is because the annual DMY was high at Foothills most likely due to better water availability, especially during the first growth cycle (April–June) in 2016 (Figure A1). In practice, chopped biomass conserved as silage is usually used as biogas substrate; however, problems with ensilaging of this fibrous material can occur. To achieve better usability of late-cut grassland biomass for biogas production, mechanical, chemical and biological pretreatments can be applied [42]. Another option is dry fermentation, which would be preferable due to the technical problems caused by high fibre content in wet fermentation.

Extensive grassland biomass from later date of first cut has often been suggested as a cheap resource for solid fuel [30,43] on account of its high fibre and low N content. These reduce its forage value, cause problems during the biogas process, for example with stirring devices, and also lower the SMY. Thus, biomass from a late cut could be more suitable for combustion. However, as N content leads to NOx emissions, the threshold N value of 6 g kg^{-1} DM [12] for unproblematic combustion should not be exceeded. In our study, the N content of the biomass (calculated as CP $\times$ 0.16) from both sites was always above this limit, although it was comparable to values of dry hay meadows found in other studies e.g., [43].

Another important aspect for combustion is ash. In 2016, the ash content was very high due to soil contaminations related to the weather conditions at the time of harvest. This contamination was most likely caused by a larger area of uncovered soil this year through a higher occurrence of anthills (Foothills site) and voles (Swabian Jura site). In all other years, the ash contents were in the range of those reported in previous studies [30,43]. Mean K contents of later date of first cut were also similar to those reported in other studies [30,43]. K content was above the guideline value of 7% of ash content [12] at later date of first cut. These high K contents can lead to slagging and corrosion during the combustion process due to lower ash melting temperatures. Mean Ca content was comparable to that found in a dry hay meadow [43] and within the guideline range of 15%–35% of dry ash [44] at later date of first cut (D3, D4). However, in most cases, the Ca content was below this percentage, which can cause problems related to ash melting. Mg contents lower than 2.5% of dry ash can promote slagging [43]. At Foothills, Mg content was sufficient mainly at late dates of first cut in 2013 and 2014. At Swabian Jura, it was consistently low (1.6 ± 0.2 g kg^{-1}DM).

The quality of hay can be improved through upgrading treatments. For example, leaching can reduce unwanted contents of ash, Cl, K and Mg [45]. Contents of Cl, K and Mg can also be reduced by delaying the first cut until September, but this leads to substantial changes in species composition [46]. Therefore, this method is only suitable for maintaining the openness of the landscape and not for the preservation of species-rich hay meadows.

Another pretreatment is the IFBB (integrated generation of solid fuel and biogas from biomass) system, which was developed for the energetic use of late-mown grassland biomass. This technique separates silage into solid press cake and a liquid phase. The press cakes are rich in fibre and have better combustion properties as the detrimental mineral nutrients are concentrated in the press juice [47]. The drawback of this system is that it is only profitable if in spatial proximity to a biogas plant [48].

Our field trial showed that extensive fertilisation of species-rich hay meadows has only a minor influence on biomass quality, but harvesting time significantly influences chemical composition. Both the hypotheses that date of first cut has a strong effect on biomass quality and that there are site-specific effects were confirmed. The stage of maturity of grasses (the dominant plant functional type) needs to be considered for each site, because it depends for example on altitude.

Other newly developed conversion pathways, such as bioethanol fermentation and pyrolysis, have not yet been tested on biomass from extensively used meadows. One alternative use could be the production of paper. Late-cut biomass from the first growth cycle would be appropriate for this usage.

A first analysis identified water-soluble organic substances in wastewater as critical [49]. Further research is necessary on the usability of grassland biomass in this conversion pathway.

Where several biomass uses are possible, a combination of usage pathways could help alleviate trade-offs between agronomic and biodiversity goals, for example an early cut for forage and a late cut for energetic use. Depending on local conditions, a rotational use of several lowland hay meadows would allow an earlier cut in some years without changing their botanical composition. For the conservation of these meadows, new management and usage concepts are necessary. However, the usability of the biomass for farmers is limited and any additional income would not compensate for the extra expenditure. Therefore, the ecological benefit of extensive management concepts would need to be remunerated by public funds. With a continued increase in the price of fossil fuels, the energetic use of grassland biomass could become more economically viable.

5. Conclusions

An overview of the most recommendable uses of biomass from different cuts is provided in Table 6. The hypothesis that biomass from an early first cut is more suitable for forage use than that of a later first cut was confirmed. Early-cut biomass is suitable as forage for extensive animal husbandry systems, such as suckler cows and sheep, whereas the feeding of later harvests is limited to horses with low performance. The biomass needs to be supplemented due to its low energy content, especially at late dates of first cut. Forage quality can be improved through PK fertilisation; this leads to a higher proportion of legumes and thus higher protein content. However, it should be considered that an early cut before the main flowering period could lead to a loss of biodiversity in the long term.

Table 6. Recommendations for most suitable uses of biomass from harvesting dates of lowland hay meadows.

Harvest Time	Before Flowering (D1)	Main Flowering Period (D2)	End of Main Flowering Period (D3 and D4)
Components:			
Protein, energy content	High	low	low
Fibre content	Low	low	high
Usability for biomass			
Forage use	beef cattle, non-lactating sheep	suckler cows, sheep	as exclusive feed only for horses
Biogas use	suitable	suitable	only dry fermentation
Combustion	not suitable	not suitable	leached material suitable

A late cut is more suitable for biogas use than for feed application because any toxic species would not be problematic. The implementation is, however, restricted by economic considerations, because methane yields are comparatively low.

The most advisable use of biomass from a late cut is combustion. The hypothesis on the suitability of late-cut biomass for combustion was confirmed, the main problem here being the high N and ash contents. Other solid biofuels such as wood are more available and easier to combust than this late-cut grassland biomass. However, a late cut is more flexible than an early cut with respect to weather conditions. It was found in the field trial that the quality of the hay was not significantly reduced when the late cut was postponed by two weeks.

Author Contributions: Conceptualization, U.T. and M.E.; methodology, M.B. and J.H.; formal analysis, J.H. and M.B.; investigation, M.B.; data curation, M.B.; writing—original draft preparation, M.B.; writing—review and editing, M.B., U.T., M.E., I.L. and J.H.; visualization, M.B.; supervision, U.T. and I.L.; project administration, U.T.; funding acquisition, M.E. and U.T.

Funding: This research was supported by Stiftung Naturschutzfonds Baden-Wuerttemberg, financed by earmarked contributions of Gluecksspirale and by a Ph.D. grant from the 'Landesgraduiertenfoerderungsgesetz Baden-Wuerttemberg'.

Acknowledgments: We would like to thank the laboratories of LAZBW and Ihinger Hof for their analyses, as well as B. Truckses, D. Mezger, J. Class, S. Rothenhäusler and E. Gerster for their technical support. We are grateful to landowners for providing the species-rich meadows. We thank N. Gaudet for improving the language quality of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results

Appendix A

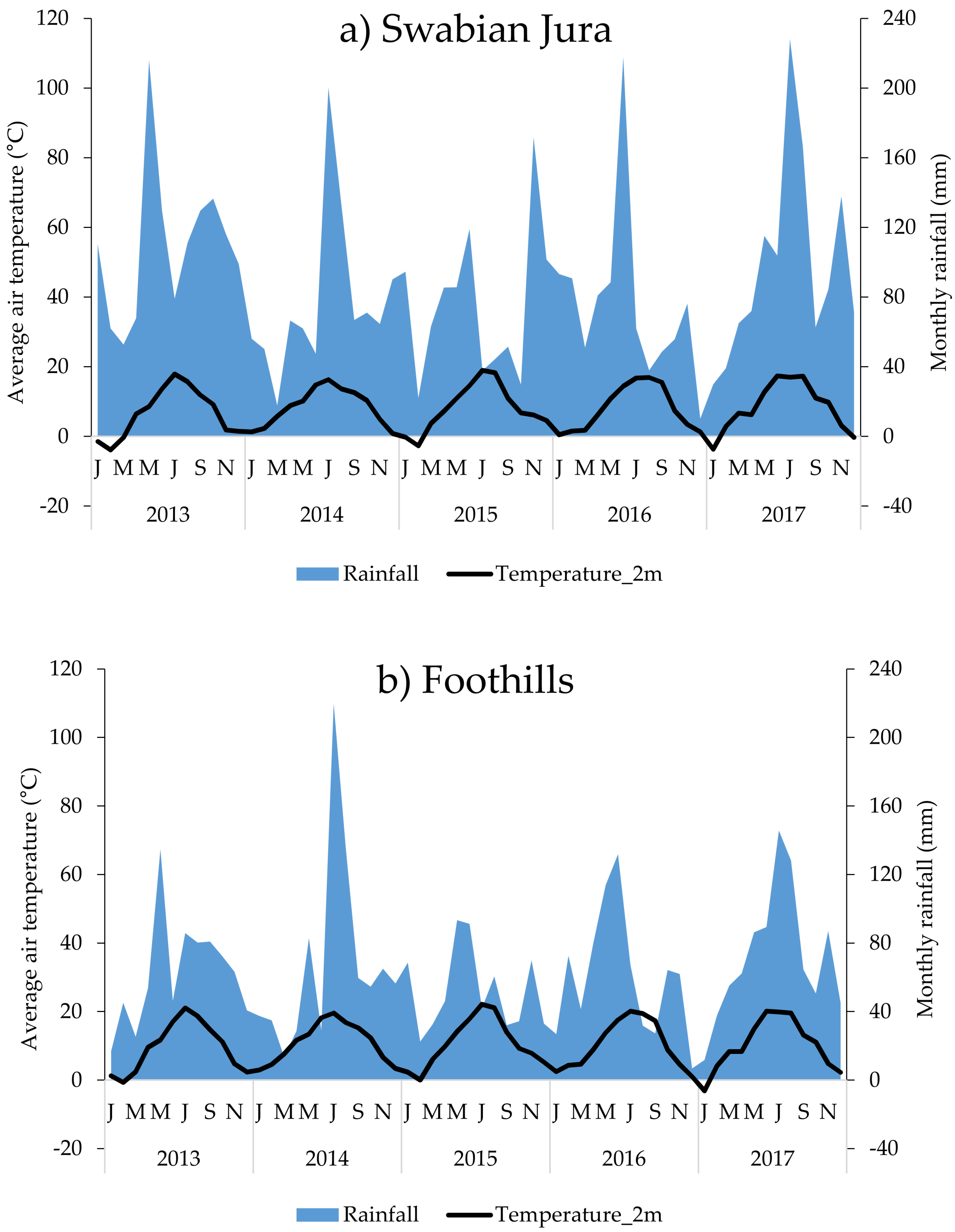

Figure A1. Mean air temperature (2 m) and total monthly precipitation [50] at (**a**) nearest weather station to Swabian Jura site, St. Johann (48°48′54.7″ N, 9°33′86.2″ E, 749 m above sea level) and (**b**) nearest weather station to Foothills site, Tachenhausen (48°64′96.1″ N, 9°38′56.5″ E, 330 m a.s.l.).

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