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Article

Deciphering Substrate-Specific Methane Yields of Perennial Herbaceous Wild Plant Species

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Abstract: The global demand for plant biomass to provide bioenergy and heat is continuously increasing because of a growing interest among many industrialized and developing countries towards climate sound and renewable energy supply. The exacerbation of land-use conflicts proliferates social-ecological demands on future bioenergy cropping systems. Perennial herbaceous wild plant mixtures (WPMs) represent an approach to providing social-ecologically more sustainably produced biogas substrate that has gained increasing public and political interest only in recent years. The focus of this study lies on three perennial wild plant species (WPS) that usually dominate the biomass yield performance of WPM cultivation. These WPS were compared with established biogas crops in terms of their substrate-specific methane yield (SMY) and lignocellulosic composition. The plant samples were investigated in a small-scale mesophilic discontinuous biogas batch test for determining the SMY. All WPS were found to have significantly lower SMY (241.5–248.5 l_N kgVS^{-1}) than maize (337.5 l_N kgVS^{-1}). This was attributed to higher contents of lignin (9.7–12.8% of dry matter) as well as lower contents of hemicellulose (9.9–11.5% of dry matter) in the WPS. Only minor, non-significant differences to cup plant and Virginia mallow were observed. Thus, when planning WPS as a diversification measure in biogas cropping systems, their lower SMY should be considered.

Keywords: anaerobic digestion; *Artemisia vulgaris* L.; biodiversity; biogas production; brown knapweed; *Centaurea nigra* L.; common tansy; mugwort; perennial crops; *Tanacetum vulgare* L.

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1. Introduction

Supplying "clean" energy is a major component of the growing bioeconomy, the core goal of which is the complete replacement of fossil and nuclear resources with renewable energy and bioenergy [1]. The full extent of this challenge can be seen in the fact that the share of renewable energy in total global energy consumption seems to have stalled at between 12 and 14% over the last 20 years, despite various efforts and scientific progress. While the amount of renewable energy has increased from 54.4 to 82.7 EJ, the amount of fossil fuels, such as coal, oil, and gas, has also increased significantly over the same period, from 337.7 EJ to 486 EJ [1]. Apart from the end use sectors heat and transport, bioenergy makes up only a small share of 2.4% of total renewable energy production [1]. However, bioenergy cropping systems are assumed to have a promising future for two important reasons:

1. By growing bioenergy crops, unused land can be returned to agricultural production and, if necessary, even protected from further degradation by adhering to best management practices.
2. Bioenergy production enables a stable basis for the reliable provision of electricity and heat compared to wind and solar energy, which are subject to strong fluctuations.

Many other ecosystem functions besides the provision of biomass are currently only being discovered bit by bit or investigated in connection with bioenergy cropping systems. The additional ecosystem services resulting from these ecosystem functions could be a turning point in the history of bioenergy cropping systems, as monetization of them could increase land conversion many times over. For example, the monetary value of all ecosystem services of growing Miscanthus (*Miscanthus* ANDERSSON), a very well-known perennial bioenergy crop [2–4], in a case study region in Germany varies between 1200 and 4183 € per hectare and year [5]. Several other perennial second generation lignocellulosic crops such as switchgrass (*Panicum virgatum* spp.) [6,7], willow (*Salix* spp.) [8–11], cup plant (*Silphium perfoliatum* L.) [12–16] and Virginia mallow (*Sida hermaphrodita* L. Rusby) [13,17] have been intensively researched worldwide for decades [18]. All these bioenergy cropping systems have one thing in common: they are monocultures. Therefore, it is to be expected that agricultural biodiversity could be better promoted by a more diverse bioenergy cropping system. In the search for more diverse bioenergy cropping systems, the first reports were published during the last nine years on how species-rich flowering mixtures of annual, biennial, and perennial wild plants can significantly enhance many nursery services compared with the abovementioned mono-perennials [19–22]. These so-called "perennial wild plant mixtures" (WPM) were investigated by several German institutions over the past decade for their use as second generation co-substrates in anaerobic digestion [19–21,23–30]. Whether WPMs are also suitable for other bioenergy production pathways such as combustion, pyrolysis or bioethanol production has not yet been explored [22].

It was found that WPM cultivation for anaerobic digestion, under the best circumstances, provides both a notable farm productivity, as indicated by a five-year average annual dry matter yield (DMY) of 12.5 Mg ha^{-1} at an annual nitrogen fertilization of 50 kg ha^{-1} [28,31,32], and an improvement of various social-ecological services [20,25,27,33–35]. However, the successful cultivation of WPMs strongly depends on several factors such as the seed-bed preparation, the sowing procedure, the weather conditions, the soil heterogeneity and the weed pressure [22,23,31,36]. After successful establishment, WPM cultivation provides high biomass yields each year accompanied by a dynamic change in the WPM species composition over the years [31]. Annual species dominate the plant stand in the first year of cultivation, biennial species in the second year, and perennial species from the third year onwards [25,31,36]. Therefore, perennial wild plant species (WPS) such as common tansy (*Tanacetum vulgare* L.), common knapweed (*Centaurea nigra* L.) and mugwort (*Artemisia vulgaris* L.) have the highest impact on the overall yield performance of the WPM in the long-term [22]. This is because the WPM can grow up to 5 years and even longer [22,25,33–35], and the perennial WPS have the highest share of total accumulated DMY [22,31,36].

Despite the fact that the DMY is the main determinant for the methane yield per hectare (MYH) of biogas crops [37–39], the substrate-specific methane yield (SMY) also plays a vital role in biogas plant management, with regard to (i) the organic loading of the fermenter (the higher the SMY the better the organic loading efficiency), (ii) the retention time of the co-substrate in the fermenter (the higher the SMY, the shorter the retention time in the biogas plant), and (iii) the secondary effects on the digestibility of the other fermentation substrate components, for example through the provision of essential trace elements [22,25,40–42]. However, little is known about the substrate-specific methane yield (SMY) of perennial WPS [19,43,44]. In most of the few studies on the methane yield potential of WPM, the mixtures are considered as a whole (plant stand level) and not examined for individual plant performance [21,23,36,45,46]. In addition, there are large differences within the limited data available. For example, SMY values from 287.5 [19] to 362.0 l_N kgVS^{-1} [47] are reported for common and brown knapweed, respectively. For the other promising WPS, only single values are available, accounting for 233 l_N kgVS^{-1} (common tansy) and 346 l_N kgVS^{-1} (mugwort) [19]. Therefore, this study aims at investigating the potential SMY of relevant perennial WPS and compare them with relevant annual and perennial alternative biogas co-substrates. The results are expected to help better understanding the relevance of the WPM species composition dynamics [31] towards the development of social-ecologically more sustainable bioenergy cropping systems.

2. Materials and Methods

2.1. Origin and Harvest of Plant Material

The investigations in this study are based on above-ground biomass harvested from common tansy, brown knapweed, mugwort, cup plant, Virginia mallow, and maize (*Zea mays* L.) (Table 1). Cup plant, maize and Virginia mallow served as reference crops. All biomass samples were taken from the same field trial in Hohenheim, southwest Germany (407 m AMSL, N 48°42′57.024″, O 9°12′52.956″) (Figure 1).

Table 1. Overview of the crops (sorted alphabetically) used in this study.

Trivial Name	Botanical Name	Life Cycle	Origin
Common knapweed	*Centaurea nigra* L.	Perennial	Temperate Europe
Common tansy	*Tanacetum vulgare* L.	Perennial	Temperate Europe and Asia
Cup plant	*Silphium perfoliatum* L.	Perennial	Northern America
Maize	*Zea mays* L.	Annual	Central America
Mugwort	*Artemisia vulgaris* L.	Perennial	Temperate Europe, Alaska, Northern Africa and Asia
Virginia mallow	*Sida hermaphrodita* L. Rusby	Perennial	Northern America

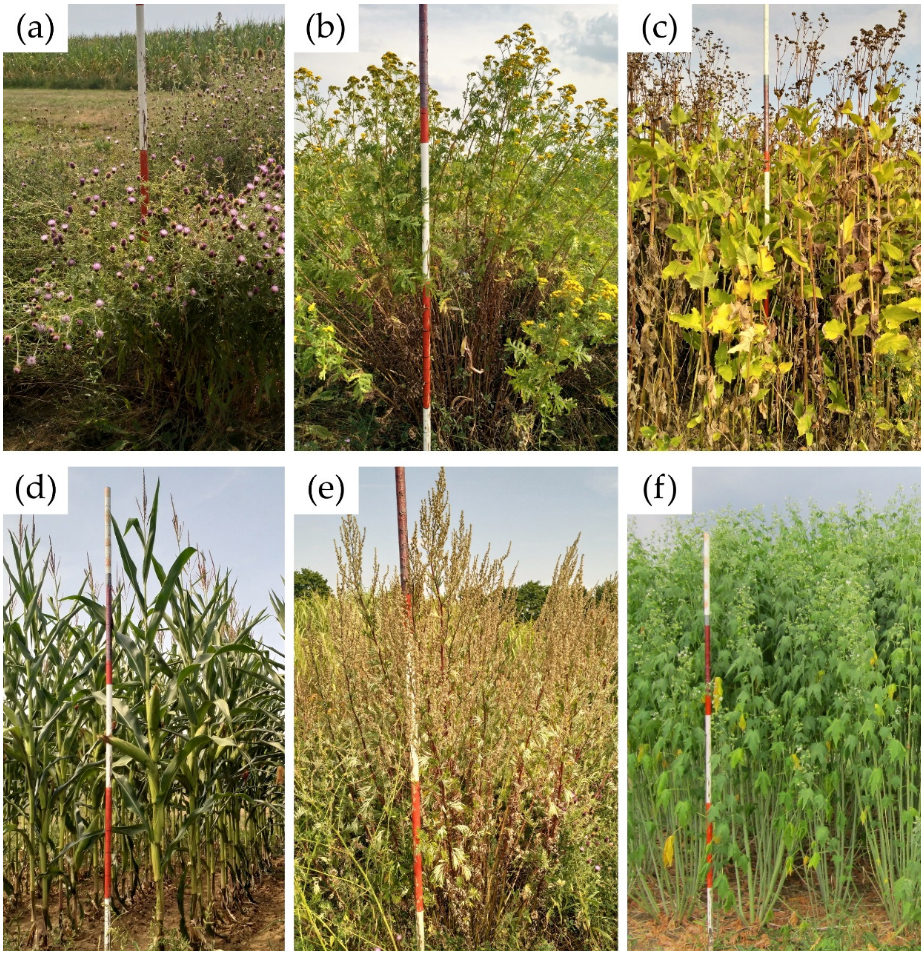

Figure 1. Overview of the crop species investigated in this study: (**a**) common knapweed (**b**) common tansy (**c**) cup plant (**d**) maize (**e**) mugwort and (**f**) Virginia mallow.

This field trial was established in a randomized block design with three (maize, Virginia mallow, cup plant) and five replicates (WPM), respectively, in 2014. The plots were of square shape and their gross area was 36 m^2. The distance between the plots was 1.5 m, and the distance between the blocks was 5 m. The site is characterized by homogeneous favorable abiotic growth conditions, such as (i) clayey loam (Luvisol) [36], (ii) an average annual air temperature of 10.1 °C in 2016 (Figure 2), 1.4 °C higher compared with long-term data, and (iii) an annual precipitation of 595 mm in 2016 (Figure 2), which was 103 mm less compared with long-term data. The harvest dates of the biomass samples for this study varied according to the crop-specific demands. The WPS (common tansy, common knapweed and mugwort) and Virginia mallow were harvested in August 2016. Cup plant and maize were harvested in October 2016. Only fully developed individual plants from the WPM plots were selected for harvest of the WPS, with three plots each found for common tansy and common knapweed, but only one plot for mugwort. For cup plant, only plant samples of two randomly selected representative plots of the three existing plots were chosen due to technical reasons. For all crops, harvesting was done by hand using a pruning shear.

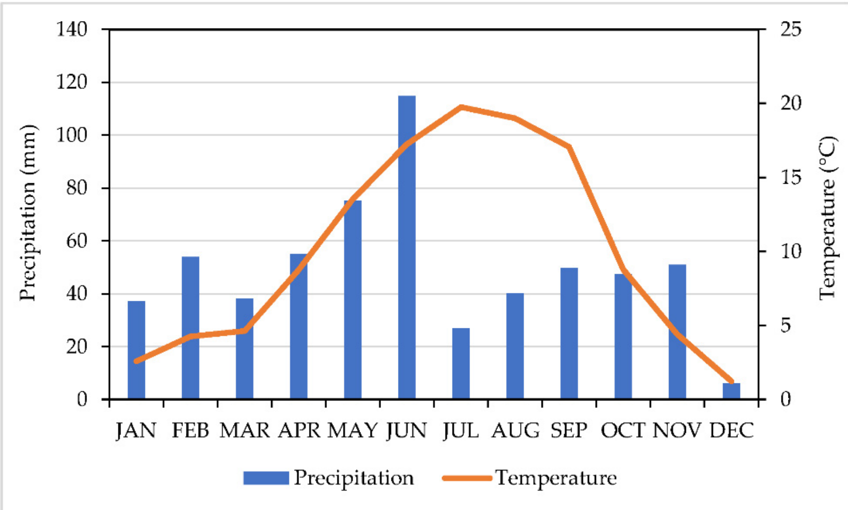

Figure 2. Overview of monthly precipitation and monthly average temperature conditions at the field trial site (407 m AMSL, N 48°42′57.024″, O 9°12′52.956″) in the year of harvest (2016).

2.2. Determination of C- and N-Content, Fibre Analyses

After harvesting and drying to constant weight (at 58 °C), the samples were milled using a cutting mill (SM 200, Retsch, Haan, Germany) with a 1 mm sieve for further analysis (including the biogas batch test). For the following analyses, the plant sample material was not pre-treated, e.g., through enzymatic hydrolysis. To measure nutrient detergent fiber content (NDF), acid detergent fiber (ADF), lignin (ADL), total carbon (C_T) and total nitrogen (N_T) all samples were prepared as follows: The ash content of plant samples was estimated according to Kiesel and Lewandowski [48], by drying a 1 g subsample at 105 °C in a cabinet dryer (to determine residual moisture) and burning at 550 °C in a muffle furnace to constant weight. After that, the contents of NDF, ADF and ADL were analyzed according to VDLUFA Method Book III, methods 6.5.1, 6.5.2 and 6.5.3 [49]. The contents of cellulose (CL) and hemicellulose (HC) were calculated using the following Equations:

$$CL = ADF - ADL \tag{1}$$

$$HC = NDF - ADF. \tag{2}$$

The contents of N_T and C_T were measured according to DIN ISO 5725 using the elemental analyzer 'Vario Max CNS' (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.3. Biogas Batch Test

The biogas batch test was conducted according to Von Cossel et al. [50]. The test commenced on 8 April 2019 and ended on 13 May 2019 with the duration of the experiment fixed in the implementation protocol of the biogas batch test. For the biogas batch test (wet fermentation), 200 mg of organic dry matter of the plant samples was mixed with 30.0 $\pm$ 0.1 g inoculum (4% DMC, origins from a biogas plant, degassed under the conditions intended for the biogas batch test) in 100 mL air-tight bottles and kept at 39 °C for 35 days, a standard procedure according to VDI guideline 4630 [48,51,52]. The substrate to inoculum ratio accounted for 1:3 on a volatile solids (VS) basis. The actual plant material per batch flask ranged from 229.2 mg DM (Virginia mallow) to 234.5 mg DM (cup plant) due to differences in ash content. Therefore, the DMC in the test bottles was about 4.7%. Each field replicate of the plant samples was repeated four times within the biogas batch test, and gas was collected a total of four times. After each gas collection, each bottle was emptied with a hollow needle. A hand-held pressure gauge for external pressure sensors (HND-P pressure gauge, Kobold Messring GmbH, Hofheim, Germany) was used to measure the pressure rise in order to calculate the gas production, taking into account the respective ambient air pressure. At the beginning of the biogas batch test, measurements were taken daily, while towards the end measurements were taken every three days due to decreasing gas production. The pressure increase was measured 19 times during the batch test and converted into standardized values (standard conditions: 0 °C and 1013 hPa). The control (inoculum without plant material) and ambient atmospheric pressure was required to calculate the accumulated substrate-specific net biogas yield (SBY). This is because biogas production still occurs even when the inoculum is starved, and its volume must be subtracted from the total volume per plant sample. A thermal conductivity detector (gas chromatograph GC-2014, Shimadzu, Kyoto) was used to determine the methane content (MC) of the collected biogas at a detection temperature of 120 °C. Under an oven temperature of 50 °C and the carrier gas argon, two columns (Haye-Sep and Molsieve column) were used [48]. All gas samples were injected with a Combi-xt PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) [48]. The substrate-specific methane yield (SMY) was calculated following Equation (3):

$$\text{SMY} = \text{SBY} \times \text{MC}. \tag{3}$$

2.4. Statistical Analysis

Data curation was conducted using MS Excel. The biogas batch test was analyzed in accordance with [50]. The F-tests for the effects of the different crops on SMY and the biochemical constituents were conducted as adapted from according to [50] following Equation (4):

$$y_i = \mu + \tau_i + e_i \tag{4}$$

where μ is the intercept and e_i is the error of observation y_i with crop-specific variance. τ_i is the fixed effect for the ith crop species.

If differences were found, a multiple *t*-test was performed to create a letter display [53]. The assumptions of normality and homogeneous error variance were checked graphically. The Akaike information criterion (AIC) [54] was used to selected the best model. All analysis run using the PROC MIXED procedure of the SAS® Proprietary Software 9.4 TS level 1M5 (SAS Institute Inc., Cary, NC, USA). For the correlation matrix and SMY prediction, PROC CORR and PROC REG (SAS® Proprietary Software 9.4 TS level 1M5, see above) were used. Both degrees of freedom and standard errors were approximated using the Kenward-Roger method [55].

3. Results and Discussion

Both the lignocellulose composition studies, and the biogas batch tests showed significant differences between the WPS and the reference crop species. Only results from one crop year are available here, which means that there is not yet any information on the possibility of an interaction between crop type and climatic variations with respect to SMY. This could be assumed, since seasonal climatic conditions usually have a large influence on crop-specific biomass yield and quality [51]. However, no information is yet available on this with regard to WPS and it was not possible to investigate this in this study. Therefore, the use of plant samples from two or more seasons would be appropriate in future studies to examine the year effects on both specific biomass yield and quality of different biogas crops or biogas cropping systems. In the following, the results of the two categories lignocellulose and biogas batch test are presented and discussed separately.

3.1. Lignocellulosic Composition

The analyses of lignocellulosic composition revealed a large variation across plant species in contents of DM of lignin (3.2–12.6%), cellulose (25.8–48.8%) and hemicellulose (5.0–27.4%) (Table 2).

Table 2. Lignocellulosic composition of the biogas crops (sorted alphabetically) investigated in this study. Additionally, the standard error is provided. The color scaling indicates per parameter the meaning of the value for the use of biomass as biogas substrate from good (dark green) to bad (deep red).

Crop	NDF (% of DM)	ADF (% of DM)	ADL (% of DM)	Cellulose (% of DM)	Hemicellulose (% of DM)
Common knapweed	57.6 + 1.9 ab	47.3 + 1.9 a	9.7 + 0.7 b	37.6 + 1.3 a	10.3 + 0.6 b
Common tansy	62.4 + 1.9 a	50.9 + 1.9 a	12.8 + 0.7 a	38.1 + 1.3 a	11.5 + 0.6 b
Cup plant	52.0 + 2.4 b	44.6 + 2.3 a	6.7 + 0.9 c	37.9 + 1.6 a	7.4 + 0.7 c
Maize	52.7 + 1.9 b	29.0 + 1.9 b	3.3 + 0.7 d	25.8 + 1.3 b	23.7 + 0.6 a
Mugwort	61.9 + 3.4 a	52.0 + 3.3 a	12.6 + 1.3 ab	39.4 + 2.3 a	9.9 + 1.0 bc
Virginia	58.7 + 1.9 ab	47.8 + 1.9 a	7.0 + 0.7 c	40.8 + 1.3 a	10.9 + 0.6 b

NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, DM = dry matter, n = number of field replicates. Different lower case letters denote for significant ($p < 0.05$) differences between crops within parameter.

The C:N ratio was highest for mugwort (127.1) and lowest for maize (55.2) (Table 3). Considering that a C:N ratio of 15–30:1 is required for a stable anaerobic digestion process in the biogas plant [56], all crops show too high a C:N ratio (Table 4). While there are no data in the literature for mugwort, common tansy and common knapweed that could be used for comparison, the values for maize compare well with those in the literature [57], although they appear somewhat too high (>36.2:1). This may be due to the difference in sample preparation, as the values in the literature are based on maize silage [57], whereas in this study dried maize samples were available that had not been ensiled beforehand. In any case, it can be seen that with an increasing share of WPS in the biogas crop rotation [58], attention should be paid to appropriate N supply to the fermenter in the biogas production process, which can usually be realized by adding residues from animal husbandry (slurry, manure). The C:N ratio of mugwort was thus much higher than that of straw, which is 69.5:1. But still, the SMY of mugwort was notable higher than that of straw, which is about 189 l_N $kgVS^{-1}$ [59]. This could be due to the low ash content and mediocre hemicellulose content of mugwort (Tables 2 and 3) compared to the other crops studied. However, the C:N-ratio alone does not allow an evaluation for or against one of these wild plant species in comparison with maize.

The ash content of dry matter was highest for cup plant (9.7%) and intermediate in wild plant species (5.2–6.4%) indicating the highest ash dry matter content (Table 3).

Table 3. Contents of nitrogen, carbon, C_T:N_T ratio, ash and dry matter content (right before entering the biogas batch test) within the plant material (sorted alphabetically). Additionally, the standard error is provided. The color scaling indicates per parameter the meaning of the value for the use of biomass as biogas substrate from good (dark green) to bad (deep red).

Crop	N_T (% of DM)	C_T (% of DM)	C_T:N_T Ratio	Ash (% of DM)	DMC_{DS} (%)
Common knapweed	0.7 + 0.1 bc	46.1 + 0.3 bc	68.3 + 4.2 bc	6.4 + 0.3 b	93.6 + 0.3 c
Common tansy	0.6 + 0.1 bd	47.3 + 0.3 a	75.5 + 4.2 b	6.1 + 0.3 bc	93.9 + 0.3 bc
Cup plant	0.6 + 0.1 cd	44.0 + 0.3 d	77.9 + 5.2 b	9.2 + 0.3 a	90.8 + 0.3 d
Maize	0.8 + 0.1 b	45.4 + 0.3 c	57.2 + 4.2 c	4.1 + 0.3 d	95.9 + 0.3 a
Mugwort	0.4 + 0.1 d	46.8 + 0.4 ab	127.1 + 7.3 a	5.2 + 0.4 cd	94.8 + 0.4 ab
Virginia	1.2 + 0.1 a	45.7 + 0.3 bc	38.0 + 4.2 d	6.7 + 0.3 b	93.3 + 0.3 c

N_T = total nitrogen content, DM = dry matter, C_T = total carbon content, DMC_{DS} = dry matter of the dried plant substrate right before entering the biogas batch test. Different lower case letters denote for significant ($p < 0.05$) differences between crops within parameter.

Table 4. Methane content and substrate-specific methane yield of the crops (sorted alphabetically). Additionally, the standard error is provided. The color scaling indicates per parameter the meaning of the value for the use of biomass as biogas substrate from good (dark green) to bad (deep red).

Crop	CH_4 (%)	SMY (l_N kgVS^{-1})
Common knapweed	53.7 + 0.2 ab	248.5 + 4.1 c
Common tansy	54.2 + 0.2 a	243.2 + 4.1 c
Cup plant	53.3 + 0.3 bc	264.7 + 5.0 b
Maize	52.9 + 0.2 c	337.5 + 4.1 a
Mugwort	53.5 + 0.4 ac	241.5 + 7.0 c
Virginia	54.1 + 0.2 ab	267.2 + 4.1 b

$_N$ = norm conditions, CH_4 = methane content, SMY = substrate-specific methane yield, vs. = volatile solids. Different lower case letters denote for significant ($p < 0.05$) differences between crops within parameter.

3.2. Methane Content and Substrate-Specific Methane Yield

The methane content of the substrate-specific biogas was highest for common tansy (54.2%) and lowest for maize (52.9%) (Table 4). The SMY ranged from 241.5 l_N kgVS^{-1} (mugwort) to 337.5 l_N kgVS^{-1} (maize). The net velocity of biogas production was lowest for the WPS compared with maize, Virginia mallow and cup plant (Figure 3). This resulted in a lower slope of the accumulated substrate-specific net biogas production of the WPS (Figure 4). For all crops however, the duration of the biogas batch test appears to have been long enough to reach the maximum specific biogas yield potential because no significant biogas production was observed after the 34th day of the biogas batch test (Figure 4).

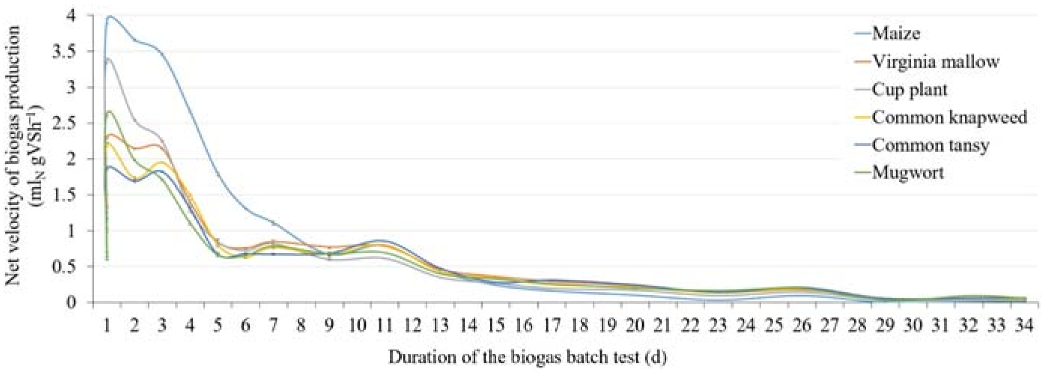

Figure 3. Net velocity of biogas production per gram volatile solids from the crops tested in this study. For each measurement and for each crop except mugwort, the error bars indicate the standard deviation for the replicates of the crop species in the field trial.

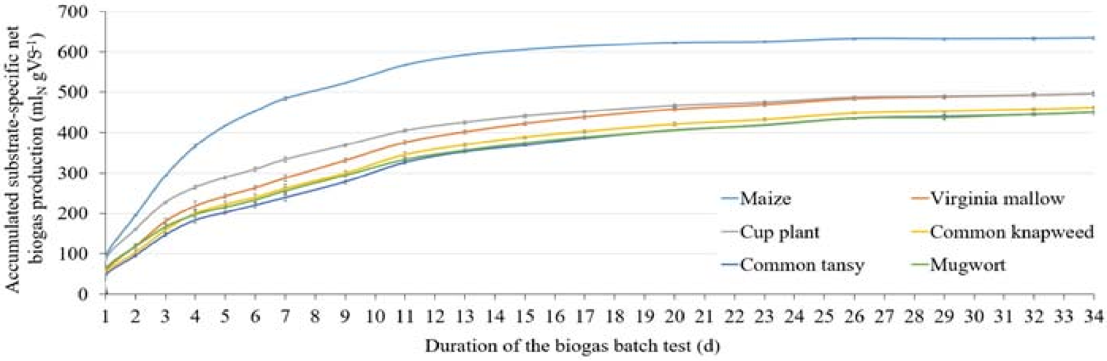

Figure 4. Accumulated substrate-specific net biogas production of the crops investigated in this study. For each measurement and for each crop except mugwort, the error bars indicate the standard deviation for the replicates of the crop species in the field trial.

Both, methane content and SMY are slightly lower than reported by [60,61]. This is likely because of variations in pre-treatment; the plant samples were ensiled before biogas batch test by [60]; whereas in our study, the plant samples were not ensiled. Ensilaging is known to increase SMY to some extend [60,62,63]. However, the results of biogas batch tests are generally not directly comparable due to large variations of methodological settings and conditions [60]. Against this backdrop, it also makes sense to compare the ratios between plant species within the studies. In [60] for example, the SMY of maize was about 1.6 times higher than for cup plant. In this study, the SMY of maize was also notably (1.3 times) higher compared with cup plant (Table 4). In [60], this was drawn back to differences in biochemical composition. This also applies to the results in this study, because maize has (i) significantly lower contents of lignin, which negatively correlates with the SMY (0.92, $p < 0.001$), and (ii) higher contents of N, which positively correlates with the SMY (0.54, $p < 0.05$) (Table 5).

Table 5. Pearson's correlation coefficients matrix of substrate-specific biochemical compositions and the key parameters of the biogas batch test. The levels of significance are indicated by asterisks. Significant Pearson's correlation coefficients were colorized to emphasize negative (dark red) and positive values (dark green).

	NDF	ADF	ADL	CEL	HC	Ash	N_T	C_T	CNR	SMY
ADF	0.78 **									
ADL	0.83 ***	0.87 ***								
CEL	0.65 *	n.r.	n.r.							
HC	n.s.	n.r.	−0.61 *	−0.89 ***						
Ash	n.s.	n.s.	n.s.	n.s.	−0.80 **					
N_T	n.s.	−0.15 *	−0.41 *	0.03 *	n.s.	n.s.				
C_T	0.67 **	n.s.	0.70 **	n.s.	n.s.	n.s.	n.s.			
CNR	n.s.	0.33 *	0.54 *	n.s.	n.s.	n.s.	n.r.	n.s.		
SMY	−0.66 *	−0.96 ***	−0.88 ***	−0.89 ***	0.90 ***	n.s.	0.26 *	n.s.	−0.39 *	
CH_4	n.s.	0.69 **	0.59 *	0.66 **	−0.51 **	0.32 *	n.s.	n.s.	n.s.	−0.64 **

N_T = total nitrogen content, C_T = total carbon content, CNR = C_T:N_T ratio, NDF = neutral detergent fiber, ADF = Acid detergent fiber, ADL = acid detergent lignin, CEL = cellulose, HC = hemicellulose, CH_4 = methane content, SMY = specific methane yield, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$, n.s. = not significant, n.r. = not relevant.

The results from the lignocellulosic analyses (Tables 2 and 3) helped to interpret the results of the biogas batch test. Across plant species, lignin content had the strongest (negative) effect on SMY. This is in line with literature [43,64,65] (Table 5). Other correlations between SMY and biochemical constituents of the crops were either weak or not significant (Table 5). Regression analyses revealed a well-fitting ($R^2 = 0.9825$, $p < 0.0001$) prediction model shown in Equation (5):

$$SMY = 305.15579 + 2.94265 \times NDF - 3.79094 \times ADF - 4.20099 \times ADL, \quad (5)$$

with NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin. As expected, the strong negative influence of lignin on SMY also has a great significance in this SMY prediction equation.

However, following the findings of [43], the high accuracy of this prediction model is very likely due to the large variation of biochemical composition between the crops (Tables 2 and 3). Overall, lignin was found to be most relevant for SMY prediction (Table 5). But this is mostly the case for so-called "across-crop" prediction models [43,64–66]. Such models may be useful for the prediction of the SMY of mixtures whose species compositions of are known, for instance regarding crop rotation planning or national biomass potential analyses [67]. But for selecting the best genotypes within individual crop species such as WPS, species-specific prediction models would be required [43]. However, lignin content is an important parameter for the SMY of WPS [43,64–66]. Therefore, it is necessary to learn more about how to reduce the lignin content of WPS through advanced agronomic practices, e.g., harvest determination and planting geometry, in the future. Breeding could probably also help further improving WPS, which is currently being investigated in a German research project that focuses on common tansy [68].

As Table 5 further shows, the SMY correlates strong positively (R = 0.90) and highly significantly ($p < 0.0001$) with hemicellulose. Since hemicellulose is relatively low in WPS, this is also another reason for the low slope of the accumulated substrate-specific net biogas production of the WPS (Figure 4). This is also in line with expectations, since hemicellulose is easily digestible in anaerobic digestion [43,64–66]. Thus, it seems reasonable to pay attention to increasing the hemicellulose content for improving the biogas substrate quality of WPS. Furthermore, lignin and hemicellulose were found to be significantly ($p < 0.05$) moderately ($R = |0.4| - |0.7|$) correlated with methane content. For lignin, the correlation was positive, and for hemicellulose, the correlation was negative. Therefore, it would be expected that a decrease in lignin content combined with an increase in hemicellulosic content could result in a reduction in methane content of the biogas produce. However, as shown by the low methane content of maize (Table 4), this should not be a hindrance to increasing the overall SMY of WPS.

If only relatively small areas, such as field margins, are to be managed with WPS in a biogas scenario, only relatively small amounts of WPS silage would be available for biogas production. These could then be mixed in the biogas plant with more fermentable biomass from other biogas crops or manure. In this case, WPS would provide an opportunity to promote agrobiodiversity in the biogas crop rotation, at least on a small scale, without causing significant net income losses. If these small quantities were to be used in the alternative utilization pathway of combustion, additional investments might be required (e.g., for pellet production), which would not be worthwhile for small substrate quantities. However, the currently still lower specific methane yield of WPS compared to maize should be carefully considered for biogas plant management. It remains to be seen how the development of new seed mixtures [58,69] or breeding of new genotypes [68] will help reduce these qualitative differences between WPS and the more established biogas crops.

4. Conclusions

In this study, those WPS which most strongly contribute to the accumulated biomass yield of WPM over the whole multi-annual growth period (five years and longer) were analyzed for their specific biogas yield. All of them yield less biogas than the comparison plant species: conventional annual (maize), or perennial (cup plant, Virginia mallow). This is mostly due to the unfavorable ratio of lignin (too high) and hemicellulose (too low) in the biomass of those perennial WPS. Therefore, other energetic end uses, such as combustion, may be more appropriate. For combustion high lignin contents are desirable and therefore the crops are harvested later and stay longer in the field [8,70,71]. This brings additional positive effects in terms of other ecosystem services, such as (i) extended protection for animals from the weather and from predators (nursery services), and (ii) extended feed provision.

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