

Methane yield and species diversity dynamics of perennial wild plant mixtures established alone, under cover crop maize (*Zea mays* L.), and after spring barley (*Hordeum vulgare* L.)

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Abstract

The cultivation of perennial wild plant mixtures (WPMs) in biogas cropping systems dominated by maize (*Zea mays* L.) restores numerous ecosystem functions and improves both spatial and temporal agrobiodiversity. In addition, the colorful appearance of WPM can help enhance landscape beauty. However, their methane yield per hectare (MYH) varies greatly and amounts to only about 50% that of maize. This study aimed at decreasing MYH variability and increasing accumulated MYH of WPM by optimizing the establishment method. A field trial was established in southwest Germany in 2014, and is still running. It tested the effects of three WPM establishment procedures (E1: alone [without maize, in May], E2: undersown in cover crop maize [in May], E3: WPM sown after whole-crop harvest of spring barley [*Hordeum vulgare* L.] in June) on both MYH and species diversity of two WPMs [S1, S2]. Mono-cropped maize and cup plant (*Silphium perfoliatum* L.) were used as reference crops. Of the WPM treatments tested, S2E2 achieved the highest ($19,296 \text{ m}^3/\text{ha}$, 60.5% of maize) and S1E1 the lowest accumulated MYH ($8,156 \text{ m}^3/\text{ha}$, 25.6% of maize) in the years 2014–2018. Cup plant yielded slightly higher than S2E2 ($19,968 \text{ m}^3/\text{ha}$, 62.6% of maize). In 2014, the WPM sown under maize did not significantly affect the cover crop performance. From 2015 onward, E1 and E2 had comparable average annual MYH and average annual number of WPM species. With a similar accumulated MYH but significantly higher number of species (3.5–10.2), WPM S2E2 outperformed cup plant. Overall, the long-term MYH performance of WPM cultivation for biogas production can be significantly improved by undersowing with maize as cover crop. This improved establishment method could help facilitate the implementation of WPM cultivation for biogas production and thus reduce the trade-off between bioenergy and biodiversity.

KEY WORDS

agricultural diversification, biodiversity, biogas production, biomass, perennial, *Silphium perfoliatum* L., wild flower, wild plant mixture

1 | INTRODUCTION

The cultivation of perennial wild plant mixtures (WPMs) is a promising new biogas cropping system for Central European conditions (von Cossel & Lewandowski, 2016), first mentioned by Vollrath et al. (2012). WPMs are seed mixtures of annual, biennial, and perennial, predominantly wild and flower-rich plant species. The annual plant species dominate the plant stands in the year of establishment, the biennial species in the second year, and the perennial species from the third year onward. Both ecosystem services (Emmerling, 2014; Emmerling, Schmidt, Ruf, von Francken-Welz, & Thielen, 2017) and landscape beauty (Daniel, 2001; Huth, Paltrinieri, & Thiele, 2019) have been proven to be much higher for WPM than for common biogas cropping systems, such as short biogas crop rotation systems with high shares of maize.

In less than a decade since the publication of the study by Vollrath et al. (2012), various types of WPMs have been developed by the breeding companies Rieger-Hofmann (Germany) and Saaten-Zeller (Germany). Both the socio-ecological and economic performance of these WPMs have been investigated at the field scale by several institutes and nonprofit associations across Germany (Friedrichs, 2013; Janusch, 2014; Vollrath, Werner, Degenbeck, & Marzini, 2016; von Cossel & Lewandowski, 2016; Wurth et al., 2016; Zürcher, 2014). These investigations found the methane yield per hectare (MYH) of WPM to be much lower than for silage maize and whole-crop cereal silage (WCCS), ranging from 40% to 60% of silage maize MYH (Friedrichs, 2013; Vollrath et al., 2016; Wurth et al., 2016; Zürcher, Stolzenburg, Messner, Wurth, & Löffler, 2014). This is mainly caused by both lower dry matter yields (DMY; 2.9–22.5 Mg/ha) and lower specific methane yields (SMY; 212–289 l_N/kg volatile solids [VS]; von Cossel & Lewandowski, 2016; von Cossel, Möhring, Kiesel, & Lewandowski, 2018; Wurth et al., 2016). This large quantitative and qualitative variation in biomass characteristics of the WPM species is caused by (a) high species diversity dynamics (von Cossel & Lewandowski, 2016) and (b) considerable differences in the species-specific substrate quality (Vollrath et al., 2012).

The low and rather variable economic performance of WPM impedes future large-scale implementations given the increasing scarcity of arable land available for industrial crops (Cosentino, Testa, Scordia, & Alexopoulou, 2012; Foley et al., 2005; Galatsidas et al., 2018) due to rising land-use demand for food and feed production, urbanization, and other land-use requirements (Tilman et al., 2009). For this reason, it has been suggested that biomass production should be restricted to those kinds of agricultural lands that are marginal in the sense that they are not suitable for food crop production (Elbersen, Van Eupen, et al., 2018; Elbersen, Van Verzandvoort, et al., 2018; Ramirez-Almeyda et al., 2017).

However, the constraining biophysical conditions often found on these marginal lands, such as poor soils and harsh climate (Terres et al., 2014) somewhat complicate the successful establishment and growth of WPM. This could lead to a further decrease in both their potential methane and dry matter yield over their whole cultivation periods (Brauckmann & Broll, 2016; Wurth et al., 2016).

In our study, however, WPMs are considered a promising option for biomass production on marginal agricultural land because they contain a mixture of on average five species (von Cossel & Lewandowski, 2016) and show features of a perennial system (Emmerling et al., 2017), for example, less soil disturbance and a well-established rooting system. This promotes soil carbon accumulation, the development of soil biodiversity, and reduces the risk of erosion (Emmerling et al., 2017; Weißhuhn, Reckling, Stachow, & Wiggering, 2017). The costs of biomass production are also lower in perennial than in annual cropping systems (Lewandowski, 2016; Lewandowski et al., 2016). In addition, WPM cultivation is expected to increase the resilience (Walker, Holling, Carpenter, & Kinzig, 2004) of the agroecosystem, because heterogeneous cropping systems are capable of reacting more flexibly to both biotic and abiotic disturbances (Bucharova et al., 2018; von Cossel & Lewandowski, 2016). This increased resilience can be mainly attributed to the wide range of species-specific demands and stress tolerances of the diverse WPM species. Given the high number of species within the WPM (von Cossel & Lewandowski, 2016), it is expected that—for the vast majority of sites (especially marginal agricultural lands)—there will be at least one species that is able to cope with these conditions. This renders WPM even more interesting when considering both unfavorable sites and the projected effects of climate change on agriculture (Cosentino et al., 2012; Pachauri et al., 2014; Tuck, Glendining, Smith, House, & Wattenbach, 2006; von Cossel, 2019).

With regard to the overall yield performance of WPM, a previously published study that investigated two different WPMs cultivated on three different sites in southwest Germany over a 5 year period came to some potentially expedient conclusions: (a) low-field emergence rates combined with low DMY levels of annual WPM species in the first production year lead to low income and this cannot be compensated in the following four cropping years, (b) incomplete canopy closure of annual WPM species can have an indirect negative effect on the establishment of biennial and perennial species through higher abundance of weed species (von Cossel & Lewandowski, 2016). Wurth et al. (2016) also found both good weed control prior to sowing and a complete canopy closure during the first WPM vegetation period to be crucial to avoid weed infestation. Consequently, to optimize the overall MYH performance of WPM biomass production, improved establishment procedures are required that lead to better canopy closure and increased yield level in the year of

establishment. An improved cultivation strategy for the establishment year in particular could help to (a) make WPM a promising tool to increase agricultural biodiversity (Pfiffner, Ostermaier, Stoeckli, & Müller, 2018; Warzecha, Diekötter, Wolters, & Jauker, 2018) and thus contribute to biodiversity conservation (Sheppard, Gillespie, Hirsch, & Begley, 2011) on marginal agricultural lands, (b) make WPM a net-profit low-input system for farms on marginal agricultural lands, and (c) accelerate the potential of large-scale implementation of WPM throughout Central European marginal agricultural lands.

Vollrath et al. (2013) proposed the use of maize (*Zea mays* L.) as a nurse crop to optimize the establishment of WPM. Maize is known to be highly suitable for intercropping and functioning as a nurse crop (von Cossel et al., 2019; von Cossel, Möhring, Kiesel, & Lewandowski, 2017). Von Redwitz et al. (2019) investigated the establishment of wild-flower strips under maize in eastern Germany. They found these to (a) reduce maize yield by about 30%, (b) benefit pollinators and ground beetles, and (c) improve the habitat quality for ground-nesting, open-land birds such as skylark (Von Redwitz et al., 2019). Brauckmann and Broll (2016) examined both the economic and ecological aspects of WPM establishment under maize in eastern Germany over a 3 year cultivation period. They concluded that it could be economically feasible to establish WPM under maize (Brauckmann & Broll, 2016). However, there are no studies available that cover the whole cultivation period of up to 5 years (as proposed by Vollrath et al., 2012). For this reason, our study aims to gain insights into the potential effects of maize as a nurse crop for WPM establishment in terms of both MYH performance and WPM species diversity dynamics over a 5 year cultivation period.

Mono-cropped maize was chosen as main reference due to its high MYH potential (Herrmann & Rath, 2012) and its predominant use as a biogas crop in Germany (Witt et al., 2012). As second reference, cup plant (*Silphium perfoliatum* L.) was taken. It is a perennial biogas crop with a rapidly increasing cultivation area in Germany (about 3,000 ha in 2018; TFZ, 2019). The reasons for this are (a) its positive effects on both biodiversity and the environment (Bufe & Korevaar, 2018), (b) its high MYH potential (Gansberger, Montgomery, & Liebhard, 2015; Haag, Nägele, Reiss, Biertümpfel, & Oechsner, 2015; Mast et al., 2014; Šiaudinė et al., 2015; Ustak & Munoz, 2018), (c) it has been accepted as a greening measure since 2018 (Buße & Korevaar, 2018), and (d) there has been a breakthrough in establishment procedure (sowing instead of planting) which makes its cultivation more cost-efficient. It is also commonly established under maize (Stolzenburg, Bruns, Monkos, Ott, & Schickler, 2016). However, despite the positive effects on the environment (Buße & Korevaar, 2018; Schorpp & Schrader, 2016), cup plant is still a monoculture. The polyculture WPM could

potentially perform much better in terms of social-ecological aspects such as landscape beauty and biodiversity conservation.

2 | MATERIALS AND METHODS

A single field trial with two WPM (S1, Rieger-Hofmann; S2, Saaten-Zeller GmbH & Co KG) and three establishment procedures was sown at Hohenheim in southwest Germany in 2014 and is being continued ever since. This study includes the results of the first 5 years of cultivation from 2014 to 2018. The WPMs S1 and S2 were also used in another field trial which was conducted during the years 2011–2015 (von Cossel & Lewandowski, 2016). But during 2011 and 2014, the breeding companies have adjusted the species compositions of WPMs S1 and S2 according to first experiences of the farmers testing the WPM. This means that the species compositions of S1 and S2 used for this study slightly vary from those used in von Cossel and Lewandowski (2016). Moreover, the differences of the species compositions of S1 and S2 have extended: While the total number of species remained about the same in S2, it was nearly doubled in S1 from 27 species in 2011 (von Cossel & Lewandowski, 2016) to 52 species in 2014 (Table S1). Detailed deviations of species mixture compositions between the WPMs used in this study and those described in von Cossel and Lewandowski (2016) are shown in Table S1.

2.1 | Field trial establishment and site characteristics

The field trial was established as a completely randomized design. Fifteen treatments were arranged in six rows each with nine plots; thus, the trial had 54 plots in total, each with 36 m² gross area (6 m × 6 m). Treatments were tested in two to five replicates. For the current study, only data coming from two WPMs (S1 and S2) under three types of establishment and the two monoculture crops, silage maize (Carolinio, KWS) and cup plant, were used. No other WPMs were included in the trial. The standard establishment (E1) of S1 and S2 was conducted as described by von Cossel and Lewandowski (2016), whereas the seed mixtures were sown directly using a pneumatic seed drill at a sowing density of 10 kg/ha and a row distance of 15 cm (Kuhn, Zeller, Bretschneider-Herrmann, & Drenckhahn, 2014). The second establishment method was simultaneous sowing of WPMs S1 and S2 (both with *Calendula officinalis* L. and *Phacelia tanacetifolia* Benth. instead of sunflower) with maize (*Zea mays* (L.) var. Carolinio [KWS, Germany]) as nurse crop. The third establishment method was direct sowing (without tillage) of S1 and S2 1 day after harvest of spring barley (*Hordeum vulgare* (L.)) in June 2014. The barley straw was removed

from the field. Each combination of WPM and establishment method was tested on five plots. The two monoculture crops were tested on two plots each.

The soil is a clayey loam (Luvisol) with a pH of 6.3 (spring 2014). The major agricultural measures and observations are listed in Table 1. The weather conditions of the whole cultivation period are presented in Figure 1.

TABLE 1 Key information on the agricultural practices applied for the cropping systems presented in this study during the years 2014–2018. Additionally, two important weather events are listed

Procedure or special event	Details	Date or value
Ploughing	Depth: 20–25 cm	February 4, 2014
Rotary harrow	Depth: 8–10 cm (two times)	February 28, 2014
Soil nitrogen (NO ₃ -N), May 2014	kg/ha (depth 0–90 cm)	109.6
Soil phosphorus (CAL), May 2014	mg/100 g (depth 0–30 cm)	9.2
Soil potassium (CAL), May 2014	mg/100 g (depth 0–30 cm)	15.8
Soil magnesium (CaCl ₂), May 2014	mg/100 g (depth 0–30 cm)	13.9
Quicklime (77% CaO) application	1.5 Mg/ha	February 26, 2014
Sowing of summer barley ^a	350 kernels/m ²	April 20, 2014
Sowing of maize ^b	9 kernels/m ²	May 15, 2014
Planting of cup plant ^c	4 plants/m ²	May 16, 2014
Sowing of E1 and E2 ^d	Plot drill (Haldrup, Germany)	May 20, 2014
Field emergence maize		May 22, 2014
Field emergence WPM		June 1, 2014
N fertilization ^e	90 kg N/ha	June 5, 2014
High radiation + temperature	8–8.5 UVR, 25–35°C	June 6–9, 2014
Weeding of thistles	By hand	June 6, 2014
Hail damage	25 mm precipitation	June 10, 2014
Harvest of summer barley	By hand	June 25, 2014
Sowing of E3 ^d	Plot drill (Haldrup, Germany)	June 27, 2014
Harvest ^d of E1, E2	Species specifically (by hand)	End of September 2014
Mulching of E3	Mechanical	End of February 2015
Harvest ^d of E1, E2, and E3	Species specifically (by hand)	Each year end of July (2015–2018)
Harvest of maize	By hand	Each year in October (2014–2018)
Harvest of cup plant	By hand	Each year in October (2014–2018)
N fertilization (maize)	90 kg N/ha	May 2014
P/K/Mg/S fertilization (all treatments)	88 kg P/ha, 176 kg K/ha 32 kg Mg/ha, 48 kg S/ha	March 2015
N fertilization (WPM, maize, cup plant)	90 kg N/ha	April–May 2015
N fertilization (WPM, maize, cup plant)	90 kg N/ha	April–May 2016
N fertilization (WPM, maize, cup plant)	90 kg N/ha	April–May 2017
N fertilization (WPM and cup plant)	25 kg ^f N/ha	April–May 2018
N fertilization (maize)	90 kg N/ha	April–May 2018

Abbreviation: UVR, ultraviolet radiation.

^a*Hordeum vulgare* (L.) var. Grace (BayWa, Germany).

^b*Zea mays* (L.) var. Carolinio (KWS, Germany).

^c*Silphium perfoliatum* ssp. (mk jungpflanzen GmbH, Germany).

^dDescribed by von Cossel and Lewandowski (2016).

^eENTEC@26N (BASF, Germany).

^fDue to technical reasons.

2.2 | Harvest and sample analysis

The evaluation of both biomass yield and biogas substrate quality of the various cropping systems required several work procedures in the field and in the lab. Harvest of monoculture crops was conducted by hand from a sampling area of 1.5 m² (maize) and 2 m² (cup plant). The WPMs

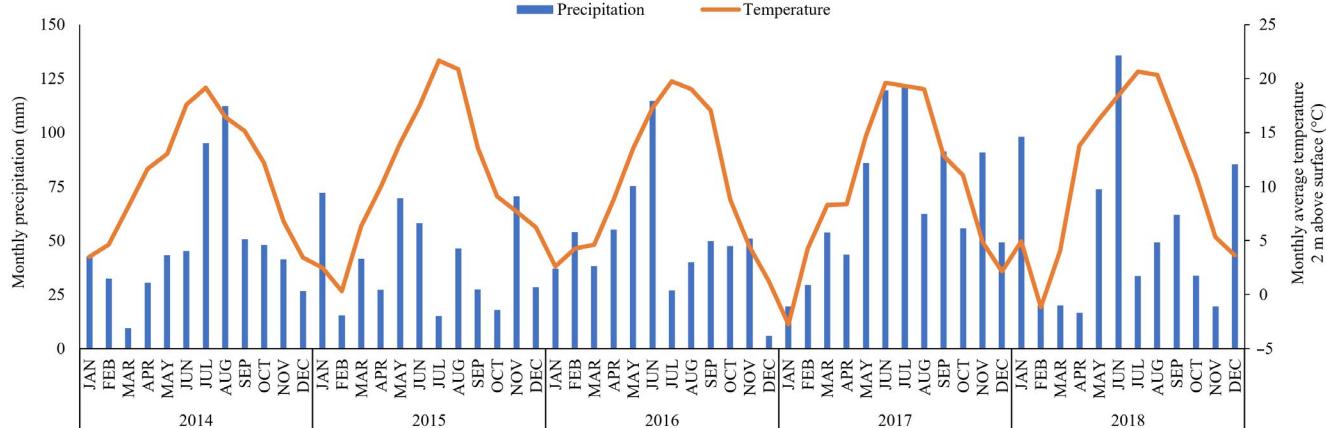


FIGURE 1 Overview of weather conditions (bars = monthly precipitation; line = monthly average temperature 2 m above ground) in the field trial site from 2014 to 2018 (LTZ, 2019)

were harvested by hand separately for each species from a sampling area of 4 m². Samples of each species were then chopped manually in the field immediately after harvest to determine the fresh matter biomass yields. For most of the wild plant species, the whole fresh matter samples were used for dry matter determination. For other dominant species, about 500 g of fresh matter was used as subsamples. The fresh matter samples were immediately put into the drying chamber and dried at 60°C to constant weight for 48 hr to determine the water content. A similar procedure was also applied by von Cossel and Lewandowski (2016). After sample preparation, biomass attributes relevant for the performance as feedstock for biogas production were determined. These attributes comprise biochemical compositions of lignin, cellulose, hemicellulose, and ash which were measured according to von Cossel et al. (2017). Additionally, the SMY was determined as described in the following subsection.

2.3 | Biogas batch tests

The biogas yield of the different raw materials was evaluated through biogas batch tests at lab scale. These tests were conducted with milled subsamples of the dry matter samples under mesophilic conditions (39°C) according to VDI directive 4630 (VDI, 2016). The biogas production was measured according to the pressure increase in bottles of hermetic digesters (100 ml) as described by von Cossel et al. (2017), and later standardized to norm conditions.

2.4 | Statistical analysis

The data were analyzed using a mixed model approach. The model was as follows:

$$y_{ijkl} = \mu + \tau_i + \varphi_j + a_k + (\tau\varphi)_{ij} + (\tau a)_{ik} + (\varphi a)_{jk} + (\tau\varphi a)_{ijk} + e_{ijkl}$$

where μ is the intercept, τ_i , φ_j , and $(\tau\varphi)_{ij}$ are the fixed effects for the i th establishment method, the j th WPM and their interaction effects, respectively. a_k , $(\tau a)_{ik}$, $(\varphi a)_{jk}$, and $(\tau\varphi a)_{ijk}$ are the effect of the k th year and its interactions with establishment method and WPM, respectively. e_{ijkl} is the error of observation y_{ijkl} with establishment procedure-specific variance. To account for possible gradients in the field, random row and column effects are included in the model if they decreased the Akaike information criterion (AIC; Wolfinger, 1993). Different error variance–covariance structures (compound symmetry, first-order autoregressive both with homogeneous and heterogeneous variances as well as unstructured) were fitted to account for temporal correlation, because repeated measures were taken from each plot across years. Again, we selected the best model based on the AIC. Assumptions of normality and deviations from homogeneous error variance (except the deviations already accounted for by the model) were checked graphically. We tested the influence of factors using a global F test. In case of significant differences, a multiple t test was conducted to provide a letter display (Piepho, 2004). Note that the number of weed-relevant species in monoculture maize and cup plant is always one. Thus, means for WPM treatments were estimated without these data. To complete the letter display, these means were tested against a fixed value of one using a simple t test. In all analysis, both standard errors and degrees of freedom were approximated based on the Kenward–Roger method (Kenward & Roger, 1997). All analyses were performed using the PROC MIXED procedure of the SAS ® Proprietary Software 9.4 TS level 1M5 (SAS Institute Inc.).

3 | RESULTS

Both maize and most of the perennial cropping systems (WPM E1, WPM E2, and cup plant) were successfully established in

2014. This was indicated by homogeneous species-rich plant stands end of July 2014 (Figure 2). The WPM establishment procedure E3 developed rather slow due to low precipitation from end-June to mid-July. However, the perennial wild plant species emerged homogeneously in all establishment procedures for both S1 and S2 (Figure S1). The plant species of E3 were growing until December 2014, and many species flowered until mid of December such as *Borago officinalis* L., *Calendula officinalis* L., *Coriandrum sativum* L., *Fagopyrum esculentum* Moench, *Malva verticillata* L., and *Phacelia tanacetifolia* Benth (Figure S1c). Overall, both S1 and S2 showed a high species diversity ranging from 3 to 22 yield-relevant species per year (Figures 2–4, Table 2; Figure S1). Significant threefold interactions (a dependency on the interaction of year, WPM, and establishment procedure) were found for DMY, DMC, the lignocellulosic components (lignin, cellulose, and hemicellulose), SMY, and the number of yield-relevant WPM species (Table S2). The inflorescences of the wild plant species attracted a high number of various insect species, some of which are shown in Figure 4. The establishment procedure did not influence the average number of yield-relevant species across years from year 2015 onward (Table 2). From 2015 onward, the most dominant species were yellow chamomile (*Anthemis tinctoria* L.), yellow melilot (*Melilotus officinalis* L.), common knapweed

(*Centaurea nigra* L.), fennel (*Foeniculum vulgare* Mill.), lucerne (*Medicago sativa* L.), mugwort (*Artemisia vulgaris* L.), and common tansy (*Tanacetum vulgare* L.; Table S3). E2 showed slightly higher weed occurrence measured by the number of weed species occurring compared to other establishment methods (E1 and E3; Table 2). However, the proportions of total DMY of weed species and thus their overall impact on the economic performance of the WPM cultivation were higher for S1 than for S2 (Table 2).

Additionally, the total DMY of S1 treatments were lower than those of S2 (Table 2). The highest DMY was reached by cup plant in 2018 (27.8 ± 1.9 Mg/ha), the lowest by WPM S1 in 2018 (4.6 ± 1.9 Mg/ha). Even though the DMY of maize was significantly lower than cup plant in 2018, maize reached a significantly higher MYH of $7,646.7 \pm 544.5$ m³_N/ha (Table 2). This was due to a much better biogas substrate quality of maize compared to cup plant (Table 2). Biogas substrate quality here means that the biomass is easier digestible during anaerobic fermentation. In this study, it was shown that maize has higher contents of hemicellulose and lower contents of both lignin and ash (Table 2) compared with all other crops and cropping systems investigated here (Table 2). A comparison of the 5 year performance of all cropping systems investigated in this study revealed that mono-cropped maize gained about 30% higher DMY and about 65% higher MYH

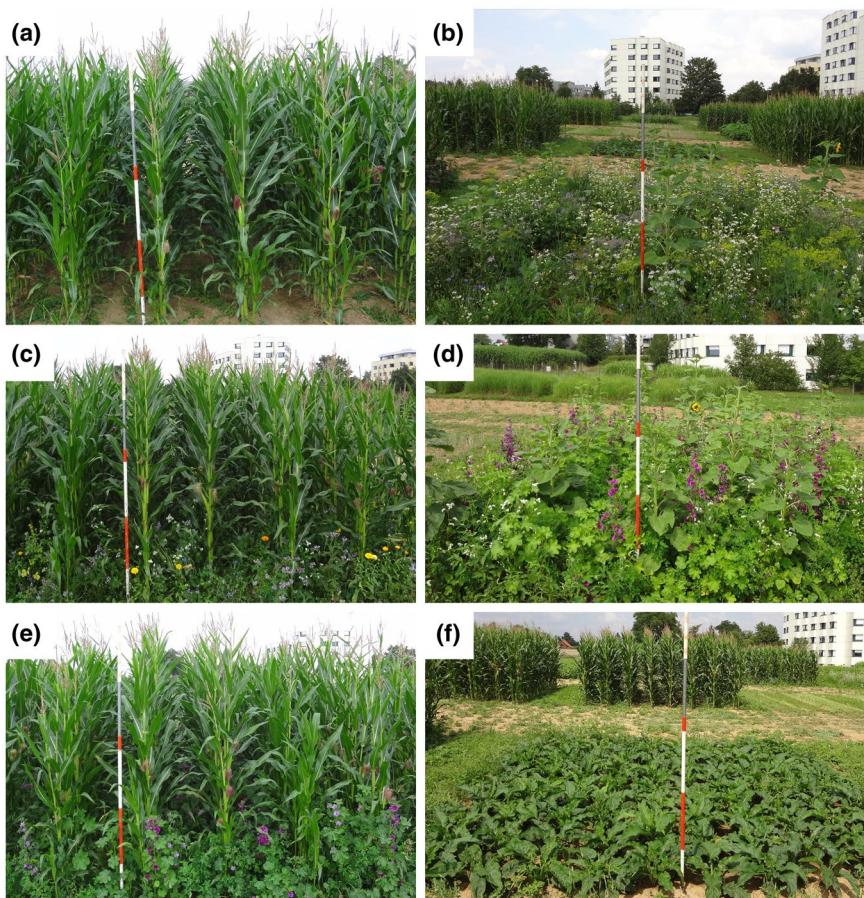


FIGURE 2 Plant stands of mono-cropped maize (a), wild plant mixture S1 sole established (b) and under maize (c), wild plant mixture S2 sole established (d) and under maize (e), and cup plant (f). Pictures taken on July 28, 2014

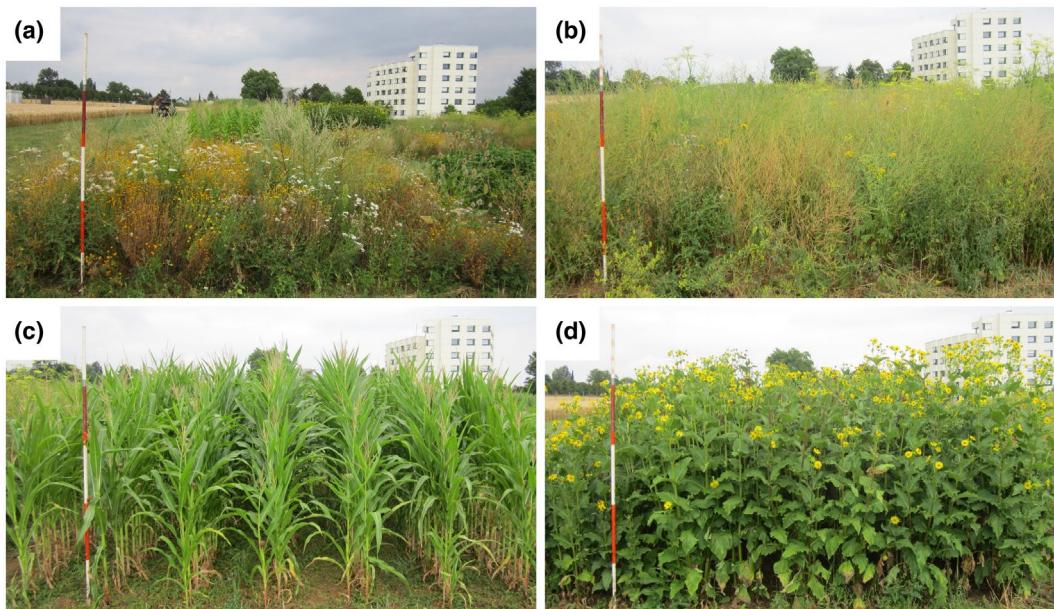


FIGURE 3 Plant stands of sole established wild plant mixtures S1 (a) and S2 (b) mono-cropped maize (c) and cup plant (d) on July 23, 2015. The other wild plant mixture establishment procedures (under maize and after barley) are not shown, because they did not differ visually from the sole establishment

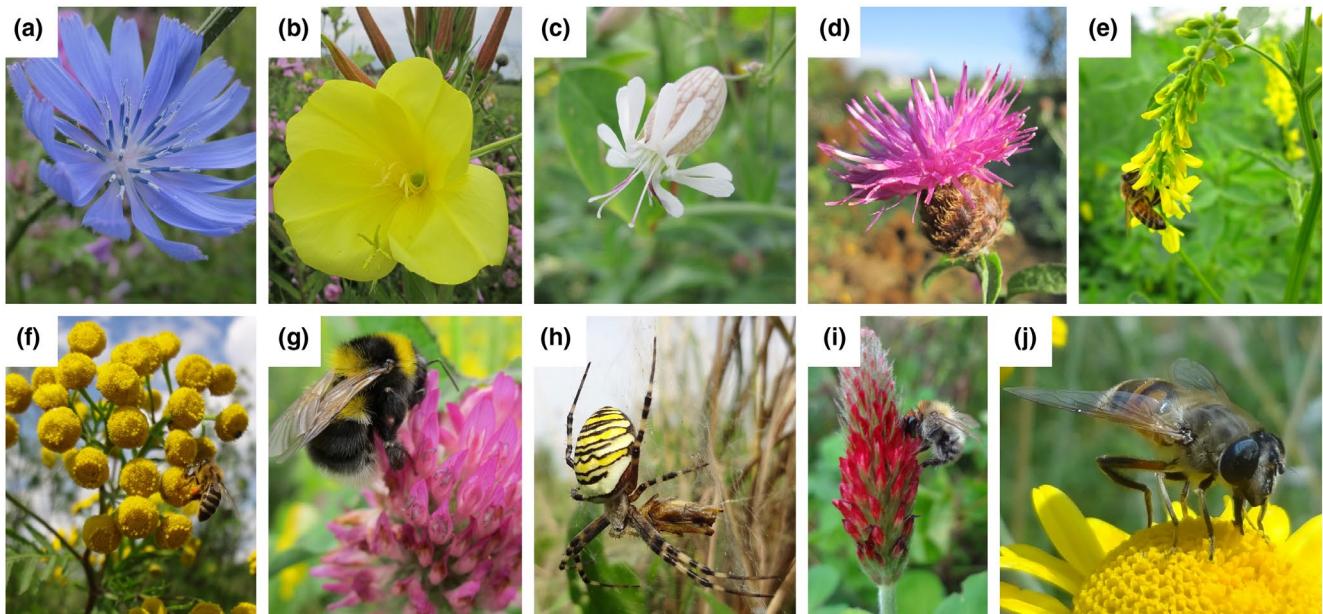


FIGURE 4 Some impressions of inflorescences and benefitting insects within the WPM stands: *Cichorium intybus* L. (a), *Oenothera biennis* L. (b), *Silene vulgaris* (MOENCH) GARNKE (c), *Centaurea jacea* L. (d), *Melilotus officinalis* (L.) PALL. with *Apis mellifera* (LINNAEUS, 1758) (e), *Tanacetum vulgare* L. with *Apis mellifera* (LINNAEUS, 1758) (f), *Trifolium pretense* L. with *Bombus terrestris* (LINNAEUS, 1758) (g), *Argiope bruennichi* (SCOPOLI, 1772) with *Gomphocerinae* (FIEBER, 1853) (h), *Trifolium incarnatum* L. with *Bombus pascuorum* (SCOPOLI, 1763) (i), *Anthemis tinctoria* L. with *Eristalis tenax* (LINNAEUS, 1758) (j). The pictures were taken in the field trial presented in this study during the years 2015–2017

compared to the best performing WPM treatment (S2E2; Figure 5). For each establishment procedure, S2 outperformed S1 in both DMY and MYH. Cup plant outperformed all WPM treatments except for S2E2 which was almost equal

with about 60% of maize accumulated 5 year MYH (Figure 5). Note that there was no MYH for cup plant during its establishment year 2014 (Figure 2f).

TABLE 2 Results of the field and laboratory analysis. Estimates are shown. Similar letters denote for nonsignificant differences between treatments within years

Year	Crop/ mixture	WPM establish- ment procedure	WPM	DMY		DMC		Ash		Lignin		Cellulose		Hemi-cell- ulose		SMY		MYH		Yield-re- levant WPM species		Weed species		Number of species		
				Mg/ha	% FM	Mg/ha	% DM	Mg/ha	% DM	Mg/ha	% DM	Mg/ha	% DM	Mg/ha	% DM	m ³ /kg VS	m ³ /ha	Number of species	Number of species							
2014	Maize	—	—	18.6a	36.4ab	4.7a	4.7a	4.7a	4.7a	4.7a	4.7a	4.7a	4.7a	4.7a	328.8a	5.837.1a	1.0d	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
	Cup plant	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	326.5a	5.608.4a	4.8c	2.2a								
	WPM S1	E1	6.5b	25.0c	12.1a	12.1a	12.1a	12.1a	12.1a	12.1a	12.1a	12.1a	12.1a	12.1a	273.6b	1.418.5c	19.0a	N.A.								
	WPM S1	E2	18.6a	34.6ab	5.1a	5.1a	5.1a	5.1a	5.1a	5.1a	5.1a	5.1a	5.1a	5.1a	322.0a	2.027.5bc	1.0d	1.0a								
	WPM S1	E3	6.6b	37.9a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	291.2b	2.582.5b	7.8b	N.A.								
	WPM S2	E1	8.8b	30.5b	9.3a	9.3a	9.3a	9.3a	9.3a	9.3a	9.3a	9.3a	9.3a	9.3a	327.4a	5.921.1a	4.4c	1.4a								
	WPM S2	E2	18.8a	34.4ab	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	5.3a	327.4a	2.346.9bc	1.0d	1.4a								
	WPM S2	E3	7.0b	38.8a	5.2a	5.2a	5.2a	5.2a	5.2a	5.2a	5.2a	5.2a	5.2a	5.2a	327.4a	4.698.0a	1.0d	N.A.								
	Maize	—	14.8ab	41.6bc	3.9b	3.9b	3.9b	3.9b	3.9b	3.9b	3.9b	3.9b	3.9b	3.9b	330.2a	3.854.6ab	1.0d	N.A.								
	Cup plant	—	17.3a	33.5d	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a	269.4b	1884.9e	17.2b	2.0a								
	WPM S1	E1	8.1ef	50.0a	7.8b	7.8b	7.8b	7.8b	7.8b	7.8b	7.8b	7.8b	7.8b	7.8b	262.0b	2080.3de	19.4ab	2.4a								
2015	WPM S1	E2	7.8f	48.1a	7.3b	7.3b	7.3b	7.3b	7.3b	7.3b	7.3b	7.3b	7.3b	7.3b	243.8c	1812.4e	20.4a	1.4a								
	WPM S1	E3	8.2ef	40.1c	9.6b	9.6b	9.6b	9.6b	9.6b	9.6b	9.6b	9.6b	9.6b	9.6b	269.6b	3.253.5bc	8.8c	1.2a								
	WPM S2	E1	13.0bc	43.0bc	6.8b	6.8b	6.8b	6.8b	6.8b	6.8b	6.8b	6.8b	6.8b	6.8b	269.5b	2.502.7ce	10.2c	2.2a								
	WPM S2	E2	10.3de	47.1ab	7.1b	7.1b	7.1b	7.1b	7.1b	7.1b	7.1b	7.1b	7.1b	7.1b	267.5b	2.788.5cd	11.2c	1.8a								
	WPM S2	E3	11.2cd	41.7c	7.2b	7.2b	7.2b	7.2b	7.2b	7.2b	7.2b	7.2b	7.2b	7.2b	333.2a	6.368.2a	1.0d	N.A.								
	Maize	—	19.8a	35.6bc	3.6a	3.6a	3.6a	3.6a	3.6a	3.6a	3.6a	3.6a	3.6a	3.6a	328.8a	4.230.9b	1.0d	N.A.								
	Cup plant	—	18.1a	33.8c	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	264.1b	1994.5cd	12.0b	3.0a								
	WPM S1	E1	10.3bc	35.6ac	8.6a	8.6a	8.6a	8.6a	8.6a	8.6a	8.6a	8.6a	8.6a	8.6a	212.6e	2.405.2cd	22.0a	1.0a								
	WPM S1	E2	11.5bc	43.4ab	9.6a	9.6a	9.6a	9.6a	9.6a	9.6a	9.6a	9.6a	9.6a	9.6a	230.5cde	1636.3d	10.0bc	2.0a								
	WPM S1	E3	8.0c	42.4ac	7.6a	7.6a	7.6a	7.6a	7.6a	7.6a	7.6a	7.6a	7.6a	7.6a	220.4de	3.122.9c	5.5c	1.0a								
	WPM S2	E1	13.4b	33.5c	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	250.1bc	2.675.3cd	6.0c	1.0a								
	WPM S2	E2	11.7bc	41.7ab	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	245.5bd	2.323.1cd	8.0bc	1.5a								
	WPM S2	E3	10.3bc	43.7a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	240.0cd											

(Continues)

TABLE 2 (Continued)

Year	Crop/ mixture	WPM establish- ment procedure	DMY	DMC		Ash	Lignin	Cellulose	Hemi-cel- lulose	SMY	MYH	Yield-rele- vant WPM species	Number of weed species
				Mg/ha	% FM							% DM	m ³ N/ha
2017	Maize	—	2.3a	30.4bc	4.4a	4.4a	4.4a	4.4a	329.6a	7,366.0a	1.0d	N.A.	
	Cup plant	—	21.7ab	29.1c	10.7a	10.7a	10.7a	10.7a	254.0b	4,922.0b	1.0d	N.A.	
	WPM S1	E1	7.8f	38.0a	8.1a	8.1a	8.1a	8.1a	237.8bc	1701.1e	11.5b	2.0a	
	WPM S1	E2	10.1ef	33.6ac	8.5a	8.5a	8.5a	8.5a	234.7bc	2,175.3de	12.0ab	1.5a	
	WPM S1	E3	11.4df	37.0ab	6.9a	6.9a	6.9a	6.9a	222.2c	2,360.0de	16.0a	1.0a	
	WPM S2	E1	13.0de	35.6ac	6.9a	6.9a	6.9a	6.9a	244.4b	2,959.1d	3.5c	1.0a	
	WPM S2	E2	18.4bc	33.6ac	6.5a	6.5a	6.5a	6.5a	245.4b	4,210.1bc	4.5c	1.5a	
	WPM S2	E3	14.6cd	34.9ac	6.6a	6.6a	6.6a	6.6a	241.2bc	3,282.0cd	5.0c	1.5a	
2018	Maize	—	24.0b	33.8c	3.4a	3.4a	3.4a	3.4a	330.4a	7,646.7a	1.0b	N.A.	
	Cup plant	—	27.8a	29.6c	9.0a	9.0a	9.0a	9.0a	253.3b	6,414.5b	1.0b	N.A.	
	WPM S1	E1	6.0de	64.4a	7.3a	7.3a	7.3a	7.3a	211.1d	1,156.7de	3.5a	1.5ab	
	WPM S1	E2	4.7e	52.0b	8.4a	8.4a	8.4a	8.4a	232.7c	1,012.5de	4.0a	3.0a	
	WPM S1	E3	4.6e	61.8a	5.9a	5.9a	5.9a	5.9a	112.1e	506.5e	6.0a	0.5b	
	WPM S2	E1	8.2de	51.2b	6.9a	6.9a	6.9a	6.9a	237.7bc	1809.2d	5.5a	1.0b	
	WPM S2	E2	17.4c	63.8a	5.5a	5.5a	5.5a	5.5a	225.3cd	3,729.3c	3.5a	0.5b	
	WPM S2	E3	9.0d	48.4b	5.8a	5.8a	5.8a	5.8a	223.2cd	1874.6d	3.0a	0.5b	

Note: Species with a DMY share of greater or equal than 1% were considered as “yield-relevant”. “Weed species” denote for spontaneous species which were not sown

Abbreviations: DM, dry matter; DMY, dry matter yield; DMC, dry matter content; FM, dry matter content; MY, methane yield per hectare; N, norm conditions; N.A., not available, level of significance = $p < 0.5$; SMY, specific methane yield; VS, volatile solid; WPM, wild plant mixture.

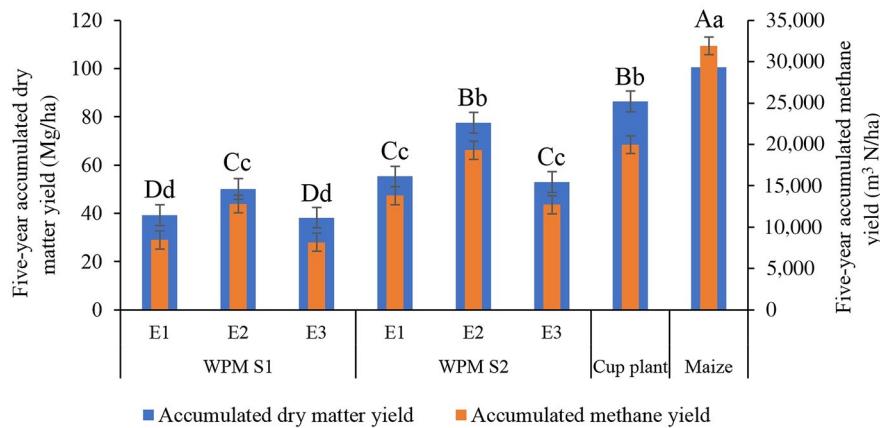


FIGURE 5 Absolute 5 year accumulated dry matter yield (DMY) and methane yield (MYH) (years 2014–2018) of the seven perennial cropping systems (WPM: wild plant mixture; S1: Rieger-Hofmann; S2: Saaten-Zeller; E1: sole establishment; E2: under maize establishment; E3: after barley establishment and cup plant: *Silphium perfoliatum* L.); and maize: *Zea mays* L. Identical lower and uppercase letters show nonsignificant DMY and MYH differences between systems, respectively

4 | DISCUSSION

The research object of this study constitutes an example of the trade-off between economic and ecosystemic performances of more diverse biogas cropping systems (von Cossel, 2019). While mono-cropped maize provides best MYH performance (Table 2 and Figure 5), WPM relevantly increase the ecosystemic functions of biomass production (Table 2; Emmerling et al., 2017) and also improve its aesthetical appearance in the landscape (Figures 2–4; Huth et al., 2019). Here, three alternative WPM establishment strategies were investigated aiming at improving the MYH performance of the WPM and reducing the risks of establishment failures for the farmers. This could enable a faster practical implementation of WPM into biogas cropping systems across Europe and thereby contribute to a more wildlife friendly agriculture (Gevers, Høye, Topping, Glemnitz, & Schroeder, 2011; Tscharntke et al., 2012).

In the following sections, the effects of the establishment procedures on (a) the MYH performance and (b) the biodiversity effects of the WPM will be discussed. Thereafter, a general outlook for WPM cultivation and the conclusions on the findings of this study will be provided.

4.1 | Methane yield performance of WPM compared to maize and cup plant

Overall, the highest accumulated MYH was observed for mono-cropped maize (Figure 5). This was expected due to the high biomass productivity of maize and its high suitability for methane production through anaerobic digestion (Herrmann & Rath, 2012; Rath, Heuwinkel, & Herrmann, 2013). Whereas, WPM and cup plant not only show a lower 5 year accumulated DMY than maize (Table 2), they are

also less suitable for anaerobic digestion. This was indicated by lower SMYs of WPM and cup plant compared to maize (Table 2). Low SMY was caused by higher contents of ash and lignin in WPM and cup plant (von Cossel et al., 2018; Table 2) which is in line with findings from available studies (Carlsson, Mårtensson, Prade, Svensson, & Jensen, 2017; Schmidt, Lemaigre, Delfosse, von Francken-Welz, & Emmerling, 2018). Both lignin and ash are not digestible during anaerobic fermentation (Oleszek, Król, Tys, Matyka, & Kulik, 2014; Triolo, Pedersen, Qu, & Sommer, 2012; von Cossel et al., 2017, 2018). Therefore, the contents of lignin and ash within the biogas substrate should be as low as possible. In this study, maize and summer barley (E3) showed the lowest contents of lignin and ash (Table 2). The optimal biochemical composition of maize results from its long breeding history (Barrière et al., 2006). For WPM, the composition was not bred at all, since the most yield-relevant perennial WPM species such as common tansy (*Tanacetum vulgare* L.) and knapweed (*Centaurea* spp. L.) are wild species (Vollrath et al., 2012). This means that they have not exclusively been bred for biogas production or forage use over decades such as maize. We propose that breeding (for cup plant) and both a better selection and combination of wild plant species (for WPM) could help improving the SMYs and thus, closing the genetic and agronomic MYH gaps of cup plant and WPM, respectively.

Among different WPM establishments, E2 (under maize) enabled the highest accumulated biomass yield of the WPM cultivation compared to the other establishment procedures E1 (solely) and E3 (after WCCS; Figure 5 and Table 2). This was mainly a result of (a) the replacement of sunflower in its function as major annual crop in both WPM with a high-bred silage maize variety and (b) a good suitability of maize as nurse crop for WPM establishment. Maize generally allows

for a constant yield and processability compared to sunflower due to several reasons:

1. The distribution of sunflower plants on the field is not comparably uniform to that of maize (Figure 2a–e). Maize can be sown precisely, and both best practice planting geometry and sowing density of maize are much better known than for sunflower. Therefore, there may be agronomic yield gaps for sunflower based on suboptimal planting geometries. A suboptimal planting geometry can cause a decreased mechanical stability of the plants (Gardiner, Berry, & Moulia, 2016), which are then more affected to wind damage.
2. One important consequence of the above-mentioned suboptimal planting geometry of sunflowers would be an increased risk of earth contaminations within the harvested biomass. This is because the plants often happen to be entangled which causes that they are pulled out of the soil including the roots instead of being cut and harvested without the roots (Frick & Pfender, 2019).
3. A higher oil content compared to silage maize renders sunflower biomass a crucial challenge for biogas processing. Therefore, it is more common to use sunflower oil cake for biogas processing rather than silages from whole sunflowers (Raposo et al., 2009).

This study focuses on reducing the establishment-related agronomic risks of WPM cultivation such as variable yields and weed infestation (von Cossel & Lewandowski, 2016). A lower risk of establishment failures is meant to help overcoming the risk adversity of those farmers who are latently motivated to give WPM cultivation a try (Frick & Pfender, 2019). In many cases, these farmers hesitate to grow WPM because of the potentially lower and variable MYH compared to maize during the first year (von Cossel & Lewandowski, 2016). Here, E2 was found to be one option for making it less risky for farmers to test WPM cultivation. Undersowing WPM with other annual industrial crops than maize such as hemp (*Cannabis sativa* L.) and false flax (*Camelina sativa* L.) should be investigated in the future.

The third WPM establishment procedure investigated in this study, sowing after barley (E3), resulted in significantly lower total accumulated MYH in the long term compared to E1 and E2. This could be explained by two reasons:

1. *Suboptimal growth conditions.* The summer-annual barley variety was sown in April 2014 and harvested end of June 2014 (Table 1) which means that the vegetation period accounted for less than 90 days. Additionally, there was evidence for heat and radiation stress for plants in June (Table 1). In combination with heterogeneously distributed precipitation events, the climatic growth conditions were suboptimal for a summer-annual crop that is harvested at the end of June.

2. *Weak weed suppressiveness.* The summer-annual barley developed slowly due to the above mentioned suboptimal climatic growth conditions and low top-soil wetness during the weeks after sowing. Similar suboptimal growth conditions were observed for the WPM sown after WCCS harvest (E3). This paved the way for several weed species such as thistle, amper, and honey grass which became dominant over time in E3 of both S1 and S2. Following Baraibar, Hunter, Schipanski, Hamilton, and Mortensen (2018), we assume that a winter-annual cereal would have been more weed suppressive than summer barley.

We assume that winter-annual cereal species such as winter rye and winter-triticale would be likely preferable for the purpose of establishing WPM after WCCS harvest. This is because winter-annuals already have an established root system in April and could use the whole vegetation period. Baraibar et al. (2018) reported that winter-annual cereal would have been more weed suppressive than summer barley. Thus, winter-annual could be more efficient compared to summer-annual cereals. Here, “more efficient” means both a higher MYH and a better suppression of weed species.

For WPM, fertilization was applied from the second year onward. The fertilization rate was set at 90 kg based on experiences from another field trial with WPM (von Cossel & Lewandowski, 2016). In other studies, higher fertilization rates of about 130 kg N/ha were used; however, no higher yields have been reported (Wurth et al., 2016; Zürcher et al., 2014). The DMYs of both WPMs (S1, S2) in this study were similar to those reported by Wurth et al. (2016) and Zürcher et al. (2014), who investigated similar wild plant seed mixtures than those presented in our study. In 2018, the DMY of WPM (S2) was even similar to those of Wurth et al. (2016) and Zürcher et al. (2014) despite the fact that the WPMs were only 25 kg N/ha fertilized in our study (due to technical reasons). Therefore, this study also revealed that WPM maybe economically viable under low-input fertilization regimes, which is in line with other studies (Carlsson et al., 2017). We suggest that the proportions of the WPM of legumes such as melilot (*Melilotus officinalis* L.) and lucerne (*Medicago sativa* L.; Table S3) are the main drivers for a successful low-input WPM cultivation regarding the aim of low nitrogen fertilization. Additionally, the deep rooting systems of many of the biennial and perennial species such as common tansy and knapweed are also of great importance. Thus, WPMs are potentially suitable for agricultural low-input practices. This renders WPM cultivation even more socio-ecologically benign under aspects of GHG mitigation (substitution of synthetic or digestate N), groundwater protection (less N leaching), and soil protection (less prone to erosion than annual crops) as was also concluded by von Cossel and Lewandowski (2016).

4.2 | Biodiversity effects of the WPM

The high species diversity of both WPMs (Figures 2–4, Tables 2; Table S3) was in accordance with both findings from other studies (Vollrath et al., 2012; von Cossel & Lewandowski, 2016; Von Redwitz et al., 2019; Wurth et al., 2016; Zürcher et al., 2014) and observations by farmers who have cultivated WPM for 8 years (Frick & Pfender, 2019). The high diversity of WPM is known to have positive effects on both above- and belowground fauna (Carlsson et al., 2017; Emmerling, 2014; Vollrath et al., 2016). Within the aboveground fauna, the potential benefits of WPM for pollinators are highly relevant, because of the ongoing great losses of pollinator abundances during the past 27 years (Hallmann et al., 2017). The extend of these potential benefits depends on the growth (and thus, the establishment success) of the wild plant species: In low-height plant stands, there will only be a weak effect on pollinator abundances than in tall plant stands due to the higher number of flowers (Frick & Pfender, 2019). In this study, there were no significant differences in plant species diversity and biomass yield between E1, E2, and E3 (Table 2) across years starting from the second year onward. Therefore, a somewhat similar amount of food and shelter was provided for the pollinators across all establishment procedures. This indicates that the establishment procedures investigated in this study will probably not influence the effects of WPM on the pollinator abundances from the second year onward. In the first year, however, the proportion of total biomass of the wild plant species was rather low in E2 (on average about 3% of total DMY). This may result in a much lower support for pollinators of E2 compared to E1 and E3. Nevertheless, the spaces between maize rows in E2 were almost covered completely by WPM species during the vegetation period of year 2014. Most of these WPM species also developed inflorescences (Figure 2c,e) and thus improved soil cover compared to mono-cropped maize (von Redwitz et al., 2019).

4.3 | General evaluation and outlook

Overall, we conclude that E2 allows for a less risky establishment of the WPM from an agronomic perspective. This conclusion is based on the following facts:

1. The MYH of maize was not significantly affected by the establishing WPM underneath—this enables both a safe income for the farmers and a sufficient supply of biomass for the local biogas plants during the initial year of WPM cultivation.
2. The WPM established well under maize and showed similar yield levels to sole establishment of WPM (E1) from the second year onward.

3. Both WPM and maize share the same seedbed preparation in one year, which (in combination with the high MYH of maize) also reduces the MYH-related establishment costs of WPM cultivation in E2 compared to E1 and E3.

von Cossel and Lewandowski (2016) and Wurth et al. (2016) found a trade-off between the number of WPM species and the biomass productivity of the WPM plant stands. The results of our study did not disprove this trade-off: While the number of yield-relevant species was significantly higher in S1 than in S2, the opposite was observed for the MYH (Table 2, Figure 5). This may have caused a stronger interspecific competition between the wild plant species which resulted in significantly lower biomass production in S1 than in S2. Furthermore, a similar trade-off between species diversity and biomass yield performance was also found by Bonin et al. (2018) who investigated a diverse mixture of forbs and grasses and compared it with mono-cropped switchgrass (*Panicum virgatum* L.) and a mixture of three grasses. We suggest that an average number of six to eight yield-relevant species in S2 already renders a great improvement of the spatial agricultural diversity (Altieri & Letourneau, 1982; Letourneau et al., 2011; von Cossel, 2019) compared with mono-cropped maize or cup plant. Furthermore, S2 appears to be a more reliable WPM compared to S1 under economic aspects. The lower number of yield-relevant species makes S2 probably much easier to handle than S1 under aspects of harvest date determination and biogas substrate quality prediction (von Cossel et al., 2018). Therefore, we draw a similar conclusion as von Cossel and Lewandowski (2016) who recommended a moderately low number of yield-relevant wild plant species of up to five for a successful WPM cultivation under both ecosystemic and economic aspects.

The silage quality of WPM was found to be suitable due to the high DMC. Instead, the DMC of cup plant is critically low in some cases given that a DMC of about 28% (Eberl, Fahlbusch, Fritz, & Sauer, 2014) is required for a successful ensilage of the harvested biomass. Further advantages of WPM over cup plant are a higher species diversity and that species in WPM are no neophytes in mid-European ecosystems.

Altogether, the establishment of WPM under maize was found being a reasonable improvement of the WPM establishment procedure under both social-ecological and economic aspects. A faster implementation of WPM cultivation into practice could help making biogas crop cultivation more environmentally benign—especially in times of dramatically decreasing pollinator abundances (Hallmann et al., 2017).

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SUPPORTING INFORMATION

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