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Methane yield of biomass from extensive grassland is affected by compositional changes induced by order of arrival

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Abstract

Low-input grassland biomass from marginal and other slightly more fertile sites can be used for energy production without competing with food or fodder production. The effect of grassland diversity on methane yield has received some attention, but we do not know how community assembly may affect methane yield from grassland biomass. However, methane yields determine the potential economic value of a bioenergy substrate. Hence, a better understanding of how plant community assembly affects methane yield would be important. We measured biomass production and methane yield in the second year of a grassland field experiment which manipulated the order of arrival of different plant functional groups (forbs, grasses or legumes sown first and all sown simultaneously) and sown diversity (9 vs. 21 species). The order of arrival of the plant functional groups significantly determined the relative dominance of each group which in turn mainly explained the variance in aboveground biomass production. Differences in area-specific methane yields were driven by differences in biomass production and which plant functional groups dominated a plot. When grasses were sown first, legumes and grasses codominated a plot and the highest area-specific methane yield was obtained. Overall, the results indicate that altering the order of arrival affected the community functional and species composition (and hence methane yields) much more than sown diversity. Our study shows that a combined use of positive biodiversity effects and guided plant community assembly may be able to optimize methane yields under field conditions. This may allow a guided, sustainable, and lucrative use of grassland biomass for biogas production in the future.

Keywords: biodiversity, bioenergy landscape, biogas, biomethane potential, community assembly, plant functional groups, priority effect

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Introduction

Permanent species-rich grasslands can be valuable sources of biomass for biogas production. Indeed, biomass from grasslands has been increasingly used for energy provision in recent years (Prochnow *et al.*, 2009) despite maize remaining dominant as a dedicated energy crop. If certain species compositions by virtue of their chemical composition are favorable for anaerobic digestion, methane yields can be optimized (Prochnow *et al.*, 2009; Khalsa *et al.*, 2014). As biomass composition is more similar in species belonging to the same plant functional group (PFG), the presence and abundance of certain PFG affect the methane yield to a large extent (Herrmann *et al.*, 2014; Stinner, 2015).

van Meerbeek *et al.* (2014) found a large range of annual biomass yield of low-input high-diversity systems including grasslands. In detail, many long-term biodiversity-ecosystem functioning (BEF) experiments, especially in grasslands, showed that higher richness of plant species and functional groups leads to higher plant productivity, due to positive biodiversity effects (Hector & Bagchi, 2007; Marquard *et al.*, 2009; Picasso *et al.*, 2011). van Meerbeek *et al.* (2015) studied the energy potential of different low-input high-diversity systems including grasslands. They found that grasslands had the highest energy efficiencies during anaerobic digestion and observed a correlation between biogas yield and functional group composition. However, it is

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still barely known how an increase in productivity as a consequence of higher species richness affects area-specific methane yields. Khalsa *et al.* (2014) studied effects of species richness and functional group composition within the Jena Experiment (Roscher *et al.*, 2004) on biomethane production and area-specific methane potential. They found a positive effect of species richness and presence of legumes on area-specific methane potential. However, methane yields were only calculated from concentrations of chemical constituents of the substrates.

In most biodiversity experiments, the diversity treatments were sown at the same time, and then, plots were weeded to maintain the desired diversity gradient; therefore, natural assembly did not occur. There is evidence, however, that positive diversity effects also occur within a natural assembly context (Bullock *et al.*, 2007). Bullock *et al.* (2007) tested the effect of sowing high- and low-diversity restoration seed mixtures on ex-arable land and found that sown diversity (after one sowing event) had effects on both productivity and diversity which persisted over many years.

The order of arrival of species can also be decisive for community assembly in that the plant species which arrive first at a site can significantly affect further assembly, with knock-on effects on diversity and biomass productivity (Diamond, 1975; Martin & Wilsey, 2012). Such so-called priority effects can drive species and functional diversity from the very early stages of a plant community (Chase, 2003; Körner *et al.*, 2008; Vaughn *et al.*, 2010; Plückers *et al.*, 2013).

In recent experiments under controlled conditions, the order of arrival of PFG was directly manipulated. These studies found that the ensuing species richness and productivity of the communities was largely contingent upon such priority effects created by order of arrival (Körner *et al.*, 2008; von Gillhaussen *et al.*, 2014). Additionally, it was found that legumes sown before grasses and forbs resulted in more productive communities aboveground (von Gillhaussen *et al.*, 2014) and less productivity belowground (Körner *et al.*, 2008). These results open up the possibility of using priority effects to steer a community's development toward desired trajectories of ecosystem function and possibly diversity. Based on the fact that the plant functional identity plays a key role for methane yields within a biogas setting (Herrmann *et al.*, 2014; Stinner, 2015), knowing more about how to direct plant functional composition toward optimal methane yield would be a valuable gain for the sustainable economic use of restored species-rich grasslands.

Knowledge from ecological experiments may allow for more sustainable management of species-rich grasslands that are highly endangered in Europe either through agricultural intensification or land abandonment. If we find that a combined use of positive biodiversity and order of arrival effects (Temperton *et al.*, 2016) shows positive results under field conditions, this may allow a sustainable and lucrative use of grassland biomass for biogas production. This would provide a win–win situation if aboveground productivity can be increased by both biodiversity and order of arrival effects whilst not diminishing biomass quality. This would also enhance the provision of a number of different ecosystem services especially in agro-ecosystems (Altieri, 1999; Bullock *et al.*, 2001; Rey Benayas & Bullock, 2012). However, in most of these ecological experiments, the effects of either richness or order of arrival of species on aboveground productivity and other ecosystems functions were investigated individually. The combined effects of sown species richness and order of arrival (priority effects) on ecosystem functions especially in a natural assembly were barely addressed whether under controlled or field conditions, but see Weidlich *et al.* (2016). Even less is known about how grassland methane yield may respond to sown diversity and the creation of priority effects.

We studied a mesotrophic grassland field experiment composed by central European native species adapted to relatively nutrient-rich soils. We analyzed methane yield in 2013 in a field experiment (the Priority Effect experiment; see Weidlich *et al.*, 2016 for details), which was set up in 2012 to investigate the effects of two main factors: the order of arrival of PFG (either forbs, grasses, or legumes sown first followed by the other two groups) and sown diversity (9 vs. 21 species) on grassland community assembly and ecosystem functions. Biomass from the September harvest in 2013 (prior to the second mowing of the growing season) was anaerobically digested, and its biomethane potential as well as area-specific methane yield was compared. The aim of this study was to test the following hypotheses:

- Methane potential of biomass will differ depending on variations in species and functional group abundances induced by order of arrival of different PFG and sown diversity.
- The dominance of legumes will positively affect both the aboveground biomass and the methane yield and therefore best results for area-specific methane yields will greatly depend on the species and functional composition.

Materials and methods

Experimental setup and design

We measured methane yields in the Priority Effect experiment, a grassland experiment in which aboveground biomass and

community composition were monitored from 2012 to 2015, see Weidlich *et al.* (2016). With this experiment, the effects of sown diversity and order of arrival of PFG on aboveground biomass and methane yield were tested on two different soil types. The factor order of arrival of PFG had four levels, with forbs sown first (F-first), grasses sown first (G-first), legumes sown first (L-first) and all PFG sown at the same time (control). The factor sown diversity had two levels: low diversity (LD: nine species) and high (HD: 21 species). Soil type A was classified as Stagnic Cambisol and soil type B as an Anthrosol according to the official German soil mapping guidelines (Sponagel, 2005).

Species selection and treatments

The seed mixtures consisted of typical central European grassland species so that the target plant community was a semi-natural, species-rich, mesotrophic grassland. Species were classified into three PFG (nonlegume forbs, grasses, and legumes) which differ significantly in functional and morphological traits (based on Roscher *et al.*, 2004). In total, a fixed set of 21 common grassland species (seven forbs, seven grasses, and seven legumes) was selected for high-diversity communities. A fixed subset of nine species (three forbs, three grasses, and three legumes) was selected to represent low diversity communities, see Weidlich *et al.* (2016).

The PFG order of arrival was created by sowing the species of one PFG first on 19.04.2012 (or all at the same time as control) and the other species of the remaining PFG at the same time on 31.05.2012. Before the second sowing, all plots were mown at a cutting height of 30 mm, to reduce initial aboveground competition and to allow subsequently sown species to establish well. None of the plots were weeded after sowing to allow assembly close to natural dynamics. All plots were mown twice per growing season (in early June and early September after biomass-sampling) as typical for such grasslands and plant material was removed from the plots.

Sampling and data collection

The core area (3.5 × 3.5 m) within every plot (4 × 4 m) was used for species specific cover assessments and biomass harvesting. Plant cover per species (community composition) and species richness (total number of species present) were estimated prior to each harvest. Cover assessments were performed using a modified decimal cover estimation method following Braun Blanquet (Londo, 1976). Total community cover was higher than 100% as the canopy was multilayered and three-dimensional. Total aboveground biomass was measured using two randomly distributed 0.1 m^2 rectangles (20 × 50 cm) within the core areas of each plot, avoiding areas where previous sampling was carried out. All aboveground plant material within the rectangle was cut approx. 5 cm above soil surface, and samples were dried at 70 °C for two days before weighing.

Biomethane potential

We measured biomethane potentials of grassland aboveground biomass harvested in September 2013 from eight low diversity plots from both soil types with forbs, grasses, or legumes sown first and all PFG sown simultaneously (first replicate of each treatment). In addition, to get a slight handle on how the high- and low-diversity treatments may have affected the methane potentials, we sampled aboveground biomass from two high-diversity plots (one of each soil type, first replicate of each treatment) on which all PFG were sown simultaneously (control plot). Batch experiments were performed using triplicate 400-g assays in an Automated Methane Potential Test System (AMPTS II, Bioprocess Control, Sweden) in accordance with VDI 4630 (VDI 4630, 2006). Mixtures of digestates from a farm-scale and a laboratory-scale biogas reactor served as inocula with total solids (TS) contents of 3.8–4.4% and volatile solids (VS) contents of 65–69%$_{TS}$. The TS and VS contents were determined as described by Sträuber *et al.* (2012). Inocula were stored at 37 °C for at least five days to allow for degassing prior to the biomethane potential tests.

Dried plant biomass was digested under anaerobic conditions with an inoculum to substrate ratio of 2 : 1 (VS basis) according to VDI 4630 (VDI 4630, 2006). Accordingly, 6.2–8.1 g of substrate and 391–394 g of inoculum were added to 500-mL bottles. Duplicate negative controls were implemented by digesting only the inoculum, and these reactors were filled up with distilled water to 400 g in total. Furthermore, microcrystalline cellulose (2.5 g) was digested as reference substrate together with the inoculum in a single bottle. Inocula showed sufficient activity as at least 90% of the theoretical methane yield of cellulose (VDI 4630, 2006) was achieved. The AMPTS system was set up and operated as described previously (Popp *et al.*, 2015). After 30 days, the daily methane production was lower than 1% of the total methane production and experiments were stopped on day 35. The area-specific methane yield for the second cut (September 2013) was calculated as product of aboveground biomass (g_{TS} m^{-2}) and biomethane potential (L_N CH_4 g_{VS}^{-1}) taking into account the VS content.

Statistical analyses

Response variables were biomethane potential as well as area-specific methane yield for the plots sampled for methane yield and the relative dominances (RD) of the PFG and community aboveground biomass of all plots. Beside order of arrival and sown diversity, soil type was included in the analyses as factor to test for interactions with the other two factors. Average values for both soil types were given when differences of a response variable for both soil types and interactions with other factors were not statistically significant. Statistical analyses were performed with R version 3.2.0 using the packages 'LSR', 'STATS', and 'CAR' (R Development Core Team, 2014). Normal distributions and homogeneity of variance of variables were tested by Shapiro–Wilk and Levene's test, respectively. There was no collinearity between RD of the PFT according to the variance inflation factors which were calculated using the R package 'USDM'.

RD of PFG were analyzed by three-way multivariate ANOVA (analysis of variance) for effects of order of arrival of PFG, sown diversity, and soil type. As soil type had no significant influence on RD of PFG, results are given as average values for

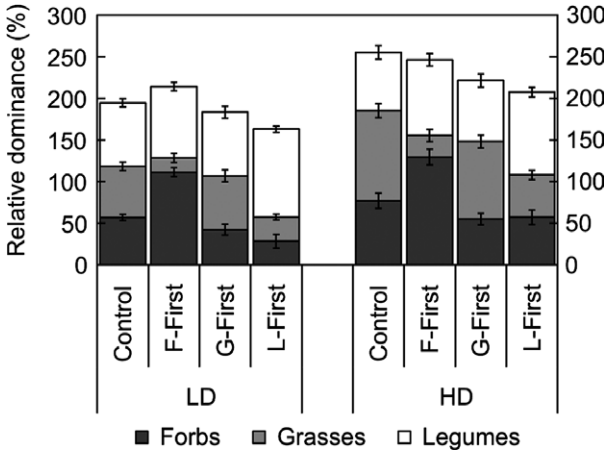

Fig. 1 Mean relative dominances (n = 8, ±SE) of plant functional groups (PFG) forbs, grasses, and legumes found growing in September 2013 depending on their order of arrival (control: all PFG sown simultaneously, F-first: forbs sown first, G-first: grasses sown first, L-first: legumes sown first) and on sown diversity (LD: low diversity, HD: high diversity). Note that the sum of RD is higher than 100% as plant species overlapped within canopies. One can see that in both HD and LD treatments the PFG that was sown first in 2012 still dominated the vegetation in September 2013 (except for LD G-first where legumes codominated with grasses).

both soil types (Fig. 1, Table 2). Furthermore, effects of the factors order of arrival and sown diversity on the RD of the individual PFG were tested by two-way univariate ANOVA. Observed species richness was analyzed by ANOVA for effects of order of arrival and sown diversity. Community aboveground biomass was analyzed by three-way ANOVA for the effects of the RD of the PFG (Table 1). Biomethane potentials and area-specific methane yields were analyzed by three-way ANOVA for effects of the order of arrival of PFG and sown diversity. In addition to these two effects, effects of soil type on area-specific methane yields were analyzed. Furthermore, effects of RD of PFG on biomethane potentials and area-specific methane yields were studied by three-way ANOVA (Table 1). Effect sizes were given as (partial-) η^2 representing the power of the analysis based on the replication. High values denote high percentages of variance of the response variable explained by the source variable. Normality and homogeneity of residuals of the models were tested by qq-plots and by plotting the residuals against the fitted values.

Results

In this study, when we sampled in September 2013 low diversity plots for methane potential determination, legumes dominated all treatments except when forbs were sown first (Fig. 1 and Table 2). On high-diversity plots, the PFG was sown first dominated and when all PFG were sown simultaneously, grasses were most abundant (Fig. 1).

In detail, the order of arrival and sown diversity affected the RD of the three different PFG in the community composition in September 2013 (MANOVA with Pillai's trace: order of arrival $V = 1.12$, $F_{9,168} = 11.2$, $P < 0.001$ and sown diversity $V = 0.36$, $F_{3,54} = 10.1$, $P < 0.001$), see Fig. 1. However, only the factor order of arrival significantly affected the RD of each PFG as revealed by separate univariate ANOVA (RD forbs: $F_{4,59} = 16.8$, $P < 0.001$; RD grasses: $F_{4,59} = 29.9$, $P < 0.001$; RD legumes: $F_{4,59} = 7.0$, $P < 0.001$).

The sown diversity had a small but significant influence on the RD of forbs ($P = 0.015$, $\eta^2 = 5\%$). Grasses dominated on high-diversity plots when grasses were sown first or all PFG were sown simultaneously (order of arrival: $P < 0.001$, $\eta^2 = 53\%$). On low diversity plots, RD of grasses was lower than on high-diversity plots (sown diversity: $P < 0.001$, $\eta^2 = 14\%$, Fig. 1). RD of legumes was high when legumes were sown first as well as on all low-diversity plots except when forbs were sown first (order of arrival: $P < 0.001$, $\eta^2 = 32\%$) with sown diversity having no effect ($P = 0.556$, Fig. 1). Furthermore, as expected species richness was strongly determined by sown diversity ($P < 0.001$, $\eta^2 = 52\%$). On average, 13 (±0.4 standard error) and 8 (±0.4) species were found on HD and LD plots, respectively (compared to 21 and 9 sown species). In contrast, order of arrival had only a small influence on species richness ($P = 0.011$, $\eta^2 = 8\%$).

The community aboveground biomass had a mean of 574 g_{TS} m^{-2} in September 2013 with a large range from 229 g_{TS} m^{-2} to 951 g_{TS} m^{-2} (Fig. 2a). Variances in the aboveground biomass can partly be explained by the RD of the PFG (Table 1). However, only the RD of legumes explained the observed aboveground biomass significantly.

Biomethane potentials

In this study, the effects of order of arrival (eight low sown diversity plots) and of sown diversity (two control plots with all PFG sown at the same time) on biomethane potentials were explored. Biomethane potentials, given as feedstock-specific methane yield, had a range from 231 to 278 mL_N g_{VS}^{-1} across all subsampled plots (Fig. 2b and Table 2). No significant differences between biomethane potentials due to order of arrival of PFG (as measured in the low sown diversity plots, $P = 0.236$) or due to sown diversity (control plots, $P = 0.810$) were found. However, the RD of PFG from each plot determined the biomethane potentials significantly (Table 1). In detail, the RD of forbs, legumes, and the interaction of the three PFG had significant effects on the biomethane potential ($P < 0.05$).

Table 1 ANOVA table of aboveground biomass, biomethane potentials, and area-specific methane yield as explained by the relative dominance (RD) of forbs, grasses, and legumes (as well as their interactions) growing in the sampled plots in September 2013. Significant factors ($P < 0.05$) are highlighted in bold. Effect size partial-η^2 shows the power of the analysis based on the replication. High values denote high percentages of variance of the response variable explained by the source variable

Response	Source: Relative dominance	df	SS	MS	*F*	*P*	η^2 (%)
Aboveground biomass	**Legumes**	1	139633	139633	4.484	**0.039**	6.5
	Grasses	1	25027	25027	0.804	0.374	0.8
	Forbs	1	7212	7212	0.232	0.632	0.4
	Legumes × Grasses	1	19569	19569	0.628	0.431	1.3
	Legumes × Forbs	1	655	655	0.021	0.885	0.4
	Grasses × Forbs	1	14898	14898	0.478	0.492	0.7
	Legumes × Grasses × Forbs	1	54875	54875	1.762	0.190	2.7
	Residuals	56	1743960	31142			86.9
Biomethane potential	**Forbs**	1	840	840	24.100	**0.008**	36.5
	Legumes	1	749	749	21.500	**0.010**	32.6
	Grasses	1	168	168	4.818	0.093	7.3
	Legumes × Forbs	1	10	10	0.299	0.613	0.5
	Grasses × Forbs	1	82	82	2.351	0.200	3.6
	Legumes × Grasses	1	42	42	1.201	0.335	1.8
	Legumes × Grasses × Forbs	1	355	355	10.194	**0.033**	15.4
	Residuals	4	139	35			6.1
Area-specific methane yield	**Forbs**	1	7644	7644	138.133	**0.000**	41.5
	Grasses	1	4516	4516	81.602	**0.001**	24.5
	Legumes	1	4110	4110	74.276	**0.001**	22.3
	Grasses × Forbs	1	1479	1479	26.726	**0.007**	8.0
	Legumes × Forbs	1	172	172	3.101	0.153	0.9
	Legumes × Grasses	1	4532	4532	81.905	**0.001**	24.6
	Legumes × Grasses × Forbs	1	2285	2285	41.291	**0.003**	12.4
	Residuals	4	221	55			1.2

Table 2 Mean relative dominances (RD) of plant functional groups (PFG) in plots in September 2013, mean biomethane potentials and mean area-specific methane yields in relation to sown diversity and order of arrival of PFG. Note that the sum of RD is higher than 100% as plant species overlapped within canopies. Means with same letter (superscript) are not significantly different

Sown diversity	Order of arrival	RD forbs (%, ±SE)	RD grasses (%, ±SE)	RD legumes (%, ±SE)	Biomethane potential (L_N CH_4 g_{VS}^{-1}, ± range)	Area-specific methane yield (L_N CH_4 m^{-2}, ± range)
Low diversity	F-first	111 (±10)^{a}	16 (±5)^{a}	86 (±5)^{a,b}	234 (±2)^{a}	106 (±13)^{a}
	G-first	42 (±15)^{b,c}	65 (±7)^{b}	77 (±7)^{b}	250 (±5)^{a}	148 (±17)^{a}
	L-first	28 (±11)^{c}	29 (±8)^{a}	106 (±4)^{a}	256 (±13)^{a}	101 (±3)^{a}
	Control	57 (±5)^{b,c}	62 (±4)^{b}	77 (±5)^{b}	262 (±16)^{a}	121 (±8)^{a}
High diversity	Control	77 (±12)^{a,b}	109 (±9)^{c}	70 (±8)^{b}	264 (±1)^{a}	114 (±31)^{a}

Area-specific methane yield

Aboveground biomass and biomethane potential were taken into account to evaluate methane yield per area unit. The area-specific methane yields had a mean of 119 L_N CH_4 m^{-2} and a large range which was solely due to the variations in aboveground biomass ($P < 0.001$, $\eta^2 = 97\%$) and not due to biomethane potentials ($P = 0.600$, $\eta^2 = 1.8\%$). The variance of the area-specific methane yields can be explained by order of arrival ($P = 0.168$, $\eta^2 = 23\%$), sown diversity ($P = 0.135$, $\eta^2 = 14\%$), and soil type ($P < 0.05$, $\eta^2 = 27\%$) based on the effect size rather than significance. Biomass from soil B had a slightly higher methane yield than from soil A (127 (±10) L_N CH_4 m^{-2} compared to 110 (±9) L_N CH_4 m^{-2}). This reflects the higher aboveground plant biomass results, which however were not found to be significantly different. As the factor soil type was not

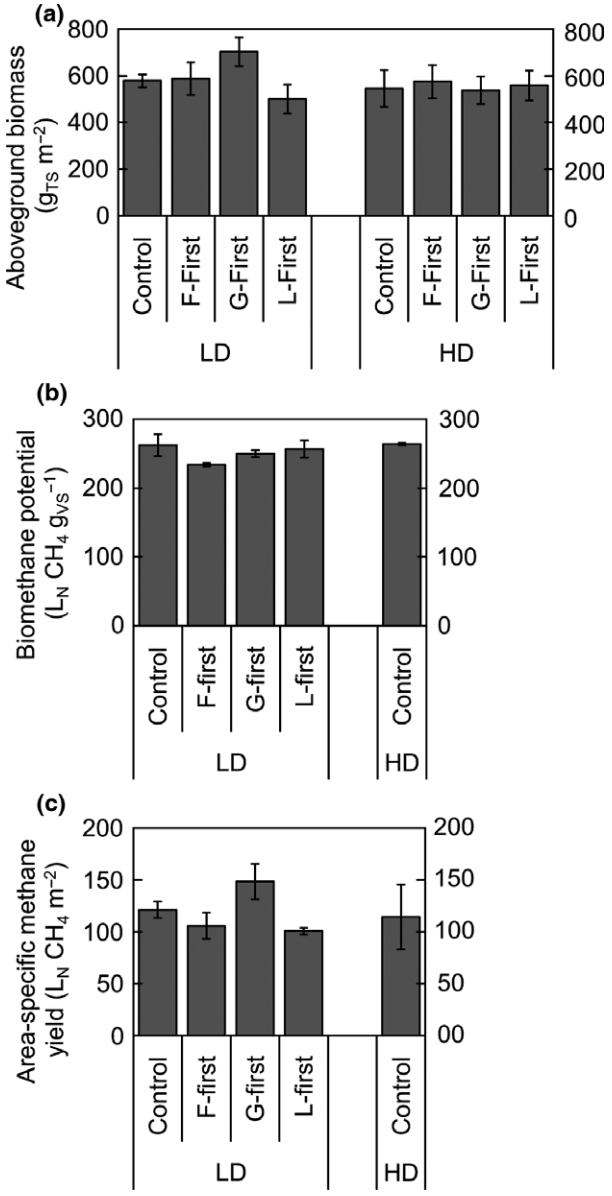

Fig. 2 (a) Mean aboveground biomass of all plots harvested in September 2013 (n = 8, ±SE), (b) mean biomethane potentials (n = 2, ± range), as well as (c) mean area-specific methane yield of subsampled plots (n = 2, ± range) according to the order of arrival of PFG (control: all PFG sown simultaneously, F-first: forbs sown first, G-first: grasses sown first, L-first: legumes sown first) and sown diversity (LD: low diversity, HD: high diversity).

the focus of this study, mean values for both soil types were given hereafter and in Fig. 2c as well as Table 2. The highest area-specific methane yield of 148 L_N CH_4 m^{-2} was obtained from plots where grasses were sown first (Table 2 and Fig. 2c). When PFG were sown simultaneously (control), the mean area-specific methane yields were 121 L_N CH_4 m^{-2} and 114 L_N CH_4 m^{-2} at low and high sown diversity, respectively. Lowest area-specific methane yields were obtained from plots where forbs and legumes were sown first (106 and 101 L_N CH_4 m^{-2}, respectively).

Differences in the area-specific methane yield can also be explained by the RD of the PFG (Table 1). Most of the variance was explained by the RD of forbs, grasses, and legumes as well as the interaction of grasses and forbs. Methane yields were higher when RD of legumes was between 70% and 77% and RD of grasses was as high as 62% – 109% (Table 2). Higher RD of legumes (>86%) and low RD of grasses (≤29%) resulted in lowest methane yields. Species richness had no significant effect on the area-specific methane yield.

Discussion

The biomass production and methane yield data presented in this study are from September 2013, when legumes were the most dominant functional group (see Fig. 1). In more detail, forbs were dominating in the F-first plots (both HD and LD levels) and grasses were dominating in control and G-first plots in high-diversity plots. Our biomethane potential study shows clearly that the order of arrival affected the RD of specific PFG and this in turn significantly affected the aboveground biomass, the biomethane potential, and the area-specific methane yield. Furthermore, we obtained an indication for an effect of sown diversity on the RD of PFG and hence, methane yields.

Our reported aboveground biomass production was well within the range described in the literature for similar grasslands consisting of typical European species (Bullock *et al.*, 2007; Marquard *et al.*, 2013). Henschell *et al.* (2015) reported an even greater range from 80 to 1070 g m^{-2} annual aboveground biomass production for low-input grasslands. However, the second cut usually yields less biomass than the first cut within a year (Amon *et al.*, 2007; Khalsa *et al.*, 2014). Biomethane potentials expressed as feedstock-specific methane yields represent the potential energy of a certain biomass which can be exploited by anaerobic digestion. Biomethane potentials found in this study were similar as reported for low-input conservation areas (Herrmann *et al.*, 2014; van Meerbeek *et al.*, 2015) and as the theoretical potentials of low-input grassland (Corton *et al.*, 2013) or lower than reported earlier for typical European grasslands and different meadows (Melts *et al.*, 2013; Khalsa *et al.*, 2014). Even though species compositions of the subsampled plots were different, no significant differences between biomethane potentials were found which is in contrast to other studies reporting clear differences (Herrmann *et al.*, 2014; Khalsa *et al.*, 2014). Significant differences between biomethane

potentials were also found between single grass species (Seppälä *et al.*, 2009; McEniry & O'Kiely, 2013). Furthermore, Melts *et al.* (2014) observed a higher biomethane potential of grasses than of legumes and forbs. However, in our study herbs and legumes contributed to the biomethane potential as well and an averaging effect might occur when grassland communities of different composition are utilized for biogas production.

The aim of supplying biomass for biogas production is a maximized area-specific methane yield comprising biomass production and biomethane potential which are both influenced by many factors. Literature on area-specific methane yields of grassland communities is rare. Khalsa *et al.* (2014) reported yields of 56 to 111 L_N CH_4 m^{-2} which is low compared to our results. McEniry & O'Kiely (2013) and Seppälä *et al.* (2009) reported methane yields from 116 to 350 L_N CH_4 m^{-2} for single grass species which are similar or higher than yields obtained in this study. Area-specific methane yields were influenced strongly by biomass production of different plant functional groups and not by biomethane potentials, which is consistent with results from Khalsa *et al.* (2014).

Furthermore, Khalsa *et al.* (2014) described a positive correlation between legume abundance and area-specific methane yields. In turn, they found a negative correlation with the abundance of grasses. These correlations were linked to lower crude fiber and higher crude protein content of legumes compared to grasses. Lignocellulosic fibers are hard to degrade under anaerobic conditions in contrast to proteins thus lowering the biomethane potential (Klimiuk *et al.*, 2010; Herrmann *et al.*, 2016). Furthermore, community biomass production is increased by legumes due to nitrogen fixation (Roscher *et al.*, 2011). This is supported here, in that we found that the RD of legumes and grasses positively affected area-specific methane yields by increased biomass production. Furthermore, this may be due to general differences in chemical composition of PFG (not measured in this study) or to competition for light between PFG leading to different fiber contents as hypothesized by Khalsa *et al.* (2014).

The sown diversity affected the ensuing species richness of the plots as well as the relative dominance of PFG. In contrast to other studies (Bezemer & van der Putten, 2007; Bullock *et al.*, 2007; Mangan *et al.*, 2011; Khalsa *et al.*, 2014), sown diversity did not affect aboveground biomass or area-specific methane yields.

The main driver of the methane potential and yield, however, was clearly the indirect effect of assembly treatments on the relative dominance of the plant functional groups. As the differences in methane yields are not large between the treatments where grasses were sown first and the other treatments (and in particular are not significantly different from the control), looking at the composition of the community is probably more important to methane yield than which PFG was sown first. The relative dominance of the PFGs that manage to establish in the grassland plots is actually what was driving the area-specific methane yield. According to our data, sown diversity seemed to have a minor influence on methane yield although it should be noted here that we only compared HD with LD in the assembly control plots (sown at the same time) such that a general conclusion here would require further research.

In our study at this time point (September 2013) highest area-specific methane yields were obtained when grasses were sown first and where grasses and legumes codominated. One should take into account however, that this result is only one time point in the experiment. If further studies also find the same pattern, then one would have to consider the possible implications of this outcome in relation to restoration goals. A restoration process aiming at a high biodiversity might be different than aiming at a high methane yield. When grasses were sown first and highest methane yields were observed, grasses dominated and a lower species diversity compared to the control was observed which is not desired for a high biodiversity. Further research is necessary to see whether biodiversity and bioenergy goals can be balanced by a specific restoration strategy.

Interestingly, we found that the assembly treatment determined the relative dominance of the plant functional groups and that the highest area-related methane yield was obtained when legumes and grasses codominated. These effects may persist for several years as observed by Bullock *et al.* (2007) and Plückers *et al.* (2013). However, in the Priority Effect experiment, on which this biomethane study is based, priority effects were not persistent over time (Weidlich *et al.*, 2016). These conflicting results show that the assembly process needs to be further investigated. Priority effects might depend on the year of sowing, on impact of harvesting and weather conditions. According to our results, it is likely that grassland communities can be steered toward high area-specific methane yields using priority effects. This seems to be a potentially valuable tool to maximize methane yields from grasslands. As feedstock-specific methane yields are secondary (own data and literature), efforts to optimize area-specific methane yields can be simplified by focusing on assembly and plant biomass production.

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