agriculture

MDPI

Article

Effect of Sowing Method and Weed Control on the Performance of Maize (*Zea mays* L.) Intercropped with Climbing Beans (*Phaseolus vulgaris* L.)

Liina Nurk [1,*], Rüdiger Graß [1], Carola Pekrun [2] and Michael Wachendorf [1]

1 Organic Agricultural Sciences, Department of Grassland Science and Renewable Plant Resources, University of Kassel, Steinstraße 19, 37213 Witzenhausen, Germany; grass@wiz.uni-kassel.de (R.G.); mwach@uni-kassel.de (M.W.)

2 Agronomy and Quality Management, Nuertingen-Geislingen University, Neckarsteige 6-10, 72622 Nuertingen, Germany; Carola.Pekrun@hfwu.de

* Correspondence: liina.nurk@uni-kassel.de or gnr@uni-kassel.de

Academic Editor: Elizabeth Baggs

Received: 6 February 2017; Accepted: 14 June 2017; Published: 23 June 2017

Abstract: Maize is grown on a large area in Germany and there is a growing concern in society about negative environmental effects related to this. The objective of the study was to test the performance of mixtures of maize and climbing beans as an alternative to monocropped maize under different site and management conditions. The effects of sowing density of maize and beans as well as the sowing time of beans on total dry matter (DM) yield and bean DM contribution to the total yield were investigated. Further, various mechanical and chemical weed control methods were tested and the resulting total DM yield was compared with that of a reference treatment (manual weeding). Hardly any consistent yield difference between maize/bean mixtures and monocropped maize occurred. The proportion of beans varied over a wide range among sites and was consistently higher when beans were sown at an early growth stage of maize. Mixtures did not suppress weeds efficiently and at two of the three sites their yield clearly declined with increasing weed coverage in the mixtures. A weed coverage of up to circa 10% may be tolerated, as the corresponding yield reduction is less than 1 t ha^{-1}. Considering the additional effort (i.e., two sowings, high costs for bean seeds, complicated weed control) in managing such mixtures, it can be concluded that maize/bean mixtures can currently hardly be recommended as an alternative to monocropped maize for feedstock production.

Keywords: crop production; crop performance; monoculture; plant competition; biodiversity; legume

1. Introduction

The amount of monocropped maize in Germany increased from 0.8 million (m) ha in 1980 to 2.5 m ha in 2015 [1]. Maize is primarily used as fodder for livestock and, in the last 10 years, has been increasingly cultivated to produce silage as an input for biogas plants due to its high yields and advanced breeding activities. This dominance and the cultivation of maize as a monocrop can cause ecological problems like loss of biodiversity, soil erosion, and nitrogen leaching [2,3]. The reduction of diversity may be accompanied by an increasing vulnerability to climatic and other stresses, which raises the risk of harvest losses for individual farmers and undermines the stability of agriculture [4].

Cultivation of maize in mixtures with other crops attracted great research attention in the 1970s and 1980s; however, this was mostly in tropical and subtropical regions [5–8]. Intercropping is considered a suitable management strategy for small farmers [9] to increase the diversity of their products and the stability of their annual output through the effective use of land and other resources. The main purpose of intercropping is to minimize the risk of crop failure and reduce income risks due to unstable market prices for a given commodity. Much research has dealt with the improvement of forage quality through intercropping [10–19] by investigating the effects of different proportions of leguminous plants. Legume-maize mixtures can be used as a local, protein-rich forage [20], while also having a positive ecological impact by increasing the soil fertility due to the N fixation by rhizobia in symbiosis with legumes [21,22]. Various researchers have proved that intercropping provides more effective use of land area than monocropping [17,23,24]. Many other benefits result from intercropping, such as erosion control [2] and ecosystem stability due to the diversity of organisms [25,26], further emphasizing the importance of plant diversity in agriculture. However, no experiments have been carried out thus far to assess the performance of maize/bean mixtures as an alternative energy crop for biogas production.

In maize/bean mixtures the climbing bean can make use of the maize plant to grow in height. Therefore, the installation of bars is not needed. The rooting systems of maize and beans are also compatible, because they utilize the nutrients of different soil depths [2,27]. To exploit the potential of maize/beans mixtures, Francis et al. [7] concluded from previous research that climbing beans need to be planted after maize to prevent the latter from being smothered. In this context, weed control is an important issue in intercropping, as chemical control is challenging. Generally, intercropping a dicotyledonous crop with a monocotyledonous crop reduces herbicide options. In the case of climbing beans and maize, only three active ingredients are potentially useful: pendimethalin, dimethenamid-P, and clomazone. All three are pre-emergence herbicides, thus resulting in sub-optimal weed control of late emerging weeds. Weed control of cropping mixtures in organic farming systems, which rely on mechanical weed control, is also a challenge due to the different sowing dates and emergence times of the two crops.

The aim of this study was (i) to measure the effect of sowing density of maize and beans on total dry matter (DM) yield and bean contribution to the mixture, (ii) to investigate the effect of bean sowing time on total yield and bean contribution to the mixture, and (iii) to determine the effect of weed control methods on weed coverage and total DM yield.

2. Material and Methods

2.1. Experimental Sites and Field Experiments

2.1.1. Experiment I—Intercropping

Field experiments were conducted at three sites in Germany with contrasting soil conditions in two consecutive years (Table 1). Composite topsoil samples were taken for determination of pH, P, and K [28]. Soil properties, preceding crops, tillage, fertilizer use, and sowing and harvest dates are shown in Tables 1 and 2. A maize hybrid (FAO 250) (*Z. mays* L., '*Fernandez*') and a late-maturing cultivar of climbing bean (*P. vulgaris* L. '*Anellino Verde*') were intercropped. The maize variety was recommended by a local breeder and the climbing bean variety was selected based on previous experiments with several bean varieties [29]. In order to analyse the effect of beans on maize and the total yield, both plants were sown with different densities and sowing dates. As a control, maize was sown with a seed density of 10 seeds m^{-2}, which was common practice in previous experiments. In mixtures, both crops were sown with densities of 7.5 seeds m^{-2}, 5 seeds m^{-2}, and combinations of these two densities. All crops were sown at a higher density and then thinned to the intended density. Two sowing times of beans were examined: early sowing (at the 2–3 leaf stage of maize) and late sowing (at the 5–6 leaf stage of maize). In total, there were nine different treatments (Table 3), each with four field replicates, laid out in a fully randomised block design. Each plot was 10 m long and 3 m wide with a sub plot that was 1.5 m wide in the centre. Thus, there were two buffer rows of maize and beans between each harvesting plot. Maize was sown during mid-April to early May (Table 2) depending on weather conditions and common practice at the experimental site. Row spacing for maize was 0.75 m, the seeding rate was 10, 7.5, or 5 seeds m^{-2}, and sowing depth was 0.04–0.06 m. Climbing beans were sown at a distance of 0.125 m on either side of the maize row, with densities of 7.5 or 5 seeds m^{-2} at two different sowing dates.

Table 1. Characteristics of the three experimental sites.

Name	Witzenhausen (WIZ)		Tachenhausen (TAC)		Grub (GRU)	
Geographical location	51°23′ N, 9°54′ E		48°39′ N, 9°23′ E		48°09′ N, 11°47′ E	
Height above sea level (m)	228		361		526	
Soil type *	Luvisol		Luvisol		Cambisol	
Farming system	organic		conventional		conventional	
Year	2014	2015	2014	2015	2014	2015
Preceding winter crop	Wheat	Rye	Wheat	Wheat	Barley	Barley
Fertilizer (kg ha^{-1})	none	none	100 N_m	106 N_m	100 N_m, 30 P, 35 S	N_o 51, N_m 70
Soil parameters:						
pH	6.4	6.6	6.6	6.4	6.3	7
P (mg 100 g^{-1})	9	10	17	4	24	24
K (mg 100 g^{-1})	9	11	33	19	16	34
Annual total rainfall (mm)	563	506.4	816.5	456.5	814.8	561.5
Average rainfall 1985–2015 (mm)	629		718.1		884.7	
Annual mean temperature (°C)	10.7	10.8	11	11.7	9.9	10.9
Average temperature 1985–2015 (°C)	8.2		10.4		8.9	

N_o = Organic N fertilizer, N_m = Mineral N fertilizer, * Classification according to [30].

Table 2. Sowing and harvest dates as well as phenological stage BBCH * of crops in experiment I over two years at three experimental sites.

	WIZ		TAC		GRU	
	2014	**2015**	**2014**	**2015**	**2014**	**2015**
Maize						
Sowing date	29 April	06 May	15 April	10 April	06 May	08 May
Harvest date	06 October	30 September	01 October	28 August	29 September	01 September
(days after sowing)	(160)	(147)	(169)	(140)	(146)	(116)
BBCH	83	83	83	80	83	80
Beans						
Sowing date early	02 June	29 May	22 May	11 May	06 June	01 June
Sowing date late	13 June	26 June	04 June	29 May	13 June	13 June
Harvest date	06 October	30 September	01 October	28 August	29 September	01 September
(days after early sowing)	(126)	(124)	(132)	(109)	(115)	(92)
Harvest date	06 October	30September	01 October	28 August	29 September	01 September
(days after late sowing)	(115)	(96)	(119)	(91)	(108)	(80)
BBCH of early (late) sown beans	76 (62)	76 (62)	76 (62)	59	76 (62)	59

* Phenological development of the crops was assessed by determining the developmental stages during growth of crops according to the BBCH scale [31]

Table 3. Details of the intercrop treatments carried out in experiment I over two years at three experimental sites.

Abbreviation	Maize Seeds m^{-2}	Bean Seeds m^{-2}	Bean Sowing Time
10M	10		
7.5M + 7.5B (e)	7.5	7.5	early
7.5M + 5B (e)	7.5	5	early
5M + 7.5B (e)	5	7.5	early
5M + 5B (e)	5	5	early
7.5M + 7.5B (l)	7.5	7.5	late
7.5M + 5B (l)	7.5	5	late
5M + 7.5B (l)	5	7.5	late
5M + 5B (l)	5	5	late

2.1.2. Experiment II—Weed Control

The same maize hybrid (FAO 250) (*Z. mays* L. '*Fernandez*') and late-maturing cultivar of climbing bean (*P. vulgaris* L. '*Anellino Verde*') were cultivated at three different experimental sites. The maize was sown with a density of 7.5 seeds m^{-2} and the beans were sown in the 2–3 leaf stage of maize at a distance of 0.125 m on either side of the maize row, also at a density of 7.5 seeds m^{-2}. Planting densities were chosen according to results of previous experiments. Row spacing between maize plants was 0.75 m and each plot was 10 m long and 3 m wide.

Four different organic weed control methods were tested in Witzenhausen (WIZ). For the purpose of comparison, one treatment received no weed control (NWC). Another treatment, managed by multiple episodes of hand-hoeing (HHM), was maintained completely weed-free in order to assess yield potential in the absence of weed competition and damage by weed control. In a further treatment, weeds were controlled multiple times with a mechanical hoe (MH) with goosefoot sweeps until the maize plants grew too high. Additional treatments provided two episodes of weeding, once before beans were sown and once after bean sprouting, either with a hand hoe (HH2) or mechanical hoe (MH2), also with goosefoot sweeps. In all treatments, weed control was conducted according to the appearance and quantity of weeds. All five treatments had four field replicates, resulting in a total of 20 plots.

In Tachenhausen (TAC) and Grub (GRU), chemical weed control was tested and compared with mechanical weed control methods as well as the two extreme treatments mentioned above (i.e., no weed management and completely weed-free). As chemical weed control, two different strategies were tested. Both were pre-emergence herbicides applied immediately after sowing: (SA + S) Stomp Aqua (2.8 l ha^{-1}, active ingredient pendimethalin) + Spectrum (1.4 l ha^{-1}, active ingredient dimethenamid-P) and (C36) Centium 36 CS (0.25 l ha^{-1}, active ingredient clomazone). Total weed coverage (as a percentage) was estimated visually in three 0.75 m^2 areas per plot. Measurements were conducted at three different growth stages of maize: in the leaf-development stage, when five leaves had unfolded (BBCH 15) [31]; at the beginning of stem elongation (BBCH 30); and when five nodes were detectable (BBCH 35) and the canopy was closing. The three most important weed species were *Galium aparine*, *Chenopodium album*, and *Cirsium arvense* in WIZ, *Echinochloa crus-galli*, *Chenopodium album*, and *Fallopia convolvulus* in TAC, and *Solanum nigrum*, *Chenopodium album*, and *Galinsoga parviflora* in GRU.

2.2. Harvest

2.2.1. Experiment I

The aim was to harvest the crops when the maize DM content was above 30% (early dent stage of maturity, BBCH 83), which is common practise for silage maize in Germany (Table 2). For measuring the percentage of beans in the mixture, plants were sampled from a 4×1.5 m sub-plot in the two middle rows of each plot, then bean and maize plants were separated and weighed to calculate the bean percentage. For DM analysis, one representative sub-sample was chopped and thoroughly mixed, then 1 kg was weighed, dried at 105 °C for 48 h, and weighed again.

2.2.2. Experiment II

Experiment II was harvested at the same time as experiment I. In WIZ, a 3×1.5 m sub-plot from the two middle rows of each plot was sampled and weighed. In TAC, a field harvesting machine (Claas Jaguar 70 SF) harvested the two middle rows and weighed the harvested biomass by a machine-mounted scale. In GRU, a field harvesting machine (Hege 212 with Kemper maize headers) and scale (Hege DK 800 HMP-2) were used. For DM analysis, 1 kg of thoroughly mixed fresh matter was weighed, dried at 105 °C for 48 h, and weighed again.

2.3. Statistical Analysis

Statistical analysis was conducted using R Software [32]. As the site effects were intermingled with farming system effects (organic versus conventional farming), every experimental year and site was analysed individually. The underlying assumptions for analysis of variance (ANOVA), i.e., normal distribution of residuals and homogeneity of variance, were met. For both experiments, a one-factorial ANOVA was conducted. Significant differences ($p < 0.05$) were evaluated with the Bonferroni least significant difference post-hoc test. Linear regression analysis was performed with Microsoft Excel® software.

3. Results

3.1. Experiment I

In 2014, significant treatment effects on total crop yield only occurred at WIZ and TAC (Figure 1). In WIZ, the northernmost research site, total DM yield of the control treatment (pure maize crop) was 25.4 t ha^{-1}. DM yield of the mixtures was significantly lower, with 20.7 t DM ha^{-1} on average and a DM content of 280 g kg^{-1}. In TAC and GRU in 2014, the control treatment achieved total yields of 17.3 and 19.1 t DM ha^{-1}, respectively, with a DM content of 380 g kg^{-1} and 350 g kg^{-1}, respectively. In TAC, the average total yield of the mixtures was 2.8 t DM ha^{-1} lower than the control. In GRU, there were no significant differences between the control and mixtures, though DM yield of the treatment 5M + 7.5B (e) was as low as 16.4 t ha^{-1}. In WIZ in 2014, the highest bean DM proportion was 33% in treatment 5M + 5B (e). In contrast, the lowest proportion (11%) was found in 7.5M + 5B (l). In 2014 in both TAC and GRU, the highest bean proportions (45% and 44%, respectively) were achieved in the 5M + 7.5B (e) and lowest were found in 7.5M + 7.5B (l) (16% and 17%, respectively).

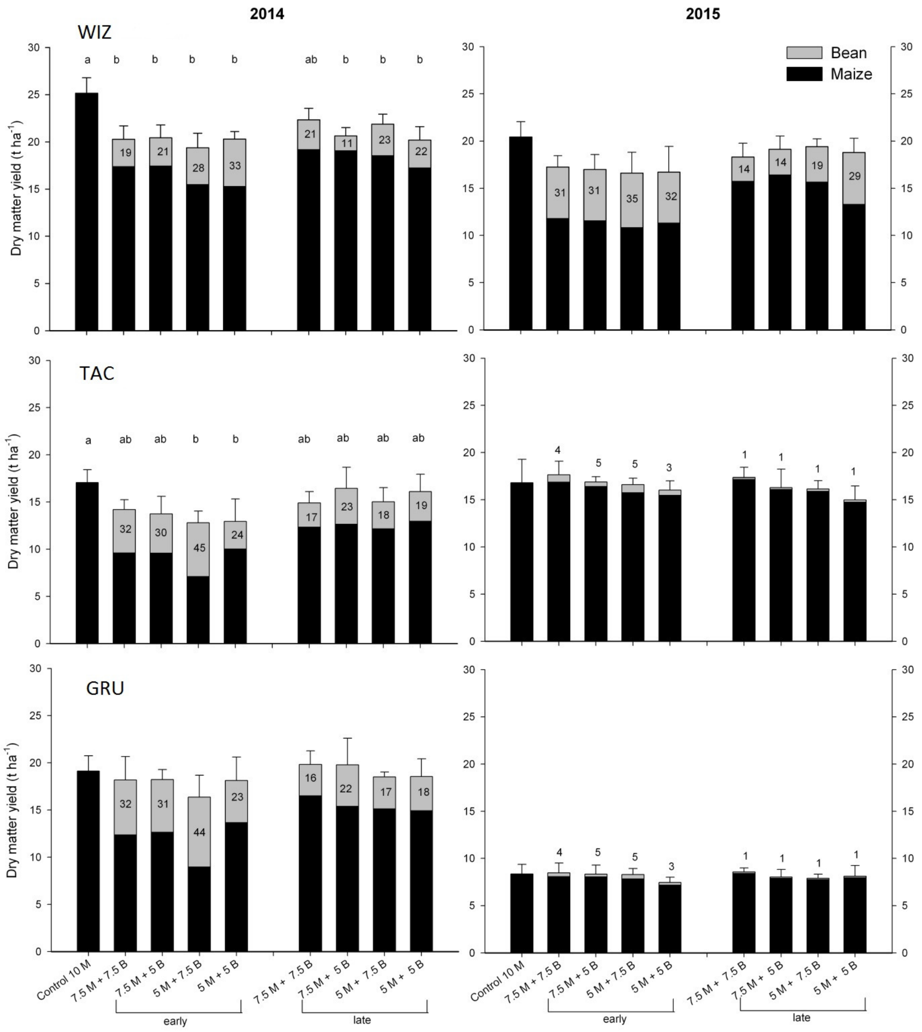

Figure 1. Total dry matter yield (t ha^{-1}) in a pure stand of maize (control) and intercropped with early and late sown beans in experiment I over two years at three experimental sites. Letters indicate significant differences found by Bonferroni Least Significance Tests ($p < 0.05$). Numbers inside or above bars indicate bean contribution (% DM) to the intercrop. M = maize, B = bean. Treatment names indicate sowing densities of the crops (e.g., 5M + 5B = 5 maize plants m^{-2} with 5 bean plants m^{-2}). Whiskers indicate standard errors of the means.

In 2015, average DM yield in WIZ was only 3 t ha^{-1} lower than in 2014 and differences among treatments were not statistically significant (Figure 1). In TAC and GRU, all mixtures achieved extremely low bean proportions (<5%) in 2015. Nevertheless, DM yields in TAC (16.5 t DM ha^{-1} on average) were similar to or higher in 2015 than in the previous year. In GRU, average DM yields were 8.2 t DM ha^{-1}, which is 10 t ha^{-1} less than in the previous year. Across all sites and both years, mixtures with 5 maize plants m^{-2} produced a similar total DM yield as with 7.5 maize plants m^{-2}. While DM content in harvested maize was between 320 g kg^{-1} and 380 g kg^{-1}, DM content of beans was 200 g kg^{-1}, with little variation among sites and years.

Regression analysis of the bean DM contribution data from intercropped plots at all experimental sites revealed a highly significant relationship:

$$y = 0.565x + 0.6989, R^2 = 0.72 ***, n = 24 (*** = p < 0.001) \quad (1)$$

where x = mean bean DM contribution (% of total DM yield) in mixtures with early sown beans (at the 2–3 leaf stage of maize), y = mean bean DM contribution (%) in mixtures with late sown beans (at the 5–6 leaf stage of maize).

Bean DM contribution in mixtures was higher when beans were sown at a time when the maize plants were smaller and less competitive. While the difference between sowing dates was negligible at low levels of bean DM contribution, the regression coefficient in equation (1) indicates an increasing difference, which was 26% at the highest measured value of bean contribution to DM in mixtures with early sown beans (45%).

3.2. Experiment II

Omission of weed control in WIZ resulted in 30% and 58% weed coverage for 2014 and 2015, respectively, as estimated around the time of row closure by maize (BBCH 35). The corresponding yield reduction was 25% and 60% for 2014 and 2015, respectively, compared with the weed-free treatment (HHM, Table 4). HHM resulted in high yields with no weed coverage in both years, whereas the yield of MH2 was low in both years, despite low (0% in 2014) and high (26% in 2015) weed coverage. Although weed coverage in the non-weeded control in TAC was higher than with mechanical or chemical weed control, yields were uniform, at around 16 t DM ha^{-1}, irrespective of the type of weed control (Figure 2). For both TAC and GRU, weed coverage was lowest for HHM and HH2. Although weed infestation in GRU was higher in 2014, mixtures performed much better (39.5% weed coverage, 14.95 t DM yield ha^{-1} on average) in this year than in 2015 (10.7% weed coverage, 7.34 t DM yield ha^{-1} on average). In 2014, MH and C36 resulted in similar levels of weed coverage and total DM yield as in the uncontrolled treatment, which was also observed in 2015 but at much lower levels.

Table 4. Estimated mean weed coverage (%) at three measuring dates in weed control experiment (II) over two years at three experimental sites.

Year		**2014**			**2015**	
Maize BBCH	15	30	35	15	30	35
				WIZ		
Date	17 June	9 July	28 July	16 June	9 July	16 July
Treatment						
NWC	38.0 ^{a}	29.0 ^{a}	30	17.9 ^{a}	58.2 ^{a}	58.2 ^{a}
HHM	6.9 ^{b}	3.8 ^{b}	0	7.3 ^{b}	7.2 ^{b}	7.2 ^{b}
MH	9.0 ^{b}	4.5 ^{b}	0	8.9 ^{b}	18.3 ^{b}	18.3 ^{b}
HH2	10.5 ^{b}	4.3 ^{b}	0	12.1 ^{a,b}	27.3 ^{a,b}	27.3 ^{a,b}
MH2	9.8 ^{b}	6.8 ^{b}	0	10.4 ^{a,b}	26.4 ^{a,b}	26.4 ^{a,b}

Table 4. *Cont.*

Year	2014			2015		
Maize BBCH	15	30	35	15	30	35
				TAC		
Date	6 June	1July	15 July	25 June	6 July	30 July
Treatment						
NWC	1.7 [a]	10.5 [a]	16.7 [a]	9.5 [a]	17.2 [a]	19.1 [a]
HHM	0.0 [b]	0.0 [b]	1.8 [b]	0.0 [b]	2.8 [c]	0.0 [c]
MH	1.0 [a,b]	5.2 [a,b]	12.4 [a,b]	7.9 [a]	14.4 [a,b]	13.6 [a,b]
SA + S	0.3 [b]	0.9 [b]	2.3 [b]	2.1 [b]	3.5 [b,c]	3.5 [b,c]
C36	0.0 [b]	2.5 [b]	5.3 [a,b]	7.7 [a]	12.9 [a,b,c]	14.1 [a,b]
				GRU		
Date	12 June	2 July	17 July	23 June	15 July	4 August
Treatment						
NWC	18.4	16.8 [a]	47.2 [a]	6.1 [a,b]	10.5 [a]	10.4 [a]
HHM	1.2	5.7 [a,b]	2.7 [b]	3.5 [b,c]	4.7 [b]	1.0 [a]
MH	33.9	14.1 [a]	35.1 [a]	6.7 [a]	11.2 [a]	11.0 [a]
SA + S	0.1	1.5 [b]	0.3 [b]	1.4 [c]	2.5 [b]	2.0 [b]
C36	19.5	15.2 [a]	36.8 [a]	6.6 [a]	11.0 [a]	10.8 [a]

Different letters indicate statistically significant differences between the values found by Bonferroni Least Significance Tests ($p < 0.05$).

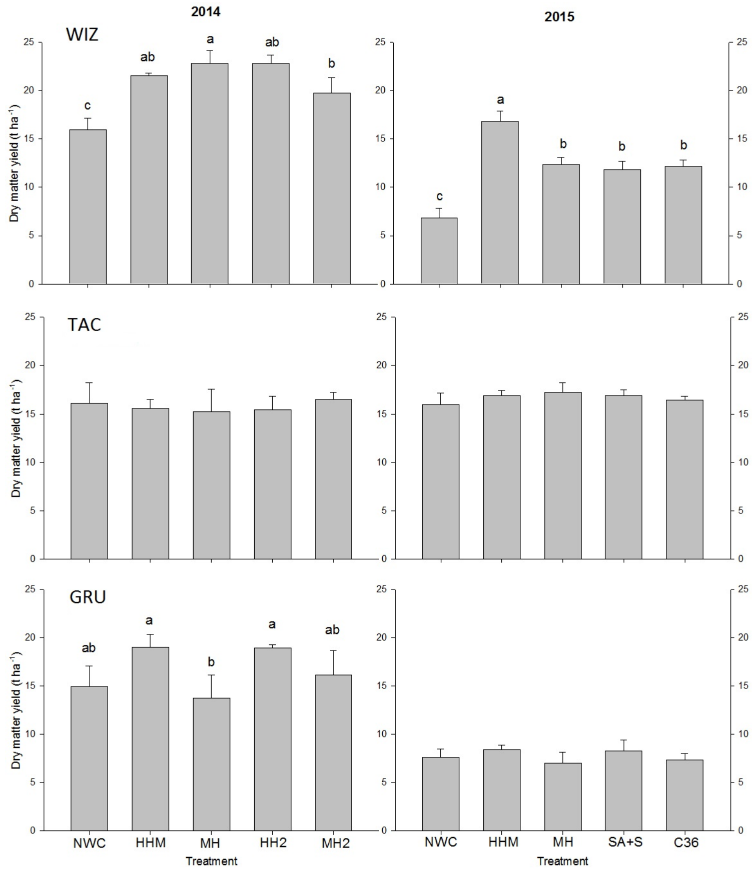

Figure 2. Total dry matter yields (t ha^{-1}) of maize/bean intercrops in the weed control experiment (II) over two years at three experimental sites. Letters indicate statistically significant differences found by Bonferroni Least Significance Tests ($p < 0.05$). Whiskers indicate standard errors of the means. (NWC = no weed control; HHM = multiple hand hoeing; MH = mechanical hoeing; HH2 = hand hoeing twice; MH2 = mechanical hoeing twice; SA + S = Stomp Aqua + Spectrum; C36 = Centium 36 CS).

4. Discussion

While a lot of research has been done on crop mixtures of cereals (e.g., wheat and oats) with grain legumes (e.g., field beans, peas) [33–35] and this is common practise in organic and traditional farming, cultivating maize in mixtures with legumes is not common either in organic or conventional agriculture. Under temperate European climate conditions, there may be three major reasons for this: (i) relatively low competitiveness of maize in spring compared with 'C3' cereals, especially in higher latitudes and altitudes, as low temperatures slow maize growth [36], (ii) mixing dicotyledonous and monocotyledonous crop species complicates the use of herbicides, (iii) maize grows tall at later stages of growth and exerts strong competitive pressure on the partner crop, which is why only tall-growing crops, e.g., sunflower [37] and sorghum [38], or climbing crops, like climbing (*Phaseolus vulgaris*) or runner beans (*P. coccineus*) [39], were considered in recent mixture trials with maize.

In addition, climbing beans may compete with maize for resources at early stages of growth, which is why the present field trials focused on the effect of a delayed sowing of beans. Across all experimental sites, results of the regression analysis show that the contribution of beans to the total DM yield was higher with early sowing, which is consistent with findings of Francis et al. [7]. This effect was enhanced by a reduction of the sowing density of maize, however, maize could not compensate for reduced plant density with increased growth of single plants, which could be expected due to an increased light incidence and nutrient supply, as reported by Lithourgidis et al. [2].

At all sites, maize contributed substantially to the total DM yield of the mixtures. Although bean proportions achieved values up to 45%, beans were hardly able to compensate for the yield losses of maize caused by a decreased sowing density. Pure stands of maize were sown with 10 plants m^{-2} in the present study; however, the question arises as to whether a better comparison for the maize-bean mixtures could be made with a control treatment utilizing the same planting density of maize as in the mixtures, i.e., 5 or 7.5 plants m^{-2}. Fischer et al. [40] conducted a field experiment with such a design and found increased yields of maize in mixed stands compared with the respective pure maize stands. However, consistent with our results, they found no yield advantage of mixtures compared with pure maize stands when plant density was not reduced. Since the goal of our study was to consider cropping systems with practical relevance, we designed our experiment based on the assumption that under practical conditions, farmers would probably not reduce the plant density of pure maize stands. Therefore, we chose the standard plant density of 10 plants m^{-2} for monocropped maize as a reference for comparisons with mixtures.

In 2015, the TAC and GRU treatments were strongly affected by unusual weather conditions, which were characterized by longer periods of drought and strongly reduced annual rainfall. Beans performed badly in all treatments and it seems that the dominance of maize increases with unfavourable weather conditions at the expense of bean development. The annual rainfall in WIZ in 2015 was not as low as in GRU and TAC, and the distribution of rainfall throughout the growing season was favourable for the growth of maize and beans. However, it is remarkable that the highest crop yields in both years were at the organically managed WIZ site, which did not receive any N fertilization, whereas 100 kg N ha^{-1} were applied in both TAC and GRU. This superiority is probably caused, on the one hand, by more suitable soil (i.e., a deep soil profile and a high share of loess) and, on the other hand, high amounts of available soil N at the time maize was sown (64 kg ha^{-1} in 0–90 cm depth in 2014 and 23 kg ha^{-1} in 2015; data not shown). This high soil N content, in turn, was probably due to the fact that the experimental field had been converted to organic management only in the year before the experiment. Furthermore, it was only in WIZ that active nodules of *Rhizobium leguminosarum* [41] were identified using the purple colour of the nodules' interior as an indicator for bacterial activity. This may be a further reason for higher yields of beans at this site. However, Graham and Ranalli [39] and Camisao [41] reported relatively low rates of N fixation by climbing beans compared with other grain legumes. Considering that maize has a high demand for N following the appearance of the eighth leaf until the beginning of ripening [36], it is only then that beans are typically beginning with N fixation. Thus, a direct transfer of fixed N from beans to maize is unlikely. It is rather the following crops within the crop rotation that may benefit from the N fixed by the beans.

The results of the weeding experiment show that maize/bean mixtures are as sensitive to weeds as maize grown in monoculture. Apparently, neither cropping system can supress weeds efficiently if there is a high prevalence of weeds, e.g., from the soil seed bank. Repeated hand hoeing resulted in very low weed coverage and allowed high crop yields at many sites, though differences in crop yield between this and other weeded treatments were not always significant. Hand hoeing before sowing and after sprouting of beans resulted in similar effects, although this treatment demanded much less effort. Apparently, with only two episodes of weeding, weeds were weakened to such an extent that crops were given a competitive edge. Regression analysis showed that total DM yield declined with increasing weed coverage in the mixtures at two sites (WIZ and GRU) (Figure 3), and that this relationship was particularly strong in WIZ when weed coverage was assessed at later growth stages (shortly before row closure) [42]. The regression equation for WIZ demonstrates that with the increase of weed coverage by 1%, DM yield of the mixture declined by 1% compared with the maize yield in the completely weed-free treatment. Furthermore, it seems that weed coverage of up to circa 10% may be tolerated, as the corresponding yield reduction was not very large. However, this finding cannot be readily transferred into practical farming management, as this relationship depends to a great extent on the type of weeds present. While the weeds in this case were mainly seed-propagated species, weeds that propagate vegetatively may need to be controlled already at much lower rates. It should be noted that mechanical weeding not only reduces the competitive pressure of weeds, but also stimulates crops by triggering additional mineralisation of soil N through turbation by cultivation tools [43–45]. However, the net benefit of this N priming effect cannot be quantified with the present experimental approach, as both effects of weed control (i.e., reduced competition and increased N supply) are inextricably linked. Sufficient weed control appears to be possible when applying a pre-emergence mixture of pendimethalin (Stomp Aqua) with dimethenamid-P (Spectrum). However, it has to be stated that many weeds were still present in the plots that received this combination. Therefore, sites with a heavy infestation of *Amaranthus* spec. or *Chenopodium* spec. should not be chosen for intercropping maize with climbing beans. Sub-optimal weed control is not seen as a hindrance to adoption of the system, as limited weed growth and reproduction can be tolerated in the fields, particularly because weeds enhance biodiversity and therefore potentially improve habitats for insects and birds [4,46].

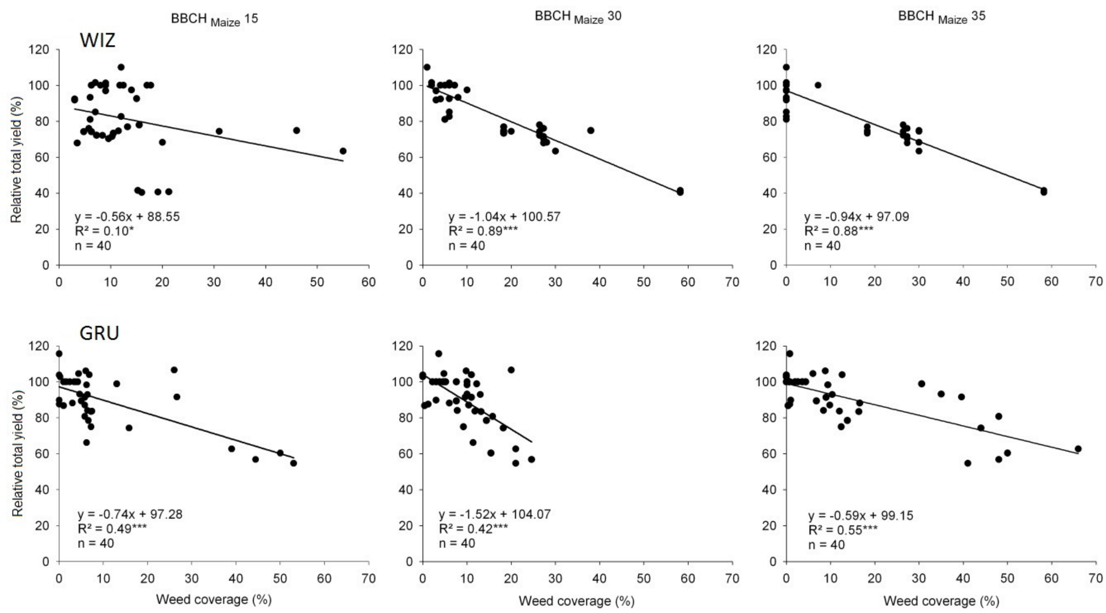

Figure 3. Graphics and statistics for the regression function $y = ax + b$, where y = total dry matter (DM) yield as a% of the DM yield in the weed-free treatment HHM and x = weed coverage in %, based on data from all plots at two sites in experiment II (*** <0.001, ** <0.01, * <0.05).

5. Conclusions

Considering the additional effort (i.e., two sowings, high costs for bean seeds, complicated weed control) in managing maize/bean mixtures it can be concluded that maize/bean mixtures can currently hardly be recommended as an alternative to monocropped maize for feedstock production. However, the results do not allow a final judgement on the potential of maize/bean mixtures as an alternative to monocropped maize. Although ruminants generally show positive response to increased protein content and higher digestibility of feed [47], there is little information on the feeding value of climbing beans. In terms of energy production, Nurk et al. [48] found a rather low methane yield when using climbing beans in biogas plants. Further testing under various site conditions and further development of sowing technology (contemporary sowing of both crops), exploiting the large genetic diversity of beans in combination with feeding trials would be desirable to comprehensively evaluate the benefit of maize/bean mixtures in temperate climates in Europe.

Acknowledgments: We thank Sabine Hubert, Andrea Gerke, and Wolfgang Funke for excellent technical help, as well as Walter Schmidt for inspiring and critical discussions. We thank Isabel Greenberg for thorough proofreading. Financial support by the Federal Ministry of Food and Agriculture of Germany is gratefully acknowledged.

Author Contributions: All four authors have made substantial contributions to the conception and design of the work and helped with the acquisition, analysis, and interpretation of data for the work. They all have helped drafting the work or revising it critically for important intellectual content. They all have made final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of Interest: The authors declare no conflicts of interest

Abbreviations

B	bean
DM	dry matter
e	early
GRU	Grub
l	late
M	maize
TAC	Tachenhausen
WIZ	Witzenhausen

References

1. Statistisches Bundesamt. Anbaufläche. Available online: https://www-genesis.destatis.de (accessed on 6 July 2016).
2. Lithourgidis, A.S.; Dordas, C.A.; Damalas, C.A.; Vlachostergios, D. Annual intercrops: An alternative pathway for sustainable agriculture. *Aust. J. Crop Sci.* **2011**, *5*, 396.
3. Herrmann, A. Biogas Production from Maize: Current State, Challenges and Prospects. 2. Agronomic and Environmental Aspects. *Bioenergy Res.* **2013**, *6*, 372–387. [CrossRef]
4. Thrupp, L.A. Linking Agricultural Biodiversity and Food Security: The Valuable Role of Agrobiodiversity for Sustainable Agriculture. *Int. Aff.* **2000**, *76*, 283–297. [CrossRef]
5. Clawson, D.L. Harvest Security and Intraspecific Diversity in Traditional Tropical Agriculture. *Econ. Bot.* **1985**, *39*, 56–67. [CrossRef]
6. Davis, J.; Roman, A.; Garcia, S. The effects of plant arrangement and density on intercropped beans (*Phaseolus vulgaris*) and maize II. Comparison of relay intercropping and simultaneous planting. *Field Crops Res.* **1987**, *16*, 117–128. [CrossRef]
7. Francis, C.A.; Prager, M.; Tejada, G. Effects of relative planting dates in bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) intercropping patterns. *Field Crops Res.* **1982**, *5*, 45–54. [CrossRef]
8. Searle, P.; Comudom, Y.; Shedden, D.C.; Nance, R.A. Effect of maize + legume intercropping systems and fertilizer nitrogen on crop yielding and residual nitrogen. *Field Crops Res.* **1981**, *4*, 133–145. [CrossRef]

9. Peksen, G. Intercropping Efficiency and Yields of Intercropped Maize (*Zea mays* L.) and Dwarf Bean (*Phaseolus vulgaris* L.) Affected by Planting Arrangements, Planting Rates and Relative Time of Sowing. *Int. J. Curr. Microbiol. Appl. Sci.* **2013**, *2*, 290–299.
10. Anil, L.; Park, J.; Phipps, R. The potential of forage-maize intercrops in ruminant nutrition. *Anim. Feed Sci. Technol.* **2000**, *86*, 157–164. [CrossRef]
11. Contreras-Govea, F.E.; Muck, R.E.; Armstrong, K.L.; Albrecht, K.A. Fermentability of corn–lablab bean mixtures from different planting densities. *Anim. Feed Sci. Technol.* **2009**, *149*, 298–306. [CrossRef]
12. Contreras-Govea, F.; Marsalis, M.; Angadi, S.; Smith, G.; Lauriault, L.M.; VanLeeuwen, D. Fermentability and Nutritive Value of Corn and Forage Sorghum Silage When in Mixture with Lablab Bean. *Crop Sci.* **2011**, *51*, 1307. [CrossRef]
13. Dawo, M.I.; Wilkinson, J.M.; Sanders, F.E.T.; Pilbeam, D.J. The yield and quality of fresh and ensiled plant material from intercropped maize (*Zea mays*) and beans (*Phaseolus vulgaris*). *J. Sci. Food Agric.* **2007**, *87*, 1391–1399. [CrossRef]
14. Dawo, M.I.; Wilkinson, J.M.; Pilbeam, D.J. Interactions between plants in intercropped maize and common bean. *J. Sci. Food Agric.* **2009**, *89*, 41–48. [CrossRef]
15. Flores-Sanchez, D.; Pastor, A.; Lantinga, E.A.; Rossing, W.A.H.; Kropff, M.J. Exploring Maize-Legume Intercropping Systems in Southwest Mexico. *Agroecol. Sustain. Food Syst.* **2013**, *37*, 739–761. [CrossRef]
16. Gebeyehu, S.; Simane, B.; Kirkby, R. Genotype×cropping system interaction in climbing beans (*Phaseolus vulgaris* L.) grown as sole crop and in association with maize (*Zea mays* L.). *Eur. J. Agron.* **2006**, *24*, 396–403. [CrossRef]
17. Javanmard, A.; Nasab, A.D.M.; Javanshir, A.; Moghaddam, M.; Janmohammadi, H. Forage yield and quality in intercropping of maize with different legumes as double-cropped. *J. Food, Agric. Environ.* **2009**, *7*, 163–166.
18. Maasdorp, B.V.; Titterton, M. Nutritional improvement of maize silage for dairying: Mixed-crop silages from sole and intercropped legumes and a long-season variety of maize. 1. Biomass yield and nutritive value. *Anim. Feed Sci. Technol.* **1997**, *69*, 241–261. [CrossRef]
19. Titterton, M.; Maasdorp, B.V. Nutritional improvement of maize silage for dairying: Mixed crop silages from sole and intercropped legumes and a long season variety of maize. 2. Ensilage. *Anim. Feed Sci. Technol.* **1997**, *69*, 263–270. [CrossRef]
20. Stoltz, E.; Nadeau, E. Effects of intercropping on yield, weed incidence, forage quality and soil residual N in organically grown forage maize (*Zea mays* L.) and faba bean (*Vicia faba* L.). *Field Crops Res.* **2014**, *169*, 21–29. [CrossRef]
21. Cong, W.-F.; Hoffland, E.; Li, L.; Six, J.; Sun, J.-H.; Bao, X.-G.; Zhang, F.-S.; van der Werf, W. Intercropping enhances soil carbon and nitrogen. *Glob. Chang. Biol.* **2015**, *21*, 1715–1726. [CrossRef] [PubMed]
22. Tsai, S.M.; Da Silva, P.M.; Cabezas, W.L.; Bonetti, R. Variability in nitrogen fixation of common bean (*Phaseolus vulgaris* L.) intercropped with maize. *Plant Soil* **1993**, *152*, 93–101. [CrossRef]
23. Albino-Garduno, R.; Turrent-Fernandez, A.; Isabel Cortes-Flores, J.; Livera-Munoz, M.; Carmen Mendoza-Castillo, M. Root distribution and solar radiation in maize-bean intercropping systems. *Agrociencia* **2015**, *49*, 513–531.
24. Sadeghi, H.; Kazemeini, S.A.; Edalat, M. Effect of plant density ratios and weed control on the performance of maize-bean intercropping. *J. Ecol. Field Biol.* **2012**, *35*, 313–322. [CrossRef]
25. Coll, M.; Bottrell, D.G. Predator-prey association in mono- and dicultures: Effect of maize and bean vegetation. *Agric. Ecosyst. Environ.* **1995**, *54*, 115–125. [CrossRef]
26. Smith, H.A.; McSorley, R. Intercropping and Pest Management: A Review of Major Concepts. *Am. Entomol.* **2000**, *46*, 154–161. [CrossRef]
27. Zhang, F.; Li, L. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. *Plant Soil* **2003**, *248*, 305–312. [CrossRef]
28. Naumann, C.; Bassler, R.; Seibold, R.; Barth, C. *Methodenbuch*; VDLUFA-Verlag: Darmstadt, Germany, 1976.
29. Hoppe, C. Development of Biogas Maize Cultivars for Intercropping with Climbing Beans. Ph.D Thesis, Georg-August-Universität Göttingen, Göttingen, Germany, 2016.
30. IUSS Working Group. *World Reference Base for Soil Resources 2014*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2014.

31. Meier, U. Biologische Bundesanstalt für Land- und Forstwirtschaft. In *Growth Stages of Mono- and Dicotyledonous Plants. BBCH-Monograph*; Blackwell Wissenschafts-Verlag: Berlin, Germany; Boston, MA, USA, 1997.
32. R Core Team. R: A Language and Environment for Statistical Computing. Available online: https://www.R-project.org (accessed on 25 May 2016).
33. Hauggaard-Nielsen, H.; Jørnsgaard, B.; Kinane, J.; Jensen, E.S. Grain legume-cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *RAF* **2008**, *23*. [CrossRef]
34. Malézieux, E.; Crozat, Y.; Dupraz, C.; Laurans, M.; Makowski, D.; Ozier-Lafontaine, H.; Rapidel, B.; Tourdonnet, S.; Valantin-Morison, M. Mixing plant species in cropping systems: Concepts, tools and models. A review. *Agron. Sustain. Dev.* **2009**, *29*, 43–62. [CrossRef]
35. Mariotti, M.; Masoni, A.; Ercoli, L.; Arduini, I. Forage potential of winter cereal/legume intercrops in organic farming. *Ital. J. Agron.* **2006**, *1*, 403. [CrossRef]
36. Böttinger, S. *Handbuch Mais. Grundlagen, Anbau, Verwertung, Ökonomie*; DLG-Verl.: Frankfurt, Germany, 2013.
37. Graß, R.; Heuser, F.; Stülpnagel, R.; Piepho, H.-P.; Wachendorf, M. Energy crop production in double-cropping systems: Results from an experiment at seven sites. *Eur. J. Agron.* **2013**, *51*, 120–129. [CrossRef]
38. Schittenhelm, S. Effect of Drought Stress on Yield and Quality of Maize/Sunflower and Maize/Sorghum Intercrops for Biogas Production. *J. Agron. Crop Sci.* **2010**. [CrossRef]
39. Graham, P.H.; Ranalli, P. Common bean (*Phaseolus vulgaris* L.). *Field Crops Res.* **1997**, *53*, 131–146. [CrossRef]
40. Fischer, J.; Höppner, F.; Böhm, H. Gemengeanbau von Mais mit Phaseolus-Bohnen: Einfluss von Sorte und Saatdichte der Bohnen auf die Bestandszusammensetzung. *GPW* **2015**, *27*, 177–178.
41. Carranca, C. Legumes: Properties and symbiosis. In *Symbiosis: Evolution, Biology, and Ecological Effects*; Camisão, A.F., Pedroso, C.C., Eds.; Nova Biomedical: Hauppauge, NY, USA, 2013; pp. 67–94.
42. Hall, M.R.; Swanton, C.J.; Anderson, G.W. The Critical Period of Weed Control in Grain Corn (*Zea mays*). *Weed Sci.* **1992**, *40*, 441–447.
43. Dierauer, H.-U.; Stöppler-Zimmer, H. *Unkrautregulierung ohne Chemie. 28 Tabellen*; Ulmer: Stuttgart, Germany, 1994.
44. Melander, B.; Rasmussen, I.A.; Bàrberi, P. Integrating physical and cultural methods of weed control—Examples from European research. *Weed Sci.* **2005**, *53*, 369–381. [CrossRef]
45. Silgram, M.; Shepherd, M.A. The Effects of Cultivation on Soil Nitrogen Mineralization. *Adv. Agron.* **1999**, *65*, 267–311.
46. Marshall, E.J.P.; Brown, V.K.; Boatman, N.D.; Lutman, P.J.W.; Squire, G.R.; Ward, L.K. The role of weeds in supporting biological diversity within crop fields. *Weed Res.* **2003**, *43*, 77–89. [CrossRef]
47. Aulrich, K.; Meyer, U.; Fischer, J.; Böhm, H. Futterwert von Mais-Bohnen-Silagen: Stangen- und Feuerbohnen im Vergleich. In *Ökologischen Landbau Weiterdenken: Verantwortung Übernehmen, Vertrauen Stärken*; Wolfrum, S., Heuwinkel, H., Reents, H.J., Hülsbergen, K.J., Eds.; Verlag Dr. Köster: Berlin, Germany, 2017; pp. 96–100.
48. Nurk, L.; Graß, R.; Pekrun, C.; Wachendorf, M. Methane Yield and Feed Quality Parameters of Mixed Silages from Maize (*Zea mays* L.) and Common Bean (*Phaseolus vulgaris* L.). *Bioenergy Res.* **2016**. [CrossRef]