Effects of Stand Density and N Fertilization on the Performance of Maize (Zea mays L.) Intercropped with Climbing Beans (Phaseolus vulgaris L.)

Daniel Villwock 1,*, Sabine Kurz 1, Jens Hartung 2 and Maria Müller-Lindenlauf 1

1 Institute for Applied Agricultural Research, Nürtingen-Geislingen University, Neckarsteige 6-10, 72622 Nürtingen, Germany; sabine.kurz@hfwu.de (S.K.); maria.mueller-lindenlauf@hfwu.de (M.M.-L.)
2 Institute of Crop Science, Biostatistics, University of Hohenheim, Fruwirthstr. 23, 70599 Stuttgart, Germany; jens.hartung@uni-hohenheim.de
* Correspondence: daniel.villwock@hfwu.de

Abstract: Maize is Germany’s most important fodder and energy crop. However, pure maize cultivation has ecological disadvantages. Moreover, its yield is low in crude protein, an important feed quality parameter. Maize–bean intercropping can potentially address both issues. A bean variety specially developed for intercropping was first introduced in 2016. Using this variety, a network of institutions conducted 13 field trials from 2017 to 2020 on four sites in Germany. We sought to determine the effects of stand density and nitrogen (N) fertilization on dry matter yield, crude protein yield, and soil mineral N content (N$_{\text{min}}$) at harvest of intercropped vs. pure maize. The three intercropping bean densities we tested (7.5, 5.5, and 4 plants/m$^2$) produced non-significantly different yields of dry matter or crude protein, given a maize density of 7.5–8 plants/m$^2$. Intercropping was inferior to pure maize in dry matter yield, but non-significantly different in crude protein yield. Under neither cropping strategy were significant losses in dry matter or crude protein yield recorded with reduced compared to full N fertilization. At full fertilization, however, both pure maize systems and the 8/4 maize–bean intercrop system left significantly higher N$_{\text{min}}$ at harvest than the other variants of the corresponding system or N fertilization level and thus an increased risk of nitrate leaching. We encourage further optimization of yield performance in maize–bean intercropping, e.g., through breeding or promotion of biological N fixation via rhizobia inoculation. Furthermore, we recommend reducing N fertilization levels in maize cultivation.

Keywords: maize; Zea mays; climbing bean; Phaseolus vulgaris; intercropping; stand density; nitrogen fertilization; crude protein

1. Introduction

Maize (Zea mays L.) is Germany’s most important fodder and energy crop, cultivated on roughly 20% of the total arable land [1]. However, pure maize cultivation is criticized for its negative ecological effects, including both high erosion risk due to poor soil cover for great parts of the season and low biodiversity, a common phenomenon in intensively managed agricultural landscapes [2,3].

In addition, pure maize cultivation is susceptible to nitrate leaching, especially when inappropriate fertilization strategies lead to nitrogen (N) over-fertilization, as in some regions of Germany [4,5]. Nitrate concentrations in groundwater are still frequently, albeit decreasingly, above the permissible limits, especially in agricultural regions [6]. Nitrate leaching problems have led to regulations limiting N use and increasingly strict limits on fertilization strategies [6], such as the German Fertilizer Ordinance (GFO) [7].

Where pure maize is grown for fodder, its cultivation additionally taxes the ecosystem indirectly. While maize silage is a superior feed for dairy cattle because it provides roughage with high food energy, its crude protein content, another important measure of feed quality,
is too low to permit its use without supplements. As Germany is not self-sufficient in crude protein, part of the demand must be covered by imports [8]. This can have negative ecological effects in countries from which the feed components rich in crude protein are imported.

Each of these shortcomings of maize cultivation can potentially be addressed by intercropping maize with other species. Intercropping can reduce erosion risk by providing greater soil cover, while the added roots of the intercrop help bind the soil on the surface [2]. Intercropping promotes biodiversity, not only through crop diversification but also by providing a habitat for a variety of insects and soil organisms that would not be present in a single crop environment [2,3]. Intercropping maize with legumes can reduce N fertilization requirements due to their ability to fix N via a symbiotic relationship with rhizobia, and also lessen the threat of nitrate leaching [9]. Maize–legume intercropping has also been shown to increase crude protein content and yield, reducing the need for protein supplements compared to pure maize [10].

The combination of maize with climbing bean (Phaseolus vulgaris L.) for human consumption has a long tradition in South America, where the mixture is often supplemented by squash and other species in the Milpa system [11]. In many countries of the tropics and subtropics, maize–bean intercropping has been tested and optimized, always with the aim of increasing use efficiency of limited resources, such as crop land and nutrients [12–19]. In particular, the climbing bean has been shown capable of meeting more than 60% of its N requirements through biological N fixation [16,17].

In temperate climates, maize and beans have been combined only in rare traditional cropping systems [20]. Maize–bean intercropping for silage production was first tested in Europe barely two decades ago, such as in promising field trials in the UK in 2000 and 2001 [21,22]. Since 2011, the system has been optimized in Germany [8,23–33]. In field trials, different agronomic parameters such as bean varieties, seed rates, bean seed dates, sowing techniques, and weed control methods have been assessed against yield performance. The dry matter yields have been mostly comparable between intercropping and pure maize cultivation, both in conventional [23–25] and organic farming [8,26], but crude protein yield with intercropping has been higher when sufficiently high bean yields were achieved [8,21].

Other studies have focused on the use of maize–bean silage as animal feed. An initial study reported that the feed value of maize–bean silage can be higher than that of pure maize due to the increased crude protein content provided by the bean [27]. A further study showed good ensilability and fermentability as well as good digestibility and feed quality of the maize–bean silage [28]. Dairy cattle feeding experiments have also demonstrated that maize–bean silage and pure maize silage are comparable in milk yield and quality [29].

Maize–bean silage has also been studied as a biogas substrate. An early study found that high bean proportions can negatively impact methane yield [30], but later studies with lower bean proportions more typical of those achieved in practice report comparable methane yields between the two sources of silage [24,25].

Still, despite promising results in yield and silage quality of maize–bean intercropping, the system suffered from a major disadvantage in its first years of use, namely, the faster juvenile development and shorter maturing time of the bean varieties in comparison to maize. For this reason, beans were sown considerably later than maize, so that the beans would not compete with the maize in its juvenile development and would reach maturity around maize harvest times. However, this resulted in relatively low bean yields, especially when early-maturing bean varieties were used with late sowing dates, as shown by Fischer et al. 2020 [8] based on field trials conducted in 2011 and 2012. They recommended late-maturing bean cultivars with a longer growth period, similar to maize, to achieve higher bean yields and crude protein content.

This improvement was made by Nurk et al. 2017 [23], who conducted field trials in 2014 and 2015 with the late-maturing and high-yielding bean cultivar “Anellino verde,” intercropped with the stable and competitive maize hybrid “Fernandez,” both recommended by commercial breeders. They tested the effects of sowing density, bean sowing time, and
several weed control methods on the yield performance across two years and at three sites. They found that intercropping with plant densities of 7.5 maize plants/m² and 5 or 7.5 bean plants/m² with an early sowing date can produce comparable dry matter yields to pure maize at 10 plants/m², while reaching bean proportions of 20–30%. Their results also indicated that maize plant density should be at least 7.5 plants/m²; otherwise, interspecific competition is too strong, resulting in considerably reduced yields. They also identified the pre-emergence herbicides Stomp (2.8 l/ha, active ingredient pendimethalin) and Spectrum (1.4 l/ha, active ingredient dimethanamid-P) as acceptable for chemical weed control. However, they concluded that the intercropping system they investigated was not practicable because, despite the early sowing date of the bean, sowing had to be done in two steps due to the different thousand-seed weight of maize and beans [23]. This causes additional workload and reduces the profitability of the system.

This obstacle was overcome by a breeding project seeking varieties especially tailored for intercropping. Maize varieties like the hybrid “KWS Figaro” were selected due to their high competitiveness and stability, allowing for high yields when planted with beans close to each other and carrying high proportions of bean mass without collapsing in later stages under the high fresh-matter weight of the bean [31]. In addition, breeders identified bean genotypes like “SAT512” with a thousand-seed weight comparable to that of maize, allowing for simultaneous sowing from one seed hopper. The new bean varieties showed good frost tolerance, slow youth development, and late maturity, allowing for simultaneous sowing with maize at the usual maize sowing dates and high biomass yields at the usual maize harvest dates [32].

What remains unclear is the stand density best used with these new maize and bean varieties to maximize both dry matter and crude protein yields and if these new maize and bean varieties achieve yields comparable to pure maize. Additionally, it has remained unclear how the maize–bean intercropping system will respond to different levels of N fertilization. Beans were expected to balance intercropping yields across N fertilization levels by taking advantage of biological N fixation. In contrast, pure maize yields were expected to decline with lower N fertilization. If both were the case, maize–bean intercropping would have an advantage over pure maize at reduced or without N fertilization.

Given this dynamic, the question arises whether common N fertilization levels would cause higher soil mineral nitrogen content ($N_{\text{min}}$) at harvest in intercropped vs. pure maize because of the additional N brought into the soil via biological fixation. If so, that would mean a higher risk of nitrate leaching than with pure maize. We assumed biological N fixation only when the soil supply was low, and further that bean plants would take up and transfer the fixed N into the aboveground biomass. When the supply was high, beans would take up N from the soil, as the uptake is energetically more efficient than biological N fixation. Hence, we assumed that intercropping would not increase, but rather decrease, $N_{\text{min}}$ at harvest compared to pure maize.

To answer these questions, a network of institutions conducted three series of trials with a total of 13 field trials with different stand densities and N fertilization levels on four sites in Germany between 2017 and 2020. The specific objectives were (i) to study the effect of bean density in the maize–bean intercropping system on dry matter and crude protein yield, (ii) to compare these intercropping yields with those of pure maize (iii) to determine the effect of N fertilization on dry matter and crude protein yield and (iv) to investigate and compare $N_{\text{min}}$ at harvest of maize–bean intercropping and pure maize.

2. Materials and Methods

2.1. Study Sites

The four sites belong to a network of research institutions in Germany, namely Nürtingen-Geislingen University (Tachenhausen), Centre for Renewable Resources of the Experimental and Education Center Agriculture Haus Düsse of the North Rhine-Westphalia Chamber of Agriculture (Haus Düsse), and Lower Saxony Chamber of Agriculture (Wehnen...
and Obershagen). The sites were distributed among Germany (Figure 1) and differ in their site conditions (Table 1).

Figure 1. Location of the four study sites Tachenhausen (TH), Haus Düsse (HD), Wehnen (WN), and Obershagen (OH) in Germany.

Table 1. Conditions of the four study sites.

| Parameter                               | Site          |
|-----------------------------------------|---------------|
|                                        | Tachenhausen (TH) | Haus Düsse (HD) | Wehnen (WN) | Obershagen (OH) |
| Elevation above sea level (m)           | 360           | 70             | 10          | 43             |
| Long-term mean annual temperature (°C)  | 10.2          | 10.5           | 9.3         | 9.2            |
| Long-term total annual rainfall (mm)    | 802           | 651            | 759         | 652            |
| Soil texture, according to [34]         | clay/silty loam (CL/SiL) | silty clay (SiC) | sand (S) | loamy sand (LS) |

2.2. Experimental Design

Three series of trials totaling 13 field trials were used in this study (Table 2). Trial series were performed in one to three years at four different sites (Table 3). Each series used a two-factor design with factors “System” and “N Fertilization” (Table 2). Treatment combinations were allocated to field plots according to a randomized complete block design or a block design with two structures of complete blocks. In the latter design, plots were arranged in two rows and twelve columns, where half-rows (six plots) and units of three columns and two rows (resulting in six plots, too) formed complete block structures. All trials were performed with four field replicates.

Table 2. Factor levels of the field trials.

| Factor          | Level       | Trial Series |
|-----------------|-------------|--------------|
|                 |             | 1  | 2  | 3  |
| System¹         | MB 7.5/7.5  | X ³ |   |   |
|                 | MB 7.5/5.5  | X  |   |   |
|                 | MB 8/4      | X  |   |   |
|                 | M 7.5–8     | X  | X |   |
|                 | M 10        | X  |   |   |
| N Fertilization² | Without     | 0% | 0% | 0% |
|                 | Reduced     | 50%| 66%| 40%|
|                 | Full        | 100%| 100%| 100%|

¹ Maize–bean intercropping (MB) or pure maize (M), numbers are plant densities (per m²). ² Percentages indicate shares of the maximum permitted amount according to GFO (100%). ³ X indicates that the level was tested within the trial series.
Table 3. Soil characteristics, N fertilization data and crop management dates of the 13 field trials.

| Parameter                                      | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial | Trial |
|------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Trial series ¹                                 | 1     | 1     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     |
| Year                                           | 2017  | 2017  | 2018  | 2018  | 2018  | 2019  | 2019  | 2020  | 2020  | 2019  | 2019  | 2020  | 2020  | 2020  | 2020  | 2020  | 2020  | 2020  | 2020  |
| Site ²                                         | TH    | HD    | TH    | WN    | OH    | WN    | OH    | WN    | OH    | TH    | HD    | TH    | HD    | TH    | HD    | TH    | HD    | TH    | HD    | TH    |
| Soil texture, according to ³ [34]              | CL    | SiC   | CL    | Si    | LS    | LS    | LS    | LS    | SiL   | SiC   | SiC   | SiL   | SiL   | SiL   | SiC   | SiC   | SiC   | SiC   | SiC   | SiC   |
| pH                                             | 6.7   | 6.6   | 6.9   | 4.9   | 6     | 5     | 6.3   | 5     | 6.1   | 6.2   | 6.6   | 6     | 6.6   | 6.5   | 6.5   | 6.5   | 6.5   | 6.5   | 6.5   | 6.5   |
| P₂O₅ (mg/100 g)                                | 24    | 33    | 21    | 12    | 4     | 10.5  | 7     | 8     | 5.7   | 10    | 18    | 9     | 23    | 23    | 23    | 23    | 23    | 23    | 23    | 23    |
| K₂O (mg/100 g)                                 | 25    | 14    | 23    | 5     | 8     | 6.1   | 14.5  | 7.9   | 19.6  | 38    | 22    | 14    | 19    | 19    | 19    | 19    | 19    | 19    | 19    | 19    |
| MgO (mg/100 g)                                 | 7.75  | 7     | 9.47  | 2     | 9     | 5.6   | 7     | 6     | 6.9   | 13    | 8     | 13    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    |
| N demand (kg/ha) ⁴                              | 230   | 242   | 230   | 177   | 206   | 159   | 180   | 189   | 216   | 230   | 230   | 230   | 230   | 230   | 230   | 230   | 230   | 230   | 230   | 230   |
| Nₘᵦspring (kg/ha) ⁴                            | 122   | 53    | 76    | 23    | 51    | 27    | 104   | 12    | 42    | 98    | 70    | 56    | 59    | 59    | 59    | 59    | 59    | 59    | 59    | 59    | 59    |
| Nₗₜg (kg/ha) ⁴                                 | 0     | 0     | 0     | 0     | 0     | 0     | 17    | 26    | 0     | 38    | 0     | 26    | 26    | 26    | 26    | 26    | 26    | 26    | 26    | 26    | 26    |
| Full N fertilizer amount, according to GFO ⁴ (kg/ha) | 108   | 189   | 154   | 154   | 154   | 154   | 132   | 76    | 160   | 148   | 132   | 122   | 174   | 145   | 145   | 145   | 145   | 145   | 145   | 145   |
| Reduced N fertilizer amount ⁵ (kg/ha)           | 54    | 95    | 103   | 102   | 102   | 87    | 50    | 107   | 97    | 53    | 49    | 70    | 58    | 58    | 58    | 58    | 58    | 58    | 58    | 58    | 58    |
| Nₘᵦsampling date                               | Apr. 20 | Apr. 26 | Apr. 24 | Mar. 23 | Apr. 13 | Mar. 22 | Mar. 28 | Mar. 15 | Apr. 27 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 | May 18 |
| Sowing date                                     | May 11 | May 9  | Apr. 27 | May 16 | May 14 | May 13 | May 15 | May 7  | May 13 | May 17 | May 7  | Apr. 16 | Apr. 27 | Apr. 27 | Apr. 27 | Apr. 27 | Apr. 27 | Apr. 27 | Apr. 27 | Apr. 27 |
| Harvest date                                    | Sep. 13 | Sep. 21 | Aug. 31 | Sep. 12 | Sep. 4 | Oct. 10 | Oct. 12 | Sep. 30 | Sep. 17 | Sep. 18 | Sep. 15 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 | Sep. 24 |
| Plant age at harvest (Days after sowing)        | 125    | 135   | 126   | 119   | 113   | 150   | 140   | 158   | 140   | 122   | 123   | 152   | 150   | 150   | 150   | 150   | 150   | 150   | 150   | 150   |

¹ trial series refer to Table 2. ² Tachenhausen (TH), Haus Düsse (HD), Wehnen (WN) or Obershagen (OH). ³ Clay loam (CL), silty clay (SiC), sand (S), loamy sand (LS), or silty loam (SiL). ⁴ The full nitrogen (N) fertilizer amount is the N demand minus the amounts of soil mineral N (Nₘᵦ) and organic N (Nₗₜg). ⁵ The reduced N fertilizer amount is 66%, 50% or 40% of the full N fertilizer amount, depending on the respective trial series.
The factor “System” comprised three maize–bean intercropping systems with varying bean densities and two pure maize systems used for reference (Table 2). The maize hybrids “KWS Figaro” (S 250) or “Benedictio KWS” (S 230) were grown. For intercropping systems, the bean variety “WAV512” (also known as “SAT512”) was used.

Data were imbalanced. In the first series, the density for intercropping was 7.5 plants/m² for both maize and beans (MB 7.5/7.5). This was adopted from a 2016 pre-trial showing good plant development and yields comparable to pure maize (data not published). Pure maize was tested with 7.5 and 10 plants/m² (M 10). The former replicates the maize density in the intercropped case, while the latter is the commonly used plant density in Germany. In the two following trial series, maize density remained almost constant at 7.5–8 plants/m² in pure maize (M 7.5–8) and intercropped, whereas bean density was reduced to 5.5 (MB 7.5/5.5) and 4 plants/m² (MB 8/4).

The factor “N fertilization” comprised three conditions defined by their percentage of the maximum amount of N fertilizer permitted by GFO [7]. Levels were categorized into “without fertilization”, meaning no N fertilizer was applied, a reduced level, and a full fertilization level. The latter was conducted with the maximum permitted amount of N fertilizer according to GFO. The reduced level used 66%, 50% or 40% of the maximum amount. The three were assumed to be similar in terms of crop performance (Table 2).

2.3. Crop management and Measurements

All trials were preceded by a winter cereal crop. In the trials in Haus Düsse, these main crops were followed by an intercrop. In spring, soil samples were taken from 0–30 cm depth for determination of phosphorus, potassium and magnesium contents; results are presented in Table 3. Common basic fertilization except for N took place prior to soil cultivation.

N fertilization amounts were calculated according to GFO to be those in Table 3. This calculation assumes a crop- and site-specific N demand, from which available quantities of $N_{\text{min}}$ in spring and organic N ($N_{\text{org}}$) are subtracted to give the full fertilization demand. Crop- and site-specific N demands were estimated for silage maize using local yield expectations. $N_{\text{min}}$ in spring was measured from soil samples taken from depth of 0–60 cm (trial series 1 and 2) or 0–90 cm (trial series 3); dates are reported in Table 3. $N_{\text{org}}$ subtractions came from culture-specific values for the preceding crops (main crops and catch crops), e.g., 20 kg/ha N for a non-freezing non-legume catch crop, and 10% of the organic N fertilization from the previous season (Table 3). N fertilization took place using one dose of the mineral N fertilizer calcium ammonium nitrate at the time of sowing.

Sowing was performed at usual local maize sowing dates (Table 3). Within one trial, all variants were sown with maize seeding machinery, with maize and bean seeds mixed prior to seeding and sown together in the same row. Seed rates were always higher than the targeted plant density. Exact plant densities were achieved by removing excess plants after field emergence. In most trials, chemical weed control was through the pre-emergence herbicides Stomp (2.8 l/ha, active ingredient pendimethalin) and Spectrum (1.4 l/ha, active ingredient dimethanamid-P), as recommended by [23]. If necessary, mechanical weed control was applied to keep the trials free from weeds.

Harvest took place at usual local harvest dates for silage maize at a plant age between 113 and 158 days after sowing (Table 3). Prior to machine harvest of mixed yields, bean and maize proportions were measured via manual harvest. Maize and bean plants from randomly selected subplots were separately cut, weighed and analyzed for moisture and N content. Afterwards, the remaining plot was harvested with maize harvest machinery, weighed, and analyzed for moisture content. Total dry matter yield was calculated by adding the dry matter yields from manual and machine harvest. Bean and maize dry matter fractions were then calculated from, respectively, bean and maize proportions. In the trials at Haus Düsse, the entire plots were harvested manually and used for determination of bean and maize fractions.

Crude protein content was calculated separately for maize and bean fractions by multiplying their N content by the standard factor of 6.25 [35]. Crude protein yield of
the fractions could then be obtained by multiplying the respective dry matter yield by its corresponding crude protein content. Total crude protein yield was then the sum of these two figures, and total crude protein content was calculated from that yield divided by the total dry matter yield.

After harvest, $N_{\text{min}}$ was measured in soil samples from 0–60 cm depth.

2.4. Statistical Analysis

Data were analyzed in a two stage approach [36] averaging across replicates in the first stage and then submitting estimated means and their standard error to the second stage analysis across the 13 trials. In the first stage, each trial was analyzed according to its design with the following model:

$$y_{ijk} = \mu + b_k + \tau_i + \varphi_j + (\tau \varphi)_{ij} + e_{ijk}$$  \hspace{1cm} (1)

where $y_{ijk}$ is the observation of system $i$ in N fertilization level $j$ and block $k$, $\mu$ is the intercept, $b_k$ is the fixed effect of block $k$, $\tau_i$ is the fixed effect of system $i$, $\varphi_j$ is the fixed effect of N fertilization level $j$, $(\tau \varphi)_{ij}$ is the fixed effect of the interaction of system $i$ and N fertilization level $j$, and $e_{ijk}$ is the error of $y_{ijk}$. In case of a block design with two structures of complete blocks, $b_k$ was replaced by the two complete block effects. Again, both block effects were taken as fixed, as blocks were complete.

Estimated mean values $\overline{y}_{ij}$ and their standard error $\hat{f}_{ij}$ for each site $m$ and year $l$ were then submitted to a common multi-environmental trial analysis [37] in the second stage. The model can be described as:

$$\overline{y}_{ijlm} = \mu + a_l + s_m + (as)_{lm} + \tau_i + \varphi_j + (\tau \varphi)_{ij} + (a \tau)_{il} + (a \varphi)_{jl} + (a \tau \varphi)_{ijl} + (s \tau)_{im} + (s \varphi)_{jm} + (s \tau \varphi)_{ijm} + (a \tau \varphi)_{ijlm} + \hat{f}_{ijlm}$$  \hspace{1cm} (2)

where $\overline{y}_{ijlm}$ is the estimated mean of system $i$ and N fertilization level $j$ at year $l$ and site $m$; $a_l$, $s_m$, and $(as)_{lm}$ are the random effects of year $l$, site $m$, and the trial in year $l$ and site $m$; $(a \tau)_{il}$, $(a \varphi)_{jl}$, $(a \tau \varphi)_{ijl}$, $(s \tau)_{im}$, $(s \varphi)_{jm}$, $(s \tau \varphi)_{ijm}$, $(a \tau)_{ilm}$, $(a \varphi)_{jlm}$, and $(a \tau \varphi)_{ijlm}$ are the random two-, three-, and four-way interaction effects of the corresponding main factors and $\hat{f}_{ijlm}$ is the estimated error from stage one. Note that $(a \tau \varphi)_{ijlm}$ and $\hat{f}_{ijlm}$ include the same indices, but a separation is possible because the latter is already known prior to second stage analysis. Pre-requisites of statistical analysis were checked in both stages graphically via residual plots.

In the case of finding significant differences via global F-test, means were compared using Fisher’s LSD test. Results of multiple comparisons were presented via letter display [38]. All analyses were performed using the procedure PROC MIXED in SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC, USA).

3. Results

For all yield-related dependent variables (dry matter yield, dry matter bean yield, crude protein content, crude protein yield, and crude protein bean yield), non-significant interactions between the factors system and N fertilization were found. Thus, marginal means for levels of each factor were presented (Table 4).
Table 4. Results for all yield-related dependent variables.

| Factor Level | DMY (t/ha) | DMYB (t/ha) | CPC (%) | CPY (t/ha) | CPYB (t/ha) |
|--------------|------------|-------------|---------|------------|-------------|
| System       |            |             |         |            |             |
| MB 7.5/7.5  | 18.7 ± 0.8 | 3.5 ± 0.9   | 7.4 ± 0.5 | 1.4 ± 0.1  | 0.4 ± 0.1   |
| MB 7.5/5.5  | 17.9 ± 0.6 | 2.8 ± 0.8   | 7.6 ± 0.4 | 1.3 ± 0.1  | 0.4 ± 0.1   |
| MB 8/4      | 19.2 ± 0.5 | 1.6 ± 0.6   | 6.7 ± 0.4 | 1.3 ± 0.1  | 0.2 ± 0.0   |
| M 7.5–8     | 20.3 ± 0.5 | 6.4 ± 0.3   | 1.3 ± 0.1 |            |             |
| M 10        | 22.3 ± 0.8 | 6.1 ± 0.5   | 1.4 ± 0.1 |            |             |
| p-value      | 0.0026     | 0.0859      | 0.0859  | 0.1194     |             |

N fertilization

| N fertilization | DMY (t/ha) | DMYB (t/ha) | CPC (%) | CPY (t/ha) | CPYB (t/ha) |
|-----------------|------------|-------------|---------|------------|-------------|
| without         | 18.8 ± 0.6 | 2.3 ± 0.6   | 6.2 ± 0.3 | 1.1 ± 0.1  | 0.3 ± 0.1   |
| reduced         | 20.3 ± 0.6 | 2.6 ± 0.6   | 6.8 ± 0.3 | 1.4 ± 0.1  | 0.3 ± 0.1   |
| full            | 19.9 ± 0.6 | 3.0 ± 0.6   | 7.5 ± 0.3 | 1.5 ± 0.1  | 0.4 ± 0.1   |
| p-value         | 0.0041     | 0.0024      | 0.0024  | 0.2235     |             |

System × N fertilization

| p-value | 0.3536 | 0.707 | 0.7841 | 0.8646 | 0.7258 |

Dry matter yield (DMY), dry matter bean yield (DMYB), crude protein content (CPC), crude protein yield (CPY), crude protein bean yield (CPYB). Means with at least one identical letter are not significantly different from each other (LSD-test, \( p < 0.05 \)). Derivations are standard errors of the mean. \( p \)-values refer to the global F-test of the corresponding factors.

3.1. Total Dry Matter Yield

Dry matter yield was significantly influenced by both system and N fertilization. The three intercropped systems did not differ significantly in dry matter yield. Both pure maize systems produced significantly higher yields than the intercropped systems and yield increased significantly with higher plant density in pure maize case. The system MB 8/4 displayed the highest yield among the intercropped systems. It was 1.1 t/ha (5.4%) lower than M 7.5–8 and 3.1 t/ha (13.9%) lower than M 10. M 7.5–8 was 2.0 t/ha (9%) lower than M 10 (Table 4). Full and reduced N fertilization did not differ significantly in dry matter yield, and only the level without fertilizer was significantly lower (Table 4).

3.2. Dry Matter Bean Yield

Dry matter bean yield was not significantly influenced by any factor studied and showed high variance (Table 4). Larger bean yield was observed with increasing bean densities (Table 4). However, despite having the lowest bean yield, the 8/4 maize–bean configuration had the highest total dry matter yield and therefore the highest maize yield. Thus, total dry matter yield remains constant, but bean proportions increase from 8% in MB 8/4 to 16% in MB 7.7/5.5 and 19% in MB 7.5/7.5 (Figure A1 in Appendix A).

There was an increase in dry matter bean yield with increasing N fertilization. The highest bean yield was reached in MB 7.5/7.5 under full fertilization, but with extremely low maize yield. In the trial at Tachenhausen, bean proportions in yield in MB 7.5/7.5 reached up to 45% (data not shown), causing parts of the maize plants to collapse under the high fresh matter weight of the bean, making harvest difficult.

3.3. Crude Protein Content

Crude protein content was significantly influenced by N fertilization (Table 4). Crude protein content significantly increased with higher N fertilization, both in stepping from the level without N fertilization to reduced fertilization and from that level to full fertilization (Table 4). Comparing systems, we can see that crude protein content tends to be smaller in pure maize systems, but variance is high, so the global F-test was not significant (Table 4).

Overall, crude protein content in beans was around 12–14%, whereas in maize, crude protein content was only around 5–7% (data not shown).
3.4. Crude Protein Yield

Crude protein yield was significantly affected by N fertilization (Table 4). Similar to dry matter yield, full and reduced N fertilization did not differ significantly in crude protein yield, whereas without N fertilization did produce significantly lower yields. In contrast to dry matter yield, the systems did not significantly differ in crude protein yield (Table 4). Crude protein yield of the pure maize systems was within the range of the intercropping systems.

3.5. Crude Protein Bean Yield

As with dry matter bean yield, crude protein bean yield was not significantly influenced by any factor under investigation (Table 4). Higher crude protein bean yields were observed with increasing bean density (Table 4). Due to the higher crude protein content of the bean, bean proportions in crude protein yield increased as compared to the bean proportions in dry matter yield, ranging from 16% in MB 8/4 to 28% in MB 7.7/5.5 and 29% in MB 7.5/7.5 (Figure A2 in Appendix A). Crude protein bean yield also tended to increase with higher N fertilization (Table 4). For MB 7.5/5.5 and MB 8/4, crude protein bean yield was only slightly higher under full N fertilization, for MB 7.5/7.5 full N fertilization led to very high bean yields (data not shown).

3.6. $\text{N}_{\min}$ Content at Harvest

Table 5 shows the result for $\text{N}_{\min}$ at harvest. Since we observed significant interactions between the factors system and N fertilization, we analyzed differences only for the combination of levels from both factors. Across all variants with the N fertilization levels without and reduced, $\text{N}_{\min}$ at harvest ranged from about 20 to 40 kg/ha with relatively high variances and no significant differences among these variants (Table 5). Under full fertilization, the two pure maize systems and MB 8/4 showed significantly higher $\text{N}_{\min}$ at harvest, compared to the other two intercropping systems as well as compared to the other two N fertilization levels within the respective system, with values of around 54 to 60 kg/ha. The difference in $\text{N}_{\min}$ at harvest between full and reduced N fertilization level was greatest in the M 10 system, over 40 kg/ha, followed by the M 7.5–8 system. Differences then narrow across intercropping systems with increasing bean plant density, so at MB 7.5/5.5 a tendency towards an increased $\text{N}_{\min}$ at harvest at full N fertilization exists, but not at MB 7.5/7.5.

Table 5. Result for $\text{N}_{\min}$ at harvest (kg/ha).

| System    | N fertilization |
|-----------|-----------------|
|           | Without         | Reduced | Full      |
| MB 7.5/7.5| 28.2 ±10.2      | 40.1 ±10.2 | 37.6 ±10.2 |
| MB 7.5/5.5| 23.1 ±7.5       | 25.2 ±7.5 | 37.8 ±7.5 |
| MB 8/4    | 25.3 ±6.2       | 33.7 ±6.1 | 54.2 ±6.1 |
| M 7.5–8   | 23.3 ±5.7       | 33.4 ±5.7 | 59.2 ±5.6 |
| M 10      | 20.9 ±10.2      | 19.6 ±10.2 | 60.3 ±10.2 |

$p$-value 0.0027

Means with at least one identical lower-case letter within a column indicate non-significant differences between systems; means with at least one identical upper-case letter within a row indicate non-significant differences between N fertilization levels (LSD-test, $p < 0.05$). Derivations are standard errors of the mean. The $p$-value refers to the global F-test of the factor interactions.

4. Discussion

From 2017 to 2020, a network of German research institutions conducted a total of 13 field trials on four sites to determine the effects of stand density and N fertilization on dry matter yield, crude protein yield, and $\text{N}_{\min}$ at harvest of intercropped vs. pure maize.
4.1. Effects of Stand Density on Yield Performance of Maize–Bean Intercropping

Our first objective was to study the effect of bean density in the maize–bean intercropping system on dry matter and crude protein yield. We found that the three different bean densities we tested (7.5, 5.5, and 4.0 plants/m²) did not produce significantly different yields of dry matter or crude protein, given a maize density of 7.5–8 plants/m².

Bean contributions to yields trended higher with increasing bean density, although not significantly because bean yields in the study were highly variable, both between and within trials. This was probably caused by adverse conditions for beans, such as drought, scarcity of naturally occurring rhizobia, or poor accuracy of seeding. However, increases from bean contributions were offset by decreases from maize contributions, precluding further yield gain through higher bean density. This can be explained by increasing competition for nutrients, water, and light as overall plant density increases.

Maize seems especially sensitive to such competition because it suffered from yield losses under all and especially under high bean densities. This was particularly evident in the intercropping trial in Tachenhausen with maize–bean plant densities of 7.5/7.5, where the bean yields were highest while maize yields were so reduced that by the end of the season, the maize plants actually collapsed under the high fresh matter weight of the bean, making harvest technically impossible. From this experience, we would not recommend to use MB 7.5/7.5 in an agronomic application.

Similar results were reported by Nurk et al. 2017 [23], who compared plant densities of 7.5 and 5.5 plants/m² for both maize and bean in all combinations. They report similar yields for maize plant densities of 7.5 plants/m² mixed with bean plant densities of 7.5 or 5.5 plants/m² in all of their four trials, where beans reached dry matter yield proportions of 15 to 30%. With maize plant density reduced to 5.5 and bean plant density at 7.5 plants/m², dry matter bean yield increased, but maize and total dry matter yield both decreased, confirming the findings in our study.

With regard to our first objective, we conclude that none of the intercropping stand density studied significantly maximized both dry matter and crude protein yields. We cannot recommend MB 7.5/7.5 for agronomic practice, and the other two intercropping systems, MB 7.5/5.5 and MB 8/4, have reciprocal yield performance advantages. The dry matter yield is slightly better using MB 8/4, which might be more important when using silage as a biogas substrate. But if the emphasis is on the use of silage as fodder for dairy cattle, then the slightly better crude protein yield of MB 7.5./5.5 is indicated.

4.2. Comparison of Yields between Maize–Bean Intercropping and Pure Maize

Our second objective was to compare maize–bean intercropping and pure maize with respect to dry matter and crude protein yield. We found that all three intercrop systems produced significantly lower dry matter yields than both the M 10 pure maize system, representing common agricultural practice in Germany, and the M 7.5–8 system, replicating the intercrop maize density. We also found significant differences in dry matter yield between M 10 and M 7.5–8 with larger yields coming from M 10. To consider intercropping as an alternative to pure maize, a comparison to M 10 more fairly represents the alternative against current practice. The yield losses from the intercrop system with the highest yield (MB 8/4) compared to M 10 were around 3.1 t/ha, a loss of 13.9%.

This is in line with Nurk et al. 2017 [23], who compared intercropping systems with maize density of 7.5 plants/m² and bean densities of 5 or 7.5 plants/m² to an M 10 system. In all four of their trials, where beans reached relevant proportions in the dry matter yield (15–30%), the yields of the maize–bean systems were around 1 to 5 t/ha lower compared to M 10. This finding held despite their having used the late-maturing bean variety “Anellino verde”, whose sowing date was later than the one of maize.

Fischer et al. 2020 [8] used maize–bean systems with five different climbing bean cultivars with sowing densities of 8 maize plants/m² and 6 bean plants/m² and again bean sowing dates later than maize. They compared intercropped to pure maize, both with common (M 11) and reduced (M 8) sowing densities and reported no significant differences
between intercropping systems with late-maturing bean cultivars and either M 8 or M 11. Again, different bean varieties and sowing dates limit the comparability of their findings to our study.

The only study using the same varieties as this study in an intercropping system where maize and bean are sown at the same time is that of Schulz et al. 2020 [25]. Additionally, they tested the bean variety “Anellino verde”. They applied intercropping sowing densities of 8 maize plants/m² and 4.5 bean plants/m² and compared the yields to a pure maize system at a reduced sowing density of 8 plants/m² (M 8). Both intercropping systems and M 8 did not significantly differ in dry matter yield. This shows that “Anellino verde” is comparable with the new varieties in terms of dry matter yield. However, the bean proportion in the yields obtained by Schulz et al. 2020 [25] were low, with 16.6% in one trial and only around 5% or lower in the other five trials.

Here we should note that in our trials and in those in [23], stand densities were achieved through excess sowing densities and subsequent removal of excess plant. In contrast, Refs. [8,25] used fixed sowing rates. We observed in our trials that field emergence rates are almost always below 100%, and generally lower for beans than for maize. This is in line with Fischer et al. 2020 [8], who report a high variance in field emergence rates for both maize and beans. In one year, they had very low maize emergence rates, resulting in a very low overall yield with high proportions of beans. In the second year, the early-sown beans had very low field emergence rates, resulting in low bean contributions to yield. In both years, M 11 displayed lower field emergence rates than M 8. This may explain why the systems did not differ significantly in yield. Due to the low bean proportions reported by [25], we suspect a low field emergence rate, especially for the bean, and thus a reduced bean plant density compared to the applied sowing density. Under this assumption, maize–bean and pure maize systems had comparable maize plant densities with just minor competition from beans in the intercrop systems. This might explain why the yields were not different in their study.

While we found significant dry matter yield loss in the intercrop systems, non-significant differences were found in crude protein yield between intercropped and pure maize. This can be explained by the higher crude protein content of the bean that causes a slightly increased protein content in intercropping as compared to pure maize that offsets the lower dry matter yield of the intercropping systems. The higher contribution of the bean to crude protein yield as compared to dry matter yield can also be seen by higher proportions of the bean to crude protein yield as compared to dry matter yield. However, the bean contributions to crude protein yield were not high enough to achieve a significantly higher crude protein yield in the total yield of maize–bean intercropping as compared to pure maize, independent of bean density. Since the maize plants cannot carry the higher fresh matter weights of the bean plants, bean contributions to dry matter yield cannot increase. This means that with these varieties it is hardly possible to outperform pure maize in terms of crude protein.

Fischer et al. 2020 [8] achieved higher crude protein yield in maize–bean intercropping compared to pure maize only in one year where maize suffered from very low field emergence rates, resulting in poor overall yield level and allowing for bean proportions of 34–39%. This is in the range where maize plants in our study collapsed under the fresh matter weight of the bean at a normal overall yield level. For the other year, where bean proportions were much lower, crude protein yield in maize–bean intercropping was comparable to that of pure maize, matching the results of our study.

Concerning our second objective, we must say that intercropping is inferior to pure maize in terms of dry matter yield and comparable in terms of crude protein yield, and these relations hold irrespective of stand density. Therefore, we encourage research to further optimize yield performance for maize–bean intercropping and continue breeding activities on adapted maize and bean varieties. Recent breeding activities demonstrated that genetic potentials for yield increases, both in dry matter and crude protein yield, are still high, so maize–bean might possibly outperform pure maize in the future, especially
under reduced N fertilization. In a single field trial carried out by Leiser et al. (2021) [39], with new genotypes in the density of 8 maize and 4 bean plants/m² and pure maize with 10 plants/m² as reference, both at reduced N fertilization (65 kg/ha N), intercropping with the best bean genotypes achieved dry matter yield of 116%, crude protein content of 133%, and crude protein yield of 167% as compared to pure maize.

4.3. Effects of N fertilization on Yield Performance of Maize–Bean Intercropping and Pure Maize

Our third objective was to determine the effect of N fertilization on dry matter and crude protein yield in maize–bean intercropping and pure maize. In our study, dry matter and crude protein yield performance of both intercropping and pure maize systems were affected in the same way by N fertilization levels. Yields did not significantly differ under full and reduced N fertilization whereas without N fertilization, yields were significantly lower. This was in contrast to the assumption that the beans would balance intercrop yields across all N fertilization levels by taking advantage of biological N fixation, thereby offsetting yield losses in maize through higher bean yields. However, in our study bean yields of both dry matter and crude protein tended to decline with lower N fertilization.

That our intercrop systems did not show the expected independence of yield performance from N fertilization levels could be explained as a result of symbiosis with rhizobia not being reliably achieved in all our trials. At the study’s Tachenhausen site in 2019, high amounts of bean root nodules were found in a companion study in the direct neighborhood of trials 10 and 12 [40]. At this site and year, yields in the intercrop system were constant at all N fertilization levels, whereas pure maize yields increased with increasing N fertilization [41]. In contrast, only a few nodules were found in random samples taken at our other study sites at Wehnen and Obershagen [42], as well as at Haus Düsse. This could indicate a scarcity or complete absence of naturally occurring rhizobia. In any case, we were not able to show that intercrop systems generally have a yield advantage under reduced N fertilization over pure maize systems.

The key factor here in achieving stable yields at reduced N fertilization might be effective symbiosis with rhizobia. To this end, the Nürtingen-Geislingen University will conduct this year further field trials at both Tachenhausen and Haus Düsse within the project “GEMABO” [43] to investigate whether an inoculation with specific rhizobia strains can promote biological N fixation in beans under different N fertilization levels.

Our results are in line with Schulz et al. 2020 [25], who also applied three N fertilization levels in one of their trials, namely 0%, 50%, and 100% of the required N demand of maize. As we report, they found that N fertilization had a significant effect on dry matter yield of both intercropped and pure maize. The 100% and 50% levels were not significantly different, whereas 0% produced significantly reduced yields.

Cossel et al. 2017 [24] also applied three N fertilization levels. Their levels were absolute amounts of N, namely 135, 90, and 0 kg/ha. These levels are in the range of the levels studied here. Although they applied the three N levels only to pure maize systems, limiting N applications in intercrop systems to the mid-level N, the pure maize effects they recorded agree with the results of our study. They report no differences in dry matter yield for all three levels in one year and a significantly reduced yield in the level without N fertilization in the other year.

The results of our study, bolstered by the findings in the literature, demonstrate that a sizable reduction in N fertilization from the maximum rate permitted by GFO does not lead to significant yield losses in either an intercropping or pure maize system. This shows that reduced N fertilization levels are sufficient for maize to reach its yield potential. Our findings of increased N_{min} at harvest from full N fertilization in the pure maize systems show that full fertilization under GFO exceeds the demands of maize. N overfertilization in maize cultivation is also known from practice and leads directly to a high risk of nitrate leaching [5]. Therefore, we encourage policy makers to adjust the GFO numbers and practitioners to reduce N fertilization rates in maize cultivation.
4.4. Effects of Stand Density × N fertilization on N$_{\text{min}}$ at Harvest after Maize–Bean Intercropping and Pure Maize

Our fourth objective was to investigate and compare N$_{\text{min}}$ at harvest of maize–bean intercropping and pure maize. Under full fertilization, the two pure maize systems and MB 8/4 showed significantly higher N$_{\text{min}}$ at harvest, compared to the other two intercropping systems as well as compared to the other two N fertilization levels within the respective system. This shows that the bean does not add additional N to the soil, as had been expected in advance, and that under full fertilization, beans contribute to the uptake of mineral N from fertilization, thereby reducing, rather than increasing, the risk of nitrate leaching with increasing bean density.

Schulz et al. 2020 [25] also compared N$_{\text{min}}$ at harvest and at the end of the growing period between intercropped and pure maize. Intercropping did not lead to significantly higher N$_{\text{min}}$ in four of the five trials they performed. In the one trial, where a significant difference was measured, no mineral N fertilizer had been applied, resulting in low N$_{\text{min}}$ levels overall. In the trials with fertilization, N$_{\text{min}}$ at harvest were much higher but without differences between intercropped and pure maize, confirming the results of our study. They further found that from harvest to the end of the growing period, N$_{\text{min}}$ increased in most trials or remained the same. At the end of the growing period, N$_{\text{min}}$ was equal in intercropped and pure maize.

However, nitrate leaching predominantly happens within the period after the growing period. Until then, N$_{\text{min}}$ levels can still accrue from mineralization of crop residues, thereby increasing the risk of nitrate losses over the leaching period. On the other hand, N$_{\text{min}}$ levels can also decrease up to the end of the growing period, either due to N uptake of the following crop or immobilization via soil microorganisms, which can lead to a higher N supply for the following crop in the following year.

The actual risk of nitrate leaching after intercropped and pure maize under varying N levels, as well as the potential effects of crop residues on the following crop, are the focus of current studies at the Nürtingen-Geislingen University within the project “GEMABO” [43]. Preliminary results from our study site at Tachenhausen, the site with abundant nodule symbiosis, show no increased risk of nitrate leaching and a slight positive effect on the yield of the following crop after maize–bean intercropping compared to pure maize [41].

In view of our findings, the advantages of intercrop systems are currently most likely found in ecological benefits. Further research at Nürtingen-Geislingen University focuses on the effects of intercropping on soil erosion [43] as well as the impact on biodiversity [44]. According to the first study, biodiversity in maize–bean intercropping is only slightly increased as compared to pure maize [45].

5. Conclusions

After intensive research and optimization in the last decades, maize–bean intercropping has reached an important step in agronomic development. The simultaneous sowing of both intercropping partners is technically feasible, and there are only minor differences to the cultivation of pure maize.

The three intercropping bean densities we tested (7.5, 5.5, and 4 plants/m$^2$) produced non-significantly different yields of dry matter or crude protein, given a maize density of 7.5–8 plants/m$^2$, but, at a bean density of 7.5 plants/m$^2$, the mass of the beans makes it impractical to harvest. Hence, from the systems under investigation, we recommend an intercrop maize density of 7.5–8 plants/m$^2$ with bean densities of 5.5 or 4 plants/m$^2$.

The first commercially available seed mixtures usually contain a mixing ratio of two-thirds maize and one-third bean, allowing, for example, a sowing rate of 8/4 plants. We encourage seed companies to analyze field emergence rates in practice and adjust bean proportions in seed mixtures so that the desired stand density can actually be achieved.

Our study demonstrated that intercrop systems are inferior to pure maize in dry matter yield and comparable in crude protein yield. Therefore, we encourage research to further
optimize yield performance of maize–bean intercropping, especially under low N fertilization, e.g., through breeding or promotion of biological N fixation via rhizobia inoculation. Under neither cropping strategy were significant losses in dry matter or crude protein yield recorded with reduced compared to full N fertilization. With full fertilization, both pure maize systems and the 8/4 maize–bean intercrop system left significantly higher $N_{\text{min}}$ at harvest than the other variants of the corresponding system or N fertilization level. These higher levels mean an increased risk of nitrate leaching. Therefore, we encourage policy makers to adjust N fertilization guidelines, but we urge practitioners to reduce N fertilization in current maize production without waiting for new policy guidance.

Author Contributions: Conceptualization, methodology, validation, investigation and data curation, D.V. and S.K.; formal analysis, J.H. and D.V.; visualization and writing—original draft preparation, D.V.; writing—review and editing, S.K., J.H. and M.M.-L.; funding acquisition and project administration, M.M.-L. and S.K.; supervision, M.M.-L.; All authors have read and agreed to the published version of the manuscript.

Funding: KWS SAAT SE & Co. KGaA funded the field trials 1–9. Field trials 10–13 and the APC were funded by the German Federal Ministry of Food and Agriculture (BMEL) within the project “GEMABO” (grant number: 22027716).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to third-party usage rights.

Acknowledgments: We thank KWS SAAT SE & Co. KGaA for the permission to use the data from field trial 1–9 and the donation of seeds for the field trials 10–13. We also thank Thekla-Karina Niehoff for management of field trials at Wehnen and Obershagen, Michael Dickeduisberg for management of field trials at Haus Düsse, and Khaliun Sukhbaatar for support with data collection and preparation at Tachenhausen. Special thanks to Walter Schmidt for the idea and his passionate accompaniment of the maize–bean intercropping research. Charles Duquette deserves credit for his editorial assistance.

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

Appendix A

Figure A1. Proportions of maize and bean (%) in dry matter yield.
Figure A2. Proportions of maize and bean (%) in crude protein yield.

References

1. Statistisches Bundesamt. Arable Land after the Main Groups and Crops. Available online: https://www.destatis.de/EN/Themes/Economic-Sectors-Enterprises/Agriculture-Forestry-Fisheries/Field-Crops-Grassland/Tables/arable-land-after-the-main-groups-and-crops.html (accessed on 15 March 2022).

2. Lithourgidis, A.S.; Dordas, C.A.; Damalas, C.A.; Vlachostergios, D.N. Annual intercrops: An alternative pathway for sustainable agriculture. *Aust. J. Crop Sci.* 2011, 5, 396–410.

3. Hufnagel, J.; Reckling, M.; Ewert, F. Diverse approaches to crop diversification in agricultural research. A review. *Agron. Sustain. Dev.* 2020, 40. [CrossRef]

4. Herrmann, A. Biogas Production from Maize: Current State, Challenges and Prospects. 2. Agronomic and Environmental Aspects. *Bioenerg. Res.* 2013, 6, 372–387. [CrossRef]

5. Volkers, K.C. Auswirkungen einer variierten Stickstoff-Intensität auf Leistung und Stickstoff-Bilanz von Silomais in Monokultur sowie einer Ackerfutterbau-Fruchtfolge auf sandigen Böden Norddeutschlands. Ph.D. Thesis, Christian-Albrechts-Universität zu Kiel, Kiel, Germany, 2005.

6. Bundesministerium für Umwelt, Naturschutz und nukleare Sicherheit BMU; Bundesministerium für Ernährung und Landwirtschaft. Nitratbericht 2020. Available online: https://www.bmu.de/fileadmin/Daten_BMU/Download_PDF/Binnengewaesser/nitratbericht_2020_bf.pdf (accessed on 15 March 2022).

7. Bundes-Ministerium der Justiz. Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen: Düngeverordnung vom 26 Mai 2017 (BGBl. I S. 1305), die zuletzt durch Artikel 97 des Gesetzes vom 10 August 2021 (BGBl. I S. 3436) geändert worden ist, 2017; Bundes-Ministerium der Justiz: Berlin, Germany, 2017.

8. Fischer, J.; Böhm, H.; Heß, J. Maize-bean intercropping yields in Northern Germany are comparable to those of pure silage maize. *Eur. J. Agron.* 2020, 112, 125947. [CrossRef]

9. Hauggaard-Nielsen, H.; Ambus, P.; Jensen, E.S. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. *Nutr. Cycl. Agroecosyst.* 2003, 65, 289–300. [CrossRef]

10. Abdollah, J.; Adel, D.; Mohammadi, N.; Aziz, J.; Hosein, J. Forage yield and quality in intercropping of maize with different legumes as double-cropped. *J. Food Agric. Environ.* 2009, 7, 163–166.

11. Moreno-Calles, A.I.; Casas, A.; García-Frapolli, E.; Torres-García, I. Traditional agroforestry systems of multi-crop “milpa” and “chichipera” cactus forest in the arid Tehuacán Valley, Mexico: Their management and role in people’s subsistence. *Agrofor. Syst.* 2012, 84, 207–226. [CrossRef]

12. Nassary, E.K.; Baijukya, F.; Ndadikemi, P.A. Assessing the Productivity of Common Bean in Intercrop with Maize across Agro-Ecological Zones of Smallholder Farms in the Northern Highlands of Tanzania. *Agriculture* 2020, 10, 117. [CrossRef]

13. Morgado, L.B.; Willey, R.W. Optimum plant population for maize-bean intercropping system in the Brazilian semi-arid region. *Sci. Agric.* 2008, 65, 474–480. [CrossRef]

14. Albino-Garduño, R.; Turrent-Fernández, A.; Cortés-Flores, J.L.; Livera-Muñoz, M.; Mendoza-Castillo, M.C. Root distribution and solar radiation in maize-bean intercropping systems. *Agrociencia* 2015, 49, 513–531.

15. Molatudi, R.L. Grain yield and biomass response of a maize/dry bean intercrop to maize density and dry bean variety. *Afr. J. Agric. Res.* 2012, 7, 3139–3146. [CrossRef]

16. 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. In *Enhancement of Biological Nitrogen Fixation of Common Bean in Latin America: Results from an FAO/IAEA*
Agriculture 2022, 12, 967

Co-ordinated Research Programme, 1986–1991; Bliss, F.A., Hardarson, G., Eds.; Springer: Dordrecht, The Netherlands, 1993; pp. 93–101. ISBN 978-94-011-2100-2.

17. Moreira, L.P.; Oliveira, A.P.S.; Ferreira, E.P.d.B. Nodulation, contribution of biological N2 fixation, and productivity of the common bean (Phaseolus vulgaris L.) inoculated with rhizobia isolates. *Aust. J. Crop Sci.* 2017, 11, 644–651. [CrossRef]

18. Ndungu-Magiroi, K.W.; Wortmann, C.S.; Kibunja, C.; Senkoro, C.; Mwangi, T.J.K.; Wamie, D.; Kifuko-Koech, M.; Msakyi, J. Maize-bean intercrop response to nutrient application relative to maize sole crop response. *Nutr. Cycl. Agroecosyst.* 2017, 109, 17–27. [CrossRef]

19. Latati, M.; Bargaz, A.; Belarbi, B.; Lazali, M.; Benlahrech, S.; Tellah, S.; Kaci, G.; Drevon, J.J.; Ounane, S.M. The intercropping common bean with maize improves the rhizobial efficiency; resource use and grain yield under low phosphorous availability. *Eur. J. Agron.* 2016, 72, 80–90. [CrossRef]

20. Schmidt, W. Gemengeanbau von Mais mit Bohnenarten—Neuer koevolutiver Züchtungsansatz. Available online: https://www.landwirtschaftskammer.de/duessel/znr/pdfs/2014/2014-06-26-energiepflanzentag-03.pdf (accessed on 15 March 2022).

21. 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]

22. 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]

23. Nürk, L.; Graß, R.; Pekrun, C.; Wachendorf, M. Effect of Sowing Method and Weed Control on the Performance of Maize (Zea mays L.) intercropped with Climbing Beans (Phaseolus vulgaris). *Agricultrure* 2017, 7, 51. [CrossRef]

24. von Cossel, M.; Möhring, J.; Kiesel, A.; Lewandowski, I. Methane yield performance of amaranth (Amaranthus hypochondriacus L.) and its suitability for legume intercropping in comparison to maize (Zea mays L.). *Ind. Crops Prod.* 2017, 103, 107–121. [CrossRef]

25. Schulz, V.S.; Schumann, C.; Weisenburger, S.; Müller-Lindenlauf, M.; Stolzenburg, K.; Möller, K. Row-Intercropping Maize (Zea mays L.) with Biodiversity-Enhancing Flowering-Partners—Effect on Plant Growth, Silage Yield, and Composition of Harvest Material. *Agriculture* 2020, 10, 524. [CrossRef]

26. Fischer, J.; Höppner, F.; Böhm, H. Gemengeanbau von Mais mit Phaseolus-Bohnen: Einfluss von Sorte und Saatdichte der Bohnen auf die Bestandszusammensetzung. *Mitt. Ges. Pflanzenbauwiss.* 2015, 27, 177–178.

27. Fischer, J.; Böhm, H. Ertrag und Futterwert von Mais-Bohnen Gemengen als Ganzpflanzensilage in der Milchviehfütterung. In *Beiträge zur 12. Wissenschaftstagung Ökologischer Landbau: Ideal und Wirklichkeit: Perspektiven ökologischer Landbewirtschaftung*; Neuhoff, D., Ed.; Köster: Berlin, Germany, 2013; pp. 470–471. ISBN 9783895748158.

28. Böhm, H.; Aulrich, K.; Barth, K.; Bussemas, R.; Fischer, J.; Höppner, F.; Kälber, T.; Meyer, U.; Weißmann, F. Verbesserung der Protein- und Energieversorgung bei Wiederkäuern und Monogastriern durch Gemengeanbau von Mais mit Stangen- und Feuerbohnen. In *Tagungsband: Kongress „Hülsenfrüchte— Wegweiser für eine nachhaltigere Landwirtschaft”*; 3. und 4. November 2016 in Berlin; Bundesanstalt für Landwirtschaft und Ernährung, Ed.; Bundesministerium für Ernährung und Landwirtschaft: Bonn, Germany, 2016; pp. 46–48.

29. Jilg, T.; Jilg, A.; Ismail, M.; Brugger, D. Einsatz von Mais-Stangenbohnen-Silage in der Milchviehfütterung, Ergebnisse des Silocontrollings. In *Mais-Bohnen-Gemenge*; Tagung des Ausschusses für Futterkonservierung und Fütterung im Deutschen Maiskomitee e.V. (DMK); DMK, Ed.; Deutsches Maiskomitee e.V.: Bonn, Germany, 2021; pp. 45–51.

30. Nürk, L.; Graß, R.; Pekrun, C.; Wachendorf, M. Maize-Bean intercrop response to nutrient application relative to maize sole crop response. *Nutr. Cycl. Agroecosyst.* 2017, 109, 17–27. [CrossRef]

31. Hoppe, J.; Höppner, F.; Böhm, H. Development of biogas maize cultivars for intercropping with climbing beans. Ph.D. Thesis, Nürtingen-Geislingen University, Nürtingen, Germany, 2018.

32. Starke, M. Selection of climbing bean varieties (Phaseolus vulgaris L.) for mixed cropping with maize. Ph.D. Thesis, University of Göttingen, Göttingen, Germany, 2015.

33. Möhring, J.; Piepho, H.-P. Comparison of Weighting in Two-Stage Analysis of Plant Breeding Trials. *Crop Sci.* 2009, 49, 1977–1988. [CrossRef]

34. Piepho, H.-P.; Michel, V. Considerations on regional evaluation of cultivar trials (in German: Überlegungen zur regionalen Auswertung von Landessortenversuchen). *Inform. Biom. Und Epidemiol. Med.* 2000, 31, 123–136.

35. Peter, R. Effekte von N-Düngung auf die Ausbildung von Knöllchen bei Stangenbohnen (Phaseolus vulgaris) im Gemengeanbau mit Mais (Zea mays). Master’s Thesis, Nürtingen-Geislingen University, Nürtiingen, Germany, 2021.
41. Villwock, D.; Müller-Lindenlauf, M. Auswirkungen des Mais-Stangenbohnen-Gemengeanbaus auf den Stickstoffhaushalt. In *Mais-Bohnen-Gemenge*; Tagung des Ausschusses für Futterkonservierung und Fütterung im Deutschen Maiskomitee e.V. (DMK); DMK, Ed.; Deutsches Maiskomitee e. V. (DMK): Bonn, Germany, 2021; pp. 28–32.

42. Niehoff, T.-K. Einfluss der N-Düngung auf Ertrag, Protein und Rest Nmin-Werte des Mais-Stangenbohnen-Mischbestandes. In *Mais-Bohnen-Gemenge*; Tagung des Ausschusses für Futterkonservierung und Fütterung im Deutschen Maiskomitee e.V. (DMK); DMK, Ed.; Deutsches Maiskomitee e. V. (DMK): Bonn, Germany, 2021; pp. 23–27.

43. Fachagentur Nachwachsende Rohstoffe e.V. Ökologische und ökonomische Bewertung des Gemengeanbaus von Mais (Zea mays L.) mit Stangenbohnen (Phaseolus vulgaris L.) unter besonderer Berücksichtigung der Auswirkungen auf Stickstoffbilanz und Biodiversität—Akronym: GeMaBo. Available online: https://www.fnr.de/index.php?id=11150&fkz=22027716 (accessed on 4 March 2022).

44. Landwirtschaftliches Technologiezentrum Augustenberg. Diversifizierung im Silomaisanbau. Available online: https://ltz.landwirtschaft-bw.de/pb/,Lde/1816954_1933290_1921233_5014658_4689642_5908116 (accessed on 4 March 2022).

45. Hüber, C.; Zettl, F.; Hartung, J.; Müller-Lindenlauf, M. The impact of maize-bean intercropping on insect biodiversity. *Basic Appl. Ecol.* 2022, 61, 1–9. [CrossRef]