Laboratory and field experiment evaluation of alternatives for maize in biogas production

Dissertation to obtain the academic degree

Doctor of Natural Sciences (Dr. rer. nat.)

Doktorin der Naturwissenschaften (Dr. rer. nat.)

Department of Grassland Science and Renewable Plant Resources

University of Kassel

Liina Nurk

Witzenhausen, February 2017

This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree Doctor of Natural Sciences (Dr. rer. nat.)

Supervisor: Prof. Dr. Michael Wachendorf (University of Kassel)

Co-Supervisor: Prof. Dr. Carola Pekrun (Hochschule für Wirtschaft und Umwelt Nürtingen-Geislingen)

Day of Defence: 3rd May, 2017

List of papers

Chapter 2: Nurk L., Bühle L., Wachendorf M. (2016) Degradation of fibre and non-fibre fractions during anaerobic digestion in silages of maize, sunflower and sorghum of different maturities. *BioEnergy Research*, 9, 720–730

Chapter 3: Nurk L., Graß R., Pekrun C., Wachendorf M. (2017) Effect of sowing method and weed control on the performance of maize (*Zea mays* L.) intercropped with climbing beans (*Phaseolus vulgaris* L.). *Agriculture,* submitted

Chapter 4: Nurk L., Graß R., Pekrun C., Wachendorf M. (2016) Methane yield and feed quality parameters of mixed silages from maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.). *BioEnergy Research*, 10, 64-73

Table of contents

Tables

Figures

Abbreviations

ADF	acid detergent fibre
ADL	acid detergent lignin
B	bean
BBCH	a scale used to identify the phenological development stages of a plant
CS	crop species
DM	dry matter
e	early
FM	fresh matter
GRU	Grub
l	late
l_N CH_4^{-1} kg oDM	standard litre methane per kilogram organic dry matter
M	maize
N	total nitrogen
NDF	neutral detergent fibre
NDO	non-determined organics
OM	organic matter
SD	sampling date
TAC	Tachenhausen
XP	crude protein
WIZ	Witzenhausen

1. General introduction

In Germany the agricultural area occupied by maize in 2015 comprises of approximately 2.5m ha (Statistisches Bundesamt 2015) making up 21 % of the entire arable land. In comparison to that wheat was cultivated on 3.3m ha covering 28 % of the arable land, however, the surface area productivity of maize is 1.7 times higher than of wheat. The high incidence of maize in Germany can be explained by a political decision that has set the goal to increase the amount of renewable energy resources up to 20 % of the total produced electricity until 2020 (EU 2013). Thus, it is not surprising that 2.1m ha of maize were cultivated for silage, whereby 0.9m ha (that is 35 % of the whole maize cultivating area) were grown for biogas production and 1.2m ha were used for animal feed. As a renewable resource maize is an energy-efficient crop. It is possible to gain 110 m^3 methane t^{-1} FM or 5.000 m^3 methane ha^{-1} resulting in 47.000 kWh ha^{-1} (FNR 2016). Another advantage of maize, in contrast to other renewable energy resources like wind and solar energy, is its ability to be used as an energy carrier at any time of the year as the biomass can be easily stored and applied on demand.

Maize has been intensively cultivated in Germany since the beginning of the 1970s (Statistisches Bundesamt 2015). Since that time, the breeding has been successful in growing cultivates that can vegetate in these latitudes and achieve high total yields. Moreover, the breeders have also managed to cultivate maize that serves the needs of farmers to use the maize either as animal feed or for the production of biogas. As a C4 plant, maize has higher water use efficiency than C3 plants such as wheat, potatoes or sugar beet, but it also needs warmer conditions in the youth development. Nevertheless the photosynthesis mechanisms of C4 plants allow the plants to make use of the warm conditions in summer leading to high biomass yields (Amon et al. 2007b). The farming techniques for seeding and harvesting maize are well established and no special machinery is needed as it is the case for other alternative energy crops like sunflower, sorghum, cup plant or miscanthus (Amon et al. 2007a). After proper harvest and ensilage, maize can be used as a feed or for biogas production any time over the year (Herrmann 2013).

Recently the increased cultivation of maize has been criticised because of its associated role in changing the landscape scenery by its growth habit and the cultivation as a monoculture (Herrmann 2013). From a societal perspective maize is seen to decrease the landscape's aesthetic aspects as well as its ecosystem and recreational services (van Zanten et al. 2014; van Vliet et al. 2015; Lupp et al. 2011; Gutzler et al. 2015). Besides the aesthetic aspect of landscape transformation by monocultures, also ecological changes can be noticed which have negative effects on the soil biology and the biodiversity of the agroecosystem, like degradation of species richness and deterioration of soil properties (Lithourgidis et al. 2011). One negative influence of maize cropping on the landscape is the increased risk of soil erosion because of the late seeding time of maize and its long youth developing time (Böttinger 2013). Further, growing maize requires a high nitrogen availability which can only be ensured by the application of fertilizers. This increases the risk of a nitrogen contamination of the ground water, if overfertilization takes place (Herrmann 2013).

In order to minimize the negative impacts of maize one can consider different strategies. The problem of N leaching can be targeted by a proper application of fertilizer and site specific optimisation of the cultivation method. Another important management aspect to reduce negative ecological influences is to follow proper crop rotation recommendations. Cross compliance (European Commission 2016) as a document with sustainable management strategies also helps suppress the risk of pests and even promote the soil to maintain its fertility.

In literature, various experiments can be found focussing on other energy crops that might be used to substitute maize like cup plant (*Silphium perfoliatum*), sunflower (*Helianthus annuus* L.), sorghum-sudangrass (*Sorghum sudanense* L. x *S. bicolor* L.). These studies aimed to investigate the potential of these plants to be used as renewable energy resources. The key finding of those studies is that up to now difficulties in finding the proper cultivation practice, harvesting these plants with common techniques and the lower total yield than maize still limit their broad cultivation. Additionally, the biogas production potential of these crops is lower than of maize (Appels et al. 2011). In order to increase the biodiversity and enrich the landscape aesthetics it is also feasible to seed flowering stripes at the edges of the maize fields. The biomass of those flowering strips could also be used for biogas production, even though the biogas potential of the flowering strips is 50-75 % less than that of maize (Döhler 2013).

Another alternative to mono-cropping is the application of a double-cropping system. This is the practice of growing two or more crops on one field during a single growing season. After the first crop has been harvested the second crop is planted (Graß et al. 2013). As described by Graß et al. (2013), double-cropping systems tend to have significantly higher yield stability than sole copping systems. The cultivation of two crops within one year also lowers the risk of harvest losses due to weather extremes, because the risk is shared among two crops. This advantage of double cropping systems is getting increasingly important with regard to climate change (Graß et al. 2013). This system could contribute to more sustainable energy crop production and higher total yield stability in the light of fluctuating weather conditions due to climate change.

A second alternative could be intercropping, i.e. growing two or more crops together. Intercropping maize with other plants is already known from ancient Mayas, who planted corn, beans and pumpkins on the same field (Böttinger 2013). Intercropping of compatible plants also encourages biodiversity by providing a habitat for a variety of insects and soil organisms that would not be present in a single-crop environment. Up to now intercropping has still been applied mostly by small farmers in tropic and subtopic regions to increase the diversity of their products and to ensure a stable annual output through the effective use of land and other resources (Clawson 1985; Davis et al. 1987; Searle et al. 1981). Maize intercropped with different legumes is a natural way to fix nitrogen from the air and an alternative way to produce protein rich feed. Maize itself has a good energy content but little protein. Therefore numerous experiments have been carried out with maize and protein rich crops, such as soybean (*Glycine max L. Merr.*), different lupines (*Lupinus ssp.*), lablab bean (*Lablab purpureus L.*), velvet bean (*Mucuna pruriens L. D.C.*), cowpeas (*Vigna unguiculata L. Walp*), lucerne (*Medicago sativa L.*) (Titterton, Maasdorp 1997), faba bean (*Vicia faba L.*), scarlet runner bean (*Phaseolus coccineus L.*) (Armstrong et al. 2008) peas (*Pisum sativum L.*) (Mustafa et al. 2000) and bush beans (*Phaseolus vulgaris L.*) (Dawo et al. 2007). However, only a few studies dealt with climbing beans. Previous results showed that the nutritive value of maize-bean silage was similar to maize silage, indicating that maize-bean silage mixtures could be used in dairy cow rations (Contreras-Govea et al. 2009a). Investigations based on calculations and feeding trials have been carried out by numerous researchers (Titterton, Maasdorp 1997; Anil et al. 2000; Mustafa et al. 2002; Contreras-Govea et al. 2009b), but there is a lack of research considering the potential of maize-bean silages for biogas production.

In different parts of Germany field trials have been conducted. In a cooperative work the KWS SAAT SE as a maize breeder and the University of Göttingen tried to find a suitable bean variety for mixed cropping stands (Hoppe 2016). Also the Nürtingen-Geislingen University in the south of Germany has done preliminary experiments to find a suitable cultivation method for conventional cultivation. In the northern part in Germany the Johann Heinrich von Thünen-Institut is conducting field trials with different maize and bean varieties and also feeding trials with different animals (Fischer et al. 2015).

Bearing in mind the previous information the first part of the thesis aims to investigate the double cropping system as an alternative to maize and to explore alternative crop biogas production yields in different maturity stages. In the second part the possibility to cultivate maize and beans has been examined studying the effects of different seeding densities and seeding times of beans as well as the competitive effect on the total yield. In the third part the feed quality and the methane potential of differently mixed maize-bean silages was investigated.

Research objectives

The objective of this thesis was to investigate possible alternatives to monocropped maize for the production of biogas.

The strategy of double-cropping for the production of biogas substrates is addressed by degradation experiments with maize as the standard crop and the two alternative crops sunflower and sorghum-sudangrass. Biological degradation experiments were made in biogas batch reactors for all three crops in each maturity stage. The remaining material was analysed for different chemical parameters to determine the degree of degradation of and their influence on biogas production.

As the second objective of this thesis the investigation of an alternative farming practice to monoculture of maize was tried out. Mixed crop experiments with maize and climbing beans were conducted at three research sites in over the course of 3 years. One site was managed under organic conditions and two under conventional conditions. In all research sites treatments with different seeding density and seeding time of beans were tested. Additionally different weed regulation methods were tested. In all research sites also the influence of weed coverage on total yield was measured.

Moreover the biogas potential of a mixture of climbing bean and maize was measured based on artificially mixed crop-silages. The silages were made with varying proportions of maize and bean. The chemical parameters from all silages were analysed and verified for influences on biogas production potential.

The specific objectives of this thesis were to

i) examine maize, sunflower and sorghum-sudangrass for biogas production potential, when cut at harvesting times other than fully ripened as it can occur in double cropping systems,

ii) measure the influence of four different crop seeding densities of maize and bean on the total yield as an alternative for maize monocrop,

iii) investigate the impact of bean sowing time on the total dry matter yield in the aforementioned mixed crop system,

iv) explore various methods of weed controlling methods under organic and conventional cultivation of maize-bean mixed crop and

v) analyse the silage quality of various artificially made maize-bean mixed silages and the influence of the silages' chemical parameters on biogas production potential.

2. Degradation of fibre and non-fibre fractions during anaerobic digestion in silages of maize, sunflower and sorghum of different maturities

2.1 Abstract

This study investigated the temporal dynamics of the degradation of organic matter (OM), fibre, protein and remaining organic constituents during anaerobic digestion of silages from maize, sunflower and sorghum-sudangrass that were harvested at different maturity stages. The tests were conducted using an *in sacco* method in 20 l digesters. The degradation kinetics of fibres showed some similarity for maize and sorghum-sudangrass with a continuous progress, whereas in sunflower it developed with a strong degradation right after incubation to reach a quasi-constant level for the rest of the 35 days lasting fermentation time. The degradation process for crude protein was usually intense from the very beginning and levelled off after only 5 days. Methane yield after 35 days amounted to 358, 278, and 320 l_N CH_4 kg^{-1} OM for maize, sunflower and sorghum-sudangrass respectively but could not be predicted with high accuracy by regression models based on organic constituents.

2.2 Introduction

Nowadays biogas is an important green energy source. Considering the total electricity production in Germany, 23.9 % are produced by renewable energy sources whereat the production of electricity from biogas made up 25.4 TWh (16.6 %) in 2013 (FNR 2014). Besides manure, there is a high variety of possible biogas substrates either for mono- or co-digestion. Several C3 (wheat, barley, rye and most grassland species) and C4 plants (maize, millet, sugar cane and sorghum) are suitable for biogas production (Appels et al. 2011). In view of societies' demand to lower the dominance of maize in some parts of Europe, there is a growing interest in alternative crops, like sugar beet or different oleiferous plants (e.g. sunflower). A multitude of cropping systems (e.g. double cropping, mixed crop cultivation) exist, which provide opportunities to increase landscape's diversity and allow an efficient and environmental friendly energy crop production (Graß et al. 2013). However, in double cropping systems, the second crop (i.e. a summer crop like maize, sorghum or sunflower), which is sown after an early harvest of a winter crop (e.g. winter rye) may not gain an adequate ripeness due to the limited length of the vegetation period.

The anaerobic digestion process in a biogas plant is, in some respects, very similar to the processes in the digestive tract of ruminants. However, an essential difference between rumen and biogas plants is the time, during which the substrate remains in the digestion system (Amon et al. 2007a; Amon et al. 2007b). While in grazing animals a maximum passage time of 72 hours was observed (Ørskov et al. 1980), the retention time in large-scale commercial biogas plants varies in a wide range from 35 to 100 days (Angelidaki et al. 2005). Batch tests for experimental purposes are standardized to last until the daily biogas production rate is equivalent to 1 % of the total volume of biogas (VDI 4630). Amon (Amon et al. 2007b) stated that the longer retention times in biogas plants allow higher methane yields from biological material than can be achieved by the digestion in the rumen. Furthermore, the bacterial composition and conditioning of rumen and biogas plant are different, which was shown to influence the rate and amount of substrate degradability (Bayané, Guiot 2011).

Buswell (Buswell, Mueller 1952) and Boyle (1977) developed a formula by which the biogas potential and its composition (CH_4, CO_2, NH_3 and H_2S) can be determined based on the chemical composition of the raw material (biomass). This formula shows the theoretical but not necessarily the real potential (Cone et al. 2002; Herrmann, Rath 2012; Labatut et al. 2011). Chemical composition and biogas potential has been measured chemically and more recently also by methods that make use of near infrared reflection spectroscopy (NIRS), which is a cost-efficient possibility to predict quality traits in substrates (Jacobi et al. 2011).

There is extensive knowledge on the digestion of protein and soluble carbohydrates in animal rumen (Sniffen C.J. et al. 1992; Dewhurst et al. 1995), which are more rapidly digested by ruminal microorganisms than are the structural polysaccharides such as hemicellulose, cellulose and lignin, or the storage polymers such as starch (Cherney et al. 1988; Chesson 1988; Hall 2003; Triolo et al. 2011). Understanding of ruminants' digestion and net energy utilisation was greatly improved using rates of digestion and passage to calculate discount values for net energy in feeds (van Soest, Peter 1994). Accurate estimates of these kinetic parameters and knowledge of factors that may affect them were determined by *in sacco* techniques, i.e. the assessment of disappearance of organic matter (OM) or specific compounds in bags while implemented in the animals' rumen (Cone et al. 2002; Getachew et al. 2004).

Similar to rumen digestion increased lignin content due to enhanced crop maturity was postulated to be the major factor in reducing substrate degradability in biogas (Cherney et al. 1988; Moore, H.-J. G. Jung 2001). The reduction of the particle size to a certain degree (< 6 mm) was shown to increase digestibility and biogas yield (Emanuele, Staples 1988; Surendra, Khanal 2015). The conservation of feedstock as silage leads to the same effects, compared to feeding fresh material (Amon et al. 2007a; Richter et al. 2009). Organic matter (OM) digestibility and substrate-specific methane yield is often predicted using acid detergent fibre (ADF) and acid detergent lignin (ADL) (Chandler, Jewell 1980; Labatut et al. 2011; Triolo et al. 2011). However, findings are not consistent and sometimes hardly any relationship was found (Gunaseelan 2007; Amon et al. 2007a). To the authors' knowledge, there exist no data on temporal degradation kinetics of fibre and non-fibre fractions of crops during anaerobic digestion in batch tests, which might provide new insights into the processes involved in the degradation of these highly complex organic compounds.

In the present study silages from maize, sunflower and sorghum-sudangrass were subjected to anaerobic digestion using batch fermenters over 35 days. Fibre and non-fibre fractions were determined from samples taken at 8 dates during digestion time. The aim of the study was to i) investigate temporal degradation kinetics of fibre and non-fibre fractions from different energy crops, ii) determine effects of crops maturity on fibre digestion processes, and to iii) evaluate the relationship of degradability and methane yields.

2.3 Material and Methods

Energy crops

The experiment was carried out on three different energy crops (CS), i.e. maize (*Zea mays* L.) \`Atletico\`, sunflower (*Helianthus annuus* L.) \`Metharoc\` and sorghum-sudangrass (*Sorghum bicolour* (L.) Moench) \`Zerberus\`. The breeder of all crops was KWS SAAT SE. Energy crops were grown in 2012 on the Neu-Eichenberg experimental farm of the University of Kassel (51°23´ N, 9°54´ E, 227 m above sea level; soil type: sandy loam; soil pH-value: 6.4; average annual rainfall: 550 mm; annual mean temperature: 9.9 °C). The plants were sampled on four different dates (SD; Table 1). Maize was harvested at two flowering stages, i.e. in flowering stage (BBCH 61) (Federal Biological Research Centre for Agriculture and Forestry 2001), where the male flowers evolve stamens in the middle of the tassel, and on the female flowers the tip of the ear is emerging from the leaf sheath. At BBCH stage 65 the entire tassel is flowering and the silk is fully emerged. At SD 3 the plants were in BBCH 75, where development of fruit occurs and kernels in the middle of the cob are yellowish-white (variety-dependent) with a milky content and a dry matter content of about 40 %. SD 4 was conducted in the ripening stage (BBCH 85), which is a dough stage, where kernels are yellowish to yellow (variety dependent) and their dry matter content is about 55 %.

Sunflower was sampled and ensiled at similar BBCH stages as maize, i.e. at flowering stage (BBCH 61) at the onset of flowering, where ray florets extended and disc florets are visible in the outer third of inflorescence. At fruit development stage (BBCH 71), the seeds on the outer edge of the inflorescence are grey and have reached final size. At ripening stage (BBCH 81) the seeds on outer third of anthocarp are dark and hard but the back of anthocarp is still green. Further at BBCH 85 stage, the seeds on middle third of anthocarp are dark and hard and the back of anthocarp is yellow, the bracts have brown edged and the seeds have a dry matter content of ca. 60 %.

Table 1 Phenological development stage (BBCH) and harvest date of investigated energy crops

			1st SD	2nd SD	3rd SD	4th SD
Maize	Date of seeding	29/04				
	Date of harvest		01/08	07/08	28/08	01/10
	Days after sowing		94	100	121	155
	BBCH		61	65	75	85
Sunflower	Date of seeding	18/05				
	Date of harvest		10/08	28/08	10/09	01/10
	Days after sowing		84	102	115	136
	BBCH		61	71	81	85
Sorghum	Date of seeding	18/05				
	Date of harvest		01/08	07/08	10/09	01/10
	Days after sowing		75	81	115	136
	BBCH		35	55	61	69

SD sampling date, *BBCH* identification key (FBRCAF, 2001)

Due to Sorghum's distinct plant architecture, maturity stages at sampling differed from the other crops. SD 1 was already conducted at BBCH 35, i.e. at stem elongation, where the 5th node is at least 2 cm above the 4th. The second sampling was at inflorescence emergence stage (BBCH 55) in the middle of heading, where half of the inflorescence has emerged. SD 3 was at a similar stage as maize and sunflower at the first sampling stage (BBCH 61), where first anthers are visible. The last sampling date was at the end of flowering (BBCH 69), where all spikelets have completed flowering but some dehydrated anthers may remain.

After each sampling date, samples from all crops were chopped to a particle size of 10 mm using a drum chopper and separately ensiled to ensure proper conservation for further investigations. Ensiling took place in PVC-U tubes with rubber caps with a volume of 2.7 l.

In sacco incubations

Digestion trials were performed as batch experiments in accordance with the German standard procedure (VDI 4630) and based on a method described by Zerr (Zerr 2006). Anaerobic fermentation of the substrates took place in airtight 20 l polyethylene containers (Speidel, Germany) with 8 kg of inoculum, which initially originated from a secondary digester of a biogas plant, operated with swine manure and maize. The inoculum was sieved through a 2mm sieve and stored until application under continuous feeding with ground hay from typical local grassland (appr. two times per week) in order to keep the bacteria alive without increasing the methane potential of the inoculum itself. Four kg of 37°C tap water was added to the container to reduce the headspace volume. The containers were placed in heated water basins to keep the process temperature at 37°C. Fifty grams of fresh matter (FM) of energy crop silages were weighed in 10 x 20 cm nylon bags (50 ± 15 µm pore size; ANKOM Technology, USA), following the *in sacco* procedure described by Ørskov (Ørskov et al. 1980; Dewhurst et al. 1995). The bags were closed with cable ties and attached to the stirrers. The containers were closed airtight to create an anaerobic environment for the digestion. Consecutive test runs comprised the digestion of silage from one energy crop at one sampling date. 16 containers were filled with inoculum and silage, 2 containers served as a blank (inoculum with only water) and another 2 containers served as a standard (inoculum with 50 g cellulose) to assess the biological activity of the inoculum. Containers in which silage was digested were opened after different fermentation times. For each fermentation time (1, 2, 3, 4, 7, 15, 21 and 35 days), two containers were operated serving as replicates. The containers were stirred for 15 min at 75 min intervals. Containers from which samples were to be removed on days 1, 2, 3, and 4 (with 100 g FM for each sample) were equipped with only two bags, whereas four bags were put into the containers, from which samples were to be removed on days 7, 15, 21 and 35 (200 g of sample FM). Gas bags (20 l Tecobags, Tesseraux Spezialverpackungen GmbH, Germany) were connected to the containers with rubber tubing. Biogas production and methane content was measured only from samples which stayed in the containers for 35 days and methane (CH_4) production was normalised to standard temperature and normal pressure conditions (i.e. dry gas, 273.15 K, 1013.25 hPa). After 35 days of degradation the daily biogas production from all samples had proportionately decreased to approximately 1 % of the total biogas volume produced up to this time [46]. This was in accordance with experiences with these substrates in preliminary studies. The produced biogas was measured with a wet drum gas meter (TG 5, Ritter, Gemany) and analysed for CH_4 content by an infrared gas analyser (GS IRM 100, GS Messtechnik GmbH, Germany). Before sample measurements, a control gas with a content of 60 % CH_4 and 40 % N_2 was measured to check the correctness of measurements.

At each sampling day the nylon bags, containing undigested sample residues and inoculum liquid, were removed from the container and directly put into ice-cold water to stop microbial activity from inoculum. The bags were then put into a washing machine (WA 9330 G, Bauknecht, Germany) and washed three times for 30 min with 20 l of cold tap water to remove the inoculum from the undigested sample residues. Subsequently, the bags with the sample residues were spin-dried for 3 min at 600 rpm and dried at 65°C (Ørskov et al. 1980; Dewhurst et al. 1995).

Chemical analyses

Before and after incubation samples were dried at 65°C for 48 h in a drying oven and weighted to determine the dry matter (DM) content. Next, the samples were ground in a cyclone mill (Cyclotec 1093, FOSS, Germany) to pass a 1 mm sieve for fibre analysis, (neutral detergent fibre (NDF), (Hall, Mertens 2012; Hristov et al. 2010), acid detergent fibre (ADF), acid detergent lignin (ADL)), total nitrogen (N) and ash. Ash was determined as residue after incineration for 2 h at 550°C. OM was calculated as DM loss during incineration. NDF, ADF and ADL were determined by the method of Van Soest (van Soest, Wine 1967) using an ANKOM 200 Fiber Analyzer (ANKOM Technologies, USA) and expressed exclusive of residual ash. As suggested by M.B. Hall (Hall 2003) the concentration of hemicellulose was calculated from the difference between NDF and ADF concentration. The concentration of cellulose was calculated as the difference between ADF and ADL concentration. Heat stable alpha amylase and sodium sulphide were added for NDF analysis (DePeters et al. 1997). For ADF concentration measurement, a separate sample was prepared and measured. Total N concentration was determined by rapid combustion using an elemental analyser (Vario MAX CHN, Elementar Analyseseysteme GmbH, Germany) and used to calculate crude protein (CP, CP= N*6.25). After subtracting NDF and CP from OM, the rest is referred to as none-determined organics (NDO). It contains non-fibre carbohydrates, fat and organic material that was not determined otherwise.

Calculation of degradation kinetics

Degradation data from OM, NDF, ADF, ADL, CP and NDO were fitted to the first order exponential decay model in SigmaPlot 9.0 (SigmaPlot 9.0). The model is of the form:

$$y = y_0 + a\, e^{-bx}$$

where y is the total indigested residue at any time x, y_0 is the fraction not digested after 35 days of digestion, a is the degradable fraction, b is the fractional disappearance rate (day^{-1}) of a and x is the time incubated in the digester in days (DePeters et al. 1997; Rodrigues et al. 2009).

Statistical analyses

Statistical analyses were conducted using R Software (R Core Team 2016). The design of the experiment followed a 3x4 factorial layout with three crop species (CS) and four sampling dates (SD) with two replicates. As the factors were completely independent from each other, a fixed-effects model of analysis of variance (ANOVA) was used to evaluate the importance of CS and SD on the measured parameters and the effects model was:

$$Y= a_0 + a_1 * CS + a_2 * SD + a_3 * CS * SD + e$$

where CS and SD are categorical variables, a_0 is the intercept, a_1 is the effect of the CS, a_2 is the effect of SD, a_3 is the interaction and e is the error term.

Bonferroni Least Significant Difference test was used as post-hoc test for comparisons among mean values, because it controls the Type I error appropriately and is conservative. Further, the test is particularly appropriate for smaller number of comparisons (Field et al. 2012). The homogeneity of variances of the model was verified graphically; the normal distribution of the residues was tested with the Shapiro–Wilk test.

2.4 Results

Maturation in maize and sunflower resulted in a typical pattern of fibre contents with an initial increase followed by a decline during the development of kernels (Table 2). This was mirrored in the opposing trends in the NDO fraction at the latest maturity stage, reflecting the relocation of carbohydrates from leaves and stems into grains and the development of storage substances (e.g. oil, starch), which were not determined exclusively in the present study. In contrast, sorghum-sudangrass showed a continuous increase in fibre till 3 SD, whereas NDO declined. This corresponds to the phenological development of the crop, which is not able to reach the grain-filling stage under the prevailing climate conditions. Visual and olfactory evaluation showed that silages from all energy crops were well fermented. pH in maize silage was 3.70 ± 0.08, in sunflower silage 4.15 ± 0.01 and in sorghum-sudangrass silage 3.89 ± 0.06. Figure 1 presents the results from OM composition of the parent material that was left in the *in sacco* bags after the digestion process of 35 days. The differences in OM composition between the crops are visually distinguishable. However, no clear differences occur among SD's. Fractions show similar patterns of degradation over the fermentation time. ADL concentration increased over the course of development in all crops and at all SD. However, there are remarkable differences among other fractions. Cellulose was mostly degraded in sorghum and in maize, but less in sunflower. Distinct differences occurred for hemicellulose concentrations between maize/sorghum-sudangrass and sunflower. Whereas sorghum and maize were showing a strong decline over time sunflower, which contained less hemicellulose in the parent material, showed no decline during fermentation. Further, species differ in the change of protein content in OM during fermentation. While protein in sunflower persisted on a rather constant and low level (< 18.5 %), it accumulated in maize and sorghum-sudangrass up to 24 % on average of the remaining OM.

Table 2 Contents of fibre and non-fibre fractions in silages from maize, sunflower and sorghum

SD	Maize					Sunflower					Sorghum				
	NDF	ADF	ADL	CP	NDO	NDF	ADF	ADL	CP	NDO	NDF	ADF	ADL	CP	NDO
	% of OM					% of OM					% of OM				
First	54.8	33.4	3.3	8.5	36.7	42.5	40.6	6.5	10.2	47.3	54.4	36.4	2.3	11.6	34.1
Second	54.6	34.0	3.9	8.3	37.1	47.5	46.7	8.7	8.7	43.8	56.6	37.6	2.7	11.9	31.6
Third	54.6	34.4	4.6	8.3	37.1	53.3	48.3	9.3	7.7	38.9	68.0	45.1	5.4	6.4	25.6
Fourth	45.5	25.9	3.2	7.9	46.6	44.1	39.1	8.7	7.7	48.2	64.6	42.5	5.5	5.8	29.5

SD sampling date, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin, *CP* crude protein (total nitrogen x 6.25), *NDO* none-determined organics, *OM* organic matter

Degradation behaviour of the chemical constituents over time was very different for the investigated crops (Table 3 and Table 4). Overall, first order exponential decay models fitted very well to the measured data with a coefficient of determination (R^2) larger than 0.96. Considering the OM fraction, the significant CS x SD interaction for the non-digestible fraction (y_0), the degradable fraction (a) and for the relative degradation rate (b) reflects the variable degradation kinetics (Figure 2, Table 3). While OM degradability was similar for sorghum-sudangrass and maize (73- 83 %), lower values were obtained for sunflower (59-76 %). There was a common trend for the non-degradable fraction to increase with crop maturity. However, degradation curves for the 2nd sampling date showed divergent patterns. Although OM of sunflower was less degradable, fractional degradation rate was considerably higher (0.27- 1.38 versus 0.1-0.40 for maize and sorghum-sudangrass). Visual inspection of the remaining material showed that the softer and better degradable material (leaves) was degraded quickly, whereas more resistant organic material (fibrous stems) remained.

Despite a significant CS x SD interaction, degradation kinetics of NDF and ADF showed some similarity for maize and sorghum-sudangrass. The non-degradable fraction was 2-25 % and degradation showed a continuous progress, which is reflected in similar relative degradation rates of 0.06-0.18. Like for OM, degradability of NDF and ADF tended to decline as the maturity of crops progressed. However, NDF and ADF degradation at the 2nd SD differed substantially from the other SD's both in shape and ultimate value (Figure 2, Table 3). Degradation of fibre fractions in sunflower developed very differently. After a strong degradation in the first 5-10 days after incubation, reflected by relatively high degradation rates of 0.15-1.21, values remained on constant levels for the rest of the fermentation time. Sunflower exhibited the least NDF degradation (40 – 62 %) with a pronounced decline with increasing crop maturity. SD effects were also strong for ADF, which after 35 days of fermentation remained undigested by 30-62 %. Degradation kinetics of ADL differed less among SD levels and species effects were primarily significant as main effects. For all species approximately 60 % of ADL remained non-degradable, except for sunflower at the 2nd and 4th SD, where 80 % could not be degraded. At all dates, 68-85 % of CP was degraded in maize and sunflower, whereas at the 3rd and 4th SD of sorghum-sudangrass half of CP remained non-degradable. Generally, degradation was intense from the very beginning and levelled off after day 5. Degradability of NDO was generally greater than 75 % and reached maximum values of up to 90 %.

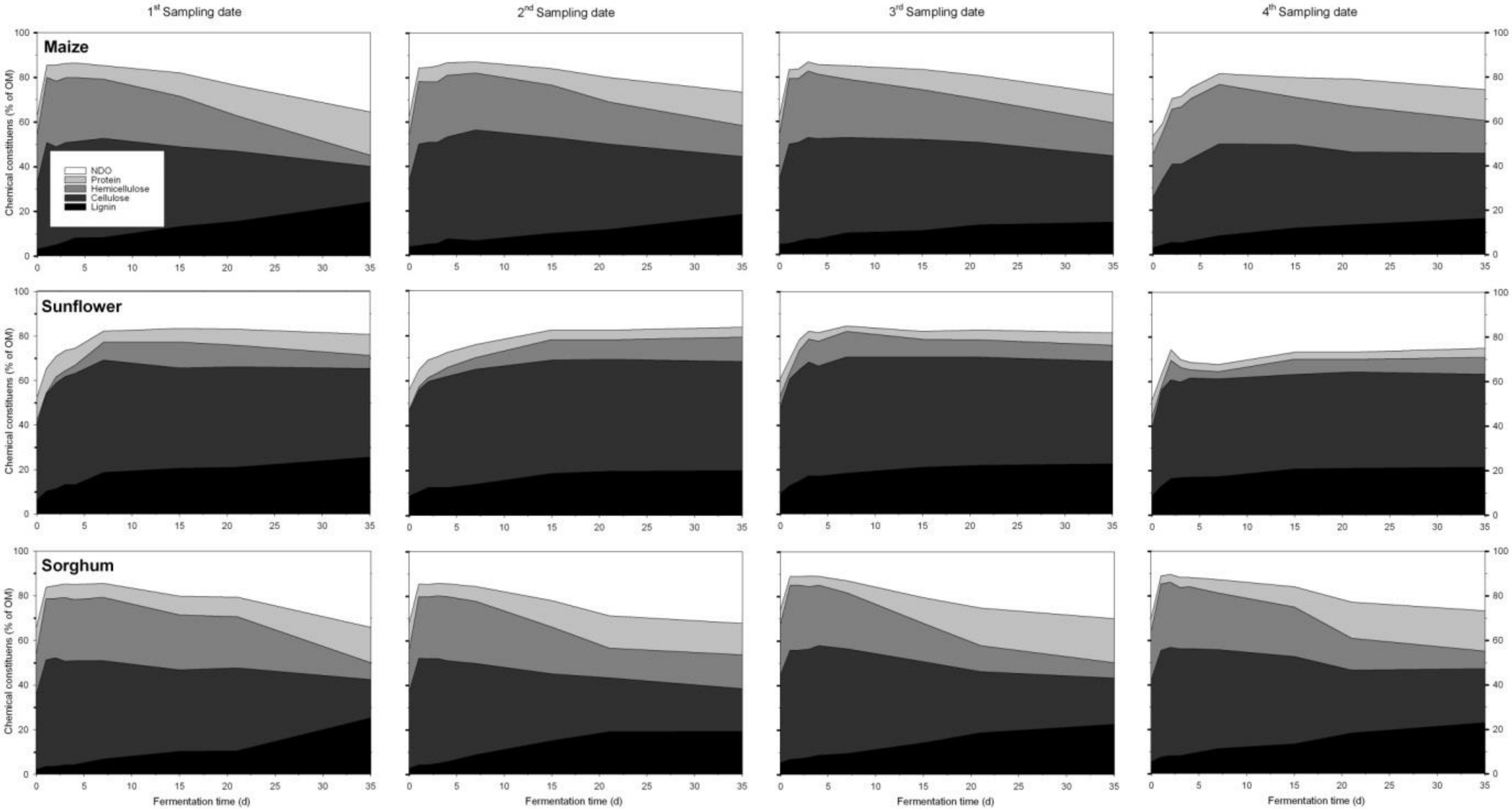

Figure 1 Change of fibre and non-fibre fractions in maize, sunflower and sorghum (*top, intermediate* and bottom rows respectively) during anaerobic digestion in batch fermenters, expressed as ash-free percentage of the respective constituent in the parent material. Crops were sampled at four successive phenological stages. *Hemicellulose* (NDF-ADF), *cellulose* (ADF-ADL), *lignin* (ADL), *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin, *NDO* non-determined organics

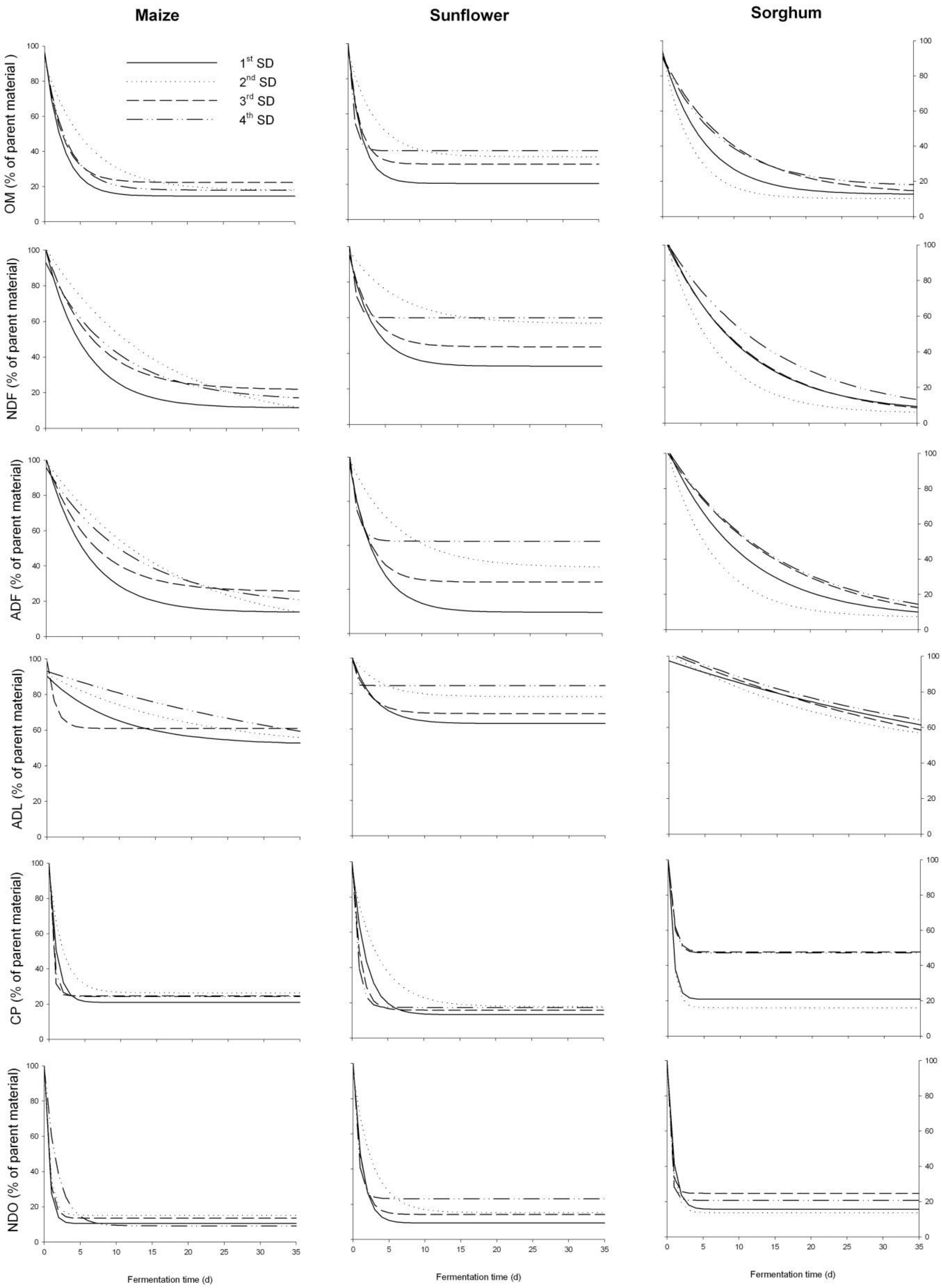

Figure 2 Digestion curves from organic matter (*OM*), fibres (*NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin), protein (*CP*) and non-determined organics (*NDO*) in crops harvested at different sampling dates (*SD*) and expressed as percentage in the parent material

Methane yield was steadiest for sorghum-sudangrass ranging from 310 to 326 l_N kg^{-1} OM with a tendency to increase with increasing maturity. Values for maize ranged from 335 to 389 l_N kg^{-1} OM and steadily increased with progressing crop maturity. Sunflower exhibited the biggest difference in methane production with 116 l_N kg^{-1} OM over the sampling dates. From the digestion experiments a multiple linear regression equation was derived that estimates methane production from the composition of crops. Starting the modelling with a saturated model including all chemical constituents as main effects and in two-way- and three-way interactions only NDF, ADF and ADL proved substantial:

Methane yield (l_N CH_4 kg^{-1} OM) = 5.4729 x NDF (% of OM) - 4.0630x ADF (% of OM) - 1.2422x ADL (% of OM).

The R^2 of the model was 0.50 with a mean standard error of 33.8 l_N CH_4 kg^{-1} OM.

Table 3 ***In sacco*** **degradation kinetic parameters of fibre and non-fibre fractions and CH_4 yield during anaerobic digestion of maize, sunflower and sorghum silages harvested at four maturity stages and fitted to first order exponential decay model** $y = y_0 + a\ e^{-bx}$**.**

	SD	OM			NDF			ADF			ADL			CP			NDO			CH_4
		y_0	a	b	y_0	a	b	y_0	a	b	y_0	a	b	y_0	a	b	y_0	a	b	$l_N kg^{-1}$ OM
Maize	First	14.63	81.51	0.40	11.47	88.55	0.18	13.65	87.10	0.17	51.72	38.52	0.10	20.90	76.99	0.96	10.54	89.20	1.65	335
	Second	17.93	73.07	0.17	1.91	99.26	0.07	1.93	97.48	0.06	49.26	42.35	0.05	26.44	68.38	0.54	15.21	84.07	1.42	340
	Third	22.36	73.43	0.40	21.60	78.86	0.16	25.45	74.49	0.16	60.94	37.51	0.89	24.69	75.29	2.37	13.50	86.17	1.55	369
	Fourth	17.96	76.97	0.33	15.22	77.88	0.11	17.10	78.65	0.09	27.76	65.31	0.02	24.28	75.53	1.73	9.15	89.79	0.56	389
Sunflower	First	20.43	76.21	0.55	32.77	62.10	0.30	29.63	66.56	0.28	63.11	36.49	0.32	13.27	85.37	0.52	9.34	88.88	0.82	274
	Second	35.61	59.51	0.27	56.67	40.85	0.15	49.97	48.89	0.15	78.26	22.16	0.27	17.87	75.75	0.27	15.30	79.75	0.38	231
	Third	31.40	66.81	0.65	43.68	51.69	0.34	43.30	54.56	0.39	68.70	29.76	0.44	15.81	83.94	0.92	14.19	85.60	0.92	260
	Fourth	39.64	60.19	1.21	60.35	38.72	0.82	55.93	45.35	0.82	82.21	18.09	0.34	17.31	82.76	1.31	23.14	77.15	1.49	347
Sorghum	First	12.53	80.58	0.17	5.78	98.98	0.10	6.62	97.62	0.10	17.12	80.21	0.02	20.90	78.31	1.53	15.69	82.77	1.17	310
	Second	10.17	83.67	0.25	5.46	98.96	0.15	7.04	96.59	0.16	41.47	59.63	0.04	16.06	83.32	1.42	13.74	85.41	1.35	326
	Third	12.71	78.30	0.11	4.11	98.63	0.09	1.69	99.80	0.06	18.30	83.59	0.02	47.69	52.32	1.31	24.70	75.21	2.12	323
	Fourth	16.93	73.14	0.12	3.84	99.21	0.07	4.96	98.33	0.07	40.18	63.07	0.03	47.25	52.34	1.41	20.71	79.25	2.33	323
CS		***	***	***	***	***	***	***	***	ns	***	***	**	***	***	*	***	***	***	ns
SD		**	***	***	**	*	**	**	**	ns	ns	ns	**	***	***	*	***	***	***	*
CS*SD		**	**	***	***	**	***	**	*	ns	ns	ns	***	***	***	ns	***	***	***	ns

SD sampling date, *OM* organic matter, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin, *CP* crude protein, *NDO* non-determined organic, *% CH_4* methane content in biogas, *l_N CH_4 kg^{-1} OM* methane yield, *CS* crop species, *ns* not significant

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 4 Results from Bonferroni Least Significant Difference test for In sacco degradation kinetic parameters, describing the degradation of chemical compounds of energy crops harvested at different maturity stages (α=0.05).

	SD	OM			NDF			ADF			ADL			CP			NDO			CH_4
		y_0	*a*	*b*	y_0	*a*	*b*	y_0	*a*	*b*	y_0	*a*	*b*	y_0	*a*	*b*	y_0	*a*	*b*	
Maize	1st	c	a	bcd	ef	ab		cde	abc		abc	abcd	b	bcde	abc		cd	ab	bc	bc
	2nd	c	abc	cd	f	ab		de	ab		abc	abcd	b	b	d		c	bcd	cde	bc
	3rd	bc	abc	bcd	de	bc		bcd	bcd		abc	abcd	ab	bc	cd		cd	abc	bc	ab
	4th	c	ab	bcd	ef	bc		cde	abcd		abc	bcd	b	bcd	bcd		d	a	fg	a
Sunflower	1st	bc	ab	bc	cd	cd		bc	cde		abc	abcd	b	e	a		d	ab	efg	de
	2nd	a	d	bcd	ab	e		a	ef		ab	cd	b	cde	bcd		c	de	g	f
	3rd	ab	bcd	b	bc	de		ab	def		abc	bcd	b	e	ab		cd	abc	defg	ef
	4th	a	cd	a	a	e		a	f		a	d	a	cde	abc		a	e	bcd	bc
Sorghum	1st	c	a	cd	f	ab		de	a		bc	ab	b	bcde	abc		bc	cd	cdef	cd
	2nd	c	a	bcd	f	a		de	ab		abc	abc	b	de	abc		cd	abc	cde	cd
	3rd	c	ab	d	f	a		e	ab		abc	abcd	b	a	e		a	e	ab	cd
	4th	c	abc	d	f	a		e	ab		c	a	b	a	e		ab	de	a	c

Numbers followed by different letters within columns indicate significant differences (α=0.05) among *In sacco* degradation kinetic parameters of energy crops shown in Table 3.

SD sampling date, *OM* organic matter, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin, *CP* crude protein, *NDO* non-determined organic, CH_4 methane yield

2.5 Discussion

The energy crops were harvested at different dates during crop growth to understand the effect of maturity stage on degradation of various chemical constituents and on biogas production. Although harvesting at pre-mature stage is not a common practice it may be necessary due to unfavourable weather conditions, which prevent a proper ripening of crops or in double cropping systems under moderate climate conditions (Graß et al. 2013; Graß et al. 2015), where a limited duration of the growing season may not allow the second plant to reach the stage of preferred maturity. NDF and ADF concentrations in maize at the first three dates were slightly over 54 and 34 % of OM, respectively, whereas at the last harvest date, when the crop was in the dough stage, concentrations were almost 10 % lower. This is due to the fact that with the ripening of grains starch is accumulating in the cob, which is also suggested by the higher proportion of the NDO fraction (Buxton 1996; Ferreira, Mertens 2007). ADL and CP concentrations found in this experiment were similar to studies by Amon (Amon et al. 2007b) for different maize varieties.

Irrespective of crop species degradation of CP and NDO matter was very pronounced in the first five days of fermentation time where nearly 80 % of these two constituents were degraded. Fractional disappearance rates (b ranged between 0.27 and 2.37) were much higher than for fibres, which showed b-values between 0.02 and 0.82. High degradation rates of CP and NDO correspond with findings of Stefanon et al. (1996), who analysed digestion kinetics of the neutral detergent-soluble (NDS) material from legumes and grasses, which is supposed to contain the soluble and easily digestible materials like minerals, protein, organic acids, various simple sugars and their short-chain polymers. Similar to the NDO fraction, NDS is also less define, because different forages release different proportions of total pectins, fructans, and starches into the aqueous phase (Stefanon et al.

1996). However, this fraction is of nutritional value, because the water-soluble fraction is easily accessible and rapidly digested. Particularly the soluble carbohydrates are more rapidly digested by microorganisms than the structural polysaccharides such as cellulose or the storage polymers such as starch (Chesson 1988). Thus, the “soluble” pool may play an important role in the early stages of forage digestion.

Degradation rates of fibre constituents were rather high in the first half of the fermentation time and differences among harvest dates were relatively small after 35 days of fermentation. Approximately 40 % of the ADL, initially contained in maize, could not be discovered with this measuring method by the end of the fermentation time. This is remarkable, as lignin as a main fraction of the ADL fraction is considered as the most recalcitrant component of the plant cell wall and particularly difficult to biodegrade (Hendriks, Zeeman 2009; Triolo et al. 2012). Mauseth (1988) stated that the low biodegradability of lignocellulose (i.e. a physical and chemical association of cellulose, hemicelluloses and lignin) in biogas reactors is due to lignin being non-degradable in anaerobic environments because the extracellular enzymes involved require oxygen for the depolymerisation. Chandler (Chandler, Jewell 1980) observed a strong correlation between the biodegradable fraction and lignin content in the volatile solids (R^2 = 0.94) from diverse organic wastes and Triolo et al (2011) found that lignin was the strongest predictor for biochemical methane potential (BMP) of energy crops (R^2 = 0.76). Gunaseelan (2007) contrarily reported a weak relationship between BMP and the lignin content of several fractions of fruit and vegetable solids (R^2 = 0.49). It is well known that lignin structure and degradability is very different among crop species (Mussatto et al. 2008), which does not preclude that it may contribute — albeit limited—to the supply of lower-molecular organics available for anaerobic digestion. Not to mention, that lignin is a complex organic polymer, that is difficult to define and there are different methods to measure the content of it in crops (Moore, H.-J. G. Jung 2001). Furthermore, hydrolysis of cellulose in lignocellulosic materials may have been reduced by lignin and hemicelluloses, since these constituents act as a protective coat, making the cellulose resistant to enzymatic digestion (Mussatto et al. 2008), which may explain the marked maturity effect on NDF and ADF degradability in sunflower.

Contrary to maize fibre concentrations in sorghum-sudangrass continued to increase over the four sampling dates whereas protein and NDO fractions declined. Under the climate conditions of north-west Europe sorghum-sudangrass does not produce grains, which would have increased the proportion of non-structural carbohydrate at later stages of the growing season (Sambusiti et al. 2012). Lower b coefficients in the equations describing the degradation of fibre fractions in sorghum-sudangrass indicate slightly lower initial rates of degradation than in maize. However, as the amount of fibre degradation after 35 days is very similar to that in maize, the difference in degradation kinetics does not explain the different methane yields in both crops.

Methane yields found in the present study were at similar magnitudes as those reviewed in Appels et al. (2011). The considerably larger range of values and moderate fitting of methane production fitting model in this study may be explained by the fact that it comprised silages of variable plant maturity, whereas values from Appels et al. (2011) are only based on mature plants. Chemical parameters with a statistically significant relationship to the methane production across all crops and maturity stages were the fibre fractions NDF and ADF. It was expected that ADL concentration would also influence methane production, but the data did not reveal such a relationship. Moore and Jung (2001) reported that across forage samples of different maturities large differences in lignin concentration and digestibility were observed, but lignin and digestibility were often not correlated. Amon (Amon et al. 2007a; Amon et al. 2007b; Hendriks, Zeeman 2009) presented methane energy models for maize, cereals and grasses separately, but not for sunflower. The parameters that were explanatory in predicting methane production in maize were crude protein, crude fat, crude fibre and N-free extracts. Moreover: it was found that across species the fibre fractions were most important, which is in accordance with the findings of the experiment presented in this paper. In contrast to the aforementioned studies, the present study comprised different maturity stages of crops creating additional variation. The prediction accuracy obtained from the all-species model (R^2=0.50) showed that the explanatory power of fibre concentration is not big enough to produce a robust model that allows the prediction of methane yields across various crops and maturity stages.

2.6 Conclusions

Based on an *in sacco* methodology, the present study provides insight into the temporal dynamics of degradation processes of various constituents of crops. The results suggest, that degradation kinetics of fibres are similar for maize and sorghum-sudangrass with a continuous progress, whereas in sunflower degradation starts impetuously right after incubation to reach a quasi-constant level for the rest of the 35 days lasting fermentation time. Degradation of crude protein is intense at the beginning and levels off soon. Sampling date effects were significant for all fractions except ADL, indicating that sorghum-sudangrass can be harvested earlier without impact, whereas for maize and sunflower methane yields strongly declined at earlier harvest dates. Regression analysis showed a moderate relationship between methane yield and organic fractions.

3. Effect of sowing method and weed control on the performance of maize (*Zea mays* L.) intercropped with climbing beans (*Phaseolus vulgaris* L.)

3.1 Abstract

Experiments with mixtures of maize and climbing beans as well as purely sown maize stands were performed during two years at three experimental sites in Germany. One experiment investigated the effect of sowing density of maize (7.5 and 5 seeds m^{-2}) and the sowing density of beans (7.5 and 5 seeds m^{-2}) as well as the sowing time of beans (2-3 versus 5-6 leave stage of maize) on total dry matter yield. The second experiment tested a variety of mechanical and chemical weed control methods in a maize/bean mixture and compared the resulting total DM yield with that of a reference treatment which was held completely free of weeds by manual weeding. Although there was a tendency for mixtures to produce lower DM yields hardly any consistent yield difference between maize/bean mixtures and purely sown maize occurred. The proportion of bean DM varied over a wide range among sites. However, it was consistently higher when beans were sown at 2-3 leave stage of maize than when sown at 5-6 leave stage of maize. Mixtures did not supress weeds efficiently, especially if there is a high prevalence e.g. from the seed bank. It was at two of the three sites (WIZ and GRU) that total DM yield clearly declined with increasing weed coverage in the mixtures and based on data from these sites it can be concluded, that with the increase of weed coverage by 1 %, DM yield of the mixtures declined by 1 % compared to DM 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 is less than 1 t ha^{-1}. Care must be taken in transferring these findings to other sites, as the effects of weed control largely depend on the type of weed species. Further testing under various site conditions would be desirable to comprehensively evaluate the performance and also ecological implications of maize/bean mixtures compared to maize monoculture.

3.2 Introduction

In Germany the amount of mono-cropped maize increased from 0.8 million ha in 1980 to 2.5m ha in 2015 (Statistisches Bundesamt 2015), among fodder production particularly during the last ten years due to the cultivation of silage maize for utilization in biogas plants. Maize is the most used energy crop for biogas production due to high yields and advanced breeding activities. This dominance and the cultivation of maize as monocrop could cause ecological problems like loss of biodiversity, soil erosion or nitrogen leaching (Lithourgidis et al. 2011; Herrmann 2013). 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 (Thrupp 2000). Many other benefits exist underpinning the importance of plant diversity in agriculture, such as erosion control (Lithourgidis et al. 2011) or ecosystem stability due to the diversity of organisms (Coll, Bottrell 1995; Smith, McSorley 2000).

Mixtures with maize attracted great attention in research in the 70’s and 80’s of the 20th century, however, this was mostly in tropic and subtopic regions (Searle et al. 1981; Francis et al. 1982; Clawson 1985; Davis et al. 1987). Intercropping is considered suitable for small farmers (Peksen 2013) 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 minimizing the risk of crop failure or to reduce income risks due to unstable market prices for a given commodity. Much research was dealing with the improvement of forage quality through intercropping (Maasdorp, Titterton 1997; Titterton, Maasdorp 1997; Anil et al. 2000; Gebeyehu et al. 2006; Dawo et al. 2007; Contreras-Govea et al. 2009a; Dawo et al. 2009; Javanmard et al. 2009; Contreras-Govea et al. 2011; Flores-Sanchez et al. 2013) by investigating the effects of different proportions of leguminous plants. Mixtures with maize can also be used as forage with the benefit of providing a local protein rich feed (Stoltz, Nadeau 2014) with positive ecological impact by increasing the soil fertility due to the N fixation of legumes (Cong et al. 2015; Tsai et al. 1993). Various researchers proved that intercropping provides more effective use of land area than monoculture (Albino-Garduno et al. 2015; Javanmard et al. 2009; Sadeghi et al. 2012). However, so far no experiments have been made to assess the performance of maize/bean mixtures as an alternative energy crop for the biogas production.

Like written in the preceding paragraph there could be lot of benefits of maize/bean mixtures. The concept itself is not new and the two plants have been grown together for centuries. The climbing bean can make use of the maize plant to grow in height and this way the installation of bars is not needed. The different rooting systems of both plants spread in different depths and thus each plant can use the nutrients of the different soil layers (Lithourgidis et al. 2011; Zhang, Li 2003). To exploit the potential of maize/beans mixtures Francis et al. (1982) concluded from previous research that climbing beans need to be planted some time after the 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 herbicidal options. In the case of climbing beans and maize only three herbicidal active ingredients are potentially possible: pendimethalin, dimethanamid-P and clomazone. All three are pre-emergence herbicides, thus potentially resulting in sub-optimal weed control under conditions of late emerging weeds. Whereas in organic farming systems mechanical weed control of intercropping is a challenge, when crops will be sown at different sowing dates and crops will emerge at different times.

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

Table 5 . 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	
Experimental year	2014	2015	2014	2015	2014	2015
Preceding 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

3.3 Material and Methods

Experimental sites and field experiments

Experiment I - Intercropping

Field experiments were conducted at three sites in Germany with contrasting soil conditions in two consecutive years (Table 5). Composite topsoil samples were taken for determination of pH, P, K (Naumann et al. 1976). Soil properties, preceding crops, tillage, fertilizer, sowing and harvest dates are shown in Table 5 and Table 6. An energy maize hybrid (FAO 250) (Z. mays L. 'Fernandez') and a late-maturing cultivar of climbing bean (P. vulgaris L. 'Anellino Verde') were cultivated as a mixed crop. The maize variety was recommended by local breeder and the climbing bean variety was selected due to results of previous experiments with several bean varieties. In order to analyse the influence of beans on maize and on the total yield, maize and beans were sown with different densities and also sowing time of the bean was varied. As control, maize was sown with a seed density of 10 seeds m^{-2}, which is common practice in German agriculture. In mixtures, maize and beans were sown with densities of 7.5 and 5 seeds m^{-2}. All crops were sown in superset and 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. That results in 9 different treatments (Table 7), each with four field replicates (in total 36 plots in each experiment) in a fully randomised block design. Each plot was 10 m long and 3 m wide. Maize was sown during mid-April to early May (Table 6) depending on weather conditions and common practice at the experimental site. Row spacing for maize was 0.75 m, seed 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 to the maize row on both sides of rows with densities of 7.5 and 5 seeds m^{-2} at two different sowing dates.

Table 6 Sowing and harvest dates and phenological stage (BBCH) of crops in experiment I over two years at three experimental sites.

	Witzenhausen		**Tachenhausen**		**Grub**	
	2014	**2015**	**2014**	**2015**	**2014**	**2015**
Maize						
Sowing date	29.04.	06.05.	15.04.	10.04.	06.05.	08.05.
Harvest date	06.10.	30.09.	01.10.	28.08.	29.09.	01.09.
BBCH	83	83	83	80	83	80
Beans						
Sowing date early	02.06.	29.05.	22.05.	11.05.	06.06.	01.06.
Sowing date late	13.06.	26.06.	04.06.	29.05.	13.06.	13.06.
Harvest date	06.10.	30.09.	01.10.	28.08.	29.09.	01.09.
Harvest date	06.10.	30.09.	01.10.	28.08.	29.09.	01.09.
BBCH of early (late) sown beans	76 (62)	76 (62)	76 (62)	59	76 (62)	59

Table 7 Details of intercrop treatments carried out in experiment I over two years at three experimental sites.

Treatment	Maize seeds m^{-2}	Bean seeds m^{-2}	Bean sowing time	Abbreviation
1	10			10M
2	7.5	7.5	early	7.5M + 7.5B
3	7.5	5	early	7.5M + 5B
4	5	7.5	early	5M + 7.5B
5	5	5	early	5M + 5B
6	7.5	7.5	late	7.5M + 7.5B
7	7.5	5	late	7.5M + 5B
8	5	7.5	late	5M + 7.5B
9	5	5	late	5M + 5B

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 leave stage of maize with a distance of 0.125 m to the maize row on both sides of maize also with the density of 7.5 seeds m^{-2} which was chosen due 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) (Table 8). As two extremes served treatments with no weed control (T 1) and a completely weed free treatment for assessing yield potential in the absence of weed competition and damage by weed control (T 2), which was managed by multiple hand-hoeing. In a further treatment (T 3) weeds were controlled only with a mechanical hoe (until the maize plants grew too high). Additional treatments were: mechanical hoeing before the beans were sown to maize and after sprouting of beans once with the hand hoe (T 4) and (T 5) with the mechanical hoe once again. In all treatments weed control was conducted according to requirements due to appearance and quantity of weeds. All five treatments had four field replicates i.e. gives a total of 20 plots.

Table 8 Weed control treatments carried out at the experimental sites in experiment II.

Treatment number	Treatment description
Witzenhausen (organic farming)	
T1	No weed control
T2	Multiple hand hoeing
T3	Weed control by mechanical hoeing only
T4	Hand hoeing before sowing of beans into maize and after sprouting of beans
T5	Mechanical hoeing before sowing of beans into maize and after sprouting of beans
Tachenhausen and Grub (conventional farming)	
T1	No weed control
T2	Multiple hand hoeing
T3	Weed control by mechanical hoeing only
T4	Stomp Aqua (2.8 l ha^{-1}) + Spectrum (1.4 l ha^{-1})
T5	Centium 36 CS (0.25 l ha^{-1})

In Tachenhausen (TAC) and Grub (GRU) (Table 8) chemical weed control was tested and compared with mechanical weed control methods on site as well as the two extremes mentioned above. As chemical weed control, two different strategies were tested. Both herbicides were applied as pre-emergence herbicides, so immediately after sowing: (T 4) Stomp Aqua (2.8 l ha^{-1}, active substance pendimethalin) + Spectrum (1.4 l ha^{-1}, active substance dimethanamid-P) and (T 5) Centium 36 CS (0.25 l ha^{-1}, active substance clomazone). Total weed coverage was estimated visually in percentages within three areas per plot within the row with 0.75 m^2 each three times during the vegetation period at different growth stages of maize. First during the leaf developing stage, where 5 leaves are unfolded (BBCH 15) (Federal Biological Research Centre for Agriculture and Forestry 2001); second in beginning of stem elongation (BBCH 30) and third when five nodes were detectable (BBCH 35) and the canopy was closing. The three most important weeds were at WIZ *Galium aparine, Chenopodium album, Cirsium arvense*; at TAC *Echinochloa crus-galli, Chenopodium album, Fallopia convolvulus*; and at GRU *Solanum nigrum, Chenopodium album, Galinsoga parviflora*.

Harvest

Experiment I

The aim was to harvest when the maize DM content was above 30 % (early dent stage of maturity), which is common practise for silage maize in Germany (Table 6). Crops were harvested when maize reached BBCH 83. For measuring the percentage of beans in the mixture, plants were sampled from 4 x 1.5 m of the two middle rows (except 1.5 m plot edge) in each plot, then bean and maize were separated and weighed again to calculate the bean percentage. For dry matter analyses, one representative sub-sample was chopped, thoroughly mixed and one kg was dried at 105° C for 48 h.

Experiment II

The experiment II was harvested at the same time as experiment I. The aim was to assess the effect of weed control on total DM yield. In WIZ 3 x 1.5 m from the two middle rows (except 1.5 m plot edge) were sampled and weighed. In TAC a field harvesting machine (Claas Jaguar 70 SF) was used, harvesting the two middle rows and weighing them on the scale mounted on the machine. In GRU a field harvesting machine Hege 212 with Kemper maize headers and a Hege DK 800 HMP-2 scale was used. For dry matter analyses, one kg of thoroughly mixed fresh matter was weighted and dried at 105° C for 48 h.

Statistical analysis

Statistical analysis was conducted using R Software (R Core Team 2016). As the site effects were intermingled with farming system effects (organic versus conventional farming), every experimental year and site was analysed individually. The underlying assumption for analysis of variance (ANOVA), i.e. normal distribution of residuals and homogeneity of variance, were checked. For both experiments a one-factorial ANOVA was conducted. In experiment I to evaluate the effect of treatment (in four replicates) on total DM yield. In experiment II to evaluate the effect of weed control on weed coverage and total crop yield. Significant results were evaluated with the Bonferroni least significant difference post-hoc test. Linear regression analysis was performed with Microsoft Excel® software.

3.4 Results

Experiment I

In 2014 significant treatment effects on total crop yield only occurred at WIZ and TAC (Figure 3). In WIZ, as the northernmost research site, total dry matter (DM) yield of the control treatment (pure maize crop) was 25.4 t ha^{-1}. With 20.7 t DM ha-1 on average DM yield of mixtures was significantly lower and DM content was 28 %. In TAC and GRU the control achieved total yields of 17.3 and 19.1 t DM ha^{-1}, respectively, with a DM content of 38 % and 35 %, respectively. In TAC average total yield of 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 treatment 4 (5 maize (M)/7.5 beans (B) – early sown (e)) was as low as 16.4 t ha^{-1}. In WIZ highest bean DM proportion was 33 % in treatment 5M/5B (e). Contrarily, the lowest proportion (11 %) was found in 7.5M/5B – late sown (l). Both in TAC and GRU highest values (45 and 44 % respectively) were achieved in 5M/7.5B (e) and lowest were found in 7.5M/7.5B (l) (16 and 17 % respectively).

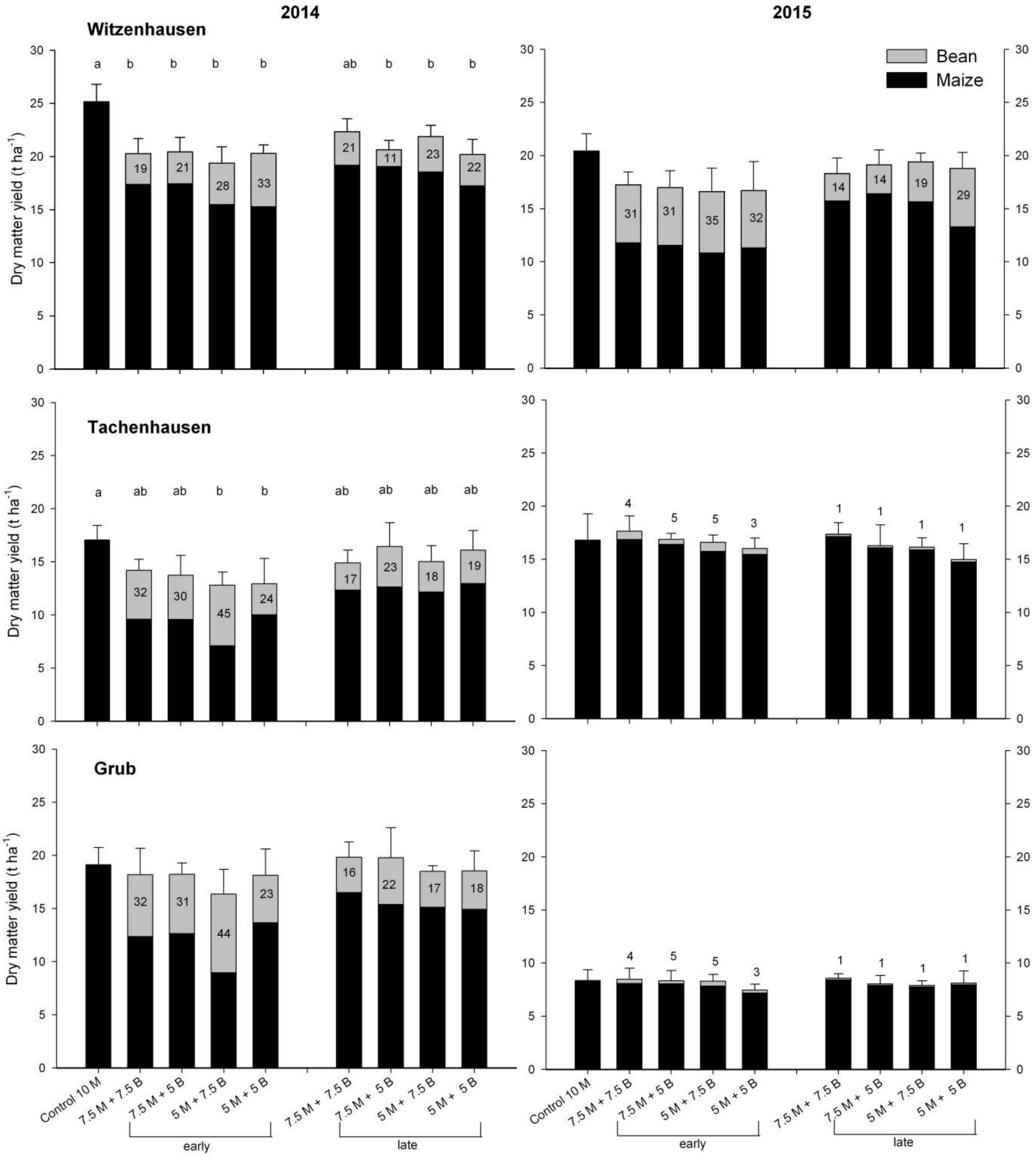

Figure 3 Total dry matter yield (t ha^{-1}) in pure stand of maize (control) and intercropped with early and late sown beans in in experiment I over two years at three experimental sites. Letters indicate significant differences in Bonferroni Least Significance Tests ($p<0.05$). Numbers inside or above bars indicate bean contribution (% DM) in the intercrop. M=maize, B=bean. Treatment names indicate sowing density of crops (e.g. 5M / 5B = 5 maize plants m^{-2} with 5 bean plants m^{-2}).

In 2015 average DM yield in WIZ was 3 t ha^{-1} lower than in 2014 and, thus, overall comparable with the results from the previous year. However, differences among treatments were not statistically significant (Figure 3). In TAC and GRU all mixtures achieved extremely low bean proportions (<5 %). Nevertheless, DM yields in TAC (16.5 t DM ha^{-1} on average) were similar to or higher than in the

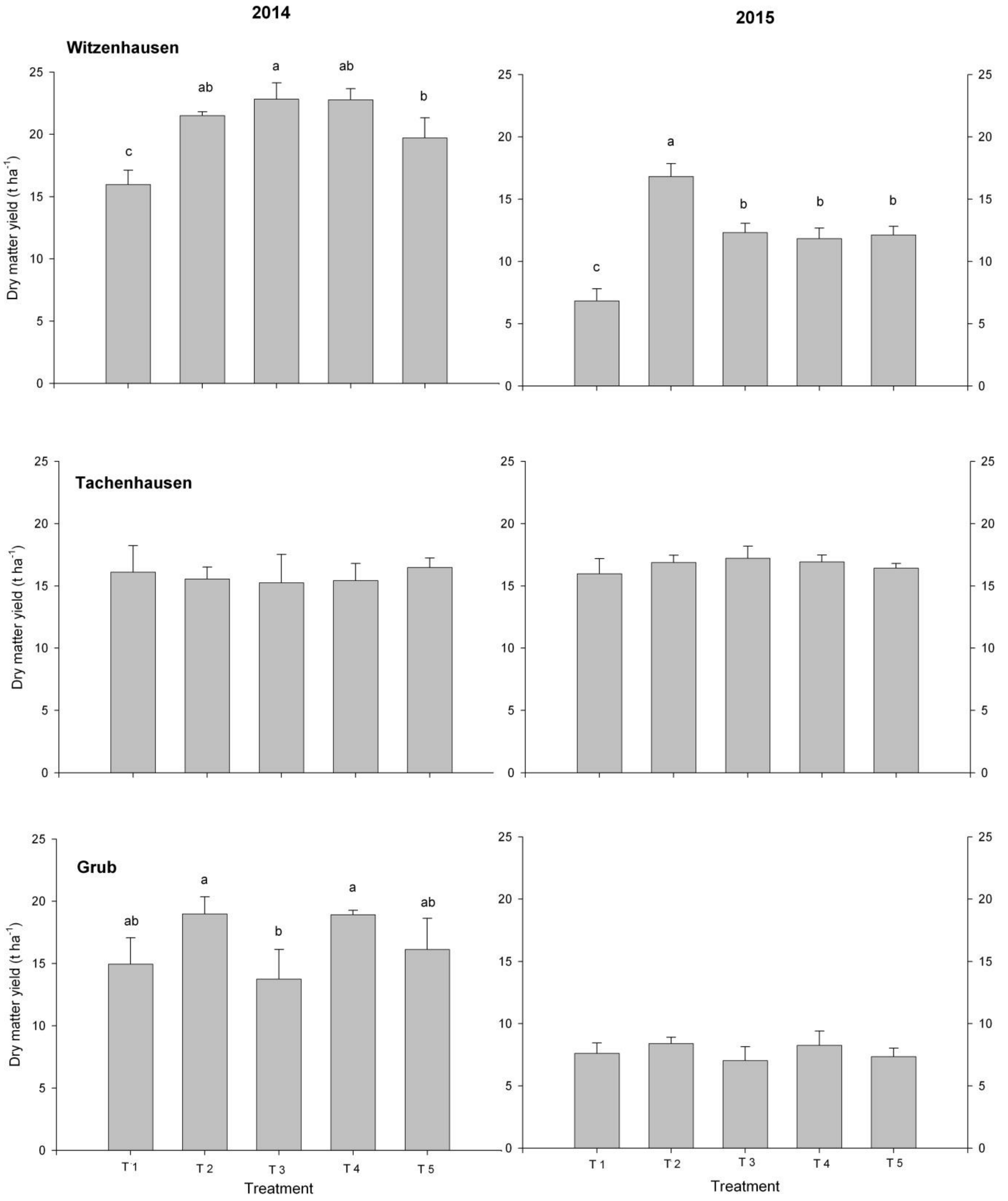

Figure 4 Total dry matter yield (t ha^{-1}) of maize-bean intercrop in experiment II over two years at three experimental sites. Letters indicate statistically significant differences in Bonferroni Least Significance Tests (p<0.05). Whiskers indicate standard errors of mean.

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 4 % less than with 7.5 maize plants m^{-2}, however, the difference could not be statistically proven. While DM content in harvested maize was between 32 and 38 %, DM content of beans was 20 % with little variation among sites and years.

Regression analysis based on bean DM contribution data from mixture plots at all experimental sites revealed a highly significant relationship of the type

$y = 0.565x + 0.6989$, $R^2 = 0.72$ ***, $n = 24$ (*** = $p<0.05$) (1)

with

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).

Obviously, 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 45 % bean DM contribution in mixtures with early sown beans.

Experiment II

Omission of weed control in WIZ resulted in 30 and 58 % weed coverage for 2014 and 2015, as estimated around the time of row closure by maize (BBCH 35). Corresponding yield reduction was 25 and 60 % for 2014 and 2015 compared to the weed-free treatment (T2;Table 9). T2 showed high yields with lowest weed coverages in both years, whereas yield of T5 was low in the two years, both with 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 16 t DM ha^{-1}, irrespective of the type of weed control (Figure 4). However, weed coverage was lowest in T2 and T4, which also applies to GRU. Although weed infestation in GRU was higher in 2014, mixtures performed much better (39.5 % 14.95 t DM ha^{-1} on average) in this year than in 2015 (10.7 % 7.34 t DM ha^{-1} on average), indicating that weed effects on the yield of mixtures may vary with weather conditions. In 2014 T3 and T5 resulted in similar levels of weed coverage and total DM yield as in the uncontrolled treatment, which also goes for 2015 but at much lower levels.

Table 9 Estimated mean weed coverage (%) at three measuring dates in experiment II over two years at three experimental sites. Numbers of treatments correspond to Table 8. Letters indicate significant differences in Bonferroni Least Significance Tests (p<0.05).

Year	2014			2015		
Maize BBCH	15	30	35	15	30	35
				Witzenhausen		
Date	17.06.	9.07.	28.07.	16.06.	9.07.	16.07.
Treatment						
T1	38.0 a	29.0 a	30	17.9 a	58.2 a	58.2 a
T2	6.9 b	3.8 b	0	7.3 b	7.2 b	7.2 b
T3	9.0 b	4.5 b	0	8.9 b	18.3 b	18.3 b
T4	10.5 b	4.3 b	0	12.1 ab	27.3 ab	27.3 ab
T5	9.8 b	6.8 b	0	10.4 ab	26.4 ab	26.4 ab
				Tachenhausen		
Date	6.06.	1.07.	15.07.	25.06.	6.07.	30.07.
Treatment						
T1	1.7 a	10.5 a	16.7 a	9.5 a	17.2 a	19.1 a
T2	0.0 b	0.0 b	1.8 b	0.0 b	2.8 c	0.0 c
T3	1.0 ab	5.2 ab	12.4 ab	7.9 a	14.4 ab	13.6 ab
T4	0.3 b	0.9 b	2.3 b	2.1 b	3.5 bc	3.5 bc
T5	0.0 b	2.5 b	5.3 ab	7.7 a	12.9 abc	14.1 ab
				Grub		
Date	12.06.	2.07.	17.07.	23.06.	15.07.	4.08.
Treatment						
T1	18.4	16.8 a	47.2 a	6.1 ab	10.5 a	10.4 a
T2	1.2	5.7 ab	2.7 b	3.5 bc	4.7 b	1.0 a
T3	33.9	14.1 a	35.1 a	6.7 a	11.2 a	11.0 a
T4	0.1	1.5 b	0.3 b	1.4 c	2.5 b	2.0 b
T5	19.5	15.2 a	36.8 a	6.6 a	11.0 a	10.8 a

3.5 Discussion

While a lot of research has been done on crop mixtures with cereals (e.g. wheat and oats) assorted with grain legumes (e.g. field beans, peas) (Mariotti et al. 2006; Hauggaard-Nielsen et al. 2008; Malézieux et al. 2009) and is common practise in organic and traditional farming, cultivating maize in mixtures with legumes is not common both in organic and conventional agriculture. Under temperate European climate conditions there may be three major reasons for this: i) relatively low competitiveness of maize in spring compared to ‘C3’ cereals; this applies the more the higher latitude and altitude, as low temperatures in spring cause a retardation of maize growth (Böttinger 2013), 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 mixing partner; this is why only tall growing crops, e.g. sunflower (Graß et al. 2013) and sorghum (Schittenhelm 2010), or climbing crops, like climbing (*Phaseolus vulgaris*) or runner beans (*P. coccineus*) (Graham, Ranalli 1997) were considered in recent mixture trials with maize.

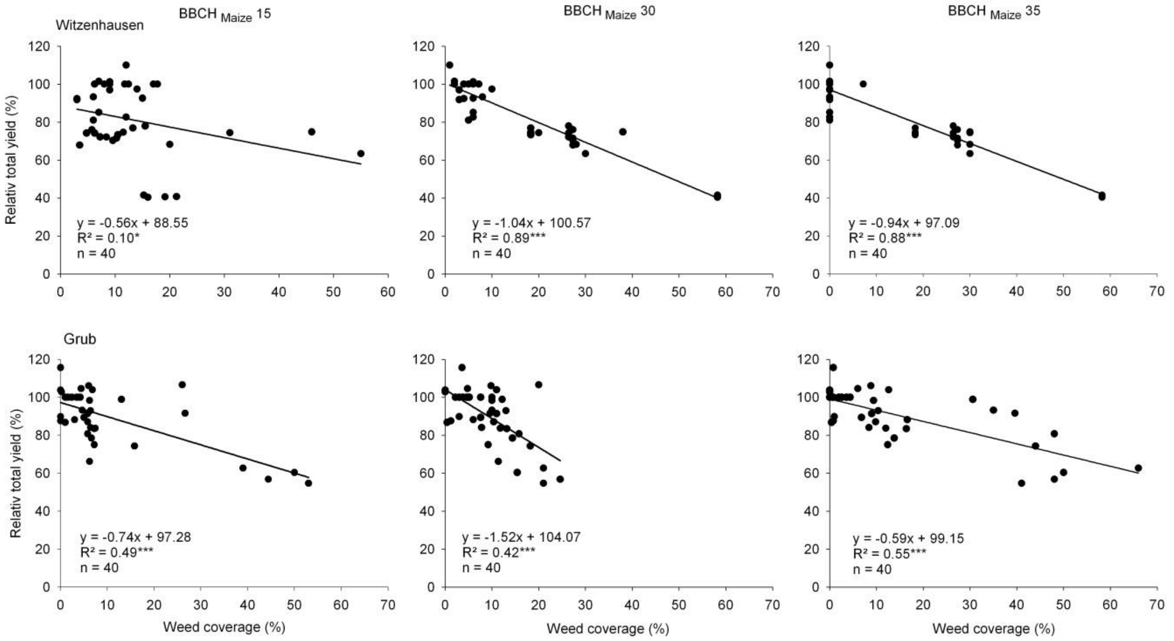

Figure 5 Graphics and statistics for the regression function y = ax + b, with y = total dry matter (DM) yield in % of the DM yield in the weed-free treatment 2 (T2, see Table 8) and x = weed coverage in % based on data from all plots in experiment II.

Contrariwise at early stages of growth climbing beans may compete with maize for resources, which is why the present field trials focused on the effect of a delayed sowing of beans (at the 5-6 leaf stage of maize compared to early sowing at the 2-3 leaf stage of maize). Across all experimental sites results of regression analysis showed that contribution of beans to the total DM yield was higher with early sowing, which is consistent with findings of Francis et al. (1982). Further this effect was enhanced by a reduction of sowing density of maize. Mixtures with early sown beans and a sowing density of only 5 maize plants m^{-2} mostly achieved higher bean shares. Obviously maize could not compensate the reduced plant density by an increased single plant growth, which could be expected due to an improved light incidence and nutrient supply through an increased distance to neighbouring plants, as reported by Lithourgidis et al. (2011).

At all sites maize contributed substantially to the total DM yield of mixtures. Although bean proportions achieved values up to 45 %, beans were hardly able to compensate the yield losses of maize caused by a decreased sowing density. This is in contrast to frequent observations that mixtures achieve higher yields (or relative yield total, RYT) compared to monocultures (Mariotti et al. 2006; Hauggaard-Nielsen et al. 2008; Urbatzka et al. 2011). Due to the design of our field trials a determination of RYT was not possible, but based on our results it must be hypothesized that mixtures of maize and beans do not lead to an increase in RYT. Pure stands of maize were sown with 10 plants m^{-2} in the present study and it can be questioned, if a comparison with maize-bean mixtures should not better be made at the same level of plant density of maize in mixtures, i.e. 5 or 7.5 plants m^{-2}. Fischer et al. (2015) conducted a field experiment with such a design and found increased yields of maize in mixed stands compared to the respective pure maize stands. However, consistent with our results they found no yield advantage of mixtures when compared to pure maize stands, whose plant density was not reduced. As the goal of our study was to consider cropping systems with practical relevance and based on the assumption that under practical conditions farmers would probably not reduce the plant density of pure maize stands, we chose the standard plant density of 10 plants m^{-2} for purely sown maize as a reference for comparisons with mixtures.

In 2015 in TAC and GRU treatment effects were strongly affected by unusual weather conditions, which were characterized by longer periods of drought and strongly reduced annual rainfall. Particularly late sown beans were affected and did hardly grow, but also early sown beans achieved very low yields. Thus, hardly any differences were found among the treatments and it seems that the dominance of maize increases with unfavourable weather conditions and at the expense of bean development. The amount of 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 very favourable for the growth of maize and beans. However it is remarkable that in both years highest crop yields occurred in WIZ as the organically managed site without any N fertilization, whereas both in TAC and GRU 100 kg N ha^{-1} were applied. This superiority is probably caused, on the one hand, by more suitable soil conditions with a deep soil profile and a high share of loess and, on the other hand, on high amounts of available soil N at the time of sowing of the maize crop (64 kg ha^{-1} in 2014 and 23 kg ha^{-1} in 2015; data not shown). This high soil N content in turn resulted probably from the fact that the experimental field had been converted to organic management only in the year before the start of the experiment and that high amounts of N were accumulated in the soil in the previous years of conventional management. Furthermore, it was only in WIZ that active nodules of *Rhizobium leguminosarum* (Camisão 2013) 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 (1997) and Camisao (2013) reported about relatively low rates of N fixation by climbing beans, compared to other grain legumes. Considering that maize has a high demand for N with the appearance of the 8th leave until the beginning of ripening (Böttinger 2013), it is only then that beans are beginning with the N fixation. Thus, an important direct transfer of fixed N from beans to maize is unlikely. It is rather the following crops within the crop rotation that may, if ever, 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 in monoculture. Apparently they do not 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 allows high crop yields at many sites, though differences to other weeded treatments were not always significant. Remarkably, hand hoeing before sowing of beans into maize and after sprouting of beans resulted in similar effects, although this treatment caused much less effort. Apparently, with only two operations weeds were weakened to such an extent, that crops were given a competitive edge. Regression analysis showed at two sites (WIZ and GRU) (Figure 5) that total DM yield declined with increasing weed coverage in the mixtures and that this relationship was particularly strong in WIZ, when weed coverage was assessed at later growth stages, shortly before row closure (Hall et al., 1992). From the equation for WIZ it can be concluded, that with the increase of weed coverage by 1 %, DM yield of the mixture declines by 1 % compared to the maize yield in the completely weed-free treatment. Further, it seems that weed coverage of up to circa 10 % may be tolerated, as the corresponding yield reduction is not very big. However, this finding cannot be readily transferred into practical farming, as it depends very much on the type of weeds. While the occurring weeds were mainly seed-propagated species, root weeds may cause much more trouble and need to be controlled already at much lower rates. It should be noted that weeding does not only reduce the competitive pressure of weeds, but also favours crops by triggering additional mineralisation of soil N through soil disturbance by cultivation tools (Dierauer, Stöppler-Zimmer 1994; Melander et al. 2005; Silgram, Shepherd 1999). 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, increased N supply) are inextricably linked with each other. Sufficient weed control appears to be possible when applying a mixture of pendimethalin (Stomp Aqua) with dimethanamid-P (Spectrum) pre-emergence. However, it has to be stated that with this combination many weeds were still present in the plots. Sites with heavy weed infestation of *Amaranthus spec*. or *Chenopodium spec.* should not be used for intercropping maize with climbing beans. Sub-optimal weed control is not seen as a hindrance of 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 (Thrupp 2000; Marshall et al. 2003).

3.6 Conclusions

Although there was a tendency for mixtures to produce lower DM yields, hardly any consistent yield difference between maize/bean mixtures and purely sown maize occurred. Bean DM contribution varied over a wide range among sites, but was higher when beans were sown at a time when the maize plants were smaller and less competitive (i.e. 2-3 leaf stage of maize). Maize/bean mixtures are as sensitive as maize in monoculture to weeds and do not supress them efficiently, if there is a high prevalence of weeds e.g. from the seed bank. It was at two from the three sites (WIZ and GRU) that total DM yield clearly declined with increasing weed coverage in the mixtures and based on data from these sites it can be concluded, that with the increase of weed coverage by 1 %, DM yield of the mixtures declined by 1 % compared to the maize yield in the completely weed-free treatment.

Further, it seems that weed coverage of up to circa 10 % may be tolerated as the corresponding yield reduction will be less than 1 t ha^{-1}. Care must be taken in transferring these findings to other sites, as the effects of weed control heavily depend on the type of weeds. The presented data do not allow a final judgement on the potential of maize/bean mixtures as an alternative for maize monocultures. There exists only few information on the feeding value of climbing beans in rations for ruminants, whereas Nurk et al. (2016) found a rather low performance when using them in biogas plants. Further testing under various site conditions exploiting the large genetic diversity of beans combined with feeding trials would be desirable to comprehensively value the benefit of maize/bean mixtures under conditions of temperate climatic conditions in Europe.

4. Methane yield and feed quality parameters of mixed silages from maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.)

4.1 Abstract

European agricultural policy increasingly focuses on environmental friendly cropping systems. Intercropping of maize (*Zea mays* L.) and common beans (*Phaseolus vulgaris* L.) has been suggested as an alternative cropping system with environmental benefits. The aim of this study was to assess methane yield potential of mixed silages. Based on material from two field experiments at three sites in Germany, mixed silages were produced with proportions of individual components varying from 0 to 100 % of fresh matter in increments of 12.5 %. Chemical parameters (neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (XP), starch, sugar and crude fat) were determined and batch tests were performed to measure methane yield potential from silages. With increasing bean proportion concentrations of XP increased while NDF, methane yield and methane content decreased. While methane yield showed a negative relationship with XP content (R^2=0.56***), a positive relation was found with NDF (R^2=0.55***). The reduction of methane yield of circa one litre of methane for each additional bean percentage in the silages could not be explained by the chemical parameters of the silages. It is hypothesized that other chemical compounds, such as lectins, which were not determined in the present study, may have influenced methane production.

4.2 Introduction

The Common Agriculture Policy (CAP) of the European Union aims at promoting environmental friendly management practices. Farmers are given incentives to implement these by EU-programmes including cross compliance regulations and greening policies (EU 2013). Additionally, national and regional regulations focus on different aspects to encourage environmental friendly management practices, as e.g. promoting the cultivation of native legumes (Legume Futures 2014).

At present maize is one of the most important crops in Europe and its utilisation as feed for ruminants or as substrate for biogas plants is common practice. In Germany nearly 70 % of all energy crops for biogas production (nearly 900.000 ha in 2014) is maize for silage (FNR 2016). This dominance is based on an efficient and well known cultivation management, high biomass yields and high contents of easily digestible constituents. However, conventional cultivation systems for maize are mostly represented by continuous cropping systems, which may increase the risk of environmental problems like decreased biodiversity, increased soil erosion and pollution of water bodies through nitrogen leaching particularly in tight crop rotations or monocultures (Koschke et al. 2013; Graß 2003; Herrmann 2013).

For this reason alternative cropping systems should be developed in agreement with the above mentioned policy strategies. These cropping systems should be productive and able to avoid or reduce these environmental problems (Dawo et al. 2007; Graß et al. 2013). Intercropping of maize (*Zea mays* L.) and common beans (*Phaseolus vulgaris* L.) has been suggested as an innovative and productive cultivation system with environmental benefits like increased biodiversity of flora and fauna, increased soil protection and leguminous N-fixation (Andersen et al. 2005; Lupwayi, Kennedy 2007). Only a few studies exist on maize/bean silage under temperate climate conditions (Mustafa, Seguin 2003; Dawo et al. 2007; Contreras-Govea et al. 2009a; Dawo et al. 2009), which mainly focussed on biomass yield and feeding value of silages for ruminants.

There exist no studies on the potential of maize/bean silage for biogas production. It is well known, that mono-digestion of maize carries the risk of lack of trace elements and nitrogen compounds with the consequence of reduced biogas and methane yields, respectively, due to malnutrition of bacteria in the digester (Demirel, Scherer 2011; Nges, Björnsson 2012; Evranos, Demirel 2015; Romero-Güiza et al. 2016). The combined digestion of several crops (Nges, Björnsson 2012) could alleviate this deficiency and increase biogas yield among other measures, like digestion of slurry or adding mineral compounds (Evranos, Demirel 2015; Romero-Güiza et al. 2016).

For the evaluation of species mixing effects the replacement series approach was used for which De Wit in year 1960 formalized the theory. This experimental design has been widely used in ecological studies to assess interference, niche differentiation, resource utilization, and productivity in simple mixtures of species (Trenbath 1974; Cousens 1991; Gibson et al. 1999). A standard replacement series is comprised of a set of pure and mixed populations in which the combined density of components is held constant (i.e. 100 % of fresh matter in our case). Although replacement series were shown to have serious limitations it is considered a valid setting for the comparison of productivity of monocultures with that of simple species mixtures (Jolliffe 2000). We considered replacement series as an appropriate experimental approach as it allows the assessment of species mixture effects—including pure maize and bean silages—on the chemical composition and biogas yield of such silages in a systematically way. With the aim to evaluate the benefits of intercropping maize and beans as an alternative for the production of biogas substrates, mixed silages with varying proportions of maize and beans were produced and tested in anaerobic digesters. The specific objectives were to

1. assess feed quality parameter of mixed maize/bean silages;
2. determine methane yield and methane percentage in biogas produced from silages;

3. investigate if there exist relationships between feed quality parameters and methane production of silages.

Figure 6 Frontal view of maize as a monocrop (*left*) and of maize-bean mixture (*right*) at the experimental site Witzenhausen at the beginning of September 2014.

4.3 Material and Methods

Field experimentation and silage production

The experiments were conducted in year 2013 and 2014 at three research sites (Table 10). Crops in Witzenhausen (WIZ; central Germany) were grown organically, whereas in Tachenhausen (TAC) and Grub (GRU), both situated in south Germany, crops were managed conventionally. While organic crops were grown without the use of mineral or organic fertilizer and pesticides, conventional crops received 100 kg mineral N ha^{-1}, 30 kg P ha^{-1} and herbicides according to local recommendations. In both years the maize variety was 'Fernandez' (FAO 250), whereas bean varieties changed. The beans from growth habit type IV were used (van Schoonhoven, Pastor-Corrales 1987): climbing if supported on a suitable tutor, with a weak, long and twisted stem and reduced branching. In 2013 in experiment I the climbing bean variety 'Neckarkönigin' was used which is an old German variety and mainly used in vegetable cultivation. It is a white blossoming and early maturing cultivar. As it turned out that this bean variety sheds the leaves soon after ripening of the first husks, which is too early compared to the maturation of maize, the bean variety 'Anellino Verde' from Italy was used in 2014 in experiment II. It grows up to 3m, has purple-pink blossoms and is a later ripening variety that stays green until the first frost and produces constantly new husks.

Table 10 Characteristics of experimental locations.

Name	Witzenhausen		Tachenhausen		Grub	
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)	227		360		525	
Soil type	Luvisol		Luvisol		Cambisol	
Experimental year	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
Annual total rainfall (mm)	522.8	563.0	744.8	816.5	914.3	814.8
Average rainfall 1985-2015 (mm)	629.0		718.1		884.7	
Annual mean temperature (°C)	8.8	10.7	9.6	11.0	8.6	9.9
Average temperature 1985-2015 (°C)	8.2		10.4		8.9	

Bean cultivars were grown in mixed stands with maize with three field replicates. Since maize was the dominating component in the stands (average across sites: 3 % of DM for 'Neckarkönigin', BBCH at harvest 97; 24 % of DM for 'Anellino Verde', BBCH at harvest 76), crops were harvested at dough ripening stage of maize (BBCH 85) (Federal Biological Research Centre for Agriculture and Forestry 2001). Figure 6 shows a pure maize crop and a maize/bean mixture at BBCH 69/69, approximately four weeks prior to harvest. In both experiments the maize was sown with a density of 7.5 seeds m^{-2} and beans were sown with same density in 2-3 leaf stage of maize with a lateral distance of 12.5 cm on both sides of maize rows. Maize and bean material was separated manually from the bulk sample and chopped to 10 mm particle size. Materials from both crops were mixed to produce mixed silages with a contribution of beans ranging from 0 to 100 % of fresh (FM) matter in increments of 12.5 %. The goal of this approach was to determine possible synergy effects between maize and beans in silages under controlled mixing ratio conditions. Three mini silos (2.7 l each) were filled with material from each level of mixing ratio, sealed gas tight and stored protected from light for 3 months at 15°C.

Biogas test and chemical analyses

With the silages batch experiments were performed in accordance with the German standard procedure (VDI 4630) as a directive and based on a procedure described by Zerr (Zerr 2006). Anaerobic fermentation of the substrates took place in airtight 20-litre polyethylene containers (Speidel, Germany) with 8 kg of inoculum which initially originated from a secondary digester of a biogas plant, operated with swine manure and maize. The inoculum (in 2013: TS 1.75 % in DM, oDM 1.15 %, pH 7.8. in 2014: TS 2.40 %, oDM 1.66 %, pH 7.9) was sieved through a 2mm sieve and stored until application under frequent feeding with ground hay from typical local grassland (appr. two times per week) in order to keep the bacteria alive without increasing the methane yield of the inoculum itself. Four kg of 37°C tap water was added to the container to reduce the headspace volume which was afterwards taken in to account in the calculation of produced methane amount. The material from three mini silos was mixed and 400 grams of fresh matter (FM) of the silage was weighed and given per container. Every silages methane production was measured in two parallels. The containers were closed airtight to create an anaerobic environment for the digestion. The containers were placed in heated water basins to keep the process temperature under mesophilic conditions at 37°C. There were containers serving as a blank (inoculum with only water) and standard (inoculum with 50 g cellulose) to assess the biological activity of the inoculum. The containers were stirred for 15 min at 75 min intervals. Gas bags of 20 litres (Tecobags, Tesseraux Spezialverpackungen GmbH, Germany) were connected to the containers with rubber tubes. The total biogas volume produced up to day 35 (VDI 4630) was normalised to standard temperature and normal pressure conditions (i.e. dry gas, 273 K, 1013 hPa). The produced biogas was measured with a wet drum gas meter (TG 5, Ritter, Germany) and analysed for CH_4 content by an infrared gas analyser (GS IRM 100, GS Messtechnik GmbH, Germany). Before sample measurements, a control gas with a content of 60 % CH_4 and 40 % N_2 was measured to check accuracy of measurements. For final calculation of the specific methane potential per kg of volatile solids the formula of Weissbach (Weißbach, Kuhla 1995) was used to take into account the losses of volatile organic compounds during dry matter analyses. All silages were analysed for dry matter (DM) at 105 °C, crude ash (XA) at 550 °C, crude protein (XP) with Kjeldahl methodology, crude fat (XL), starch and sugar, organic NDF, organic ADF after German VDLUFA methods (Naumann et al. 1976).

Statistical analyses

Statistical analyses were conducted using R Software (R Core Team 2016). In the two separate years, the design of the experiment followed a 3x9 factorial layout with three experimental sites (ES) and nine mixing ratios (MR) with two lab replicates. It is worth mentioning, that mixing ratios in the ensiled material do not refer to the mixing ratios on the field. Thus they do not reflect field variability. However, as bean DM contribution only ranged between 3 and 30 %, we worked with artificially mixed silages in a replacement series to understand the implication of increased proportions of beans in the mixture from 0 to 100 %. Both factors (mixing ratio and experimental site) were treated as independent factors in a fixed-effects model of analysis of variance (ANOVA). Bonferroni Least Significant Difference test was used as post-hoc test for comparisons among mean values, because it controls the Type I error appropriately and is conservative. Further, the test is particularly appropriate for smaller number of comparisons. The homogeneity of variances of the model was verified graphically; the normal distribution of the residues was tested with the Shapiro–Wilk test. A Gompertz model (Donoso-Bravo et al. 2010) of the type $Y=a*\exp(-b*\exp(-c*X))$ with Y = CH_4 yield (l_N kg^{-1} oDM) and X = fermentation time (d) was fitted in SigmaPlot (SigmaPlot 9.0) to observe cumulative methane production. In order to determine whether there exists any relationship between chemical composition and methane yield of silages, regression models were fitted with the chemical parameters as explanatory variables. Starting with saturated models, which included all measured chemical parameters (NDF, XP, ADF, crude fat, sugar, starch) and their two-way interactions, non-significant model effects were gradually erased until only significant parameters remained in the model.

4.4 Results

Silage quality

All silages produced with the bean cultivar 'Neckarkönigin' in 2013 (Figure 7) were well-fermented with pH-values between 3.50 and 4.40. No moulds or bad odours indicating organic matter deterioration were detected when the silos were opened. While silages from pure maize showed DM contents of 30 %, DM contents decreased to 20 % for pure bean silage. No consistent differences among sites were observed. The NDF content decreased from 49 % of DM for pure maize to 29 % of DM for pure bean silages. Ash content was from 3.8 % in DM for pure maize and increased up to 9.2 % for pure bean silage. The same pattern was observed for XP content, from 6.7 % to 12.4 % of DM in pure maize and bean silages respectively. The concentration of ADF in silages did not show any consistent change with increasing bean contribution and accounted for 25.3±1.9 % in DM. Crude fat content in silages was on a low level with somewhat lower values in WIZ (1.8±0.2 % in DM) than at the other sites with a mean value of 2.3±0.2 % in DM. The content of starch hovered around 26±3 % in DM and did not differ significantly among the experimental sites. Sugar contents were similar within the experimental sites, but differed strongly between the sites. The highest sugar content was in TAC with 2±0.5 % in DM, followed by GRU with 0.9±0.7 % and WIZ with 0.2±0.14 % in DM.

Silages of all mixing ratios produced with the bean cultivar 'Anellino Verde' in 2014 (Figure 8) were likewise well-fermented with pH ranging again from 3.50 to 4.40. Concentration of DM, ash, NDF and XP were very similar to silages with 'Neckarkönigin' and changed significantly with increasing bean contribution in the silage. ADF contents in silages from TAC were lower (<27 % in DM) than at the other sites when maize contribution was more than 25 % of FM, whereas values for silages with high bean contribution were similar for all sites (26 % of DM on average). Although starch content decreased with increasing bean contribution at sites WIZ and TAC, the changes were not statistically significant. Crude fat and sugar contents were low again over the whole range of bean contribution in the silages.

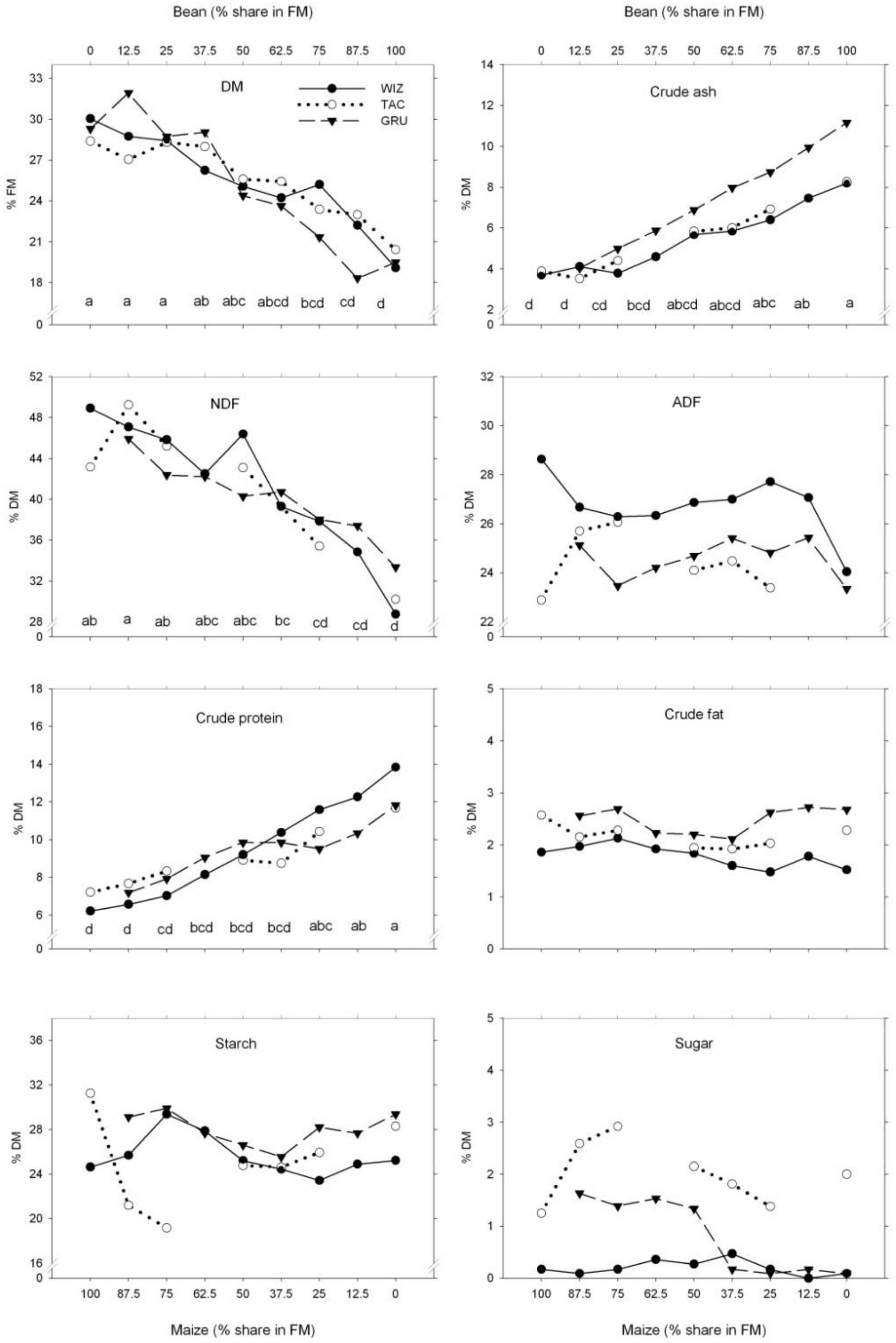

Figure 7 Experiment I (bean 'Neckarkönigin'; maize 'Fernandez'; year 2013). Dry matter (DM), crude ash and analysed chemical parameters (NDF = organic neutral detergent fibre, crude protein (XP), ADF = organic acid detergent fibre, starch, sugar and crude fat) from silages with different maize and bean proportion (% of FM) at three experimental sites (WIZ=Witzenhausen, TAC=Tachenhausen, GRU=Grub). Different letters at the bottom of diagrams indicate significant differences among mixing ratios averaged over three experimental sites (p<0.05).

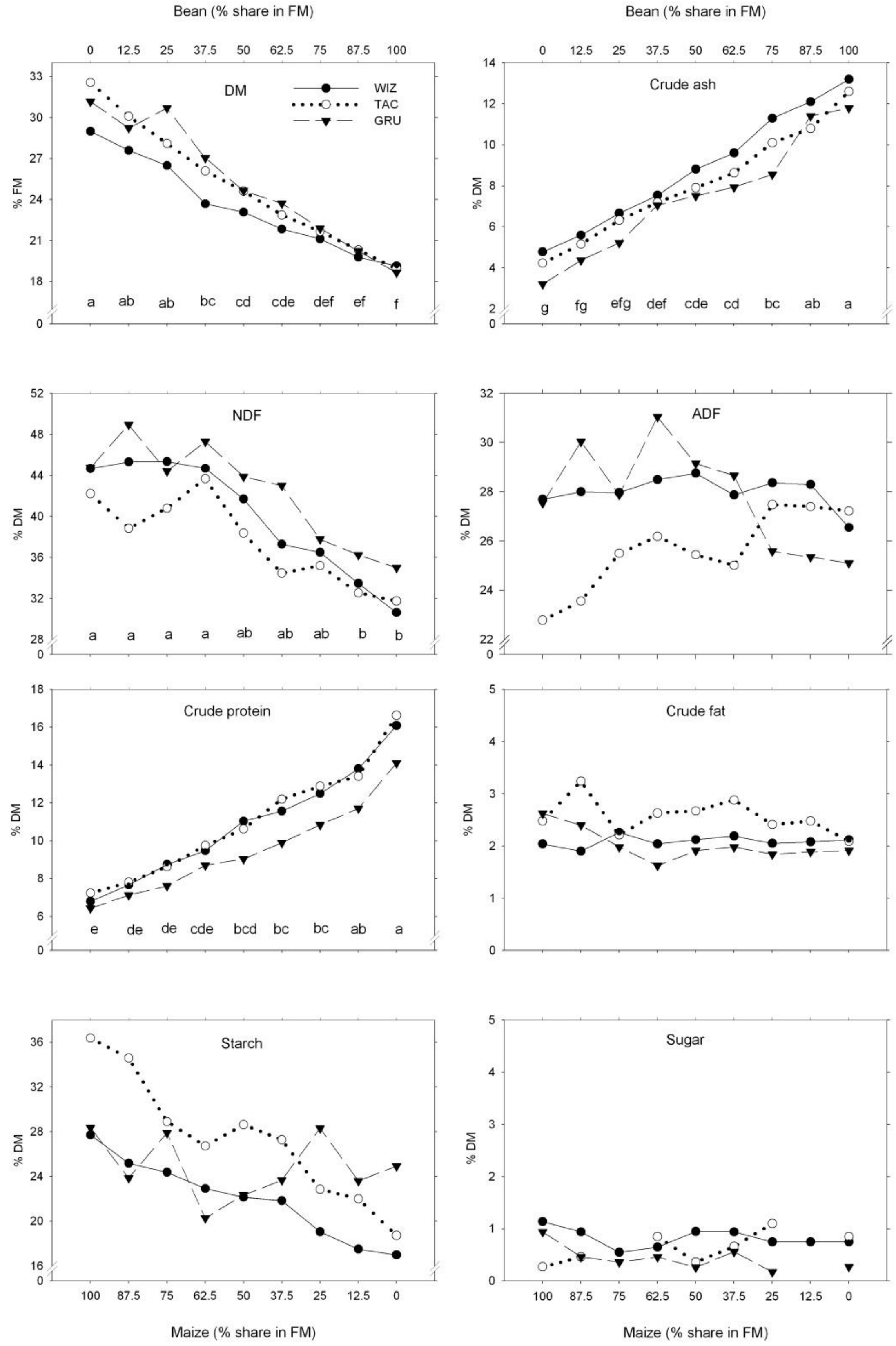

Figure 8 Experiment II (bean 'Anellino Verde'; maize 'Fernandez'; year 2014). Dry matter (DM), crude ash and analysed chemical parameters (NDF = organic neutral detergent fibre, crude protein (XP), ADF = organic acid detergent fibre, starch, sugar and crude fat) from silages with different maize and bean proportion (% of FM) at three experimental sites (WIZ=Witzenhausen, TAC=Tachenhausen, GRU=Grub). Different letters at the bottom of diagrams indicate significant differences among mixing ratios averaged over three experimental sites (p<0.05).

Methane production

Methane yield and biogas methane content generally declined with increasing bean contribution in the silage, however, in both years the scale of yield reduction was different among the three experimental sites (significant mixing ratio x site interaction with $p<0.0251$*** for 'Neckarkönigin' and $p<0.0038$*** for 'Anellino Verde') (Figure 9 and Figure 10). In silages with 'Neckarkönigin' methane yield of pure maize silages was between 340 and 360 l_N kg^{-1} oDM at all sites, whereas yields of silages purely produced from beans varied between 180 at site GRU and 250 l_N kg^{-1} oDM at site TAC. In silages with 'Anellino Verde' methane yield of pure maize silages ranged from 290 (site TAC) to 340 l_N kg^{-1} oDM (site GRU), while from pure bean silages only 200 (site WIZ) to 240 l_N methane kg^{-1} oDM (site GRU) could be produced. Based on the common dataset a relationship was found between methane yield (l_N kg^{-1} oDM) (y) and bean proportion in the silage (% of FM) (x) of the equation $y= -1.04x + 329.06$ ($R^2=0.61$***, mean square errors (MSE) =27), indicating that with each additional percent of bean proportion in the silage methane yield was reduced by one l_N kg^{-1} oDM on average.

A model of the type $Y=a*\exp(-b*\exp(-c*X))$ with Y = CH_4 yield (l_N kg^{-1} oDM) and X = fermentation time (d) was fitted to the observed cumulative methane production from all silages over both years with coefficients of determination (R^2) between 0.98 and 1.00 and MSE values of 33.7 to 15.9 l_N kg^{-1} oDM (data not shown). Drawing on the example of 'Anellino Verde' at site TAC in 2014,

Figure 11 illustrates the digestion kinetics of silages with different bean proportion. The investigation of cumulative methane production curves showed that c-values, indicating the magnitude of methane production per day, decreased with increasing bean proportion. Based on data of all sites and bean cultivars, a regression analysis showed that a relationship exists between c - value (z) and bean proportion in the silage (w) of the equation $z= 0.0013w + 0.2659$ ($R^2=0.85$***, MSE=0.0190) (data not shown).

Regression models were fitted, to evaluate relationship between chemical composition and methane yield of silages, with the chemical parameters as explanatory variables. Across both bean cultivars and all sites, only XP and NDF from all six measured parameters (NDF, XP, ADF, crude fat, sugar, starch) showed any explanatory power in multiple linear model with R^2 value of 0,59*** (

Figure 12). The standard error of prediction was rather high with 26.7 l_N methane kg^{-1} oDM. A simple linear model solely based on one of the parameters showed for XP a R^2 of 0.56*** and 0.55*** for NDF. However, standard errors of prediction were rather high with 35 and 38 l_N methane kg^{-1} oDM respectively.

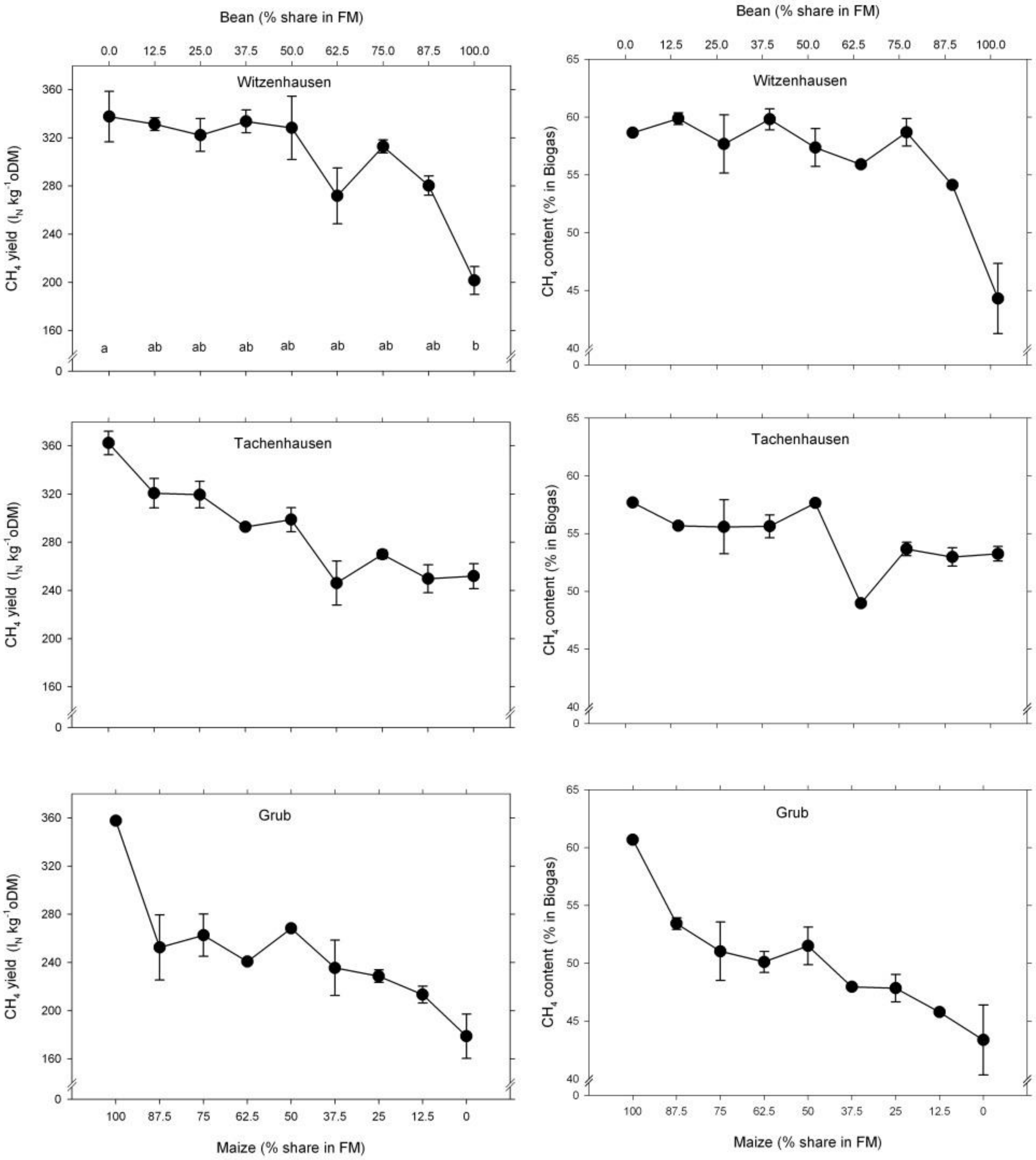

Figure 9 Experiment I (bean 'Neckarkönigin'; maize 'Fernandez'; year 2013). Methane yield and content of methane in biogas from silages with different maize and bean proportion at three experimental sites. Different letters at the bottom of diagrams indicate significant differences among mixing ratios averaged over three experimental sites ($p<0.05$).

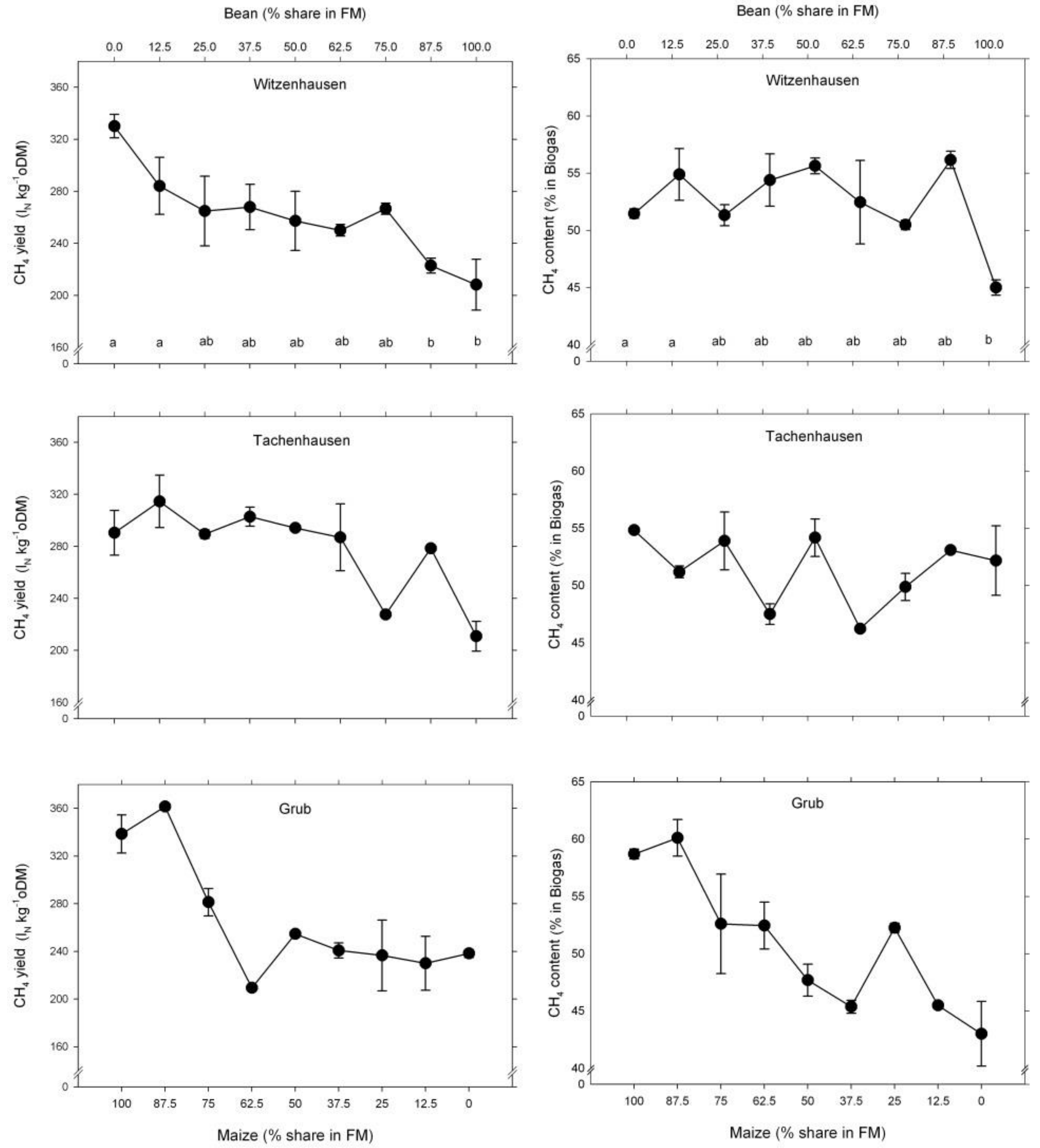

Figure 10 Experiment II (bean 'Anellino Verde'; maize 'Fernandez'; year 2014). Methane yield and content of methane in biogas from silages with different maize and bean proportion at three experimental sites. Different letters at the bottom of diagrams indicate significant differences among mixing ratios averaged over three experimental sites ($p<0.05$).

4.5 Discussion

Numerous experiments have been carried out to test the effects of intercropping maize with legumes, e. g. with soybean (*Glycine max* L. Merr.), different lupines (*Lupinus ssp.*), lablab bean (*Lablab purpureus* L.), velvet bean (*Mucuna pruriens* L. D.C.), cowpeas (*Vigna unguiculata* L. Walp), lucerne (*Medicago sativa* L.) (Titterton, Maasdorp 1997), faba bean (*Vicia faba* L.), scarlet runner bean (*Phaseolus coccineus* L.) (Armstrong et al. 2008) peas (*Pisum sativum* L.) (Mustafa et al. 2000) and bush beans (*Phaseolus vulgaris* L.) (Dawo et al. 2007). However, only few dealt with climbing beans. Previous results showed that the nutritive value of maize-bean silage was similar to maize silage, indicating that maize-bean silage mixtures could be used in dairy cow rations (Contreras-Govea et al. 2009a). Investigations based on assessing the nutritive value of mixed silages and feeding trials have been carried out by numerous researchers (Titterton, Maasdorp 1997; Anil et al. 2000; Mustafa et al. 2002; Contreras-Govea et al. 2009a), but there is a lack of research considering the potential of maize-bean silages for biogas production. The chemical compositions of the silages used in this experiment were comparable with results described in other experiments (Titterton, Maasdorp 1997; Mustafa, Seguin 2003; Armstrong et al. 2008; Contreras-Govea et al. 2009a; Contreras-Govea et al. 2009b). While DM content of pure maize silages was as the standards require (approx. 30% in FM), DM content of pure bean silages was lower than described in the literature (Mustafa, Seguin 2003). Apparently due the fact that the latter author used air dried crops with a DM content of 30% for producing pure bean silages, while silages in the present study were produced from fresh material. Armstrong et al. (2008) conducted experiments with maize/legume silages, where the percentage of the legume was so low that it did not affect DM content. In our study DM content of silages with high proportions of beans was generally not adequate as DM contents below 30% significantly increase the risk of mal-fermentation as well as energy and nutrient losses (Herrmann, Rath 2012). For a further evaluation it is yet to determine, which bean proportions can be achieved under practical conditions when intercropped with maize.

Ash content in pure maize silages was similar in both experiments, whereas it differed between the bean cultivars. This might be explained by the different maturity levels of the two bean cultivars. At the time of harvest 'Neckarkönigin' had already lost most of the leaves, as this cultivar is bred for vegetable purposes, thus, discarding the leaves to facilitate the harvest of beans for food consumption. The low proportion of leaves may have decreased the ash amount, as in turn a large leaf area in combination with the hairy surface of bean leaves may bind minerals from rainwater splashes, resulting in increased ash contents. Vice versa, this may also explain the higher ash content in the cultivar 'Anellino Verde' (experiment 2), which still had green and lush leaves at the time of harvest. Our study confirms the findings of Armstrong (2008) that NDF content of maize in under given conditions and as a grass is higher than in pure climbing bean as an annual leguminous plant.

NDF content in silages declined almost linearly with increasing bean proportion in both experiments (Figure 7 and Figure 8). A similar linear trend, but in the opposite direction, was observed for protein content, which is in good agreement with results of Armstrong et al. (2008), who found double protein contents in bean compared to maize. Fischer and Böhm (2013) also reported higher XP contents of silages with a mixture of maize and climbing beans compared to pure maize silage. There is not much information about starch, sugar and fat contents in silages from legumes. Bean proportion in the present experiment did not show a clear effect on these chemical components.

Results from the present study show that of all measured chemical components only NDF and XP were significantly correlated over both years with the methane yield (Results from the present study show that of all measured chemical components only NDF and XP were significantly correlated over both years with the methane yield (Figure 12). While there was a positive correlation with NDF, methane yield declined with increasing content of XP. It is well known that legumes and grasses differ in NDF content and that correlations of fibres and parameters indicating the magnitude of biological digestion processes (e.g. digestibility of organic matter) may differ between these plant groups depending on plant species, annuality and developing stage (Appels et al. 2011; Dandikas et al. 2014). Looking more closely at the data, it becomes obvious, that in the diagrams of both regression models the data were arranged along the x-axis with changing bean proportions from left to right. This leads to our hypothesis, that the real driver behind these relations is the bean proportion in the silage. If models were fitted to methane yield merely based on bean contribution as the only explanatory variable, model accuracy would be the same ($R^2 = 0.55^{***}$). It was thus not possible to explain methane yield decline based only on the chemical composition. The reasons could be either an insufficient characterisation of the chemical composition or additional factors as suggested below.

Remarkable differences in methane yields between bean cultivars were observed in WIZ: while the methane yield for 'Neckarkönigin' in year 2013 remained relatively high even with higher bean proportions in the silage, methane yields from silages with 'Anellino Verde' in year 2014 declined strongly already at low bean proportion. Interestingly, also biogas methane content seems to decline with increasing bean proportions. There is ample knowledge about inhibitory substances, which affect biochemical reactions constituting the anaerobic digestion process, i.e. specific organic toxicants (e.g., chlorophenols, halogenated aliphatics and long chain fatty acids), inorganic toxicants (e.g., ammonia, sulfide and heavy metals) and nanomaterials (Hilpert et al. 1984; Slifkin, Doyle R.J. 1990; Vasconcelos, Oliveira 2004; Mudhoo, Kumar 2013). Accumulation of these substances may cause reduced biogas production and/or biogas methane content.

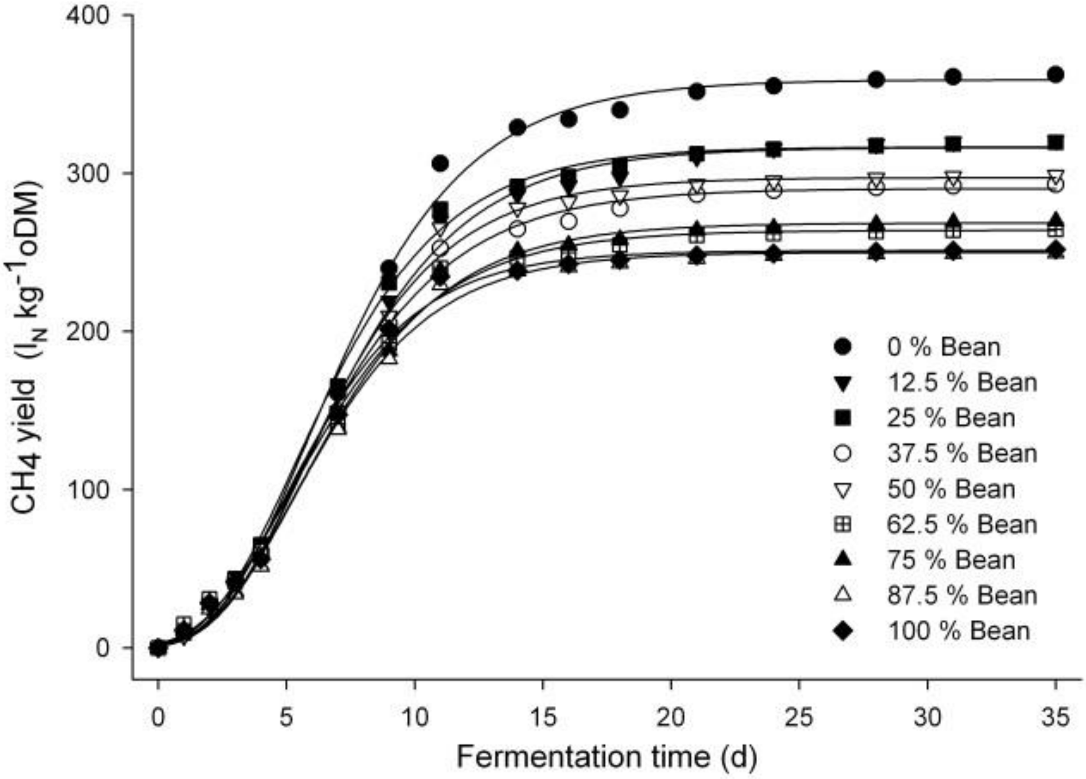

Figure 11 Mean values of specific cumulative methane yield from silages with different maize ('Fernandez') and bean ('Anellino Verde') proportion. The diagram displays data from the experimental site Tachenhausen. The equation of the fitted curve is of the type y = a*exp(-b*exp(-c*x)) (R2 ≥ 0.99*).**

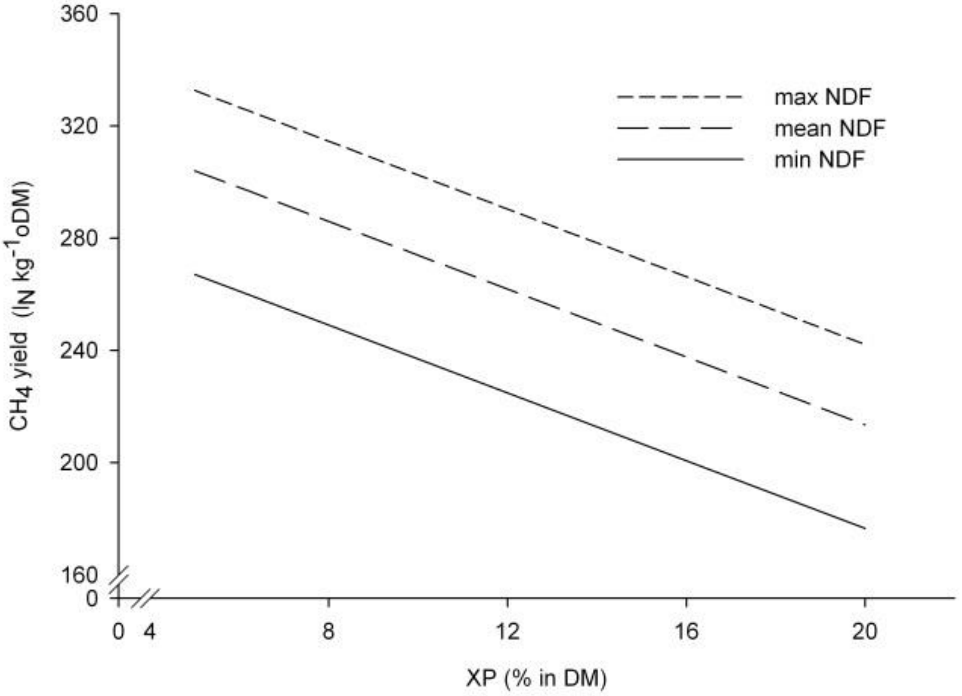

Figure 12 Relationship between methane yield, crude protein (XP) and NDF (SEM = 26.7, n = 41, R^2 ≥ 0.59*) concentrations in silages with different maize and bean proportion. The equation of the fitted curve is of the type y (methane yield) = 205.4-6.0*XP +3.2*NDF. Regression models include data from two bean cultivars grown in two separate years**

However, there is no information that beans contain high concentrations of those substances. Possibly other plant chemical compounds, which were not determined in the present study, may have caused these differences in methane yield decline. It is well-known that seeds of the kidney bean (*Phaseolus vulgaris* L.) contain Phytohemagglutinin (PHA), which belongs to the group of plant lectins. Lectins are proteins that bind in specific and reversible ways to carbohydrates and have been shown to possess a remarkable array of biological activities. The toxicity of PHA in humans and animals is well established and they also possess the ability to aggregate microorganisms (Slifkin, Doyle R.J. 1990; Vasconcelos, Oliveira 2004) and to inhibit microbial growth (Sadananda et al. 2013; Nader et al. 2015). While 'Neckarkönigin' is a cultivar with white seeds, which notoriously contain less lectins, 'Anellino Verde' is a brown shell bean cultivar, for which higher contents of tannins and lectins were recorded (Makkar et al. 1997; Muzquiz et al. 1999). However, only little knowledge exists about the effects of lectins for digestion processes both in animals and biogas digesters. Major increase of methane yield of pure maize silage started a few days earlier and with higher intensity compared to silages with higher bean proportions. Beside possible lectin effects, higher contents of easily digestible components like NDF and starch may explain the superiority of maize compared to bean (Figure 7 and Figure 8). This is in accordance with the frequently mentioned suitability of maize silage for methane production (Herrmann, Rath 2012; Rath et al. 2013) due to higher methane yields of maize silage compared to other crops (Amon et al. 2007a; Gunaseelan 2007; Appels et al. 2011).

4.6 Conclusions

The results from batch digestion experiments with mixed silages of maize and two different cultivars of climbing beans proved substantial effects of bean proportion on the chemical composition and methane yield of such silages. While XP increased with increasing bean proportion, NDF, methane yield and biogas methane content decreased. Although the increase of species diversity in energy crop production through crop mixtures would be desirable, intercropping maize with beans for biogas production does not prove promising from an energetic point of view at the moment. As the change of chemical composition with increasing bean proportion did not fully explain the decline in methane production, more information is necessary considering the role of toxic substances (e.g. lectins) in bean biomass. Further research is also needed to evaluate the potential of such crop mixtures for animal feeding.

5. General discussion

In recent years, maize has become an increasingly important substrate for biogas production in Germany. At the same time it has gotten a negative reputation (Herbes 2014). In a few regions maize is grown on more than 50 % of the arable land (Deutschen Maiskomitee e.V. 2016). This intensive cultivation is causing numerous negative effects on the environment like soil erosion, decreasing humus content and the loss of biodiversity. In order to minimize the negative environmental effects the German Renewable Energy Sources Act from 2012 (ANONYMOUS 2016) states, that the share of maize as a substrate in the newly built biogas plants may not be higher than 60 %. On the European level the Common Agricultural Policy (CAP) in the Greening policy is regulating that fields over 30 ha have to be cultivated with at least 3 different crops , and the highest share of one crop is restricted to 70 % and the two main cops are not allowed to exceed 95 % of the total area in any year (European Commission 2016). Against this background this thesis evaluated different options to find possible alternatives for maize.

There are several studies suggesting sunflower and sorghum as possible alternatives to maize (Amon et al. 2007a; Appels et al. 2011). To evaluate the potential of these suggested alternatives all three crops were harvested in four different maturity stages and conserved as silage. Furthermore, the different maturity stages were chosen based on future considerations were changing climate and different weather conditions might influence the harvesting time (Graß et al. 2013). In addition, the material was analysed for different chemical parameters – NDF, ADF, ADL, crude protein and remaining organic constituents. An *in sacco* method known from dairy production was used to determine the degradation dynamics of ingredients (Ørskov et al. 1980; Dewhurst et al. 1995). Also the effect of various chemical parameters on biogas production was evaluated based on Amon et al. (2007a). In order to examine the parameters over the time of degradation the material was analysed at various time points, however, in contrast to livestock experiments (Ørskov et al. 1980) in larger intervals. In the second chapter of this thesis it is shown that throughout the investigated maturity stages (See Figure 2), the degradation kinetics of fibres showed some similarity between maize and sorghum-sudangrass. Thereby sorghum-sudangrass can be seen as a possible alternative for maize in the younger maturity stages in biogas production with similar dry matter yields like found by Zeise et al. (2016). However sunflower had different content, degradation dynamics and a lower methane yield. Furthermore, regression models based on measured organic constituents were fitted for the methane production potential like done by Amon et al. (2007a). However, the methane potential could not be predicted with the same high accuracy. In this study the data of all three plants and maturity stages were used. Thus, the reason for the low accuracy of the models could be the differences in the plants' chemical composition, which depends on ripeness and plant species.

To find alternatives for maize monocropping field experiments were conducted with maize and climbing bean mixed culture. The reason for that is the intention to bring legumes on the field (European Commission 2016) and raise biodiversity in addition to minimizing the negative impact of erosion. Because of the long youth developing stage of maize and low ground coverage, the field is exposed to wind and rainfall much longer than in case of grain. Both of them could cause great losses of fertile soil in extreme weather conditions. Adding beans to maize increases the ground coverage and protects it from erosion and at the same time increases the Land Equivalent Ratio – the efficiency of intercropping for using the environmental resources compared with monocropping (Lithourgidis et al. 2011). In this research the climbing bean was sown in 0.125 m distance to the maize (Chapter 3). The conventional seeding density - 10 seeds m^{-2} (Deutschen Maiskomitee e.V. 2016) served as a control in the underlying study. In the experiment the seeding density of maize was reduced to complement with beans. It is known from literature that the various plants are competing over light and nutrients and verifying that the bean sowing time was varied. The results showed no statistical significant differences in the total dry matter yield (See Figure 3), but they did highlight the influence of seeding time of beans. The amount of beans was mostly higher in the treatments with early seeded beans and lower in the later seeded bean treatments like it is also stated by Francis et al. (1982). Therefore it could be concluded, that it would be possible to influence the composition of crop mix by modifying seeding time. If the target is set to have higher bean proportion then the beans should be seeded earlier to the maize, but to have higher maize yield the beans should be seeded later. In addition, it would be possible to look for bean and maize varieties that could be seeded out at the same time which would spare the drive out costs.

When seeding maize and beans together on the same field, another parameter needs to be considered. The cultivation method would need to consider weed controlling methods. In given thesis under organic conditions - enough space was left to allow the machines to drive through the rows until the maize has grown so tall that weed suppression was not necessary anymore. This approach is rather challenging as weather has a strong impact on the growth conditions of the crops and the weed. This becomes obvious when comparing the results from two years in WIZ (See Figure 4) under organic conditions. In one year suitable weather conditions ensured that maize grew so fast and supressed the weed development regardless of the weed supressing method, whereas in the next year dry weather conditions meant that there was competition between crops and weeds. The dry matter yields were lower than the year before and that regardless of treatment. In the case of conventional weed control it is not easy to find a suitable plant protection product since one of the mixture partners is a mono- and the other one a dicotyledonous crop (Lithourgidis et al. 2011). In this thesis an herbicide mix – Stomp Aqua + Spectrum – is recommended, but it is expected that it is possible to find an even more effective solution. Certain is that with every additional weed coverage percentage until the canopy closing stadium of maize and beans the reduction of dry matter that could be harvested is 1 t regardless of the achieved harvest.

Few researches have been carried out to investigate the possibility of using maize-bean silage as feed (Contreras-Govea et al. 2009a; Contreras-Govea et al. 2011). That could be a good alternative to importing foreign proteins and promoting local protein production. It is well known, that maize does not have high protein content. It varies between 60 to 90 g per kg of DM (Khan et al. 2015). Under the conditions of the underlying trials the protein content of maize silage was about 60 g pro kg DM (Figure 7 and Figure 8). This could be increased with the proportion of beans. In this thesis, it is shown (Figure 7 and Figure 8) that with increasing bean proportion the protein proportion is also linearly increasing, as expected. If we assumed that the share of beans would be 35 % in the harvested maize-bean mixture (Figure 9 and Figure 10), it would increase the protein content by 50 %. Depending on the cow, weight, age and performance, roughly 3000 g protein per day is needed for the animal (Kirchgeßner et al. 2008). To cover the need for protein with maize-bean silage, the animal should be fed one third less in comparison to pure maize silage feed. Thus, the same amount of protein from 5 kg maize-bean silage would be obtained in 1 kg of heat-treated soybeans (Kirchgeßner et al. 2008). At the same time the amount of Net Energy Lactation (NEl) decreases from 6.7 MJ to 6.3 MJ. NEl is the feed energy available for maintenance and milk production after digestive and metabolic losses. For this reason, at the moment it is economically and energetically more favourable to use pure maize silage with a high energy content supplemented with protein rich feed as animal fodder.

Regardless of higher crude protein concentrations, the experimental results of biogas production potentials obtained in this thesis are not as good as expected from the results of unpublished preliminary trials (See Figure 9 and Figure 10). A quite negative influence of beans on biogas yield was determined. With every additional percent of bean in the mixture, 1 l less methane was produced in comparison to what could be achieved from 1 kg OM of maize silage - 350 l CH_4. The reason could be the presence of certain components in the beans. It is known that beans as legumes produce a certain type of protein that could serve as defensive compound – lectins (Slifkin, Doyle R.J. 1990; Vasconcelos, Oliveira 2004; Sadananda et al. 2013; Nader et al. 2015). These substances have anti-bacterial properties and it might be possible that they are also negatively influencing the biogas production process. In this thesis, the concentration of lectins was not measured, but the results are indicating a problem that should be further investigated.

Based on the knowledge gained from this thesis regarding crop composition and alternative cultivation method it could be stated that at the time there is no better crop as biogas substrate than maize. Nonetheless, because of the difficult situation of maize as a lucrative substrate - being partially overused due to its good properties - it will be necessary to find suitable alternatives or make the cropping system better. Certain are that the maize will remain as main energy source so that seeding density under 7 seeds per m^2 is not recommended. Also a more suitable bean variety with less lectin content and same seeding time as maize would be advisable.

6. Conclusions

This thesis presents data from different approaches in the search for alternatives for maize as a biogas crop. Firstly potential of maize as a biogas crop for the methane production and alternatives like sorghum and sunflower at different harvesting time that could occur in double cropping system were investigated. Furthermore results of a three year field experiment in three research sites of maize-bean mixed culture cultivation under organic and conventional conditions are presented. Besides the total yield also weed controlling methods and the biogas production potential of the mixture were investigated. Based on these findings and subsequent laboratory investigations, the following conclusions can be drawn:

(i) As biogas substrates of double cropping systems the best results for biogas production were achieved with maize. Throughout the four different harvesting times the two alternatives sunflower and sorghum-sudangrass could not produce biogas on the same high and stable level as maize.

(ii) It is possible to cultivate maize and beans on the same field. In the tested seeding ratios there were no statistical differences in the total dry matter yield, but remarkable differences in bean proportions in the mixtures. An earlier seeding of the beans leads to a higher bean proportion in total biomass. This might allow influencing the final harvesting composition of maize-bean mixtures through the seeding strategy.

(iii) Less efficient suppression of weeds during the younger developing stages of the crops influences the total yield in such a way that each additional percent of weed coverage leads to the loss of 1 t of DM at harvest.

(iv) For the weed controlling methods in conventional farming the most effective treatment was Stomp Aqua + Spectrum. In organic farming, no statistically significant differences between treatments were found.

(v) The measured biogas production potential of maize-bean mixed silages showed a negative effect of the bean proportion. With every additional percent of beans the biogas production decreased by approximately 1 l in the comparison to pure maize silage with methane production potential of 350 l (kg oDM)$^{-1}$.

7. Summary

In Germany maize production has increased in the last years due to the need for biomass for biogas production. Despite its good properties for cultivation and usage maize also has gained a negative reputation. Particularly in dense cultivation areas it is criticized for changing the appearance of the landscape to a uniform one and also reducing species richness.

In order to find alternative crops for bioenergy production two approaches were examined: biogas production characteristics of alternative energy crops in a double-cropping system with respect to early harvesting times as well as the potential of a mixed-cropping system of maize and bean.

The degradation dynamics of sunflower and sorghum-sudangrass as alternative crop to maize were measured. To imitate the possibility to harvest earlier, like it could happen in a double-cropping system, four different maturity stages were analysed. The results imply that degradation kinetics of fibres were similar for maize and sorghum-sudangrass. Sampling date effects were significant for all fractions except lignin, indicating that sorghum-sudangrass can be harvested earlier without impact, whereas for maize and sunflower methane yields per unit of organic dry matter declined at earlier harvest dates. Regression analysis showed a moderate relationship between methane yield and organic fractions.

In search of a possible alternative to maize monoculture, field experiments with maize-bean mixtures were conducted. The interaction and competition between the crops was measured. To investigate the yield effect of mixed cropping the conventional seeding density of maize was reduced and supplemented with beans in 2-3 and 5-6 leaf stadium of maize. As a result, it can be ascertained that maize and beans can be grown together well. Between the different tested mixed seeding-treatments there was no statistical difference in the total dry matter yield, whereas maize and bean complemented each other to the average yield. Noticeable differences could be found in the amount of beans in the harvested mixture depending on seeding density and time. Moreover, different weed controlling methods were examined. A competitive effect between crops and weed could be verified: It can be stated that in the juvenile stage of plant development (until canopy closing of maize) the total yield decreases by about 1 t DM ha^{-1} with every additional percentage of weed coverage.

From the material of an intercropping experiment artificially mixed silages (100 % maize to 100 % beans in 12.5 % increments in fresh matter (FM)) were made. As a novelty, the biogas production potential of the silages was measured and the relationship to quality parameters was looked at. As a general result it could be shown, that with every additional percent of the bean approximately 1 l CH_4 kg^{-1} oDM less was produced compared to the 350 l CH_4 kg^{-1} oDM gained from pure maize silage. The reasons are unknown; it may be that lectins which are produced by the beans and which are known as compounds inhibiting vital functions in microbes are responsible.

As a result it can be stated that within the presented experiments no equal alternative for maize could be found. Still there exists development potential that should be further investigated.

8. Zusammenfassung

In den letzten Jahren hat die Maisproduktion in Deutschland aufgrund des Bedarfes von Biomasse für die Biogasproduktion stark zugenommen. Trotz seiner guten Eigenschaften im Anbau und in der Nutzung hat Mais auch einen negativen Ruf. Vor allem in Gebieten mit hoher Anbaudichte wird Mais für die Änderung des Landschaftsbildes und den Rückgang der Artenvielfalt kritisiert.

Um alternative Kulturen für die Bioenergieproduktion zu finden wurden zwei Ansätze untersucht: Biogasproduktionscharakteristika alternativer Energiepflanzen aus einem Zweikultur-Nutzungssystem vor dem Hintergrund früherer Erntezeitpunkte sowie eines gemischte Anbausystem von Mais und Stangenbohnen.

Die Abbaudynamiken von Sonnenblumen und Sorghum-Sudangrass als Alternativen für Mais wurden gemessen. Um die Notwendigkeit früher ernten müssen, wie es in Zweikultur-Nutzungssystem vorkommen kann, zu imitieren, wurden vier verschiedenen Reifestadien analysiert. Die Ergebnisse implizieren, dass die Abbaukinetiken von Fasern für Mais und Sorghum-Sudangrass ähnlich waren. Der Effekt des Erntezeitpunktes war signifikant für alle Fraktionen außer Lignin, was darauf hinweist, dass Sorghum-Sudangrass ohne Auswirkungen früher geerntet werden kann, während für Mais und Sonnenblumen Methanerträge bezogen auf die organische Trockensubstanz bei früheren Erntezeiten stark zurückgehen. Die Regressionsanalyse zeigte eine moderate Beziehung zwischen Methanausbeute und organischen Fraktionen.

Als mögliche Alternative zu Mais wurden Feldversuche mit Mais-Stangenbohnen-Mischkultur durchgeführt. Es wurden die Interaktion und Konkurrenz zwischen den Feldfrüchten geprüft. Dafür wurde die konventionelle Aussaatdichte von Mais reduziert und im 2-3 und 5-6 Blattstadium von Mais mit Bohnen ergänzt. Als Ergebnis kann festgestellt werden, dass Mais und Stangenbohne gut zusammen angebaut werden können. Zwischen den verschiedenen getesteten Aussaatvarianten gab es keine statistisch signifikanten Unterschiede hinsichtlich des Gesamttrockenmasseertrages. Merkliche Unterschiede bestanden jedoch im Anteil der Bohnen in der geernteten Mischung, welcher abhängig von Aussaatdichte und -zeit war. Außerdem wurden verschiedene Unkrautregulierungsverfahren untersucht. Ein Konkurrenzeffekt konnte auch hinsichtlich des Unkrautbewuchses nachgewiesen werden. Es wurde festgestellt, dass sich im Jugendstadium (bis zum Reihenschluss des Maises) der Gesamtertrag mit jedem zusätzlichen Prozent der Unkrautbedeckung um etwa 1 t TM ha^{-1} verringert.

Aus dem Material des Anbauexperiments wurden künstlich gemischte Silagen (von 100 % Mais zu 100 % Bohnen in Stufen von 12,5 % bezogen auf Frischmasse (FM)) hergestellt. Als Neuheit wurde

das Biogasproduktionspotential der Silagen gemessen und Wechselwirkungen mit Qualitätsparametern geprüft. Als allgemeines Ergebnis konnte gezeigt werden, dass, im Vergleich zu 350 l Methan aus reiner Maissilage, mit jedem zusätzlichen Prozentanteil Bohne etwa 1 Liter weniger Methan produziert wurde. Die Gründe dafür sind unbekannt; möglicherweise sind Lektine dafür verantwortlich, welche von den Bohnen produziert werden und inhibierend auf Mikroben wirken können.

Als Ergebnis kann konstatiert werden, dass es innerhalb der vorgestellten Experimente keine gleichwertige Alternative für Mais gefunden werden konnte. Dennoch gibt es Entwicklungspotenziale, die weiter untersucht werden sollten.

9. Publication bibliography

Albino-Garduno, Rocio; Turrent-Fernandez, Antonio; Isabel Cortes-Flores, J.; Livera-Munoz, Manuel; Carmen Mendoza-Castillo, M. (2015): Root distribution and solar radiation in maize-bean intercropping systems. In *AGROCIENCIA* 49 (5), pp. 513–531.

Amon, Thomas; Amon, Barbara; Kryvoruchko, Vitaliy; Machmuller, Andrea; Hopfner-Sixt, Katharina; Bodiroza, Vitomir et al. (2007a): Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. In *Bioresource Technology* 98 (17), pp. 3204–3212. DOI: 10.1016/j.biortech.2006.07.007\.

Amon, Thomas; Amon, Barbara; Kryvoruchko, Vitaliy; Zollitsch, Werner; Mayer, Karl; Gruber, Leonhard (2007b): Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. In *Agriculture, Ecosystems & Environment* 118 (1-4), pp. 173–182. DOI: 10.1016/j.agee.2006.05.007.

Andersen, Mette Klindt; Hauggaard-Nielsen, Henrik; Ambus, Per; Jensen, Erik Steen (2005): Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. In *Plant Soil* 266 (1-2), pp. 273–287. DOI: 10.1007/s11104-005-0997-1.

Angelidaki, I.; Boe, K.; Ellegaard, L. (2005): Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. In *Water science and technology : a journal of the International Association on Water Pollution Research* 52 (1-2), pp. 189–194.

Anil, L.; Park, J.; Phipps, R.H (2000): The potential of forage–maize intercrops in ruminant nutrition. In *Animal Feed Science and Technology* 86 (3-4), pp. 157–164. DOI: 10.1016/S0377-8401(00)00176-0.

ANONYMOUS (2016): Gesetz für den Vorrang Erneuerbarer Energien (Erneuerbare-Energien-Gesetz – EEG) (German Renewable Energy Sources Act). Available online at www.erneuerbare-energien.de, updated on 11/24/2016.

Appels, Lise; Lauwers, Joost; Degrève, Jan; Helsen, Lieve; Lievens, Bart; Willems, Kris et al. (2011): Anaerobic digestion in global bio-energy production. Potential and research challenges. In *Renewable and Sustainable Energy Reviews* 15 (9), pp. 4295–4301. DOI: 10.1016/j.rser.2011.07.121.

Armstrong, Kevin L.; Albrecht, Kenneth A.; Lauer, Joseph G.; Riday, Heathcliffe (2008): Intercropping Corn with Lablab Bean, Velvet Bean, and Scarlet Runner Bean for Forage. In *Crop Science* 48 (1), p. 371. DOI: 10.2135/cropsci2007.04.0244.

Bayané, Ali; Guiot, Serge R. (2011): Animal digestive strategies versus anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass. In *Rev Environ Sci Biotechnol* 10 (1), pp. 43–62. DOI: 10.1007/s11157-010-9209-4.

Böttinger, Stefan (2013): Handbuch Mais. Grundlagen, Anbau, Verwertung, Ökonomie. Edited by Norbert Lütke Entrup. Frankfurt: DLG-Verl.

Boyle, W. C. (1977): ENERGY RECOVERY FROM SANITARY LANDFILLS - A REVIEW. In Hans Günter Schlegel, Joseph Barnea (Eds.): Microbial energy conversion. The proceedings of a seminar. Oxford [England], New York: Pergamon Press, pp. 119–138.

Buswell, A. M.; Mueller, H. F. (1952): Mechanism of Methane Fermentation. In *Ind. Eng. Chem.* 44 (3), pp. 550–552. DOI: 10.1021/ie50507a033.

Buxton, Dwayne R. (1996): Quality-related characteristics of forages as influenced by plant environment and agronomic factors. In *Animal Feed Science and Technology* 59 (1-3), pp. 37–49. DOI: 10.1016/0377-8401(95)00885-3.

Camisão, Alejandro F. (Ed.) (2013): Symbiosis. Evolution, biology, and ecological effects. Hauppauge NY: Nova Biomedical (Animal science, issues and professions).

Chandler; Jewell (Eds.) (1980): Predicting methane fermentation biodegradability. Biotechnol. Bioeng. Symp.;(United States): Cornell Univ., Ithaca, NY (CONF-791072-).

Cherney, J. H.; Cherney, D.J.R.; Mertens, D. R. (1988): Fiber Composition and Digestion Kinetics in Grass Stem Internodes as Influenced by Particle Size. In *Journal of Dairy Science* 71 (8), pp. 2112–2122. DOI: 10.3168/jds.S0022-0302(88)79784-2.

Chesson, A. (1988): Lignin-polysaccharide complexes of the plant cell wall and their effect on microbial degradation in the rumen. In *Animal Feed Science and Technology* 21 (2-4), pp. 219–228. DOI: 10.1016/0377-8401(88)90104-6.

Clawson, David L. (1985): Harvest Security and Intraspecific Diversity in Traditional Tropical Agriculture. In *Econ Bot* 39 (1), pp. 56–67. DOI: 10.1007/BF02861175.

Coll, Moshe; Bottrell, Dale G. (1995): Predator-prey association in mono- and dicultures. Effect of maize and bean vegetation. In *Agriculture, Ecosystems & Environment* 54 (1-2), pp. 115–125. DOI: 10.1016/0167-8809(95)00582-D.

Cone, John W.; van Gelder, Antonie H.; Bachmann, Herwig; Hindle, Vincent A. (2002): Comparison of organic matter degradation in several feedstuffs in the rumen as determined with the nylon bag and gas production techniques. In *Animal Feed Science and Technology* 96 (1-2), pp. 55–67. DOI: 10.1016/S0377-8401(01)00325-X.

Cong, Wen-Feng; Hoffland, Ellis; Li, Long; Six, Johan; Sun, Jian-Hao; Bao, Xing-Guo et al. (2015): Intercropping enhances soil carbon and nitrogen. In *Glob Change Biol* 21 (4), pp. 1715–1726. DOI: 10.1111/gcb.12738.

Contreras-Govea, F. E.; Muck, R. E.; Armstrong, K. L.; Albrecht, K. A. (2009a): Nutritive value of corn silage in mixture with climbing beans. In *Animal Feed Science and Technology* 150 (1-2), pp. 1–8. DOI: 10.1016/j.anifeedsci.2008.07.001.

Contreras-Govea, Francisco; Marsalis, Mark; Angadi, Sangamesh; Smith, Gerald; Lauriault, L. M.; VanLeeuwen, Dawn (2011): Fermentability and Nutritive Value of Corn and Forage Sorghum Silage When in Mixture with Lablab Bean. In *Crop Science* 51 (3), p. 1307. DOI: 10.2135/cropsci2010.05.0282.

Contreras-Govea, Francisco E.; Muck, Richard E.; Armstrong, Kevin L.; Albrecht, Kenneth A. (2009b): Fermentability of corn–lablab bean mixtures from different planting densities. In *Animal Feed Science and Technology* 149 (3-4), pp. 298–306. DOI: 10.1016/j.anifeedsci.2008.05.009.

Cousens, Roger (1991): Aspects of the Design and Interpretation of Competition (Interference) Experiments. In *Weed Technology* 5 (3), pp. 664–673.

Dandikas, V.; Heuwinkel, H.; Lichti, F.; Drewes, J. E.; Koch, K. (2014): Correlation between biogas yield and chemical composition of energy crops. In *Bioresource Technology* 174, pp. 316–320. DOI: 10.1016/j.biortech.2014.10.019.

Davis, J.H.C.; Roman, A.; Garcia, S. (1987): The effects of plant arrangement and density on intercropped beans (Phaseolus vulgaris) and maize II. Comparison of relay intercropping and simultaneous planting. In *Field Crops Research* 16 (2), pp. 117–128. DOI: 10.1016/0378-4290(87)90002-5.

Dawo, Mohamed I.; Wilkinson, J. Michael; Pilbeam, David J. (2009): Interactions between plants in intercropped maize and common bean. In *J. Sci. Food Agric.* 89 (1), pp. 41–48. DOI: 10.1002/jsfa.3408.

Dawo, Mohamed I.; Wilkinson, J. Michael; Sanders, Francis E. T.; Pilbeam, David J. (2007): The yield and quality of fresh and ensiled plant material from intercropped maize (Zea mays) and beans (Phaseolus vulgaris). In *J. Sci. Food Agric.* 87 (7), pp. 1391–1399. DOI: 10.1002/jsfa.2879.

Demirel, B.; Scherer, P. (2011): Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. In *Biomass and Bioenergy* 35 (3), pp. 992–998. DOI: 10.1016/j.biombioe.2010.12.022.

DePeters, E. J.; Fadel, J. G.; Arosemena, A. (1997): Digestion kinetics of neutral detergent fiber and chemical composition within some selected by-product feedstuffs. In *Animal Feed Science and Technology* 67 (2-3), pp. 127–140. DOI: 10.1016/0377-8401(96)01145-5.

Deutschen Maiskomitee e.V. (2016): DMK. Available online at https://www.maiskomitee.de/web/public/Fakten.aspx/Wissenswertes, updated on 10/4/2016.

Dewhurst, R. J.; Hepper, D.; Webster, A.J.F. (1995): Comparison of in sacco and in vitro techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. In *Animal Feed Science and Technology* 51 (3-4), pp. 211–229. DOI: 10.1016/0377-8401(94)00692-3.

Dierauer, Hans-Ulrich; Stöppler-Zimmer, Holger (1994): Unkrautregulierung ohne Chemie. 28 Tabellen. Stuttgart: Ulmer.

Döhler, Helmut (Ed.) (2013): Faustzahlen Biogas. Kuratorium für Technik und Bauwesen in der Landwirtschaft; Fachagentur Nachwachsende Rohstoffe. 3. Ausg. Darmstadt: KTBL.

Donoso-Bravo, A.; Pérez-Elvira, S. I.; Fdz-Polanco, F. (2010): Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes. In *Chemical Engineering Journal* 160 (2), pp. 607–614. DOI: 10.1016/j.cej.2010.03.082.

Emanuele, S. M.; Staples, C. R. (1988): Effect of Forage Particle Size on In Situ Digestion Kinetics. In *Journal of Dairy Science* 71 (7), pp. 1947–1954. DOI: 10.3168/jds.S0022-0302(88)79765-9.

EU (2013): Regulation (EU) No 1305/2013. Available online at http://eur-lex.europa.eu/legal-content/de/TXT/?uri=CELEX:32013R1305, updated on 3/22/2016.

European Commission (2016): Cross-compliance. Available online at http://ec.europa.eu/agriculture/envir/cross-compliance/index_de.htm, updated on 9/26/2016.

Evranos, Beyza; Demirel, Burak (2015): The impact of Ni, Co and Mo supplementation on methane yield from anaerobic mono-digestion of maize silage. In *Environmental Technology* 36 (12), pp. 1556–1562. DOI: 10.1080/09593330.2014.997297.

Federal Biological Research Centre for Agriculture and Forestry (2001): Growth stages of mono-and dicotyledonous plants. With assistance of Uwe Meier. Available online at http://www.jki.bund.de/fileadmin/dam_uploads/_veroeff/bbch/BBCH-Skala_englisch.pdf, updated on 3/15/2016.

Ferreira, Gonzalo; Mertens, David R. (2007): Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. In *Animal Feed Science and Technology* 133 (3-4), pp. 335–340. DOI: 10.1016/j.anifeedsci.2006.04.010.

Field, Andy P.; Miles, Jeremy; Field, Zoë (2012): Discovering statistics using R. London: SAGE.

Fischer, J.; Böhm, H. (2013): Ertrag und Futterwert von Mais-Bohnen Gemengen als Ganzpflanzensilage in der Milchviehfütterung.

Fischer et al. (2015): Gemengeanbau von Mais mit Phaseolus-Bohnen: Einfluss von Sorte und Saatdichte der Bohnen auf die Bestandszusammensetzung. In : GPW, vol. 27, pp. 177–178.

Flores-Sanchez, D.; Pastor, A.; Lantinga, E. A.; Rossing, W. A. H.; Kropff, M. J. (2013): Exploring Maize-Legume Intercropping Systems in Southwest Mexico. In *Agroecology and Sustainable Food Systems* 37 (7), pp. 739–761. DOI: 10.1080/21683565.2013.763888.

FNR (2014): FNR (2014). Available online at https://mediathek.fnr.de/grafiken/daten-und-fakten/bioenergie/biogas.html.

FNR (2016): Fachagentur Nachwachsende Rohstoffe. Available online at http://biogas.fnr.de/, checked on 3/15/2016.

Francis, C. A.; Prager, Martin; Tejada, Gerardo (1982): Effects of relative planting dates in bean (Phaseolus vulgaris L.) and maize (Zea mays L.) intercropping patterns. In *Field Crops Research* 5, pp. 45–54. DOI: 10.1016/0378-4290(82)90005-3.

Gebeyehu, Setegn; Simane, Belay; Kirkby, Roger (2006): Genotype×cropping system interaction in climbing beans (Phaseolus vulgaris L.) grown as sole crop and in association with maize (Zea mays L.). In *European Journal of Agronomy* 24 (4), pp. 396–403. DOI: 10.1016/j.eja.2006.01.005.

Getachew, G.; Robinson, P.H; DePeters, E.J; Taylor, S.J (2004): Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. In *Animal Feed Science and Technology* 111 (1-4), pp. 57–71. DOI: 10.1016/S0377-8401(03)00217-7.

Gibson, David. J.; Connolly, John; Hartnett, David. C.; Weidenhamer, Jeffrey. D. (1999): Designs for greenhouse studies of interactions between plants. In *J Ecology* 87 (1), pp. 1–16. DOI: 10.1046/j.1365-2745.1999.00321.x.

Graham, P. H.; Ranalli, P. (1997): Common bean (Phaseolus vulgaris L.). In *Field Crops Research* 53 (1-3), pp. 131–146. DOI: 10.1016/S0378-4290(97)00112-3.

Graß (2003): Direkt- und Spätsaat von Silomais - Ein neues Anbausystem zur Reduzierung von Umweltgefährdungen und Anbauproblemen bei Optimierung der Erträge. University of Kassel.

Graß, Rüdiger; Heuser, Florian; Stülpnagel, Reinhold; Piepho, Hans-Peter; Wachendorf, Michael (2013): Energy crop production in double-cropping systems. Results from an experiment at seven sites. In *European Journal of Agronomy* 51, pp. 120–129. DOI: 10.1016/j.eja.2013.08.004.

Graß, Rüdiger; Thies, Burga; Kersebaum, Kurt-Christian; Wachendorf, Michael (2015): Simulating dry matter yield of two cropping systems with the simulation model HERMES to evaluate impact of future climate change. In *European Journal of Agronomy* 70, pp. 1–10. DOI: 10.1016/j.eja.2015.06.005.

Gunaseelan, V. Nallathambi (2007): Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. In *Bioresource Technology* 98 (6), pp. 1270–1277. DOI: 10.1016/j.biortech.2006.05.014.

Gutzler, C.; Helming, K.; Balla, D.; Dannowski, R.; Deumlich, D.; Glemnitz, M. et al. (2015): Agricultural land use changes – a scenario-based sustainability impact assessment for Brandenburg, Germany. In *Ecological Indicators* 48, pp. 505–517. DOI: 10.1016/j.ecolind.2014.09.004.

Hall, M. B. (2003): Challenges with nonfiber carbohydrate methods, pp. 3226–3232. Available online at https://www.animalsciencepublications.org/publications/jas/pdfs/81/12/0813226?search-result=1, checked on 1/6/2016.

Hall, M. B.; Mertens, D. R. (2012): A ring test of in vitro neutral detergent fiber digestibility: analytical variability and sample ranking. In *Journal of Dairy Science* 95 (4), pp. 1992–2003. DOI: 10.3168/jds.2011-4802.

Hauggaard-Nielsen, Henrik; Jørnsgaard, Bjarne; Kinane, Julia; Jensen, Erik Steen (2008): Grain legume–cereal intercropping. The practical application of diversity, competition and facilitation in arable and organic cropping systems. In *RAF* 23 (01). DOI: 10.1017/S1742170507002025.

Hendriks; Zeeman (2009): Pretreatments to enhance the digestibility of lignocellulosic biomass. In *Bioresource Technology* 100 (1), pp. 10–18. DOI: 10.1016/j.biortech.2008.05.027.

Herbes, Carsten (2014): Der gesellschaftliche Diskurs um den ,,Maisdeckel“ vor und nach der Novelle des Erneuerbare-Energien-Gesetzes (EEG) 2012 The Social Discourse on the “Maize Cap“ before and after the 2012 Amendment of the German Renewable Energies Act (EEG). In *gaia. DOI:* 10.14512/gaia.23.2.7.

Herrmann, A. (2013): Biogas Production from Maize. Current State, Challenges and Prospects. 2. Agronomic and Environmental Aspects. In *Bioenerg. Res.* 6 (1), pp. 372–387. DOI: 10.1007/s12155-012-9227-x.

Herrmann, A.; Rath, J. (2012): Biogas Production from Maize. Current State, Challenges, and Prospects. 1. Methane Yield Potential. In *Bioenerg. Res.* 5 (4), pp. 1027–1042. DOI: 10.1007/s12155-012-9202-6.

Hilpert, R.; Winter, J.; Kandler, O. (1984): Agricultural feed additives and disinfectants as inhibitory factors in anaerobic digestion. In *Agricultural Wastes* 10 (2), pp. 103–116. DOI: 10.1016/0141-4607(84)90010-6.

Hoppe, Christopher (2016): Development of biogas maize cultivars for intercropping with climbing beans. Available online at http://hdl.handle.net/11858/00-1735-0000-0028-87D0-F.

Hristov, A. N.; Mertens, D.; Zaman, S.; Vander Pol, M.; Price, W. J. (2010): Variability in feed and total mixed ration neutral detergent fiber and crude protein analyses among commercial laboratories. In *Journal of Dairy Science* 93 (11), pp. 5348–5362. DOI: 10.3168/jds.2010-3333.

Jacobi, H. Fabian; Moschner, Christian R.; Hartung, Eberhard (2011): Use of near infrared spectroscopy in online-monitoring of feeding substrate quality in anaerobic digestion. In *Bioresource Technology* 102 (7), pp. 4688–4696. DOI: 10.1016/j.biortech.2011.01.035.

Javanmard, Abdollah; Nasab, Adel Dabbagh Mohammadi; Javanshir, Aziz; Moghaddam, Mohammad; Janmohammadi, Hosein (2009): Forage yield and quality in intercropping of maize with different legumes as double-cropped. In *Journal of Food, Agriculture and Environment* 7 (1), pp. 163–166.

Jolliffe, P. A. (2000): The replacement series, pp. 371–385.

Khan, Nazir A.; Yu, Peiqiang; Ali, Mubarak; Cone, John W.; Hendriks, Wouter H. (2015): Nutritive value of maize silage in relation to dairy cow performance and milk quality. In *Journal of the Science of Food and Agriculture* 95 (2), pp. 238–252. DOI: 10.1002/jsfa.6703.

Kirchgeßner, Manfred; Roth, Franz Xaver; Schwarz, Frieder Jörg (2008): Tierernährung. Leitfaden für Studium, Beratung und Praxis. 12., neu überarb. Aufl. Frankfurt am Main: DLG-Verl.

Koschke, Lars; Fürst, Christine; Lorenz, Marco; Witt, Anke; Frank, Susanne; Makeschin, Franz (2013): The integration of crop rotation and tillage practices in the assessment of ecosystem services provision at the regional scale. In *Ecological Indicators* 32, pp. 157–171. DOI: 10.1016/j.ecolind.2013.03.008.

Labatut, Rodrigo A.; Angenent, Largus T.; Scott, Norman R. (2011): Biochemical methane potential and biodegradability of complex organic substrates. In *Bioresource Technology* 102 (3), pp. 2255–2264. DOI: 10.1016/j.biortech.2010.10.035.

Legume Futures 2014: Legume-supported cropping systems for Europe. General project report. Available online at www.legumefutures.de, checked on 5/25/2016.

Lithourgidis, A. S.; Dordas, C. A.; Damalas, C. A.; Vlachostergios, DN0 (2011): Annual intercrops: an alternative pathway for sustainable agriculture. In *Australian journal of crop science* 5 (4), p. 396.

Lupp, Gerd; Albrecht, Juliane; Darbi, Marianne; Bastian, Olaf (2011): Ecosystem Services in Energy Crop Production - A Concept for Regulatory Measures in Spatial Planning? In *Journal of Landscape Ecology* 4 (3). DOI: 10.2478/v10285-012-0043-y.

Lupwayi, Newton Z.; Kennedy, Ann C. (2007): Grain Legumes in Northern Great Plains. In *Agronomy Journal* 99 (6), p. 1700. DOI: 10.2134/agronj2006.0313s.

Maasdorp, B. V.; Titterton, M. (1997): 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. In *Animal Feed Science and Technology* 69 (1-3), pp. 241–261. DOI: 10.1016/S0377-8401(97)81639-2.

Makkar; Becker; Abel; Pawelzik (1997): Nutrient contents, rumen protein degradability and antinutritional factors in some colour- and white-flowering cultivars of Vicia faba beans, pp. 511–520.

Malézieux, E.; Crozat, Y.; Dupraz, C.; Laurans, M.; Makowski, D.; Ozier-Lafontaine, H. et al. (2009): Mixing plant species in cropping systems. Concepts, tools and models. A review. In *Agron. Sustain. Dev.* 29 (1), pp. 43–62. DOI: 10.1051/agro:2007057.

Mariotti, Marco; Masoni, Alessandro; Ercoli, Laura; Arduini, Iduna (2006): Forage potential of winter cereal/legume intercrops in organic farming. In *Ital J Agronomy* 1 (3), p. 403. DOI: 10.4081/ija.2006.403.

Marshall, E. J. P.; Brown, V. K.; Boatman, N. D.; Lutman, P. J. W.; Squire, G. R.; Ward, L. K. (2003): The role of weeds in supporting biological diversity within crop fields*. In *Weed Res* 43 (2), pp. 77–89. DOI: 10.1046/j.1365-3180.2003.00326.x.

Mauseth, James D. (1988): Plant anatomy: Benjamin/Cummings Publ. Co.: Menlo Park, Calif.

Melander, Bo; Rasmussen, Ilse A.; Bàrberi, Paolo (2005): Integrating physical and cultural methods of weed control— examples from European research. In *Weed Science* 53 (3), pp. 369–381. DOI: 10.1614/WS-04-136R.

Moore, K. J.; H.-J. G. Jung (2001): Lignin and Fiber Digestion. In *J Range Manage* 54 (4), pp. 420–430. Available online at https://journals.uair.arizona.edu/index.php/jrm/article/viewFile/9640/9252, checked on 1/6/2016.

Mudhoo, A.; Kumar, S. (2013): Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass. In *Int. J. Environ. Sci. Technol.* 10 (6), pp. 1383–1398. DOI: 10.1007/s13762-012-0167-y.

Mussatto, Solange I.; Fernandes, Marcela; Milagres, Adriane M.F.; Roberto, Inês C. (2008): Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. In *Enzyme and Microbial Technology* 43 (2), pp. 124–129. DOI: 10.1016/j.enzmictec.2007.11.006.

Mustafa, A. F.; Christensen, D. A.; McKinnon, J. J. (2000): Effects of Pea, Barley, and Alfalfa Silage on Ruminal Nutrient Degradability and Performance of Dairy Cows. In *Journal of Dairy Science* 83 (12), pp. 2859–2865. DOI: 10.3168/jds.S0022-0302(00)75186-1.

Mustafa, A. F.; Seguin, P. (2003): Characteristics and in situ degradability of whole crop faba bean, pea, and soybean silages. In *Can. J. Anim. Sci.* 83 (4), pp. 793–799. DOI: 10.4141/A03-065.

Mustafa, A. F.; Seguin, P.; Ouellet, D. R.; Adelye, I. (2002): Effects of Cultivars on Ensiling Characteristics, Chemical Composition, and Ruminal Degradability of Pea Silage. In *Journal of Dairy Science* 85 (12), pp. 3411–3419. DOI: 10.3168/jds.S0022-0302(02)74429-9.

Muzquiz, Mercedes; Burbano, Carmen; Ayet, Gemma; Pedrosa, Mercedes M.; Cuadrado, Carmen (1999): The investigation of antinutritional factors in Phaseolus vulgaris. Environmental and varietal differences. In *Biotechnologie, Agronomie, Société et Environnement* 3 (4), pp. 210–216.

Nader, N.; Hanen, B. I.; Fatma, B.A-F.; Namjun, C. (2015): In vitro assessment of Phaseolus vulgaris L. lectins activities against various pathogenic and beneficial microbes, pp. 37–47.

Naumann, Curt.; Bassler, Rolf.; Seibold, Rüdiger.; Barth, Carl. (1976): Methodenbuch. Band III, Band III. Darmstadt: VDLUFA - Verlag.

Nges, Ivo Achu; Björnsson, Lovisa (2012): High methane yields and stable operation during anaerobic digestion of nutrient-supplemented energy crop mixtures. In *Biomass and Bioenergy* 47, pp. 62–70. DOI: 10.1016/j.biombioe.2012.10.002.

Nurk, Liina; Graβ, Rüdiger; Pekrun, Carola; Wachendorf, Michael (2016): Methane Yield and Feed Quality Parameters of Mixed Silages from Maize (Zea mays L.) and Common Bean (Phaseolus vulgaris L.). In *Bioenerg. Res. DOI:* 10.1007/s12155-016-9779-2.

Ørskov, E. R.; Hovell, F.D.DeB.; Mould, F. (1980): The use of the nylon bag technique for the evaluation of feedstuffs. In *Tropical Animal Production* 5 (3), pp. 195–212, checked on 1/6/2016.

Peksen, Gulumser (2013): 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.

R Core Team (2016): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Available online at https://www.R-project.org, updated on 5/25/2016.

Rath, Jürgen; Heuwinkel, Hauke; Herrmann, Antje (2013): Specific Biogas Yield of Maize Can Be Predicted by the Interaction of Four Biochemical Constituents. In *Bioenerg. Res.* 6 (3), pp. 939–952. DOI: 10.1007/s12155-013-9318-3.

Richter, F.; Graß, R.; Fricke, T.; Zerr, W.; Wachendorf, M. (2009): Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. II. Effects of hydrothermal conditioning and mechanical dehydration on anaerobic digestion of press fluids. In *Grass and Forage Science* 64 (4), pp. 354–363. DOI: 10.1111/j.1365-2494.2009.00700.x.

Rodrigues, M.A.M.; Cone, J. W.; Ferreira, L.M.M.; Blok, M. C.; Guedes, C.V.M. (2009): Relationship between in situ degradation kinetics and in vitro gas production fermentation using different mathematical models. In *Animal Feed Science and Technology* 151 (1-2), pp. 86–96. DOI: 10.1016/j.anifeedsci.2008.12.004.

Romero-Güiza; Vila; Mata-Alvarez; Chimenos, J. M.; Astals, S. (2016): The role of additives on anaerobic digestion. A review. In *Renewable and Sustainable Energy Reviews* 58, pp. 1486–1499. DOI: 10.1016/j.rser.2015.12.094.

Sadananda; Govindappa; Ramachandra (2013): Antibacterial activity of *Viscum Album* endophytic fungal lectin, pp. 1033–1042.

Sadeghi, Hossein; Kazemeini, Seyed Abdolreza; Edalat, Mohsen (2012): Effect of plant density ratios and weed control on the performance of maize-bean intercropping. In *Journal of Ecology and Field Biology* 35 (4), pp. 313–322. DOI: 10.5141/JEFB.2012.037.

Sambusiti, C.; Ficara, E.; Malpei, F.; Steyer, J. P.; Carrère, H. (2012): Influence of alkaline pre-treatment conditions on structural features and methane production from ensiled sorghum forage. In *Chemical Engineering Journal* 211-212, pp. 488–492. DOI: 10.1016/j.cej.2012.09.103.

Schittenhelm, S. (2010): Effect of Drought Stress on Yield and Quality of Maize/Sunflower and Maize/Sorghum Intercrops for Biogas Production. In *Journal of Agronomy and Crop Science. DOI:* 10.1111/j.1439-037X.2010.00418.x.

Searle, P.G.E.; Comudom, Yuthapong; Shedden, D. C.; Nance, R. A. (1981): Effect of maize + legume intercropping systems and fertilizer nitrogen on crop yielding and residual nitrogen. In *Field Crops Research* 4, pp. 133–145. DOI: 10.1016/0378-4290(81)90063-0.

SigmaPlot 9.0: Systat Software, Inc., San Jose California USA. Available online at www.sigmaplot.com.

Silgram, Martyn; Shepherd, Mark A. (1999): The Effects of Cultivation on Soil Nitrogen Mineralization. In : Advances in Agronomy Volume 65, vol. 65: Elsevier (Advances in Agronomy), pp. 267–311.

Slifkin, Malcolm; Doyle R.J. (1990): Lectins and Their Application to Clinical Microbiology, pp. 197–218.

Smith, H. A.; McSorley, R. (2000): Intercropping and Pest Management. A Review of Major Concepts. In *American Entomologist* 46 (3), pp. 154–161. DOI: 10.1093/ae/46.3.154.

Sniffen C.J.; O'Connor J.D.; Van Soest P.J.; Fox D.G.; Russell J.B (1992): A Net Carbohydrate and Protein System for Evaluating Cattle Diets: II. Carbohydrate and Protein Availability, pp. 3562–3577. Available online at https://www.animalsciencepublications.org/publications/jas/pdfs/70/11/3562, checked on 1/6/2016.

Statistisches Bundesamt (2015): Anbaufläche. Available online at https://www-genesis.destatis.de, checked on 7/6/2016.

Stefanon, B.; Pell, A. N.; Schofield, P. (1996): Effect of maturity on digestion kinetics of water-soluble and water-insoluble fractions of alfalfa and brome hay. In *Journal of animal science*, pp. 1104–1115.

Stoltz, Eva; Nadeau, Elisabet (2014): 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.). In *Field Crops Research* 169, pp. 21–29. DOI: 10.1016/j.fcr.2014.09.004.

Surendra, K. C.; Khanal, Samir Kumar (2015): Effects of crop maturity and size reduction on digestibility and methane yield of dedicated energy crop. In *Bioresource Technology* 178, pp. 187–193. DOI: 10.1016/j.biortech.2014.09.055.

Thrupp, Lori Ann (2000): Linking Agricultural Biodiversity and Food Security. The Valuable Role of Agrobiodiversity for Sustainable Agriculture. In *Int Affairs* 76 (2), pp. 283–297. DOI: 10.1111/1468-2346.00133.

Titterton, M.; Maasdorp, B. V. (1997): Nutritional improvement of maize silage for dairying. Mixed crop silages from sole and intercropped legumes and a long season variety of maize. 2. Ensilage. In *Animal Feed Science and Technology* 69 (1-3), pp. 263–270. DOI: 10.1016/S0377-8401(97)81640-9.

Trenbath, B. R. (1974): Biomass Productivity of Mixtures. In Nyle C. Brady (Ed.): Advances in agronomy, vol. 26. New York: Academic Press (Advances in Agronomy), pp. 177–210.

Triolo, Jin M.; Pedersen, Lene; Qu, Haiyan; Sommer, Sven G. (2012): Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. In *Bioresource Technology* 125, pp. 226–232. DOI: 10.1016/j.biortech.2012.08.079.

Triolo, Jin M.; Sommer, Sven G.; Moller, Henrik B.; Weisbjerg, Martin R.; Jiang, Xin Y. (2011): A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. In *Bioresource Technology* 102 (20), pp. 9395–9402. DOI: 10.1016/j.biortech.2011.07.026.

Tsai, S. M.; Da Silva, P. M.; Cabezas, W. L.; Bonetti, R. (1993): Variability in nitrogen fixation of common bean (Phaseolus vulgaris L.) intercropped with maize. In *Plant Soil* 152 (1), pp. 93–101. DOI: 10.1007/BF00016337.

Urbatzka, Peer; Graß, Rüdiger; Haase, Thorsten; Schüler, Christian; Trautz, Dieter; Heß, Jürgen (2011): Grain yield and quality characteristics of different genotypes of winter pea in comparison to spring pea for organic farming in pure and mixed stands. In *Org. Agr.* 1 (4), pp. 187–202. DOI: 10.1007/s13165-011-0015-2.

van Schoonhoven, Aart; Pastor-Corrales, Marcial A. (1987): Standard system for the evaluation of bean germplasm. Cali, Colombia: Centro Internacional de Agricultura Tropical.

van Soest; Peter (1994): Nutritional ecology of the ruminant. 2nd ed. Ithaca, London: Comstock.

van Soest; Wine (1967): Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assn. Offic. Anal. Chem. 50:50-5, 1967. In *J. Assn. Offic. Anal. Chem* 50 (1), pp. 50–55, checked on 1/6/2016.

van Vliet, Jasper; Groot, Henri L.F. de; Rietveld, Piet; Verburg, Peter H. (2015): Manifestations and underlying drivers of agricultural land use change in Europe. In *Landscape and Urban Planning* 133, pp. 24–36. DOI: 10.1016/j.landurbplan.2014.09.001.

van Zanten, Boris T.; Verburg, Peter H.; Espinosa, Maria; Gomez-y-Paloma, Sergio; Galimberti, Giuliano; Kantelhardt, Jochen et al. (2014): European agricultural landscapes, common agricultural policy and ecosystem services. A review. In *Agron. Sustain. Dev.* 34 (2), pp. 309–325. DOI: 10.1007/s13593-013-0183-4.

Vasconcelos, Ilka M.; Oliveira, José Tadeu A. (2004): Antinutritional properties of plant lectins. In *Toxicon* 44 (4), pp. 385–403. DOI: 10.1016/j.toxicon.2004.05.005.

VDI 4630: Vergärung organischer Stoffe; Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche. Available online at https://www.vdi.de/technik/fachthemen/energie-und-umwelt/fachbereiche/energiewandlung-und-anwendung/richtlinien/vdi-4630/, checked on 3/15/2016.

Weißbach; Kuhla (1995): Substance losses in determining the dry matter content of silage and green fodder: arising errors and possibilities of correction. In *Übersicht Tierernährung 23*.

Zeise, Karen; Haller, Julia; Hartmann, Anja; Eberl, Veronika; Grieb, Michael; Haag, Jonas et al. (2016): Sorghum als Biogassubstrat – Präzisierung der Anbauempfehlungen für bayerische Anbaubedingungen. Straubing.

Zerr, Walter (2006): Versuchsanlage zur energetischen Beurteilung von Substraten und Kofermentaten für Biogasanlagen. In *Umweltwissenschaften und Schadstoff-Forschung* 18 (4), pp. 219–227. DOI: 10.1065/uwsf2006.03.117.

Zhang, Fusuo; Li, Long (2003): Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency, pp. 305–312. DOI: 10.1007/978-94-010-0243-1_26.

10. List of other publications

In reviewed journals

Melts I., Heinsoo K., Nurk L., Parn L. (2013): Comparison of two different bioenergy production options from late harvested biomass of Estonian semi-natural grasslands. Energy, 61, 6-12.

Melts I., Normak A., Nurk L., Heinsoo K. (2014): Chemical characteristics of biomass from nature conservation management for methane production. Bioresource Technology, 167, 226–231.

Other publications

Nurk L., Fricke T., Reddersen B., Wachendorf M. (2013): Einfluss der funktionellen Gruppen und Artenzahl des Grünlands auf die Silagequalität. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 25, 231-232.

Nurk L., Graß R., Hubert S., Pekrun C., Wachendorf M. (2014): Mischanbau von Energiemais mit Stangenbohnen im ökologischen Landbau: Ergebnisse des 1. Versuchsjahres. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 26, 176-177.

Nurk L., Graß R., Hubert S., Wachendorfs M., Pekrun C. (2014): Mischanbau von Mais mit Stangenbohnen - Entwicklung des Anbausystems für den konventionellen Anbau. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 26, 178-179.

Nurk, L., L. Bühle, M. Wachendorf (2015): Abbaudynamik verschiedener organischer Inhaltsstoffe von Mais-, Sonnenblumen- und Sorghumsilage bei der anaeroben Vergärung. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 27: 25–26.

Nurk L., Graß R., Hubert S., Pekrun C., Wachendorf M. (2015): Mischanbau von Energiemais mit Stangenbohnen im Ökologischen Landbau - Ergebnisse des 2. Versuchsjahres. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 27: 187–188.

Nurk L., Graß R., Hubert S., Wachendorfs M., Pekrun C. (2015): Anbau von Energiemais in Mischkultur mit Stangenbohnen Entwicklung und Optimierung des Anbausystems – Ergebnisse vom zweiten Versuchsjahr im konventionellen Anbau. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 27: 189–190.

Nurk L., Graß R., Hubert S., Pekrun C., Wachendorf M. (2016): Anbau von Energiemais in Mischkultur mit Stangenbohnen: Entwicklung und Optimierung des Anbausystems – Ergebnisse von drei Versuchsjahren an drei Standorten. In: Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften. Bd. 28, 124-125.