Effects of sorghum biomass quality on ensilability and methane yield

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Abstract
Sorghum is currently being introduced in the temperate regions of Europe. It is characterized by good digestibility and high biomass yields, which make it a useful crop for anaerobic digestion. In this study, six commercial sorghum varieties comprising four different cultivars of *Sorghum bicolor* L. Moench and two interspecific sorghum hybrids (*Sorghum sudanense* L. × *S. bicolor* L. Moench) were harvested on two different dates during the years 2016 and 2017 at two diverse soil-climate sites in Germany. The fresh harvested material and silages were analyzed to examine the ensilability of the different varieties with contrasting maturity characteristics. Subsequently, methane production experiments were performed to determine the specific methane yield (SMY) of the samples. The sorghum fresh matter (FM) varied among the sorghum types, including the parameters total solids (TS; 22.69%–46.93%FM), water-soluble carbohydrates (2.68%–11.38%TS), and nitrates (0%–0.35%TS). The excellent ensiling ability of all the sorghum types analyzed was confirmed by evaluating the fermentation profile (pH range of 3.7–4.6; dominant presence of lactic acid [LA]; acetic acid [AA] in the range of 0.70%–2.38%TS; insignificant amount of butyric acid). The SMY ranged between 231.25 and 321.31 Lm kg\(^{-1}\) VS and tended to decrease with the increasing harvest time and maturity. LA and AA were positively correlated with the SMY, while the neutral detergent fiber content was negatively correlated with it. The SMY—a key parameter reflecting the crop biomass quality for biogas production—was slightly higher for *S. bicolor* than for the sorghum hybrids. However, the results of this study confirmed that if the final purpose is biomethanation, the ensilability of different sorghum types imposes no restriction. Furthermore, different sorghum types offer a wide harvest window, which can be useful for cropping schemes, ensiling, and methane production.

KEYWORDS
anaerobic digestion, harvest date, maturity, methane yield, phenological stage, silage, sorghum, variety
1 | INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench)—an annual C4 plant of tropical origin—is currently being introduced in temperate regions of Europe. Recently, various breeding achievements have been made that are related to the cold tolerance and early maturity of sorghum. New sorghum varieties together with agricultural alterations due to climate change, such as warming and longer growing seasons, facilitate the cultivation of sorghum as feed for livestock or for bioenergy applications (Kanbar et al., 2020; Schaffasz et al., 2019; Windpassinger, 2016). Furthermore, including other (possibly new) crop species and cultivars into crop rotations promotes diversification and enhances the sustainability of crop management (Hufnagel et al., 2020; Strauß et al., 2019). Sorghum and sorghum hybrids (*Sorghum sudanense* L. × *S. bicolor* L. Moench) are generally characterized by an excellent biomass yield potential, high nitrogen and water use efficiency, pronounced abiotic stress tolerance, and no specific soil requirements (Strauß et al., 2019; Tari et al., 2013; Wannasek et al., 2017). The inherent robustness of sorghum is complemented by the ability to break disease, parasite, and weed cycles (Głąb et al., 2017; Ratnadass et al., 2012). Sorghums’ Diabrotica resistance (*Diabrotica virgifera virgifera* LeConte; Western Corn Rootworm) is particularly beneficial for the infested European maize production areas (Windpassinger et al., 2015).

Commonly, crops for biogas production are harvested seasonally as whole plants. Subsequently, wet biomass is conserved by ensiling to maintain feedstock quality for year-round availability to biogas plants (Herrmann et al., 2011; Teixeira Franco et al., 2016; Villa et al., 2020). During the fermentation process, the microflora—principally lactic acid (LA) bacteria—convert plant sugars (water-soluble carbohydrates, WSCs) into organic acids (such as LA and acetic acid [AA]), reducing the pH. The rapid decline in the pH prevents undesired microbial activity and hence spoilage of crop material. The fermentation quality of the prepared silage is mainly determined by the biomass composition during ensiling and by the bacteria species dominating the fermentation process. Inappropriate ensiling conditions may degrade the silage quality and incur large losses in yield or nutritional content (Borreani et al., 2018).

Various studies have demonstrated that sorghum is characterized by good digestibility and high biomass yields, which make it a feasible crop for anaerobic digestion (Herrmann et al., 2011; Herrmann, Idler, et al., 2016; Windpassinger et al., 2015). For this reason, in Europe, the implementation of forage sorghum for crop rotations is increasing (Sorghum (ID, 2020)). Beneficial in this process is sorghum’s high adaptability, which allows to easily modify current practices (timing of cultivation, regular recurring sequence of crops) for integrating them into crop rotations, as well as to develop alternative cropping schemes, such as double cropping systems (Goff et al., 2010; Strauß et al., 2019; Wannasek et al., 2019). However, the multiplicity of management options for maximizing the methane yield per acre presents a considerable challenge for farmers. Figure 1 shows an overview of the impact factors and interactions affecting the methane yields of biogas crops. The supply of herbaceous feedstock for anaerobic digestion aims at the highest possible methane yield per unit area (m$^3$ ha$^{-1}$). This area-specific methane yield (SMY) is determined by the biomass yield (t ha$^{-1}$) and the feedstock-SMY (energy density) per dry matter unit (m$^3$ t$^{-1}$). Both these parameters are affected by numerous factors. The feedstock-SMY depends on the feedstock quality, pretreatment method, digestion technology, and process control. The

![Figure 1](https://example.com/figure1.png)  
**FIGURE 1** Anaerobic digestion of biogas crops: factors and interactions affecting methane yields.
feedstock quality depends on the crop species, variety type, and cropping system selected and can be affected by natural site-specific conditions, crop management, and conservation. The harvest date plays a key role, because the decision to harvest for silage preparation not only affects the biomass yield but also has a significant impact on the biomass quality, that is, the ensilability, and thus on the SMY.

The nutrient and fiber composition of the crop biomass changes with plant maturity. In sorghum, the WSC content is high during the vegetative stage, but as grain filling progresses, the WSC content decreases, and the starch content increases (Piltz & Kaiser, 2004). The amount of cell wall components (cellulose [CEL], hemicellulose [HCEL], and lignin) increases with plant growth, negatively affecting degradability and biomethanation (Herrmann et al., 2011; Herrmann, Idler, et al., 2016). In contrast, less mature crops are characterized by a higher moisture content, which limits their suitability for ensiling. A low dry matter content leads to effluent production and subsequent energy and nutrient losses (Borreani et al., 2018). Clostridia—microorganisms that cause spoilage of silages—are less sensitive to pH reduction at higher water activity levels (McDonald et al., 1991). Thus, a lower pH and elevated LA concentration within the silage is needed for the inhibition of spoilage microorganisms at a low dry matter content. Hence, farmers must balance the maximum biomass yield and optimal feedstock quality for methane production. A few related studies involving regional settings and the availability of sorghum varieties were recently performed (Hassan et al., 2019; Morozova et al., 2020; Wannasek et al., 2017, 2019). However, to the best of our knowledge, no studies have focused on the impact of the sorghum biomass quality on the ensilability with regard to the SMY. In the one study on this topic, the common interaction of decreasing methane yields with increasing maturity stages could not be confirmed (Herrmann, Plogsties, et al., 2016). The presented data regarding sorghum silages indicate notable differences in the development of dry matter and the lignin content depending on the maturity stage at harvest. The nature of the correlation remains unclear (Herrmann, Plogsties, et al., 2016). Thus, there is a need for knowledge that can assist in determining the best harvest time of forage sorghum for ensiling and subsequent use as feedstock for biogas production. The objective of this study was to investigate the effects of different maturity characteristics of sorghum and different harvest dates on its ensilability and the subsequent SMY of silages. To achieve this, six commercial sorghum varieties—four varieties of Sorghum bicolor L. Moench and two sorghum interspecific hybrids (Sorghum sudanense L. × S. bicolor L. Moench)—were harvested on two different dates during the years 2016 and 2017 at two diverse soil-climate sites in Germany, to assess the fresh matter (FM) quality, fermentation profile of the silages, and SMY obtained using the silages as a substrate.

2 MATERIALS AND METHODS

2.1 Crop material

The study was conducted using four different variety types of Sorghum bicolor L. Moench, which were optimized for biomass yield (low starch content and almost no grains), that is, Amiggo, Herkules, KWS Zerberus, and RGT Gguepard, as well as two sorghum hybrids (Sorghum sudanense L. × S. bicolor L. Moench), that is, KWS Freya and Lussi (Table 1). The selected varieties covered a wide range of sorghum types and maturity tendencies, facilitating a comprehensive overview of the differences among them.

2.2 Field sites

The cultivation experiments were carried out during the years 2016 and 2017 at two experimental field sites, which were selected to represent distinct soil-climate conditions of field cropping in Germany: in the north-east, Marquardt (MQ; 52°28′01.7″N 12°57′37.2″E; 42 m above sea level; soil type: loamy sand; average annual rainfall: 585.8 mm; annual mean temperature: 9.3°C) and in the south, Straubing (ST; 48°51′48.3″N 12°36′56.4″E; 339 m above sea level; soil type: strong clayey silt; average annual rainfall: 757 mm; annual mean temperature: 8.6°C). The weather in Marquardt (2016) was characterized by dry phases during May, August, and September. Temporarily, in May, June, and September, the average temperatures increased to 4°C above the long-term average values. Overall, compared with the long-term average, 2016 was 1.2°C warmer and had less precipitation (77.6 mm). In 2017, there was above-average precipitation in June, July, and August. Overall, the year was very rainy; the precipitation of 750 mm exceeded the long-term average by approximately 160 mm. The temperatures were close to the long-term average, exhibiting slight increases in May (1.2°C) and June (1.6°C). At the Straubing site, the weather in 2017 was characterized by

| Variety type | Denomination | Maturity tendency |
|--------------|--------------|------------------|
| SBSS1        | Sorghum bicolor × Sorghum sudanense | Lussi | Very early |
| SBSS2        | S. bicolor × S. sudanense | KWS Freya | Early |
| SB1          | S. bicolor | Amiggo | Medium–early |
| SB2          | S. bicolor | KWS Zerberus | Medium–early |
| SB3          | S. bicolor | RGT Gguepard | Medium–late |
| SB4          | S. bicolor | Herkules | Medium–late |
a humid spring and a dry summer. Only August precipitation reached the long-term average. The months of June and August were significantly warmer—2.5°C and 2°C, respectively, above the long-term average (DWD, 2017, 2018).

The investigations conducted followed a uniform, standardized protocol. The trials were established as a randomized block with four replicates for each variety and plot. Each harvest plot was divided into five rows and had a size of 1.5 × 9 m², with a distance of 26.6 cm between the single rows, resulting in densities of 25 (S. bicolor) and 35 plants per m² (S. bicolor × S. sudanense). The sowing time for all the experiments and years was set for the middle of May (Table 2). Site-specific crop management (fertilization, crop protection measures) was applied. Sorghum varieties were harvested at two different harvesting dates (HD1 and HD2) and phenological growth stages (BBCH code), which were determined according to TFZ (2012; Table 2). Whole plants were chopped using a mounter forage harvester (Hege 212; Wintersteiger AG) with a Kemper Champion 1200 (Kemper GmbH & Co. KG) to a particle-size range of 5–7 mm.

### 2.3 Silage preparation

Ensiling was conducted immediately after the chopping of the sorghum, by using 2-L glass jars (J. WECK GmbH & Co. KG) as laboratory-scale silos. Chopped sorghum samples were compressed manually using a special pressing device that ensured identical conditions for all the samples. Laboratory silos were filled completely; thus, no headspace remained within the glass jars. A glass lid, rubber ring, and four metal clamps were used to close the silos for preventing air infiltration but allowed the escape of gases formed during ensiling. The storage temperature was set at 25°C for the process duration of 90 days. The ensiling was conducted without silage additives. The silage preparation was performed in duplicates for each variant. The conservation losses during the ensiling (FM losses [FML]) were determined by weighing the silos after filling and after storage via the method of Weißbach (2005), as described by Herrmann et al. (2015).

### 2.4 Chemical analyses

Crop samples of fresh material and silages were stored at −18°C directly after harvest and feed-out from the laboratory-scale silos, respectively, for further analysis of chemical composition and methane production. For characterization of the harvested parental matter before ensiling, the WSCs were determined to be monomeric and dimeric sugars, as well as fructans, in water extracts with the addition of HgCl via high-performance liquid chromatography (HPLC; UltiMate 3000 UHPLC system, Thermo Fisher Scientific Inc.) according to Hoedtke and Zeyner (2010). The nitrate content was determined in water extracts of plant material dried at 60°C via ion chromatography (ICS-1000, Thermo Fisher Scientific Inc.), as described by VDLUFA (2006). Samples from FM and silages were dried at 105°C until reaching a constant weight to determine their total solids (TS) content. The volatile solids (VS) contents of the silages were calculated by determining the ash contents of samples in a muffle furnace at 550°C, via the method of VDLUFA (2006). As described by Weißbach and Kuhla (1995), the TS content of the ensiled material was corrected to account for the losses of organic acids and alcohols (ALCs) that occurred during oven drying. All the parameters referred to as the percentage of TS are based on the corrected TS value. A Sen Tix 41 electrode (WTW) was employed to measure the pH values of the silages. Cold water extracts from sorghum silage samples were prepared for determination of the contents of LA, volatile fatty acids (VFAs), and ALC. The LA content was determined via HPLC (Dionex) with a Eurokat H column (Knauer). Gas chromatography was performed to quantify the contents of the following: AA, which included both acetic and propionic acid; butyric acid (BA), which included butyric, isobutyric, caproic, valeric, and isovaleric acid; and ALC, which included ethanol, propanol, 1,2-propanediol, and 2,3-butanediol. Further details are available in Herrmann, Idler, et al. (2016). The contents of elemental carbon and nitrogen were measured using the DUMAS catalytic combustion methodology described by VDLUFA (2006). These values were used to calculate the C/N ratio. The crude protein (CP) content was calculated by multiplying the N content by 6.25. Furthermore, fiber analyses (neutral detergent fiber [NDF], acid detergent fiber [ADF], and acid detergent lignin [ADL]) were performed using an Ankom2000 analyzer system and a fiber filter bag (Ankom Technology Corp.), as described by Herrmann et al. (2011). The CEL content was calculated as the difference between the ADF and ADL contents, and the HCEL content was obtained by subtracting the ADF content from the NDF content. Gravimetric determination was performed to measure the portion of crude fat (CF) in silages; hydrolysis was conducted with 3 N hydrochloric acid, followed by partitioning with petroleum ether for 1 h at 90°C using an AnkomXT100 extractor (Ankom Technology Corp.). The content of non-fibrous carbohydrates (NFC) was determined by subtracting the CP, CF, NDF, and crude ash contents from 100%TS.

### 2.5 Batch anaerobic digestion tests

The SMYs from the sorghum silage samples were examined by performing batch anaerobic digestion tests in 2-L glass reactors. To obtain a VS$_{	ext{substrate}}$/VS$_{	ext{inoculum}}$ ratio of 0.4–0.5, 1.5 L of inoculum and 50 g of silage were added to each reactor. The inocula (average chemical characteristics: pH, 8.04; TS content, 5.17%; VS, 72.39%; N, 4.5 g kg$^{-1}$; NH$_4$-N, 2.87 g kg$^{-1}$; and organic acids, 0.3 g L$^{-1}$) were obtained...
TABLE 2  Description of the field trial regarding the principle growth stage at harvest for the six sorghum types at two different harvest dates, sites, and years

| HD | Type | Sowing | Harvest date | Principle growth stage at harvest\(^a\) | Sowing | Harvest date | Principle growth stage at harvest\(^a\) | Sowing | Harvest date | Principle growth stage at harvest\(^a\) |
|----|------|--------|--------------|------------------------------------------|--------|--------------|------------------------------------------|--------|--------------|------------------------------------------|
| 1  | SBSS1| 19.05.16| 25.08.16     | 7: Development of fruit                  | 11.05.17| 31.08.17     | 7: Development of fruit                  | 23.05.17| 07.09.17     | 8: Ripening                               |
|    | SBSS2| 19.05.16| 25.08.16     |                                           | 11.05.17| 31.08.17     |                                           | 23.05.17| 07.09.17     |                                           |
|    | SB1  | 19.05.16| 25.08.16     | 5: Inflorescence emergence/heading        | 11.05.17| 31.08.17     | 6: Flowering, anthesis                    | 23.05.17| 07.09.17     | 7: Development of fruit                  |
|    | SB2  | 19.05.16| 25.08.16     |                                           | 11.05.17| 31.08.17     |                                           | 23.05.17| 07.09.17     |                                           |
|    | SB3  | 19.05.16| 25.08.16     | 4: Booting                                | 11.05.17| 31.08.17     | 5: Inflorescence emergence/heading        | 23.05.17| 07.09.17     | 5: Inflorescence emergence/heading        |
|    | SB4  | 19.05.16| 25.08.16     |                                           | 11.05.17| 31.08.17     |                                           | 23.05.17| 07.09.17     | 6: Flowering, anthesis                    |
| 2  | SBSS1| 19.05.16| 15.09.16     | 9: Senescence                             | 11.05.17| 12.09.17     | 8: Ripening                               | 23.05.17| 26.09.17     | 8: Ripening                               |
|    | SBSS2| 19.05.16| 15.09.16     | 8: Ripening                               | 11.05.17| 12.09.17     |                                           | 23.05.17| 26.09.17     |                                           |
|    | SB1  | 19.05.16| 15.09.16     | 7: Development of fruit                   | 11.05.17| 12.09.17     | 7: Development of fruit                   | 23.05.17| 26.09.17     | 7: Development of fruit                   |
|    | SB2  | 19.05.16| 15.09.16     |                                           | 11.05.17| 12.09.17     |                                           | 23.05.17| 26.09.17     |                                           |
|    | SB3  | 19.05.16| 15.09.16     | 6: Flowering, anthesis                    | 11.05.17| 12.09.17     | 6: Flowering, anthesis                    | 23.05.17| 26.09.17     |                                           |
|    | SB4  | 19.05.16| 15.09.16     | 8: Ripening                               | 11.05.17| 12.09.17     |                                           | 23.05.17| 26.09.17     |                                           |

\(^a\)According to BBCH code (TFZ, 2012). MQ16: Marquardt (2016), MQ17: Marquardt (2017), ST17: Straubing (2017).
from previous laboratory anaerobic digestion experiments. A water bath at a mesophilic temperature (37°C) was used for reactor incubation. Batch tests were performed for approximately 30 days (or until the daily rate of biogas during three successive days was <0.5% of the total biogas obtained up to that time [VDI 4630, 2016]). The SMYs of all variants are presented as 30-day values. A scale gas meter was used to collect the produced biogas. An acidified saturated NaCl solution was employed as a barrier solution to determine the volume of gas obtained, using the barrier solution displacement method. The biogas volumes obtained from the silages were corrected via subtraction of the biogas volume produced exclusively by the inocula. The corrected volumes were normalized to standard conditions (dry gas, 0°C, 1013 hPa). A mobile gas analyzer equipped with infrared sensors (BM5000, Geotechnical Instruments Ltd.) was used to determine the amount of methane (CH₄) in the biogas produced. The SMY of each sample was calculated as the sum of methane generated during the batch test period, in terms of the VS contents of the silages added to the reactors. Further details are available in Herrmann, Idler, et al. (2016).

2.6 Statistical analyses

To determine the significant effects of cultivation and harvest conditions on sorghum silage quality parameters, the chemical characteristics of the silages, and the SMYs, an analysis of variance (ANOVA) was conducted by setting the harvest date, sorghum type, and harvest date × sorghum type interactions as fixed effects. The analyses were conducted separately for the two experimental field sites and harvest years (only MQ). A multiple comparison of means of the SMYs was performed to determine differences between sorghum types for each harvest date. The level of significance (α) was set as 0.05. The software SAS 9.4 (SAS Institute Inc.) and the PROC MIXED procedure were used for statistical analyses. Multiple comparisons of means were conducted using the SIMULATE test procedure.

3 RESULTS

3.1 Chemical characteristics of raw materials

The harvested fresh material of the six different sorghum types revealed different chemical compositions between the two harvest dates at sites MQ16, MQ17, and ST17 (Table 3). The TS contents obtained from the samples reflected the maturity tendency of each of the six sorghum types analyzed (Figure 2). For all the samples, the TS content was higher with a later harvest date, and this trend was observed at all the sites studied. Nevertheless, the majority of the samples analyzed exhibited TS values within the optimum range for ensiling reported by Amon et al. (2007; Figure 2). Among the variety types, the early-maturity hybrids $S. bicolor \times S. sudanense$ exhibited a higher TS content than the medium–late-maturing $S. bicolor$. In accordance with this tendency, the minimum TS value belonged to MQ16 for SB3 at HD1 (22.69%FM; Table 3), whereas SBSS1 exhibited the maximum value at HD2 (46.93%FM; Table 3) at the same location. The VS content remained relatively constant, with an average value of 94.9%TS

### Table 3 Means of fresh matter characteristics of the six different sorghum types for different harvest dates, sites, and years

| HD Type   | MQ16 | MQ17 | ST17 |
|-----------|------|------|------|
|           | TS   | VS   | Nitrate | WSC | TS   | VS   | Nitrate | WSC | TS   | VS   | Nitrate | WSC |
|           | %FM  | %TS  | %TS     | %TS | %FM  | %TS  | %TS     | %TS | %FM  | %TS  | %TS     | %TS |
| 1         | SBSS1 | 33.22 | 95.71  | 0.00 | 4.53 | 33.67 | 94.91  | 0.04 | 3.18 | 32.92 | 95.15  | 0.09 | 4.13 |
|           | SBSS2 | 30.40 | 95.43  | 0.01 | 6.24 | 32.67 | 95.25  | 0.05 | 4.20 | 31.57 | 94.12  | 0.07 | 4.39 |
|           | SB1   | 26.60 | 94.84  | 0.02 | 6.55 | 30.43 | 94.91  | 0.08 | 7.27 | 29.90 | 94.44  | 0.13 | 7.80 |
|           | SB2   | 26.55 | 95.15  | 0.02 | 7.44 | 29.57 | 94.97  | 0.07 | 8.03 | 27.74 | 94.19  | 0.12 | 8.33 |
|           | SB3   | 22.69 | 94.36  | 0.05 | 7.09 | 26.91 | 94.83  | 0.14 | 5.99 | 25.56 | 94.26  | 0.20 | 6.71 |
|           | SB4   | 22.72 | 94.76  | 0.03 | 7.09 | 27.16 | 94.79  | 0.12 | 6.44 | 25.61 | 93.85  | 0.20 | 7.44 |
| 2         | SBSS1 | 45.93 | 95.59  | 0.00 | 2.68 | 37.02 | 95.17  | 0.06 | 3.00 | 35.00 | 94.56  | 0.08 | 4.33 |
|           | SBSS2 | 41.41 | 95.54  | 0.01 | 4.68 | 35.30 | 95.31  | 0.08 | 3.45 | 31.92 | 94.24  | 0.07 | 4.47 |
|           | SB1   | 37.33 | 95.70  | 0.01 | 8.96 | 32.38 | 94.98  | 0.13 | 8.94 | 31.22 | 94.49  | 0.09 | 9.57 |
|           | SB2   | 35.69 | 95.80  | 0.02 | 9.33 | 30.18 | 95.01  | 0.16 | 8.63 | 29.55 | 94.53  | 0.09 | 9.26 |
|           | SB3   | 30.68 | 95.06  | 0.02 | 10.07 | 30.21 | 94.95  | 0.19 | 7.50 | 27.11 | 94.18  | 0.35 | 11.38 |
|           | SB4   | 30.24 | 94.94  | 0.04 | 9.65 | 28.94 | 94.53  | 0.13 | 8.50 | 28.21 | 94.77  | 0.19 | 10.75 |

Note: MQ16, Marquardt (2016); MQ17, Marquardt (2017); ST17, Straubing 2017; HD, harvest date; TS, total solids; VS, volatile solids; FM, fresh matter; WSC, water-soluble carbohydrates.
for all the variants. The nitrate concentrations of the samples were <0.35%TS. A slight trend was observed concerning the sorghum types: the *S. bicolor* varieties had higher nitrate contents than *S. bicolor × S. sudanense*, particularly for HD2. According to the results presented in Table 3, the WSC content differed significantly among the sorghum varieties. In
general, *S. bicolor × S. sudanense* hybrids exhibited lower WSC contents than *S. bicolor* varieties. In almost all cases, a decreasing tendency was observed from HD1 to HD2 for the *S. bicolor × S. sudanense* hybrids, with the exception of the ST17 samples. In contrast, the WSC content of the *S. bicolor* types increased with maturity. In MQ16 and ST17, Sorghum type SB3 exhibited the highest concentration of WSC on HD2, whereas in MQ17, SB1 on HD2 ranked first (Table 3).

### 3.2 Silage fermentation characteristics

A typical silage fermentation profile was determined for all the samples (Table 4). The pH values oscillated between 3.7 and 4.6, and the BA concentrations were insignificant in all cases, indicating adequate material conservation and the absence of secondary fermentation. According to the data obtained, the LA content was the highest among the fermentation acids; it was in the range of 1.5%–6.4%TS for all cases. Only for Marquardt, sorghum variety had a statistically significant effect on the LA concentration (Table 4). Additionally, in MQ16, the results depended significantly on the HD and the effect HD × T. In contrast, the LA content in Straubing was unaffected by the factors studied. In the majority of cases, the AA concentration exhibited a slight increment from HD1 to HD2, with the exception of the *S. bicolor × S. sudanense* hybrids in MQ16. As indicated by this table, the AA contents differed significantly among the sorghum types (*p* < 0.001), with the middle–late maturity varieties *S. bicolor* containing higher concentrations than *S. bicolor × S. sudanense*. Moreover, in MQ17 and ST17, the AA content depended significantly on the HD, but this effect was not observed in MQ16. The ALC content varied widely among the samples (Table 4). In MQ16 and ST17, significant effects of HD, T, and HD × T were observed. In contrast, the results for MQ17 indicated no significant differences for the factors analyzed and remained relatively constant below 0.84%TS for all the cases (Table 4). The results for the FML differed significantly among the varieties. Nevertheless, this parameter exhibited no significant difference between the effects examined, the only exception being the HD effect in MQ16.

### 3.3 Nutrient and fiber compositions of crop silages

Chemical characterization of all six sorghum variety types after ensiling was performed for each site, year, and HD (Tables 5 and 6). The HD and T significantly affected almost all the parameters in Table 5. The TS content after ensiling was higher for *S. bicolor × S. sudanense* than for the *S. bicolor* types. Statistical analyses indicated that the TS and VS results depended significantly on the type of sorghum, HD, and the interaction of the HD and sorghum type, regardless of the location. The HD affected the CP content for the MQ16 and ST17 samples but not the MQ17 samples. Generally, *S. bicolor × S. sudanense* exhibited higher CP contents than the *S. bicolor* variants, but the effect of T was substantial only for the samples collected in Marquardt. The same tendency was observed for the CF content for both years in Marquardt, but again, no detectable differences for the Straubing samples were observed. The NFC composition was significantly affected by the HD in all the cases, but there was also a significant effect of the sorghum type on the NFC in MQ17 and ST17. The C:N ratios were always >45. The lowest value for this parameter was observed in MQ17 for the sorghum type SBSS1 harvested on HD1 (45; Table 5), and the highest value corresponded to SB1 in ST17 harvested on HD2 (76; Table 5). Nevertheless, only in Marquardt was the sorghum type a significant influencing factor. Additionally, the results indicated that the HD affected the C:N ratios for all the samples obtained at MQ16 and ST17. The variable HD had a significant impact on almost all the fiber components in the samples, with the exception of HCEL in MQ16 and ADL in MQ17 and ST17 (Table 6). Considering the T factor, there was a statistical difference in the CEL content for both the sites (whereas for the NDF and ADF contents, there was a significant difference only in MQ17 and ST17). Furthermore, the ADL content was significantly affected by the sorghum type in both harvest years in Marquardt. The ADL content was higher for the *S. bicolor × S. sudanense* hybrids than for *S. bicolor*. The effect of the HD × T interaction on the parameters was insignificant, with the exception of the ADF and ADL contents in MQ17 and HCEL in ST17 (Table 6).

### 3.4 Specific methane yields of crop silages

The SMY was negatively affected by the HD in almost all the cases in this study (Figure 3). This effect was clearly evident for the samples from Marquardt, where the values decreased from HD1 to HD2. The sorghum type significantly affected the SMY for all the Marquardt samples; however, for the Straubing samples, the sorghum type had no significant effect. Nevertheless, a trend was observed for the majority of the cases: earlier-maturing *S. bicolor × S. sudanense* hybrids had an inferior SMY (ranging from 231.25 to 294.98 Lha⁻¹ VS) than *S. bicolor* (ranging from 262.15 to 321.31 Lha⁻¹ VS). For instance, the hybrids SBSS1 and SBSS2 had the lowest SMYs at both HDs, and the SB1, SB3, and SB4 types had the highest SMYs.

### 3.5 Effects of chemical composition on methane formation

The Pearson’s correlations among all the traits are presented in Table 7. Of major interest were the interactions that
| HD | Type   | MQ16 | MQ17 | ST17 | Level of significance |
|----|--------|------|------|------|------------------------|
|    |        | pH   | LA   | AA<sup>a</sup> | BA<sup>b</sup> | ALC<sup>c</sup> | FML | pH   | LA   | AA<sup>a</sup> | BA<sup>b</sup> | ALC<sup>c</sup> | FML | pH   | LA   | AA<sup>a</sup> | BA<sup>b</sup> | ALC<sup>c</sup> | FML |
| 1  | SBSS1  | 4.2  | 2.7  | 0.89  | 0.33  | 0.82  | 1.21 | 4.2  | 3.6  | 0.87  | 0.43  | 1.21 | 0.82 | 0.33 | 0.89  | 0.33  | 0.82  | 1.21 | 3.90  | 4.96  | 0.87  | 0.33  | 0.82  | 1.21 | 3.90  | 4.96  | 0.87  | 0.33  | 0.82  | 1.21 |
|    | SBSS2  | 3.9  | 4.9  | 1.37  | 0.01  | 1.77  | 0.89 | 4.2  | 3.6  | 0.99  | 0.56  | 0.57 | 1.77 | 0.89 | 4.20  | 3.60  | 0.99  | 0.56  | 0.57 | 1.77 | 0.89 | 4.20  | 3.60  | 0.99  | 0.56  | 0.57 | 1.77 |
|    | SB1    | 3.9  | 4.7  | 1.53  | 0.07  | 0.75  | 0.54 | 4.1  | 3.8  | 1.51  | 0.83  | 0.94 | 0.75 | 0.54 | 4.10  | 3.80  | 1.51  | 0.83  | 0.94 | 0.75 | 0.54 | 4.10  | 3.80  | 1.51  | 0.83  | 0.94 | 0.75 |
|    | SB2    | 4.0  | 4.2  | 1.54  | 0.05  | 2.99  | 0.51 | 4.1  | 3.8  | 1.41  | 0.4  | 0.74 | 2.99 | 0.51 | 4.10  | 3.80  | 1.41  | 0.4  | 0.74 | 2.99 | 0.51 | 4.10  | 3.80  | 1.41  | 0.4  | 0.74 | 2.99 |
|    | SB3    | 3.8  | 6.4  | 1.59  | 0.08  | 0.69  | 0.43 | 4.0  | 4.7  | 1.44  | 0.39  | 0.41 | 0.69 | 0.43 | 4.00  | 4.70  | 1.44  | 0.39  | 0.41 | 0.69 | 0.43 | 4.00  | 4.70  | 1.44  | 0.39  | 0.41 | 0.69 |
|    | SB4    | 3.8  | 5.8  | 1.63  | 0.08  | 6.12  | 0.36 | 4.0  | 4.7  | 1.47  | 0.85  | 0.37 | 6.12 | 0.36 | 4.00  | 4.70  | 1.47  | 0.85  | 0.37 | 6.12 | 0.36 | 4.00  | 4.70  | 1.47  | 0.85  | 0.37 | 6.12 |
| 2  | SBSS1  | 4.4  | 2.1  | 0.70  | 0.20  | 0.55  | 1.52 | 4.2  | 3.6  | 0.90  | 0.27  | 0.38 | 0.55 | 1.52 | 4.20  | 3.60  | 0.90  | 0.27  | 0.38 | 0.55 | 1.52 | 4.20  | 3.60  | 0.90  | 0.27  | 0.38 | 0.55 |
|    | SBSS2  | 4.6  | 1.5  | 0.78  | 0.08  | 3.36  | 2.10 | 4.1  | 3.7  | 1.09  | 0.35  | 0.43 | 3.36 | 2.10 | 4.10  | 3.70  | 1.09  | 0.35  | 0.43 | 3.36 | 2.10 | 4.10  | 3.70  | 1.09  | 0.35  | 0.43 | 3.36 |
|    | SB1    | 4.2  | 2.1  | 1.22  | 0.05  | 0.95  | 2.43 | 4.0  | 3.7  | 1.72  | 0.42  | 0.64 | 0.95 | 2.43 | 4.00  | 3.70  | 1.72  | 0.42  | 0.64 | 0.95 | 2.43 | 4.00  | 3.70  | 1.72  | 0.42  | 0.64 | 0.95 |
|    | SB2    | 4.3  | 1.9  | 1.57  | 0.05  | 0.69  | 2.19 | 4.0  | 4.1  | 1.84  | 0.32  | 0.54 | 0.69 | 2.19 | 4.00  | 4.10  | 1.84  | 0.32  | 0.54 | 0.69 | 2.19 | 4.00  | 4.10  | 1.84  | 0.32  | 0.54 | 0.69 |
|    | SB3    | 4.1  | 3.6  | 1.92  | 0.14  | 0.70  | 1.74 | 4.0  | 3.7  | 1.77  | 0.3  | 0.47 | 0.70 | 1.74 | 4.00  | 3.70  | 1.77  | 0.3  | 0.47 | 0.70 | 1.74 | 4.00  | 3.70  | 1.77  | 0.3  | 0.47 | 0.70 |
|    | SB4    | 3.9  | 2.9  | 2.38  | 0.12  | 0.79  | 1.74 | 4.0  | 4.1  | 1.77  | 0.3  | 0.47 | 0.79 | 1.74 | 4.00  | 4.10  | 1.77  | 0.3  | 0.47 | 0.79 | 1.74 | 4.00  | 4.10  | 1.77  | 0.3  | 0.47 | 0.79 |

**Level of significance**

| HD | T | HD × T |
|----|---|--------|
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |
| ***| ***| n.s.   |

Abbreviations: AA, acetic acid; HD, harvest date; T, type; LA, lactic acid; n.s., not significant; TS, total solids.

<sup>a</sup>Sum of acetic and propionic acid, BA: butyric acid.

<sup>b</sup>Sum of butyric, isobutyric, valeric, isovaleric, caproic acid; ALC: alcohol.

<sup>c</sup>Sum of ethanol, propanol, 1,2-propanediol, 2,3-butanediol, and FML: fresh matter losses.

*p < 0.05; **p < 0.01; ***p < 0.001.
### Table 5
Silage chemical characterization of the six different sorghum types at two different harvest dates, sites, and years, along with the ANOVA results of fixed effects

| HD | Type  | MQ16          |            | MQ17          |            | ST17          |            |
|----|-------|---------------|------------|---------------|------------|---------------|------------|
|    |       | TS | %FM | %TS | %TS | %TS | %TS | %FM | %TS | %TS | %TS | %TS | %TS | C:N | %FM | %TS | %TS | %TS | %TS | %TS | %TS | C:N |
| 1  | SBSS1 |    | 32.48 | 95.41 | 5.92 | 1.60 | 22.96 | 52 | 33.60 | 94.82 | 6.78 | 2.83 | 17.77 | 45 | 33.60 | 94.49 | 5.69 | 1.86 | 22.29 | 53 |
|    | SBSS2 |    | 29.44 | 95.24 | 4.99 | 1.55 | 19.42 | 62 | 31.44 | 95.13 | 5.30 | 2.78 | 20.35 | 58 | 32.10 | 94.39 | 5.48 | 2.32 | 21.67 | 55 |
|    | SB1   |    | 25.80 | 94.81 | 5.21 | 1.48 | 20.80 | 59 | 29.17 | 94.62 | 5.07 | 1.84 | 16.74 | 60 | 30.02 | 94.54 | 5.14 | 1.41 | 20.26 | 58 |
|    | SB2   |    | 25.31 | 94.98 | 5.09 | 1.50 | 21.56 | 61 | 28.03 | 95.01 | 5.93 | 2.41 | 17.41 | 51 | 27.42 | 93.95 | 5.09 | 1.47 | 22.38 | 58 |
|    | SB3   |    | 22.44 | 94.07 | 5.48 | 1.60 | 22.64 | 56 | 26.25 | 94.44 | 5.26 | 1.95 | 17.01 | 58 | 26.37 | 93.63 | 5.38 | 1.22 | 17.10 | 55 |
|    | SB4   |    | 22.37 | 94.46 | 5.39 | 2.07 | 21.10 | 57 | 26.47 | 94.60 | 5.05 | 1.54 | 16.38 | 61 | 25.76 | 93.83 | 4.75 | 1.73 | 18.51 | 63 |

| 2  | SBSS1 |    | 44.51 | 95.39 | 5.70 | 2.12 | 24.54 | 54 | 35.29 | 94.56 | 6.21 | 2.17 | 21.27 | 49 | 33.69 | 94.63 | 3.91 | 2.19 | 25.30 | 76 |
|    | SBSS2 |    | 40.35 | 95.44 | 4.90 | 2.22 | 24.66 | 64 | 34.11 | 94.81 | 6.49 | 2.10 | 24.30 | 55 | 30.93 | 93.50 | 5.14 | 2.73 | 22.20 | 57 |
|    | SB1   |    | 34.19 | 95.38 | 4.00 | 1.88 | 24.01 | 74 | 31.40 | 94.97 | 6.08 | 1.53 | 22.84 | 59 | 30.22 | 94.21 | 4.24 | 1.67 | 24.23 | 68 |
|    | SB2   |    | 33.32 | 95.53 | 4.18 | 1.55 | 25.31 | 70 | 28.96 | 94.85 | 5.53 | 1.14 | 23.55 | 55 | 29.75 | 94.22 | 3.73 | 1.96 | 25.54 | 78 |
|    | SB3   |    | 29.44 | 94.77 | 4.82 | 1.21 | 25.63 | 58 | 29.20 | 94.93 | 5.17 | 1.57 | 18.47 | 59 | 28.62 | 94.44 | 4.19 | 2.68 | 21.39 | 69 |
|    | SB4   |    | 28.83 | 94.87 | 4.61 | 1.15 | 23.13 | 63 | 28.88 | 94.81 | 5.22 | 1.69 | 19.84 | 58 | 28.11 | 94.52 | 4.02 | 1.70 | 24.06 | 72 |

Level of significance

| HD | T | HD × T |
|----|---|--------|
| ***| ***| *** n.s. | *** | *** | *** n.s. | *** | *** | *** n.s. | *** | *** | *** n.s. | *** | *** | *** n.s. |

Abbreviations: C, carbon; CF, crude fat; CP, crude protein; FM, fresh matter; HD, harvest date; N, nitrogen; n.s., not significant; NFC, non-fibrous carbohydrates; T, type; TS, total solids; VS, volatile solids.

*p < 0.05; **p < 0.01; ***p < 0.001.
| HD | Type | MQ16 | MQ17 | ST17 |
|----|------|------|------|------|
|    |      | HCEL | CEL  | NDF  | ADF  | ADL  | HCEL | CEL  | NDF  | ADF  | ADL  | HCEL | CEL  | NDF  | ADF  | ADL  |
| 1  | SBSS1 | 27.54| 31.12| 64.95| 37.42| 5.61| 24.24| 36.60| 67.36| 43.12| 6.53| 23.01| 35.42| 64.57| 41.57| 6.15|
|    | SBSS2 | 29.60| 32.93| 69.28| 39.68| 5.64| 24.01| 35.47| 66.62| 42.61| 7.14| 23.12| 35.62| 64.81| 41.70| 6.08|
|    | SB1   | 28.56| 33.04| 67.14| 38.58| 3.97| 25.40| 39.64| 70.84| 45.44| 5.80| 24.02| 38.21| 67.57| 43.55| 5.34|
|    | SB2   | 28.84| 32.37| 66.77| 37.93| 3.85| 25.83| 36.94| 69.13| 43.30| 6.36| 23.61| 36.41| 64.78| 41.17| 4.76|
|    | SB3   | 27.13| 31.69| 64.14| 37.00| 3.70| 25.79| 38.19| 70.11| 44.31| 6.12| 25.01| 39.61| 69.72| 44.71| 5.10|
|    | SB4   | 27.39| 32.92| 65.77| 38.39| 3.60| 24.95| 40.15| 71.51| 46.56| 6.40| 23.66| 40.02| 68.64| 44.98| 4.96|
| 2  | SBSS1 | 26.53| 29.44| 62.95| 36.42| 6.11| 22.70| 35.25| 64.83| 42.14| 6.89| 22.36| 35.29| 63.09| 40.73| 5.44|
|    | SBSS2 | 26.86| 30.52| 63.58| 36.71| 5.48| 22.24| 33.87| 62.82| 40.58| 6.70| 22.59| 35.10| 63.28| 40.69| 5.59|
|    | SB1   | 28.13| 32.04| 65.35| 37.22| 4.87| 22.49| 36.41| 65.37| 42.87| 6.46| 22.52| 35.58| 63.79| 41.26| 5.69|
|    | SB2   | 28.33| 30.05| 64.24| 35.91| 5.99| 23.65| 35.16| 64.48| 40.82| 5.66| 23.15| 34.21| 62.74| 39.58| 5.37|
|    | SB3   | 28.31| 31.40| 62.62| 34.31| 4.37| 24.37| 38.37| 69.59| 45.21| 6.84| 22.31| 37.42| 65.92| 43.62| 6.19|
|    | SB4   | 30.50| 33.20| 67.44| 36.94| 4.19| 24.34| 37.44| 67.92| 43.58| 6.14| 22.30| 35.63| 64.46| 42.16| 6.52|

**Level of significance**

|    | HD   | n.s. | *** | *  | *** | *  | *** | *** | *** | *** | n.s. | **  | *** | n.s. | **  | *** | n.s. |
|----|------|------|-----|----|-----|----|-----|-----|-----|-----|------|-----|-----|------|-----|-----|------|
|    | T    | n.s. | **  | n.s. | n.s. | *  | *** | *** | *** | *** | n.s. | *  | n.s. | *  | n.s. | n.s. |
|    | HD × T | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

**Abbreviations:** ADF, acid detergent fiber; ADL, acid detergent lignin; CEL, cellulose; HCEL, hemicellulose; HD, harvest date; n.s., not significant; NDF, neutral detergent fiber; T, type; TS, total solids.

*p < 0.05; **p < 0.01; ***p < 0.001.
affected the SMY. On the one hand, the LA and AA contents were moderately correlated with the SMY. Additionally, the contents of these acids were affected by other factors, such as the TS, VS, CEL, and ADF contents in the case of LA and the ALC, TS, VS, CP, and CF contents in the case of AA. On the other hand, the SMY was strongly negatively

**FIGURE 3** Specific methane yield (SMYs) of six different sorghum types for two different harvest dates (HD) at (a) Marquardt (2016; MQ16), (b) Marquardt (2017; MQ17), and (c) Straubing (2017; ST17). Different uppercase letters indicate significant differences among sorghum types for the same harvest date at $\alpha = 0.05$. VS, volatile solids
|       | pH   | LA   | AA<sup>a</sup> | BA<sup>b</sup> | ALC<sup>c</sup> | FML  | TS  | VS  | CP   | CF   | NFC  | C/N  | HCEL | CEL  | NDF  | ADF  | ADL  |
|-------|------|------|---------------|---------------|----------------|------|-----|-----|------|------|------|------|------|------|------|------|------|------|
| LA    |      |      | -0.90         |               |                |      |     |     |      |      |      |      |      |      |      |      |      |
| AA<sup>a</sup> | -0.55 | 0.37 |
| BA<sup>b</sup> | 0.28 | -0.34 | -0.06         |               |                |      |     |     |      |      |      |      |      |      |      |      |
| ALC<sup>c</sup> | -0.06 | -0.14 | 0.54          | 0.32          |                |      |     |     |      |      |      |      |      |      |      |      |
| FML   |      |      | -0.09         | -0.09         | 0.25           | 0.27 | 0.47|
| TS    | 0.66 | -0.70 | -0.62         | 0.21          | -0.05          | 0.14 |     |     |      |      |      |      |      |      |      |      |
| VS    | 0.67 | -0.72 | -0.62         | 0.22          | -0.05          | 0.13 | 1.00|
| CP    | 0.31 | -0.11 | -0.49         | -0.01         | -0.51          | -0.59| 0.10 | 0.10 |
| CF    | 0.17 | -0.06 | -0.49         | -0.23         | -0.31          | -0.10| 0.35 | 0.34 | 0.20 |
| NFC   | 0.11 | -0.27 | 0.02          | 0.40          | 0.42           | 0.27 | 0.43 | 0.43 | -0.44 | -0.06 |
| C:N   | -0.25| 0.07  | 0.38          | -0.01         | 0.41           | 0.56 | -0.03| -0.03| -0.98 | -0.11 | 0.44 |
| HCEL  | 0.31 | -0.36 | 0.12          | 0.39          | 0.29           | -0.03| -0.12| -0.10| 0.01  | -0.39 | -0.03| -0.02|
| CEL   | -0.43| 0.51  | 0.17          | -0.56         | -0.31          | -0.06| -0.40| -0.41| 0.07  | 0.04  | -0.72| -0.10| -0.55|
| NDF   | -0.07| 0.16  | 0.11          | -0.34         | -0.29          | -0.15| -0.45| -0.45| 0.21  | -0.16 | -0.94| -0.22| 0.20 |
| ADF   | -0.30| 0.41  | -0.01         | -0.57         | -0.46          | -0.10| -0.27| -0.28| 0.16  | 0.17  | -0.73| -0.16| -0.61|
| ADL   | 0.32 | -0.28 | -0.39         | -0.30         | -0.26          | -0.05| 0.47 | 0.46 | 0.20  | 0.44  | -0.17| -0.15| -0.59|
| SMY   | -0.64| 0.59  | 0.63          | -0.09         | 0.32           | 0.12 | -0.81| -0.80| -0.34 | -0.36 | -0.13| 0.29 | 0.28 |

| 0.40–0.71 | Moderate correlation | 0.71–1.01 | Strong correlation |

Abbreviations: AA, acetic acid; ADF, acid detergent fiber; ADL, acid detergent lignin; C, carbon; CEL, cellulose; CF, crude fat; CP, crude protein; HCEL, hemicellulose; LA, lactic acid; N, nitrogen; NDF, neutral detergent fiber; NFC, non-fibrous carbohydrates; SMY, specific methane yield; TS, total solids; VS, volatile solids.

<sup>a</sup>Sum of acetic and propionic acid, BA: butyric acid.

<sup>b</sup>Sum of butyric, isobutyric, valeric, isovaleric, caproic acid; ALC: alcohol.

<sup>c</sup>Sum of ethanol, propanol, 1,2-propanediol, 2,3-butanediol, FML: fresh matter losses.
correlated with the solids content (TS and VS), but moderately negatively correlated with the pH and ADL content. These last two parameters (pH and ADL) exhibited multiple moderate correlations with other factors, particularly the fiber fractions.

Figure 4 shows the relationship between the SMY and the TS content (associated with sorghum variety: hybrids S. bicolor × S. sudanense [SBSS] or S. bicolor [SB]) and the specific BBCH code number that characterized each phenological stage of the sorghum types harvested in MQ16, MQ17, and ST17. A slight correlation is observed, indicating that the SMY decreased with crop maturity. In the diagram presented, this tendency is explained not only from the TS perspective but also from the perspective of BBCH classification, which reflects the plant’s current development stage. The S. bicolor hybrids exhibited SMYs of >250 Ln kg⁻¹ VS in all cases, even at the higher BBCH stages, with a maximum value of 314 Ln kg⁻¹ VS (for SB3 in ST17). Furthermore, the TS contents of all the S. bicolor samples remained below 35%FM. In contrast, the S. bicolor × S. sudanense hybrids exhibited BBCH numbers of >70 (development of fruit) even at the first HD, leading to higher TS contents and lower SMYs (<300 Ln kg⁻¹ VS for all cases).

4 | DISCUSSION

This study shows the ensiling ability of sorghum varieties with contrasting maturity characteristics (early, medium–late, and late). Additionally, it revealed that the silage quality varied among different sorghum types, harvest dates, and locations. Nonetheless, in all the cases examined, it was possible to produce adequate amounts of methane per unit of biomass. A discussion of the harvested biomass quality, silage fermentation profile, and methane production for all six sorghum types in MQ16, MQ17, and ST17 is presented in the following sections.

4.1 | Ensilability of raw material

One of the most important factors defining a successful silage process is the TS content of the fresh material. It is well known that an optimum TS content, along with other parameters such as the WSC content and buffer capacity of biomasses, yields favorable conditions for the desired silage fermentation (Kung et al., 2018; Teixeira Franco et al., 2016). As expected, for all six sorghum types studied, the TS content increased with a later harvest, revealing the differences between their maturity characteristics. Wannasek et al. (2017) found that four different S. bicolor sorghum types and one S. bicolor × S. sudanense, with diverse maturity tendencies, exhibited different TS fractions when testing the effects of climate, sorghum variety, and time of harvest on plant development and biomass yields. The results of the present study indicate that the early sorghum hybrids reached the optimal maturity stage at HD1 for both sites and both years (optimum TS content range is 27%–35% according to Wannasek et al., 2017). In contrast, medium-(late) S. bicolor types reached the optimum TS content at HD2. Only for HD2 in MQ16 was the TS content of S. bicolor × S. sudanense types higher than the optimum. This was confirmed by the phenological development stage at harvest (BBCH code; senescence), which was characteristic of these samples (Table 2). Nevertheless, these attributes were not observed for any sorghum type in 2017, suggesting a possible effect of the year on the results. During May, June, and July 2016, temperatures higher than the long-term average were registered at Marquardt. These favorable conditions may have positively affected crop development, particularly for the earlier maturity sorghum types. In agreement with our results, Mahmood
and Honermeier (2012) found that the *S. bicolor × S. sudanense* sorghum type “Bovital” exhibited a higher TS content than the *S. bicolor* types “Aron” and “Rona 1” when comparing the effects of row spacing on chemical composition and methane yield between different sorghum species.

In addition to the TS content, the WSC content is important during ensiling, as WSCs are the central compounds transformed into organic acids by LA bacteria (McDonald et al., 1991). Many authors specified an optimum WSC range of approximately 60–80 g kg⁻¹ TS to obtain high-quality silages (Villa et al., 2020; Woolford, 1984). The results obtained were within this range in most cases, but some *S. bicolor × S. sudanense* hybrids were below the limit, and some *S. bicolor* hybrids were above the limit (Table 3). Amer et al. (2012) found that large amounts of WSCs improved ensiling characteristics when performing experiments using two different varieties of sorghum and forage millet. In relation to these findings, *S. bicolor* types at HD2 presented the best conditions as a pre-ensiled material for both sites and years, with WSC contents reaching 11%DM in some cases. Rodrigues et al. (2020) reported a similar WSC average (10.7%DM) for *S. bicolor* types. McDonald et al. (1991) reported that the optimum nitrate content is in the range of 0.6%–1.0%TS, and the results for fresh materials were in all cases below this range. During ensiling, nitrate is usually reduced to nitrite and nitric oxide—compounds that can suppress clostridial growth, avoiding silage spoilage (McDonald et al., 1991). Nevertheless, if other ensiling parameters are favorable, nitrate is not absolutely needed, and high silage quality can be achieved.

Overall, the different maturity-tendency sorghum type biomasses differed in their TS and WSC contents at different harvest dates, but these differences did not affect the success of the ensiling process (see Section 4.2).

### 4.2 Fermentation quality

It is generally accepted that high-quality silage is characterized by low pH, high LA content, and insignificant amounts of BA (Kung et al., 2018; Teixeira Franco et al., 2016; Villa et al., 2020). As expected, according to the favorable ensiling ability of the sorghum samples, silages of all the variants analyzed in this study fulfilled these requirements and exhibited adequate silage fermentation quality. Neither the HD nor the sorghum type significantly affected the final silage quality, although these two factors significantly affected individual silage fermentation parameters, such as the AA and ALC contents (Table 4).

In a more detailed analysis, the pH values lay within the common range of 3.7–5 reported by Villa et al. (2020). Among all the products of silage fermentation, LA represented the largest proportion, indicating typical LA silage fermentation (Buxton & O’Kiely, 2003). Herrmann, Idler, et al. (2016) reported LA ranges of 2.5%–12%TS and 5.2%–13%TS for sorghum *S. bicolor × S. sudanense* and *S. bicolor × S. bicolor* silages, respectively. The results of the present study are consistent with these values, with the exception of the samples from MQ16 (HD2), which exhibited lower LA concentrations. The ANOVA results (Table 4) indicate that the sorghum type affected the LA content of the samples obtained at Marquardt. Generally, later-maturity sorghum types exhibited larger amounts of LA. This effect was not significant for the ST17 samples.

However, there were statistical differences in the AA content among the sorghum types from the different sites. This acid is typically found to be the second-highest parameter in terms of the concentration in silages, with values between 1% and 3%TS (Kung et al., 2018). The predominant number of silages analyzed in the present study was within this range. The AA amounts differed significantly between HD at MQ17 and ST17, with a visible increasing trend from HD1 to HD2. The increment of this parameter can be beneficial for the silage aerobic stability owing to the antifungal properties of AA (Kung et al., 2018).

The absence or minor amounts of BA existing in the samples indicate that clostridial metabolism did not dominate silage fermentation. The presence of BA typically results in energy losses and low-quality silages for animal feeding (McDonald et al., 1991; Pahlow et al., 2003), but Villa et al. (2020) argued that biogas-purpose silages can contain larger amounts of BA, which can lead to greater SMY production. Nonetheless, even with some BA, the FML of the samples were insignificant for the majority of the cases studied (Table 4).

The presence of ALCs is also evident in silages, with ethanol being the most common compound found (Kung et al., 2018). Researchers have evaluated the ethanol concentrations in different *S. bicolor* silages and obtained values between 1% and 3.4%TS (Miron et al., 2005). Additionally, Herrmann, Idler, et al. (2016) reported ALC contents ranging from 0.5% to 4.3%TS and from 0.6% to 4.1%TS for *S. bicolor × S. sudanense* and *S. bicolor × S. bicolor* hybrids, respectively. In agreement with the literature, the ALC content range in the present study was between 0.3% and 6.12%TS (Table 4). However, it was impossible to establish trends, owing to the high variability among the results. Given that ethanol can be produced by different microorganisms (such as heterolactic acid bacteria, enterobacteria, and yeast), this inconsistency may have been caused by the activity of diverse epiphytic microflora (Kung et al., 2018; McDonald et al., 1991).

### 4.3 Silage nutrient and fiber characteristics

The average CP content obtained for the silages studied is consistent with values reported by other authors for
**S. bicolor** and **S. bicolor × S. sudanense** types (Herrmann et al., 2011; Mahmood et al., 2013; Rodrigues et al., 2020). This parameter was significantly affected by the sorghum type for the Marquardt variants. According to previous findings, leaves are the major protein contributors in sorghum, and the CP content is significantly affected by the genotype (Beck et al., 2007; Chattha et al., 2020; Miron et al., 2005). The CF content remained below 2.8%TS, similar to previously reported results for sorghum (Mahmood et al., 2013; Theuretzbacher et al., 2013). NFC includes easily degradable carbohydrates, starches, and other compounds with simple structures. This parameter depends strongly on the sorghum genotype characteristics (Hassan et al., 2019). Furthermore, several authors have reported changes in the sugar and starch contents with maturity (Nurk et al., 2016; Theuretzbacher et al., 2013). The results of the present study for the NFC content are comparable to the aforementioned findings. Drosg (2013) reported that the optimal C/N ratio for methane formation is between 20/1 and 30/1. In this context, all the tested sorghum silages exhibited C/N ratios significantly higher than the optimum range. This outcome is contrary to that of Herrmann, Idler, et al. (2016), who observed average values of 33 and 35 for the analysis of sorghum hybrids. Control of the C/N ratio appears to be important at the stage of the anaerobic digestion of the material (particularly in continuous digestion systems) for balancing the nutrient supply and ensuring optimal biomass conversion (Chen et al., 2008; Michalska & Ledakowicz, 2013).

The plant growth stage strongly determines the digestibility of silages owing to the fiber fraction existing at harvest (McDonald et al., 1991) and can critically affect the biomethanation process (Mahmood et al., 2013; Triolo et al., 2011). The obtained fiber-fraction results were generally slightly higher than those reported by Herrmann, Idler, et al. (2016) but similar to those reported by Nurk et al. (2016). Regardless of the location or year studied, the ANOVA results revealed significant effects of the HD on the CEL, NDF, and ADF contents (Table 6). This suggests that maturity had a significant impact on the CEL content of sorghum. Dissimilarities were observed regarding the HCEL content, which was strongly influenced by the HD at MQ17 and ST17 but not at MQ17. Most of the literature refers to an increasing trend with maturity for the non-degradable fraction, particularly lignin (Hassan et al., 2019; Nurk et al., 2016; Pazderu et al., 2014). In contrast to earlier findings, no evidence of an effect of the HD on the ADL was confirmed. Nevertheless, the choice of the sorghum type appears to be a crucial factor in determining the final fiber components of silages (Beck et al., 2007; Buxton & O’Kiely, 2003; Chattha et al., 2020; Hassan et al., 2019; Thomas et al., 2019). Across all sites and years, the CEL content was the only factor affected by the sorghum type. The ADL content differed significantly among all the samples at Marquardt, but this result was not observed for Straubing. Thomas et al. (2019) observed significant genotype effects on the CEL and lignin contents in experiments involving different sorghum varieties; however, in contrast to the results of this study, only CEL was site specifically impacted.

### 4.4 Silage-specific methane yields

In the present study, the SMYs ranged between 231.25 and 321.31 L_n kg^{-1} VS. These values are intermediate in relation to previously reported SMYs for sorghum (281–312 L_n kg^{-1} VS: Hassan et al., 2019; 248–348 L_n kg^{-1} VS: Herrmann, Idler, et al., 2016; 310–326 L_n kg^{-1} VS: Nurk et al., 2016; 290–410 L_n kg^{-1} VS: Wannasek et al., 2017). The results indicate that sorghum had similar or lower SMYs in comparison with the literature values of maize, which is the standard feedstock used for methane production. For instance, Herrmann, Idler, et al. (2016) analyzed 59 different maize silages, reporting SMYs between 312 and 408 L_n kg^{-1} VS. Furthermore, with the same experimental setup, a maize reference (variety Toninio, Agromais GmbH; medium-early) was harvested at the Marquardt site (2017), exhibiting SMYs for fresh material (Toninio SMY =350 L_n kg^{-1} VS; n = 3) that are in agreement with results reported by Herrmann et al. (2015; 342–354 L_n kg^{-1} VS).

The SMY tended to decrease with the advancement of the harvest date. This finding was also reported by Hassan et al. (2019) for sorghum silages. Furthermore, significant differences among varieties were observed for the MQ16 and MQ17 samples. The inherent compositional differences of sorghum types have been reported to be crucial for the final SMY (Hassan et al., 2019; Mahmood & Honermeier, 2012; Wannasek et al., 2017). The results of this study indicate that **S. bicolor** had a higher SMY than **S. bicolor × S. sudanense**. Wannasek et al. (2017) observed different SMYs between **S. bicolor** and **S. bicolor × S. sudanense** during 3 years of experiments. In their study, three of four **S. bicolor** silages had higher SMYs than **S. bicolor × S. sudanense**. This trend was confirmed by Mahmood and Honermeier (2012), who determined the SMYs of **S. bicolor** types Aron and Rona 1 to be 12% and 37% higher than the SMY of **S. bicolor × S. sudanense** (282 L_n kg^{-1} VS). The reason for this difference could lie in the fact that **S. bicolor** sorghum types usually contain larger amounts of sugars available for fermentation during ensiling and consequently affect the methane production positively. Additionally, the ADL content tended to be lower in **S. bicolor** varieties than in **S. bicolor × S. sudanense**. This tendency was not evident in Straubing, where (at the same time) no significant differences among the SMYs obtained were observed. Contrary to these results, Herrmann, Idler, et al. (2016) reported negligible differences
between the biochemical methane potentials of *S. bicolor* and *S. bicolor × S. sudanense*, with means of 288.9 and 287.7 L_n kg⁻¹ VS, respectively.

### 4.5 Effects of silage characteristics on methane formation

Across all the sites, years, and sorghum types, the substances that positively influenced the SMYs of the samples were the volatile compounds LA and AA. VFAs are the compounds used by methanogenic microorganisms for their metabolism and subsequent methane formation. Fluctuations in the VFA level affect the dynamics of the microbial community and thus biogas and methane production (Demirel, 2014). Herrmann et al. (2011) found a positive correlation between methane yield and the contents of alcohol and acetic, propionic, and BAs when studying different biogas crops. However, only AA was shown to positively contribute to methane formation in the present study, possibly because of the high variability among the results. In contrast, ADL was an important factor negatively influencing the final SMYs of the samples studied, supporting the findings of previous investigations, which confirmed that an increasing lignocellulosic portion can alter the SMY (Hassan et al., 2019; Herrmann, Idler, et al., 2016; Triolo et al., 2011). Additionally, Thomas et al. (2019) found a negative relationship between the sum of the lignin and cellulose contents and the SMY of the sorghum studied. However, the negative CEL effect described by the aforementioned authors could not be proven in the present study. The lignin content is strongly correlated with the maturity of the plant and the TS content. In previous studies, our research group analyzed a wide range of sorghum varieties from different locations and detected an inconsistent relationship between the TS content of the sorghum and the maturity stage at harvest (Herrmann, Plogsties, et al., 2016). In the aforementioned study, the TS content of sorghum silages exhibited increments and decrements with advances in plant development. This situation can be explained by the fact that sorghum hybrids (*S. bicolor × S. sudanense*) are characterized by the continuous formation of new stocking shoots; consequently, new panicles grow at different degrees of plant development. Taking into account these previous results, during the current study, the phenological stage (or BBCH code) was estimated with consideration of the main shoots and not the whole plant. This approach increases the accuracy of BBCH code classification for sorghum development. Furthermore, the BBCH code can be used to assess the TS content at a precise moment, which is helpful to farmers. Nevertheless, further research is needed to fully understand this aspect. There are no other publications available that propose a relationship among SMY, TS, and the growth stage at harvest (BBCH code); thus, no comparison is possible. Notwithstanding, there is a remarkable trend between sorghum maturity (expressed by the BBCH code) and SMY.

### 5 CONCLUSIONS

Sorghum has high adaptability to different environmental situations, which is further enhanced by the availability of new sorghum varieties. Thus, sorghum can be locally adapted, leading to regional diversification of crop production. Furthermore, different sorghum types offer a wide harvest window, which can be useful for cropping schemes, ensiling, and methane production. This aspect is particularly beneficial for farmers, because it gives them flexibility to adapt their agronomic strategies to their final objectives. This study provides data regarding the ensiling ability of sorghum types, which differ significantly in their maturity characteristics. The SMY—a key parameter reflecting the crop biomass quality for biogas production—was slightly higher for *S. bicolor* than for *S. bicolor × S. sudanense* hybrids. However, the results of this study confirmed that if the final purpose is biomethanation, the ensilability of different sorghum types imposes no restriction. Several questions remain to be answered. In an extended approach, the factor to be optimized would be the production of biomass per unit area of different sorghum types, as it may affect the final methane yield. Additionally, further research is needed for obtaining a complete understanding of methane production of different sorghum varieties within site-specific crop rotations or cropping systems, as the biomass yield may be affected by the cropping sequence (previous and succeeding crops) and the position in the crop rotation (main or secondary crop).

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### CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available
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