agronomy

MDPI

Article

The Effects of Harvesting Period and Inoculant on Second-Crop Maize Silage Fermentative Quality

Lorenzo Serva [1], Giorgio Marchesini [1], Luisa Magrin [1,*], Arzu Peker [2] and Severino Segato [1]

[1] Department of Animal Medicine, Production and Health, Padova University, 35020 Legnaro, Italy; lorenzo.serva@unipd.it (L.S.); giorgio.marchesini@unipd.it (G.M.); severino.segato@unipd.it (S.S.)
[2] Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Ankara University, Ankara 06110, Turkey; agokdai@ankara.edu.tr
* Correspondence: luisa.magrin@unipd.it; Tel.: +39-0498-272-656

Abstract: Southern Europe's mutating weather conditions and the European environmental agenda have suggested the cropping of maize (*Zea mays* L.) after winter cereal cultivation, even if shortening the growing period could result in an immature harvesting stage, limiting its silage quality. The experimental design investigated the effects of four harvesting dry matter (DM) classes (DMvl, 23.9%; DMl, 25.3%; DMm, 26.2%; DMh, 30.4%) in two inoculant types (heterofermentative (HE) vs. homofermentative (HOM) on fermentative quality, DM losses, and aerobic stability. The early harvested DMvl and DMl classes had the lowest silage density (<130 kg m^{-3}) and resulted in an organic acids profile lowering the fermentative quality and increasing the DM losses, while no differences were detected following the use of the inoculants. The aerobic stability was more susceptible to further adverse fermentation via opportunistic microorganisms in the DMm and DMh classes, probably due to the lower moisture content, but the use of both HE and HOM lactic acid bacteria seemed to contain this silage surface damage. In summary, a shortening of the maize growing period might limit the achievement of the maturity stage ideal for high-quality silage, hampering the positive effects of both HOM and HE inoculants in the ensiling process of early harvested maize.

Keywords: second-crop maize; harvesting dry matter; microbiological inoculant; ensiling process; maize silage; fermentative quality profile; dry matter losses; aerobic stability

check for updates

Citation: Serva, L.; Marchesini, G.; Magrin, L.; Peker, A.; Segato, S. The Effects of Harvesting Period and Inoculant on Second-Crop Maize Silage Fermentative Quality. *Agronomy* **2024**, *14*, 982. https://doi.org/10.3390/agronomy14050982

Academic Editor: Katja Klumpp

Received: 11 March 2024
Revised: 26 April 2024
Accepted: 3 May 2024
Published: 7 May 2024

1. Introduction

The projection for the Mediterranean basin, specifically the climate of northeastern Italy, suggests a potential decrease in rainfall, especially in the warm season, and, consequently, lower water availability for agricultural irrigation [1]. In the case of the Po River Delta area (a surface of approximately 34,000 km^2, northern Italy), comprising 70% lowlands mainly devoted to the cultivation of cereals and legumes, in recent years (2020–2022) across the summer season (June to August), a 31% reduction in rainfall has been reported compared with the average of the preceding 25 years [2]. In this flat portion of the Po Valley, maize (*Zea mays* L.) represents the main forage crop due to its productive yield as a warm-climate plant with high temperature and water supply requirements during the growing season [3]. However, due to the risk of extreme drought events, it may be sown as a second crop in late June both to maximize the allocation of the agronomic inputs (e.g., fertilizers, herbicides, and pest control) and to mitigate the use of non-renewable resources such as irrigation water and fossil fuels [4]. Moreover, according to the suggestions to preserve the potential of fertile soil, a change of crop at least once a year at the land parcel level is recommended instead of continuous cropping; thus, maize can be conveniently sown after a preceding winter–spring cereal crop like barley or wheat, also lowering the risk of natural hazards (i.e., early frosts, chlorosis parasitic disease, and mycotoxins) and the related stress to the plant. However, a delay in maize cultivation could result in early plant and ear maturity stages, even using hybrids with a short growing season (e.g., FAO maturity group lower than the FAO 400 class) and postponing the harvest to the end of the potential growing season (e.g., middle of October in northeastern Italy). Moreover, the ensiling process requires a specific maturity stage in terms of dry matter (DM) content (e.g., around 35%) of the harvested whole plant when it is used to produce maize silage [5]. There is extended evidence that the choice of the maturity stage at harvest affects the DM losses (DMloss) across the silage-making process, since they seem to be correlated with the chemical composition of green chopped whole-plant maize (WPM) [6,7], either alone or in interaction with the use of microbial additives [8,9]. The inoculation of bacteria could improve silage quality and aerobic stability, limiting the activity of opportunistic microorganisms (i.e., yeast, molds, and acetic acid bacteria) [10,11], even though the use of homofermentative (HOM) or heterofermentative (HE) lactic acid bacteria populations is still debated [8,12].

Indeed, the fermentative quality of WPM silage is a key factor in feeding lactating dairy cows [13] and beef cattle [14]; thus, there is a need to verify whether maize grown as a double cropping would be suitable to produce ensiled forage of high fermentative quality and nutritive value for intensive ruminant farming. To test this hypothesis, after a late-spring maize cultivation and harvesting under different DM contents of the whole plant, the ensiling process was evaluated in terms of fermentative profile, DM loss, and aerobic stability according to the use of HOM or HE bacterial inoculations. This focus was based on the increasing temperature and drought conditions in southern Europe, covering the limited knowledge of the impact of varying ensiling conditions of maize as a second sowing in a sustainable double-cropping system in arable land systems.

2. Materials and Methods

2.1. Experimental Design and Ensiling Methods

After wheat, on 7 June 2022, the maize (class FAO 200) was sown in a single trial field located on a commercial farm in the Veneto Region (45°33′ lat. N; 11°47′ long. E) at a sown density of 11 seeds/m^2. The whole-plant maize (WPM) was harvested at the following four scheduled times: 1, 9, and 19 of September and 6 of October, corresponding to a harvesting pre-ensiled forage dry matter (DM) of 23.9% (DMvl), 25.3% (DMl), 26.2% (DMm), and 30.4% (DMh), respectively. Freshly harvested WPM was inoculated in a large, sterile container with a heterofermentative (HE) or a mixture of homofermentative (HOM) lactic acid bacteria (LAB) populations (Agravis Raiffeisen AG, Münster, Germany) at a concentration of 2×10^5 or 3×10^5 colony-forming units (CFU)/g of WPM, respectively. The first one was a population of *Lentilactobacillus buchneri* strain CCM 1819, and the second one was composed of *Lactiplantibacillus plantarum* strain NCIMB 30083–1k207736 (1.5×10^5 CFU/g), *Enterococcus faecium* strain 22502–1k20602 (0.9×10^5 CFU/g), *L. plantarum* strain NCIMB 30084–1k207737 (0.2×10^5 CFU/g), *Peditococcus pentosaceus* strain DSM 23688–1k1010 (0.2×10^5 CFU/g), and *Peditococcus pentosaceus* strain DSM 23689–1k1019 (0.2×10^5 CFU/g). The control group (C group) was treated with the same volume of pure water. The HE, HOM, and C groups were ensiled after 4 h of air exposure; thus, a total of 12 experimental combinations (4 harvesting DM classes × 3 inoculant types) were tested as ensiled WPM second yearly crops.

At each cropping time (DMvl, DMl, DMm, and DMh), an average of 20 maize plants were randomly sampled and harvested manually at a stubble height of approximately 2 cm, then chopped and harvested by a self-propelled forage harvester (cutting length of approximately 2 cm). The green, chopped WPM samples were prepared and inoculated for ensiling in triplicate (4 harvesting DM contents × 3 inoculant types × 3 repetitions = 36). For each of the n = 36 green chopped WPM samples, approximately 1 kg was suddenly vacuum-packed and refrigerated (4 °C) until it was analyzed (within the same day) for the chemical traits of the pre-ensiled samples. On day of the harvesting (T0), samples (n = 36) of green chopped WPM were ensiled in a 20-L truncated conic plastic bucket. The buckets were shielded using a 150 µm SealPlus film permeable to oxygen at a rate of 48 cm^3 m^{-2} 24 h^{-1} at 23 °C and 65% RH (Gamma, Mondovi, Italy) and sealed with robust tape and

10 kg of gravel used as a compressor. The sealed buckets were stored for 60 days (T60). To ensile the samples into 20-L, a 1-ton hydraulic press (141 kg cm^{-2}) was used. All buckets were kept in a darkroom at a stable temperature of approximately 23 ± 2 °C before opening and submitting the sample to analysis.

On T60, the 20-L silages were opened, and a 15 cm thick layer of silage was removed to discharge the eventually spoiled silage, which was not further considered for quality analysis. Then, 1 kg of silage was promptly submitted to analysis. The remaining portion was stored loose in a 20-L open and square polystyrene pan (495 mm × 295 mm × 140 mm), and after three days (T63), sample weights were recorded, and 1 kg of silage was promptly submitted to analysis. A data logger was positioned 7 cm under the silage surface of the 20-L buckets of the first repetition for all samples, then, at T60, repositioned 5 cm under the silage surface of the 20-L polystyrene pan, recording the temperature every 30 min with a precision of 0.1 °C. A single RC-5 data logger (Elitech Datalogger, London, UK) was used to record the room temperature throughout the period. In the survival analysis, the endpoint of interest was defined as the occurrence of aerobic instability. The outcome variable was the time (hours) elapsed until the first instance of aerobic stability failure (aerobic deterioration), which was operationalized as the moment the silage temperature exceeded the ambient room temperature of 2 °C [9].

2.2. Chemical and NIR Determination, Density, Porosity, and Dry Matter Losses

The following chemical traits (% of the DM or other specific measurement units) were instrumentally determined: DM, crude ash (CA), crude protein (CP), ether extract (EE), alpha-amylase neutral detergent (aNDF) and acid detergent (ADF) fiber fractions, sulfuric acid lignin (sa-lignin), water-soluble carbohydrates (WSC), starch, ammonia (NH_3-N, % of total N), lactic acid, acetic acid, propionic acid and butyric acid, and ethanol. These chemical variables were estimated via near-infrared (NIR) spectroscopy, scanning in triplicate before the average, using a FOSS NIRSystem 5000 scanning monochromator (FOSS NIRSystem, Silver Pring, MD, USA). The NIR calibration performances were reported in previous studies [3,15]. The reference methods used to calibrate the NIR instrument were detailed and described in previous studies [16,17] and further reported together with those for the fermentative profile. The AOAC methods [18,19] were used according to #934.01 and #942.05 for DM and CA and #2001.11, #2003.05, and #996.11 for CP, EE, and starch, respectively. aNDF and ADF were determined using an AnkomFiber analyzer (Ankom Technology Corporation, Fairport, NY, USA); aNDF was identified with sodium sulfite, heat-stable alpha-amylase, and F57 bags with a 25 µm pore size and included residual ash [20,21], while non-sequential ADF and sa-lignin were evaluated according to Vogel et al. 1999 [22]. Lactic, acetic, propionic, and butyric acids were extracted in an acid solution (0.6 N sulfuric acid) and analyzed using high-performance liquid chromatography [17], while NH_3-N (ammonia) and pH were determined as detailed in a previous study [5]. The WSC, ethanol, and mannitol were also determined using high-performance liquid chromatography after extraction in an aqueous solution. The density was calculated as the ratio of the amount of pre-ensiled fresh WPM, corrected for DM content, to the volume of the 20-L bucket and expressed as kg of DM per 1 m^3 (DM density, DMd as kg m^{-3}). The silage fermentative quality index (FQI) was determined as suggested in our previous research trial [15]. Porosity (Φ) and DM losses (DMloss) of WPM silage were calculated according to the formula reported in the following equations [23]:

$$\Phi = 1 - \rho wb \times \{[(1 - DM)/\rho w] + [(DM \times OM)/\rho OM] + [(DM \times (1 - OM))/\rho ash]\} \quad (1)$$

$$DMloss = [(DM\ of\ pre\text{-}ensiled - DM\ of\ post\text{-}ensiled)/DM\ of\ pre\text{-}\ ensiled] \times 100 \quad (2)$$

where ρwb is bulk density wet basis (g cm^{-3}); ρw is water density (1 g cm^{-3}); DM and OM are dry and organic matter, respectively (g g^{-1}); ρOM is organic matter density (1.6 g cm^{-3}); and ρash is ash density (2.5 g cm^{-3}).

2.3. Data Analysis

The twelve (12) experimental levels (theses) consisted of four harvesting DM classes (DMvl, DMl, DMm, and DMh) per three inoculants (C, HE, and HOM) and per three replicates ($n = 36$). The normal distribution for continuous variables was assessed using the Shapiro–Wilk test and visual inspection. Pre-ensiled chemical traits, density, and porosity for the harvested WPM were submitted to ANOVA, considering the fixed effect of harvesting DM class. FQI, DMloss, post-ensiled DM, and fermentative traits were submitted to an ANOVA considering the fixed effects of harvesting DM class and inoculant type, as well as their interactions both at the end of the 60 days of the ensiling process (T60) and after 3 days of silage air exposure (T63). Post hoc pairwise comparisons were run between factor levels using Bonferroni correction. The assumptions of the linearity of the model were graphically tested on the residuals. A Cox proportional hazard regression tested the effect on the event for each covariate separately (univariable approach) for pre-ensiled composition. Furthermore, a Cox proportional hazard regression for a multivariable Akaike's information criterion in the backwards (Cox-AIC-backward) model was estimated for the chemical traits of pre-ensiled maize to estimate the aerobic stability, starting from the variables selected as significative by the univariable Cox models. The proportional hazard assumption was evaluated using a visual approach (Schoenfeld, Martingala, beta, and score residuals) and Grambsch and Therneau test [24,25].

To perform these statistical analyses, the R programming language, version 4.0.2 (The R Foundation, Vienna, Austria), was used, along with Rcmdr package version 2.6–2 and RStudio version 1.2.1578 (Posit, PBC, Boston, MA, USA). Statistically significant effects were indicated if $p < 0.05$.

3. Results

The effects of the harvesting DM class on the chemical composition of the pre-ensiled WPM are reported in Table 1.

Table 1. Effect of the harvesting dry matter (DM) class on the chemical traits, bulk and DM density, and porosity of the pre-ensiled whole-plant maize (WPM).

Item	Harvesting Dry Matter Class [1]				SEM	p
	DMvl	DMl	DMm	DMh		
Dry matter (%)	23.9 ^{c}	25.3 ^{b}	26.2 ^{b}	30.4 ^{a}	0.3	<0.001
Crude protein (% DM)	9.41 ^{a}	9.08 ^{ab}	8.78 ^{b}	8.13 ^{bc}	0.1	<0.001
Ether extract (% DM)	1.84 ^{b}	1.88 ^{b}	1.66 ^{b}	2.12 ^{a}	0.1	<0.001
Crude ash (% DM)	5.57 ^{a}	5.12 ^{b}	5.18 ^{b}	4.86 ^{b}	0.1	<0.001
aNDF [2] (% DM)	46.3 ^{a}	43.2 ^{b}	44.4 ^{b}	43.1 ^{b}	0.42	<0.001
ADF [3] (% DM)	25.9 ^{a}	24.1 ^{bc}	24.6 ^{b}	23.5 ^{c}	0.3	<0.001
sa-lignin [4] (% DM)	2.37 ^{a}	2.28 ^{b}	2.30 ^{b}	2.20 ^{c}	0.01	<0.001
WSC [5] (% DM)	9.27 ^{b}	11.2 ^{a}	11.3 ^{a}	9.58 ^{b}	0.30	0.003
Starch (% DM)	23.8 ^{b}	26.6 ^{a}	26.8 ^{a}	27.6 ^{a}	0.68	<0.001
Bulk density (kg WPM m^{-3})	570 ^{a}	512 ^{b}	487 ^{b}	479 ^{b}	8.4	<0.001
DM density (kg DM m^{-3})	137 ^{ab}	130 ^{b}	128 ^{b}	146 ^{a}	2.9	<0.001
Porosity (decimals)	0.51 ^{c}	0.56 ^{b}	0.58 ^{ab}	0.60 ^{a}	0.01	<0.001

[1] DMvl, DMl, DMm, and DMh correspond to very low, low, medium, and high DM values, respectively. [2] Alfa-amylase neutral detergent fiber. [3] Acid detergent fiber. [4] Sulfuric acid lignin. [5] Water-soluble carbohydrates. ^{a,b,c} within a row indicate statistical difference ($p < 0.05$).

The harvest of WPM at an early stage of maturity, such as the very-low DM (DMvl, 23.9% of DM) class resulted in the highest ($p < 0.05$) CP, CA, aNDF, ADF, and sulfuric acid lignin (sa-lignin) contents, while in the later harvesting period, the highest EE was observed. Starch content was higher at during the low-DM harvesting stage (DMl, 25.3% of DM), while the water-soluble carbohydrates (WSC) decreased under the high-DM (DMh, 30.4% of DM) stage. The DM density (DMd) and porosity of pre-ensiled WPM are also presented in Table 1. The harvesting DM classes with lower contents of DM had lower DMd and porosity compared to those harvested later, especially in the case of high DM (DMh, 30.4% of DM). The effects of the harvesting DM class and inoculant type on pH, fermentative profile, fermentative quality index (FQI), and DMloss of the maize silage are reported in Tables 2 and 3, respectively.

Table 2. Effects of the harvesting dry matter (DM) class on pH, fermentative profile, FQI, and dry matter losses (DMloss) of ensiled whole-plant maize (WPM) (60 d of ensiling process).

Item	Harvesting Dry Matter Class [1]				SEM	*p*
	DMvl	DMl	DMm	DMh		
pH	3.86 ^{a}	3.89 ^{a}	3.80 ^{b}	3.78 ^{b}	0.02	<0.001
Dry matter (%)	19.7 ^{c}	23.6 ^{b}	24.2 ^{b}	28.3 ^{a}	0.4	<0.001
Lactic acid (% DM)	8.18 ^{a}	7.00 ^{b}	7.64 ^{ab}	7.45 ^{b}	0.24	0.021
Acetic acid (% DM)	3.41 ^{a}	2.76 ^{b}	2.40 ^{bc}	2.00 ^{c}	0.15	<0.001
Propionic acid (% DM)	1.66 ^{a}	1.32 ^{b}	1.11 ^{b}	0.83 ^{c}	0.06	<0.001
Butyric acid (% DM)	0.14 ^{a}	0.11 ^{b}	0.11 ^{b}	0.10 ^{b}	0.01	<0.001
Ethanol (% DM)	1.87	1.67	2.05	2.19	0.14	0.089
Mannitol (% DM)	1.13 ^{ab}	1.36 ^{a}	1.03 ^{ab}	0.60 ^{b}	0.15	0.007
Ammonia (% of total N)	6.81	6.65	6.45	6.77	0.25	0.151
FQI [2]	51.0 ^{bc}	49.3 ^{c}	55.9 ^{ab}	57.9 ^{a}	1.4	<0.001
DMloss (%)	19.0 ^{a}	8.8 ^{b}	9.5 ^{b}	9.6 ^{b}	1.6	<0.001

[1] DMvl, DMl, DMm, and DMh correspond to very low, low, medium, and high DM values, respectively. [2] Fermentative quality index (score 0–100). ^{a,b,c} within a row indicate statistical difference ($p < 0.05$). The interaction of harvesting DM class × inoculant type was significant ($p < 0.05$) for lactic acid and butyric acid.

Table 3. Effects of the inoculant type (heterofermentative (HE) vs. homofermentative (HOM)) on pH, fermentative profile, FQI, and dry matter losses (DMloss) of ensiled whole-plant maize (WPM) (60 d of ensiling process).

Item	Inoculant Type			SEM	*p*
	Control	HE	HOM		
Dry matter (%)	24.0	24.2	23.7	0.4	0.586
pH	3.84	3.83	3.82	0.01	0.245
Lactic acid (% DM)	7.07 ^{b}	7.71 ^{ab}	7.93 ^{a}	0.22	0.023
Acetic acid (% DM)	2.70	2.57	2.65	0.13	0.785
Propionic acid (% DM)	1.24	1.21	1.24	0.05	0.889
Butyric acid (% DM)	0.12	0.12	0.12	0.01	0.342
Ammonia (% of total N)	6.55	6.66	6.81	0.21	0.696
Ethanol (% DM)	1.82	1.88	2.14	0.12	0.174
Mannitol (% DM)	1.08	0.95	1.06	0.13	0.755
FQI [1]	53.2	54.1	53.3	1.1	0.827
DMloss (%)	12.3	11.2	11.6	1.4	0.869

[1] Fermentative quality index (score 0–100). ^{a,b} within row indicate statistical difference ($p < 0.05$). The interaction between harvesting DM class and inoculant type was never significant, except for lactic acid and butyric acid ($p < 0.05$).

The delay of the harvesting stage led to a significant increase in the silage DM content and decreased pH, while the percentages of the acids were higher in early harvested classes, especially for lactic acid and acetic acid. In the silage of the DMh class, the lowest value of all organic acids, as well as mannitol, was recorded (Table 2). Harvesting earlier mature WPM caused a lower value of the FQI, and in silage of the DMvl class, a relevant percentage of DM losses was detected (Table 2). The use of inoculant had a limited influence on the fermentative profile of the WPM silage, except for a significantly higher incidence of lactic acid in the HOM group compared to the control thesis (Table 3). The interactions of harvesting DM class per inoculant type also affected the percentages of lactate and butyrate. Lactic acid contents were highest for the HOM group in the DMvl class and the highest for

HE in the DMm class, while no differences were detected within the DMl and DMh classes (Figure S1). Butyric acid content tended to be higher in the HOM than in the HE group for the DMvl and DMl classes, and the opposite was true for the DMm and DMh classes (Figure S1). The effects of harvesting DM class and inoculant type on pH, organic acids, and ammonia in maize silage exposed to air for three days (T63), starting from the day the silo was opened, are presented in Table 4.

Table 4. Effects of the harvesting dry matter (DM) class and type of inoculant on pH, acids (% DM), and ammonia (% of total N) of maize silage after three days of air exposure (T63).

Experimental Combinations	pH	Lactic Acid	Acetic Acid	Propionic Acid	Butyric Acid	Ammonia
DMvl [1]	3.83	6.16 [a]	3.02 [a]	1.68 [a]	0.12 [a]	5.87 [a]
DMl [1]	3.88	5.14 [ab]	1.41 [b]	1.12 [b]	0.09 [b]	5.38 [ab]
DMm [1]	3.81	5.63 [ab]	1.50 [b]	0.93 [bc]	0.09 [b]	5.11 [ab]
DMh [1]	3.88	3.76 [b]	0.94 [b]	0.72 [c]	0.08 [c]	4.36 [b]
C [2]	3.91	4.42 [b]	1.13 [b]	1.01	0.08 [b]	4.42 [b]
HE [2]	3.81	5.42 [a]	1.91 [a]	1.14	0.11 [a]	5.42 [a]
HOM [2]	3.82	5.70 [a]	2.11 [a]	1.19	0.11 [a]	5.70 [a]
SEM	0.03	0.46	0.18	0.05	0.01	0.23
Significance (*p*)						
Harvesting class	0.441	0.016	<0.001	<0.001	<0.001	0.003
Inoculant	0.081	0.019	0.001	0.053	<0.001	0.001
Harvesting class × inoculant	0.463	0.341	0.046	0.338	0.086	0.076

[1] DMvl, DMl, DMm, and DMh correspond to very low, low, medium, and high DM values, respectively. [2] C = control; HE = heterofermentative; HOM = homofermentative. [a,b,c] within harvesting DM class and/or inoculant group indicate statistical difference ($p < 0.05$).

The higher the DM content of the harvesting DM class, the lower the percentages (% on DM) of acids and ammonia tended to be. During the three days of air exposure, HE and HOM inoculants significantly limited the losses of lactic acid, acetic acid, butyric acid, and ammonia. The harvesting DM class per type of inoculant interaction was significant only with respect to acetic acid, which showed the lowest value in the C group and a more marked decrease from DMvl to DMh in the HOM group (Figure 1).

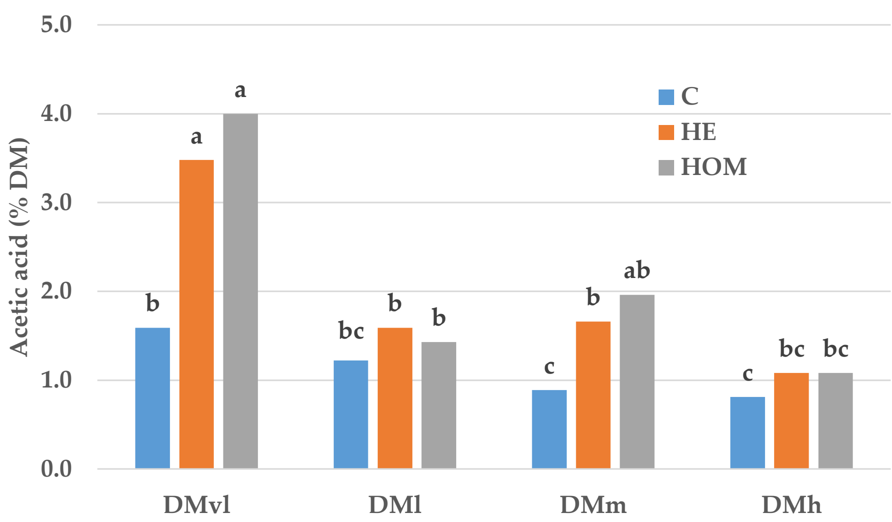

Figure 1. The effect of the interaction between harvesting dry matter (DM) class per inoculant type on acetic acid (% DM) of maize silage after three days of air exposure (T63). DMvl = very low DM; DMl = low DM; DMm = medium DM; DMh = high DM; C = control; HE = heterofermentative; HOM = homofermentative. [a,b,c] indicate statistical difference at $p < 0.05$.

The univariable hazard ratio (HR) of the pre-ensiled chemical traits predicting the aerobic stability are reported in Table 5; the main outcomes are that both EE (HR = 5.23) and DM density (HR = 1.05) resulted markedly and slightly, respectively, in predisposition to an event of aerobic deterioration (e.g., silage temperature exceeded the ambient room temperature of 2 °C), while WSC (HR = 0.69) appeared to be protective. Using the Cox-univariable significant traits as initial predictors, a Cox-AIC-backward test was performed, and, as a result, only the density was confirmed as significant. Somer's Dxy concordance index was 0.61, the likelihood ratio test had $p = 0.03$, and the Schoenfeld residuals were graphically evaluated to ensure the proportional hazard assumption.

Table 5. The effects on the aerobic stability of chemical composition, density, and porosity (average ± standard deviation) of pre-ensiled whole-plant maize (WPM) and the use of inoculants.

Variable	Dataset (n = 36)	Univariable HR (95% C.I.) [1]
Pre-ensiled traits		
Dry matter (%)	26.5 ± 2.6	1.01 (0.85–1.19)
Crude protein (% DM)	8.9 ± 0.6	1.34 (0.66–2.27)
Ether extract (% DM)	1.9 ± 0.2	5.23 (1.12–24.77)
Crude ash (% DM)	5.2 ± 0.3	1.21 (0.40–3.69)
aNDF (% DM)	44.2 ± 1.7	0.99 (0.81–1.23)
ADF (% DM)	24.5 ± 1.2	0.99 (0.72–1.37)
sa-lignin (% DM) [2]	2.3 ± 0.1	0.01 (0.01–2.49)
Water-soluble carbohydrates (% DM)	10.3 ± 1.3	0.69 (0.48–0.99)
Starch (% DM)	26.2 ± 2.4	0.93 (0.81–1.08)
Density (kg DM m^{-3})	135 ± 11	1.05 (1.00–1.09)
Porosity (decimals)	0.56 ± 0.04	0.01 (0.01–2.40)
Use of inoculants (frequency of sampling size)		
Control	12	–
Heterofermentative	12	0.67 (0.29–1.56)
Homofermentative	12	0.61 (0.25–1.51)

[1] Univariable hazard ratio with 95% confidence interval in brackets. [2] Sulfuric acid lignin.

4. Discussion

Recently, an increasing interest in dairy farmers sowing maize in late spring or even early summer has been reported to limit the adverse impact of warmer and drier summers and to meet the sustainability targets of the climate and environmental schemes (e.g., crop rotation, winter cover cropping, etc.) of the European common policy. Multiple cropping, which involves harvesting more than once a year (e.g., maize after winter cereals or legume cover crop), is an increasingly widespread land management approach aiming at to intensify agricultural production and diversify crop cultivation. As already described in the Introduction, even though yearly agricultural production could rise by up to 30%, in southern Europe, the main advantage of this strategy seems to be the promotion of agronomic sustainability related to better pest regulation, improved plant resistance to adverse climate events (e.g., crop failure due to drought), and reduced fertilizer use in association with Fabaceae [26]. However, a postponement of the growing season (so-called second crop) implies the use of maize hybrids of the FAO class with a shortened growing season and, as a consequence, a reduction in the yield in fresh maize forage [27]. Despite that, in years with unfavorable climatic and environmental conditions, the timing of the harvesting processes could be inappropriate compared with the optimal maize maturity stage, especially for those used as ensiled forage as a main source of dietary roughage in feeding highly producing dairy cows. To cover the limited knowledge on the adaptability to the ensiling process of maize cultivated as a second crop in southern Europe conditions, this trial was designed to assess the effects of the three harvesting periods and the use of inoculant on the fermentative quality, DM loss, and aerobic stability of WPM silage.

As expected, the chemical composition of green chopped, pre-ensiled WPM forage highlighted a low DM content, even in the DMh class, and a relevant amount of WSC (higher than 8% on DM basis) underlined a proximate composition of early harvested maize [5]. The progressive delays in harvest changed the chemical composition of the WPM samples. Based on the literature, the later-harvest DM classes exhibited a reduced amount of CP, while the starch content increased [28]. In contrast, conflicting trends were reported for the aNDF content in maize at 1/3 milk line to black layer, with reports indicating an increase [29] or decrease [28]. However, contrary to the literature [28,29], this study also detected a decrease in sa-lignin in the later-harvest WPM samples, likely attributed to the shorter growing season of the cultivated FAO hybrid. Notably, the fiber fractions decreased, likely due to the effect of dilution of the stem caused by the growing relevance of ears in the WPM plants. Lignin consistency changes with WPM maturity, but the observed trend was uncertain, and differences, although statistically significant, had no physiological influence on the nutritional value of this ensiled forage. Indeed, lignin is closely associated with cell wall polysaccharides [30], whose matrix results in the distribution of polymers around the cellulose microfibrils and plays a relevant role in animal feeding, as it is known to likely impair fiber digestibility [31]. Finally, WSC showed increasing content up to 11.3% in DM, confirming an intense vegetable growing rate up to the end of the trial (i.e., harvesting of maize of the DMh thesis).

In our trial, the low DM level did not allow for reliable measurement of DM density in silos compared with the minimum values of 240 kg DM m^{-3} suggested to limit juice losses during WPM compression, and the recorded DM losses were much higher than those reported in the literature [7,32]; however, the literature reports a considerable variation related to silo type [7]. The DM losses achieved very high levels across the investigated harvested WPM classes, even with an extreme value (19%) for the DMvl class. This severe DM loss was probably due to relevant effluent loss during silo preparation and silage conservation, as confirmed by the shallow levels of DM of the ensiled WPM. The DM of maize at harvest should be above 30% to avoid effluents [33,34], even though in this experimental trial, sewage decreased with increasing DM concentration (with densities lower than 150 kg DM m^{-3}). DM content plays a crucial role in silage fermentation, impacting the composition of bacterial communities with and without inoculants [35]. High DM content at harvest can hinder the growth of native lactic acid bacteria (LAB), potentially limiting efficient fermentation. Exogenous inoculants can address this limitation, leading to faster and improved ensiling outcomes [36,37]. The low post-ensiled DM content (<30%) observed across all groups, including the control, points towards an environment conducive to the proliferation of endogenous LAB, potentially resulting in rapid and efficient fermentation without the need for exogenous inoculants. This finding aligns with previous research on wheat silage, highlighting the potential influence of DM content on the efficacy of inoculants [29].

During the ensiling process, there was intense fermentative activity of the endogenous bacteria, as confirmed by the highest butyric acid content in DMvl silage. The latter allowed for the achievement of satisfactory levels of pH (<3.90) for well-fermented maize silage, with minimal although statistically significant differences among the theses. Lactic, acetic, propionic, and butyric acids showed a decreasing value for later harvests. However, despite the more intense fermentation in the wetter DMvl and DMl harvesting groups, the silage FQI significantly increased with higher DM levels at harvest (DMm and DMh classes), indicating a better composition of volatile fatty acids (VFAs) [28]. Ammonia and ethanol were unaffected by the harvesting DM classes. Finally, mannitol showed higher values in the theses with a lower pre-ensiling DM content. Mannitol is produced in secondary fermentations by yeasts or by endogenous heterofermentative LAB bacteria [38], which ferment glucose and fructose to produce mannitol [39].

The use of inoculant had a limited effect on the fermentative pattern. The increased percentage of lactic acid observed all over in HOM, even depending on the harvesting DM class, and the absence of effects on acetic acid content did not suggest a positive effect of either HE or HOM on silage FQI. However, the interaction between DM at harvest and the use of inoculants suggests complex trends. The interaction seemed to highlight a moderate role of both in the fermentative pattern and the silage quality of very early harvested maize. Besides this, the use of the HOM inoculant also did not reduce DM losses compared with the control thesis. These findings confirm the results of a meta-analysis that concluded that the use of combined LAB did not affect DM loss [40]. However, the inoculants' role in enhancing forage fermentation has yielded inconsistent results, as documented in various studies [9,41]. This variability has prompted the suggestion that developing inoculant formulations tailored to specific forage types could lead to more reliable improvements in fermentation efficiency and final silage quality [40].

The chemical traits of WPM silage after three days of exposure (T63) were compatible with well-preserved silage [42]. As expected, during the three days of air exposure, there was a reduction in VFA concentration, especially for lactic acid content, and there were slightly marked effects for the DMh class. However, acetic acid content increased in the DMvl class, confirming that silages harvested at lower DM contents might be more susceptible to a further fermentative process. Several reasons can be found for the reduced aerobic stability of silages. Opportunistic microorganisms such as yeast (e.g., *Saccharomyces*, *Candida*, *Cryptococcus*, and *Pichia*), molds, bacilli, and acetic acid bacteria become metabolically active, producing heat, consuming nutrients, and spoiling the silage quality [33,43,44]. Under certain conditions, inoculation with bacteria is recommended to avoid undesirable effects on silage and aerobic stability [11,43,45]. Inoculation can, in fact, increase the feeding intake, milk yield, dietary digestibility [13], and milk protein and fat concentrations [46]. However, the stability of silages against aerobic deterioration varies dramatically with the use of HE or HOM bacteria [47]. In addition, well-fermented silage from HOM may spoil faster because they result in a greater level of WSC and lactic acid used as a growth substrate for yeast and molds [48,49]. In contrast, the HE silages fermented by lactic bacteria (Lb) could improve the stability of silages via the anaerobic degradation of lactic acid to acetic acid [50], and Lb are commonly suggested to partially prevent aerobic deterioration at the farm level [51]. However, Lb are also associated with increased DM losses during anaerobic conservation of the silage and lower losses during the first fourteen days of air exposure in maize and sorghum silages [12]. The role of air exposure during the feed-out phase in the spoiling process of the mass is increased by the activity of lactate-assimilating yeasts, which metabolize WSC and fermentative end products into carbon dioxide and water, increasing the temperature and pH. Lactate-assimilating yeasts also decrease the silage quality and digestibility [44]. In addition, when aerobic activity begins, the temperature rises rapidly and may reach more than 20 °C above room temperature. This evidence occurs due to air infiltration into the mass, which could reach up to 1–2 m from the silage surface [52]. The application of the Cox model to predict aerobic stability showed that both the EE content at harvest and DM density at the beginning of the silage process might be predictive of aerobic instability. However, these suggestions from survival analysis should be accounted for with caution, especially for DM density. EE is also likely due also to the specific content of unsaturated fatty acids (FAs), which can promote different levels of FA oxidation and peroxidation, with adverse effects on silage aerobic stability [53]. Regarding DM density, this finding appears to contradict the literature [23,32]. However, in our study, minimal differences were observed in bulk density; meanwhile, a significant increase in porosity was detected in the harvesting DM classes with the higher values of DM, which corresponded to higher DM densities. Thus, it can be hypothesized that the positive relationship between porosity and DM density could be a predisposing factor for aerobic instability, as suggested by an HR value of 1.05 for DM density. WSC can constitute a protective chemical trait, since they promote a favorable fermentative pattern. Confirming the literature [5,23], this study found that with higher DM content, DM density and porosity increased, allowing for greater air circulation within the silage, which can promote the activity of yeasts and molds in silage [54]. This can be attributed to increasing difficulties in compacting the fresh maize forage, as evidenced by consistently higher porosity approaching the proposed limit of 0.40 and a bulk density of approximately 450 kg m^{-3}, which is far below the recommended threshold of 700 kg m^{-3} [55]. The HE inoculant, primarily *Lentilactobacillus buchneri*, was recognized to produce a higher concentration of acetic acid [56] that has the potential to inhibit yeasts, which are responsible for initiating aerobic spoilage [57]. In this study, the HE and HOM reasonably protected against aerobic instability, an outcome probably due to the low DM content as a determinant of fermentability. Therefore, despite the suggestions of survival analysis (HR of 0.67 and 0.61 for HE and HOM, respectively), their role in enhancing aerobic stability might be considered a tendency, as already observed in the literature [58].

Finally, it should be noted that the outcomes from ensiling processes based on maize hybrids with a short growing season (e.g., sown in late spring–early summer) must be analyzed cautiously, since the prediction relies on the effective achievement of an optimal ensiling phenological maturity stage rather than on the application of the best ensiling procedure. Therefore, further studies should test the effectiveness of the fermentative biochemical pathways due to the harvesting variability to decipher the optimal agronomic and ensiling conditions involved in the production of maize silage from second-crop cultivation. A potential focus might include economic parameters such as assessing production costs, inoculant efficacy, and related livestock performance in terms of feed efficiency. By examining these economic factors, farmers and stakeholders can make informed decisions regarding harvesting period and inoculant applications to optimize second-crop maize silage production and profitability.

5. Conclusions

This study evaluated the hypothesis that maize grown as a second crop in southern European conditions can reach a maturity stage suitable for ensiling as high-quality fermented forage, exhibiting resistance to aerobic deterioration upon exposure to air after silo opening and during the feeding period. The main outcomes of the experimental trial suggested that a threshold of 25% of DM of the harvested green, chopped maize ensures a minimal density to fill air pores, thus avoiding relevant effluent losses with plant juice during silage fermentation. However, the harvesting DM content of whole-plant maize should be approximately 30% to drive an optimal anaerobic biochemical pattern, increasing both the fermentative quality index and the nutritive value of the silage, which remains a challenge. Furthermore, the results highlight that to allow for a suitable ensiling process of second-crop maize, the use of lactic acid bacteria-based inoculants had a limited positive effect, mainly related to a significant increase in lactic acid concentration. Moreover, the addition of inoculants was found to play a limited role in promoting aerobic stability in the fill-out phase, since the positive increase in lactic acid was mitigated by increases in both butyric acid and ammonia, with negligible differences between hetero- and homofermentative populations. The presence of these biochemicals likely hinders the reduction of favorable volatile fatty acids, particularly in groups with lower initial dry matter content. This could be attributed to the active fermentation process facilitated by the readily available sugars in freshly harvested plants. Since ensiling is a hard-to-control fermentation process, depending on multiple parameters, the ability to ensure an optimal chemical composition at harvest, especially in terms of high sugar content, and an appropriate silage density in the silo, hold promise for improvements in silage quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14050982/s1, Figure S1: The effect of the interaction between harvesting DM class per inoculant type on the lactic acid (**panel A**) and butyric acid (**panel B**) of maize silage after 60 days of ensiling.

Author Contributions: Conceptualization, L.S. and G.M.; methodology, L.S., G.M. and L.M.; software, L.S., L.M. and S.S.; validation, L.S., G.M. and S.S.; formal analysis, S.S.; investigation, L.S., A.P. and L.M.; resources, L.M. and S.S.; data curation, L.S., A.P. and S.S.; writing—original draft preparation, L.S., G.M. and S.S.; writing—review and editing, L.S., G.M. and S.S.; visualization, A.P. and S.S.; supervision, L.S. and S.S.; project administration, L.S. and L.M.; funding acquisition, L.S., L.M. and S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by KWS Italia S.p.A, and Interconnected Nord-Est Innovation Ecosystem (iNEST) from the European Union Next GenerationEU (PNRR—Missione 4 Componente 2, Investimento 1.5—D.D. 1508 23/06/2022, ECS00000043).

Data Availability Statement: Research data will be provided upon request.

Acknowledgments: The authors thank Elisabetta Garbin, Sara Khazzar, Sandro Tenti, and Massimo Mirisola of the CNX laboratory of Padova University for their technical support in wet chemistry and NIR analyses.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Giorgi, F.; Lionello, P. Climate change projections for the Mediterranean region. *Glob. Planet. Chang.* **2008**, *63*, 90–104. [CrossRef]
2. Straffelini, E.; Tarolli, P. Climate change-induced aridity is affecting agriculture in Northeast Italy. *Agric. Syst.* **2023**, *208*, 103647. [CrossRef]
3. Marchesini, G.; Serva, L.; Chinello, M.; Gazziero, M.; Tenti, S.; Mirisola, M.; Garbin, E.; Contiero, B.; Grandis, D.; Andrighetto, I. Effect of maturity stage at harvest on the ensilability of maize hybrids in the early and late FAO classes, grown in areas differing in yield potential. *Grass Forage Sci.* **2019**, *74*, 415–426. [CrossRef]
4. Yang, J.; Cui, J.; Lv, Z.; Ran, M.; Sun, B.; Sui, P.; Chen, Y. Will maize-based cropping systems reduce water consumption without compromise of food security in the North China plain? *Water* **2020**, *12*, 2946. [CrossRef]
5. Segato, S.; Marchesini, G.; Magrin, L.; Contiero, B.; Andrighetto, I.; Serva, L. A Machine Learning-Based Assessment of Maize Silage Dry Matter Losses by Net-Bags Buried in Farm Bunker Silos. *Agriculture* **2022**, *12*, 785. [CrossRef]
6. Gallo, A.; Giuberti, G.; Bruschi, S.; Fortunati, P.; Masoero, F. Use of principal factor analysis to generate a corn silage fermentative quality index to rank well- or poorly preserved forages. *J. Sci. Food Agric.* **2016**, *96*, 1686–1696. [CrossRef] [PubMed]
7. Köhler, B.; Taube, F.; Ostertag, J.; Thurner, S.; Kluß, C.; Spiekers, H. Dry-matter losses and changes in nutrient concentrations in grass and maize silages stored in bunker silos. *Grass Forage Sci.* **2019**, *74*, 274–283. [CrossRef]
8. Serva, L.; Andrighetto, I.; Marchesini, G.; Contiero, B.; Grandis, D.; Magrin, L. Prognostic capacity assessment of a multiparameter risk score for aerobic stability of maize silage undergoing heterofermentative inoculation (*Lactobacillus buchneri*) in variable ensiling conditions. *Anim. Feed Sci. Technol.* **2021**, *281*, 115116. [CrossRef]
9. Fabiszewska, A.U.; Zielińska, K.J.; Wróbel, B. Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: A minireview. *World J. Microbiol. Biotechnol.* **2019**, *35*, 76. [CrossRef]
10. Kung, L.; Shaver, R.D.; Grant, R.J.; Schmidt, R.J. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy Sci.* **2018**, *101*, 4020–4033. [CrossRef]
11. Jia, T.; Wang, B.; Yu, Z.; Wu, Z. The effects of stage of maturity and lactic acid bacteria inoculants on the ensiling characteristics, aerobic stability and in vitro digestibility of whole-crop oat silages. *Grassl. Sci.* **2021**, *67*, 55–62. [CrossRef]
12. Tabacco, E.; Righi, F.; Quarantelli, A.; Borreani, G. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. *J. Dairy Sci.* **2011**, *94*, 1409–1419. [CrossRef]
13. Oliveira, A.S.; Weinberg, Z.G.; Ogunade, I.M.; Cervantes, A.A.P.; Arriola, K.G.; Jiang, Y.; Kim, D.; Li, X.; Gonçalves, M.C.M.; Vyas, D.; et al. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *J. Dairy Sci.* **2017**, *100*, 4587–4603. [CrossRef]
14. Coppa, M.; Martin, C.; Bes, A.; Ragionieri, L.; Ravanetti, F.; Lund, P.; Cantalapiedra-Hijar, G.; Nozière, P. Realationship between residual feed intake and digestive traits of fattening bulls fed grass silage- or maize silage-based diets. *Animal* **2023**, *17*, 101013. [CrossRef]
15. Andrighetto, I.; Serva, L.; Gazziero, M.; Tenti, S.; Mirisola, M.; Garbin, E.; Contiero, B.; Grandis, D.; Marchesini, G. Proposal and validation of new indexes to evaluate maize silage fermentative quality in lab-scale ensiling conditions through the use of a receiver operating characteristic analysis. *Anim. Feed Sci. Technol.* **2018**, *242*, 31–40. [CrossRef]
16. De Nardi, R.; Marchesini, G.; Stefani, A.L.; Barberio, A.; Andrighetto, I.; Segato, S. Effect of feeding fine maize particles on the reticular pH, milk yield and composition of dairy cows. *J. Anim. Physiol. Anim. Nutr.* **2014**, *98*, 504–510. [CrossRef]
17. Serva, L.; Andrighetto, I.; Segato, S.; Marchesini, G.; Chinello, M.; Magrin, L. Assessment of Maize Silage Quality under Different Pre-Ensiling Conditions. *Data* **2023**, *8*, 117. [CrossRef]
18. AOAC International. *Official Methods of Analysis*, 18th ed.; AOAC International: Gaithersburg, MD, USA, 2006; ISBN 0-935584-77-3.
19. AOAC International. *Official Methods of Analysis*, 20th ed.; AOAC International: Rockville, MD, USA, 2016; ISBN 0-935584-87-0.
20. Ferreira, G.; Mertens, D.R. Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. *Anim. Feed Sci. Technol.* **2007**, *133*, 335–340. [CrossRef]
21. Schlau, N.; Mertens, D.R.; Taysom, K.; Taysom, D. Technical note: Effects of filter bags on neutral detergent fiber recovery and fiber digestion in vitro. *J. Dairy Sci.* **2021**, *104*, 1846–1854. [CrossRef]
22. Vogel, K.P.; Pedersen, J.F.; Masterson, S.D.; Toy, J.J. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. *Crop Sci.* **1999**, *39*, 276–279. [CrossRef]

23. Serva, L.; Currò, S.; Andrighetto, I.; Marchesini, G.; Magrin, L. Effect of Inoculants and Sealing Delay on the Fermentation Quality of Early Harvested Wheat Forage. *Agronomy* **2023**, *13*, 508. [CrossRef]
24. Grambsch, P.M.; Therneau, T.M. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* **1994**, *81*, 515–526. [CrossRef]
25. Abeysekera, W.W.M.; Sooriyarachchi, M.R. Use of Schoenfeld's global test to test proportional hazards assumption in the Cox proportional hazards model: An application to clinical study. *J. Natl. Sci. Found. Sri Lanka* **2009**, *37*, 41–51. [CrossRef]
26. Waha, K.; Dietrich, J.P.; Portmann, F.T.; Siebert, S.; Thornton, P.K.; Bondeau, A.; Herrero, M. Multiple cropping systems of the world and the potential for increasing cropping intensity. *Global Environ. Chang.* **2020**, *64*, 102131. [CrossRef] [PubMed]
27. Berti, A.; Maucieri, C.; Bonamano, A.; Borin, M. Short-term climate change effects on maize phenological phases in northeast Italy. *Ital. J. Agron.* **2019**, *14*, 222–229. [CrossRef]
28. Filya, I. Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. *Anim. Feed Sci. Technol.* **2004**, *116*, 141–150. [CrossRef]
29. Ferraretto, L.F.; Shaver, R.D.; Luck, B.D. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. *J. Dairy Sci.* **2018**, *101*, 3937–3951. [CrossRef]
30. Massé, D.I.; Jarret, G.; Hassanat, F.; Benchaar, C.; Saady, N.M.C. Effect of increasing levels of corn silage in an alfalfa-based dairy cow diet and of manure management practices on manure fugitive methane emissions. *Agric. Ecosyst. Environ.* **2016**, *221*, 109–114. [CrossRef]
31. de Lima, E.M.; Gonçalves, L.C.; Keller, K.M.; Rodrigues, J.A.d.S.; Santos, F.P.C.; Michel, P.H.F.; Raposo, V.S.; Jayme, D.G. Re-ensiling and its effects on chemical composition, in vitro digestibility, and quality of corn silage after different lengths of exposure to air. *Can. J. Anim. Sci.* **2017**, *97*, 250–257. [CrossRef]
32. Wilkinson, J.M.; Davies, D.R. The aerobic stability of silage: Key findings and recent developments. *Grass Forage Sci.* **2013**, *68*, 1–19. [CrossRef]
33. Brüning, D.; Gerlach, K.; Weiß, K.; Südekum, K.H. Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. *Grass Forage Sci.* **2018**, *73*, 53–66. [CrossRef]
34. Gallo, A.; Bertuzzi, T.; Giuberti, G.; Moschini, M.; Bruschi, S.; Cerioli, C.; Masoero, F. New assessment based on the use of principal factor analysis to investigate corn silage quality from nutritional traits, fermentation end products and mycotoxins. *J. Sci. Food Agric.* **2016**, *96*, 437–448. [CrossRef] [PubMed]
35. Pieper, R.; Hackl, W.; Korn, U.; Zeyner, A.; Souffrant, W.B.; Pieper, B. Effect of ensiling triticale, barley and wheat grains at different moisture content and addition of *Lactobacillus plantarum* (DSMZ 8866 and 8862) on fermentation characteristics and nutrient digestibility in pigs. *Anim. Feed Sci. Technol.* **2011**, *164*, 96–105. [CrossRef]
36. Keshri, J.; Chen, Y.; Pinto, R.; Kroupitski, Y.; Weinberg, Z.G.; Saldinger, S.S. Bacterial dynamics of wheat silage. *Front. Microbiol.* **2019**, *10*, 1532. [CrossRef] [PubMed]
37. Auerbach, H.; Nadeau, E. Effects of additive type on fermentation and aerobic stability and its interaction with air exposure on silage nutritive value. *Agronomy* **2020**, *10*, 1229. [CrossRef]
38. Nishino, N.; Wada, H.; Yoshida, M.; Shiota, H. Microbial counts, fermentation products, and aerobic stability of whole crop corn and a total mixed ration ensiled with and without inoculation of *Lactobacillus casei* or *Lactobacillus buchneri*. *J. Dairy Sci.* **2004**, *87*, 2563–2570. [CrossRef] [PubMed]
39. Wilkinson, J.M.; Bolsen, K.K.; Lin, C.J. History of silage. *Silage Sci. Technol.* **2015**, *42*, 1–30. [CrossRef]
40. Zhang, F.; Wang, X.; Lu, W.; Ma, C. Meta-analysis of the effects of combined homo- and heterofermentative lactic acid bacteria on the fermentation and aerobic stability of corn silage. *Int. J. Agric. Biol.* **2018**, *20*, 1846–1852. [CrossRef]
41. McAllister, T.A.; Selinger, L.B.; McMahon, L.R.; Bae, H.D.; Lysyk, T.J.; Oosting, S.J.; Cheng, K.-J. Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of *Lactobacillus plantarum* and *Enterococcus faecium*. *Can. J. Anim. Sci.* **1995**, *75*, 425–432. [CrossRef]
42. Weiß, K.; Kroschewski, B.; Auerbach, H. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. *J. Dairy Sci.* **2016**, *99*, 8053–8069. [CrossRef]
43. Ranjit, N.K.; Kung, L. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **2000**, *83*, 526–535. [CrossRef] [PubMed]
44. Woolford, M.K.K. The detrimental effects of air on silage. *J. Appl. Bacteriol.* **1990**, *68*, 101–116. [CrossRef] [PubMed]
45. Addah, W.; Baah, J.; Groenewegen, P.; Okine, E.K.; McAllister, T.A. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. *Can. J. Anim. Sci.* **2011**, *91*, 133–146. [CrossRef]
46. Daniel, J.L.P.P.; Queiroz, O.C.M.M.; Arriola, K.G.; Daetz, R.; Basso, F.; Romero, J.J.; Adesogan, A.T. Effects of homolactic bacterial inoculant on the performance of lactating dairy cows. *J. Dairy Sci.* **2018**, *101*, 5145–5152. [CrossRef] [PubMed]
47. Tabacco, E.; Piano, S.; Cavallarin, L.; Bernardes, T.F.; Borreani, G. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by *Lactobacillus buchneri* and *Lactobacillus plantarum* inoculants. *J. Appl. Microbiol.* **2009**, *107*, 1632–1641. [CrossRef] [PubMed]
48. Schmidt, R.J.; Kung, L. The effects of *Lactobacillus buchneri* with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. *J. Dairy Sci.* **2010**, *93*, 1616–1624. [CrossRef]

49. Huisden, C.M.; Adesogan, A.T.; Kim, S.C.; Ososanya, T. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **2009**, *92*, 690–697. [CrossRef]
50. Kleinschmit, D.H.; Kung, L. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. *J. Dairy Sci.* **2006**, *89*, 4005–4013. [CrossRef]
51. Mari, L.J.; Schmidt, R.J.; Nussio, L.G.; Halladas, C.M.; Kung, L. Short communication: An evaluation of the effectiveness of *Lactobacillus buchneri* 40788 to alter fermentation and improve the aerobic stability of corn silage in farm silos. *J. Dairy Sci.* **2009**, *92*, 1174–1176. [CrossRef]
52. Borreani, G.; Tabacco, E.; Schmidt, R.J.; Holmes, B.J.; Muck, R.E. Silage review: Factors affecting dry matter and quality losses in silages. *J. Dairy Sci.* **2018**, *101*, 3952–3979. [CrossRef]
53. Han, L.; Zhou, H. Effects of ensiling processes and oxidants on fatty acid concentrations and compositions in corn silage. *J. Anim. Sci. Biotechnol.* **2013**, *4*, 48. [CrossRef] [PubMed]
54. Weiß, K.; Kroschewski, B.; Auerbach, H. Formation of volatile organic compounds during the fermentation of maize as affected by sealing time and silage additive use. *Arch. Anim. Nutr.* **2020**, *74*, 150–163. [CrossRef] [PubMed]
55. Holmes, B.J.; Muck, R.E.; Muck, R.E. Packing bunkers and piles to maximize forage preservation. In Proceedings of the 6th International Dairy Housing Conference ASABE and Harvest and Storage, Minneapolis, MN, USA, 16–18 June 2007.
56. Guo, T.; Zhang, L.; Xin, Y.; Xu, Z.S.; He, H.; Kong, J. Oxygen-inducible conversion of lactate to acetate in heterofermentative *Lactobacillus brevis* ATCC 367. *Appl. Environ. Microbiol.* **2017**, *83*, e01659-17. [CrossRef] [PubMed]
57. Zhang, G.; Fang, X.; Feng, G.; Li, Y.; Zhang, Y. Silage fermentation, bacterial community, and aerobic stability of total mixed ration containing wet corn gluten feed and corn stover prepared with different additives. *Animals* **2020**, *10*, 1775. [CrossRef]
58. Saylor, B.A.; Heinzen, C., Jr.; Diepersloot, E.C.; Ferraretto, L.F. Effect of microbial inoculation and storage length on the fermentation profile and nutritive value of high-moisture corn ensiled at 2 different dry matter concentrations. *J. Anim. Sci.* **2022**, *100*, skac254. [CrossRef]