fermentation

MDPI

Communication

Effect of Storage Period on the Fermentation Profile and Bacterial Community of Silage Prepared with Alfalfa, Whole-Plant Corn and Their Mixture

Kai Mao [1], Zhu Yu [2], Shuai Huang [1], Musen Wang [1,*] and David B. Hannaway [3]

[1] College of Animal Science and Technology, Hainan University, Haikou 570228, China
[2] College of Grassland Science and Technology, China Agricultural University, Beijing 100193, China
[3] Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, USA
* Correspondence: wangms@hainanu.edu.cn

Abstract: This study aimed to investigate the impact of storage time on the bacterial community and fermentation profile of silage prepared with alfalfa, whole-plant corn, and their mixture. Fresh alfalfa and whole-plant corn were chopped and combined in fresh weight ratios of 1:0 (alfalfa, control), 0.8:0.2 (M1), 0.6:0.4 (M2), and 0:1 (corn). Three silos of each treatment were analyzed after 30, 60, and 90 d of storage. With storage time, pH, acetic acid, propionic acid, butyric acid, and ammonia nitrogen levels increased in alfalfa silage ($p < 0.01$), whereas lactic acid level decreased ($p < 0.01$). Compared to alfalfa silage, M1, M2, and corn silages were better fermented and more stable during storage. The dominant bacteria in M1, M2, and corn silages shifted significantly from *L. plantarum*, *L. buchneri*, and *L. brevis* to *L. acetotolerans* and *L. buchneri* during 30 to 60–90 d of storage, and storage time decreased the bacterial diversity of these silages. In conclusion, storage time significantly decreased the fermentation quality of alfalfa silage and remarkably optimized the bacterial community structure of well-fermented M1, M2, and corn silages. Alfalfa should be ensiled with at least 20% whole-plant corn to improve silage fermentation quality and storage stability.

Keywords: co-ensiling; conservation duration; lactic acid bacteria; homo-fermentation; hetero-fermentation

Citation: Mao, K.; Yu, Z.; Huang, S.; Wang, M.; Hannaway, D.B. Effect of Storage Period on the Fermentation Profile and Bacterial Community of Silage Prepared with Alfalfa, Whole-Plant Corn and Their Mixture. *Fermentation* **2022**, *8*, 486. https://doi.org/10.3390/fermentation8100486

Academic Editor: Michela Verni

Received: 31 August 2022
Accepted: 23 September 2022
Published: 26 September 2022

1. Introduction

Ensiling is a forage preservation method whereby, under anaerobic conditions, lactic acid bacteria (LAB) produce organic acids from sugars present in the fresh material. The net result is a reduction in pH which prevents the growth and proliferation of spoilage microorganisms [1,2].

According to biochemical and microbiological events, ensiling can be grouped into four stages of different intensity and length, consisting of the initial aerobic stage, the fermentation stage, the stable stage during storage, and the feed-out stage [3]. In each of these stages, changes in chemical characteristics and microbial community occur to varying degrees, depending on forage properties, ensiling management, and conservation period [4,5]. The research on the dynamics of the microbial community and the fermentation parameters during storage has focused mainly on ensiled alfalfa [6–9], whole-plant corn [10–14], corn grain [15], wheat [16], sorghum [11,17], oat [18], rice straw [19], sugarcane (top) [20,21], total mixed ration [22], and alfalfa-based mixture [23,24]. To date, there is little information regarding the effect of storage period on the microbial community and fermentation parameters of silage prepared with alfalfa, whole-plant corn, and their mixture.

Our previous work evaluated the influence of mixing ratio on individual bacteria of co-ensiling alfalfa with whole-plant corn in the early stage of fermentation [25], but did not further explore the influence on bacterial composition and diversity changes that occur during storage. Therefore, this work was undertaken to investigate the effect of storage

period on the bacterial community and fermentation profile of silage prepared with alfalfa, whole-plant corn, and their mixture.

2. Materials and Methods

2.1. Ensiling

Alfalfa (eight plots, each 100 m^2) and corn (ten plots, each 50 m^2) were grown at the Yanchi research station (37°78′ N, 107°40′ E) in northwest China's Ningxia Hui Autonomous Region. The experimental design and experiment flow diagram was presented in Figure 1. In brief, the alfalfa was from the third cutting harvested at the early flowering period. The whole-plant corn was harvested at the 1/3 milk line period. Both forages were chopped to 2 cm and randomly grouped into 36 piles (400 g for each). Chopped alfalfa and corn were combined in fresh weight (FW) ratios of 1:0 (alfalfa, control), 0.8:0.2 (M1), 0.6:0.4 (M2), and 0:1 (corn). The 200 g of forages mass was placed into a plastic bag silo (20 cm × 30 cm) and was evacuated by a sealer (DS2300, Shenzhen Dingsheng Electric Appliance Co., Ltd., Shenzhen, China). Three silos of each treatment were opened for silage parameters evaluation after 30, 60, and 90 d of storage at 26–29 °C.

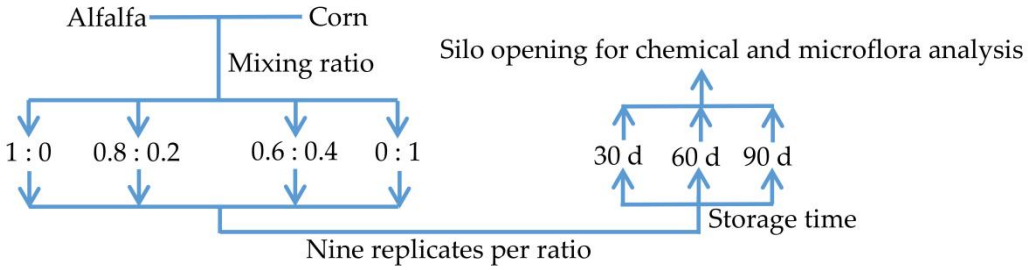

Figure 1. The experimental design and experiment flow diagram.

2.2. Analyses

A 20 g subsample from each silo was homogenized in 180 mL of distilled water and pH was determined immediately. The extract was centrifuged at 10,000× *g* for 5 min at 4 °C and passed through a 0.22 μm filter. The filtrate was analyzed by high performance liquid chromatography system (LC-20A, SHIMADZU, Shimadzu, Japan) for lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), as described by Wang et al. (2020) [25]. Ammonia nitrogen (NH_3-N) was determined by the phenol method [26] and expressed on a total nitrogen (TN) basis.

A second subsample of approximately 100 g was placed in an air dry oven for 72 h at 65 °C to determine dry matter (DM), followed by grinding to pass through a 1 mm screen. Dry matter loss (DM loss) was calculated in accordance with Wang et al. (2020) [25]. Water soluble carbohydrates (WSC) were determined by the method of Murphy (1958) [27]. Crude protein (CP) was determined according to AOAC (2001) [28] and converted to TN by a coefficient of 6.25. Silage bacterial community was analyzed by the next generation sequencing technique according to Wang et al. (2019) [29], using a universal primer pair of 806-R (5′-GGACTACHVGGGTWTCTAAT-3′) and 338-F (5′-ACTCCTACGGGAGGCAGCAG-3′).

2.3. Statistical Analysis

Statistical analysis was performed using SAS (Version 9.1, SAS Institute Inc., Cary, CA, USA). The impact of treatment, storage period, and the interaction of treatment and storage period on fermentation and chemical parameters was analyzed in a 4 × 3 factorial arrangement using the general linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed value, μ is the overall mean, α_i is the treatment impact (i = alfalfa, M1, M2, and corn), β_j is the storage period impact (j = 30, 60, and 90 d), $\alpha\beta_{ij}$ is the treatment

and storage period interaction impact and ε_{ijk} is the error. Means were compared using Duncan's multiple range test, using a significant level of $p < 0.05$.

3. Results

3.1. Fermentation Profile of Silage during Storage

Chemical composition of ensiling materials was previously reported by Wang et al. (2020). Briefly, DM and WSC concentrations in fresh forages were 205.67 g/kg FW and 60.42 g/kg DM (alfalfa), 222.09 g/kg FW and 86.83 g/kg DM (M1), 239.26 g/kg FW and 93.39 g/kg DM (M2), and 280.27 g/kg FW and 146.35 g/kg DM (corn). The CP concentrations in fresh alfalfa, M1, M2, and corn were 205.49, 172.01, 147.38, and 81.92 g/kg DM, respectively.

Dynamics of fermentation and chemical parameters of silage during storage are provided in Tables 1 and 2. Significant effects ($p < 0.01$) were observed for treatment, storage period, and their interaction on silage pH, LA, PA, and BA (Table 1). With storage time, pH, AA, PA, BA, and NH_3-N levels in alfalfa silage increased ($p < 0.01$), whereas the LA level decreased ($p < 0.01$). With more corn in the mixture, silage pH, AA, PA, BA, and NH_3-N values reduced ($p < 0.01$), irrespective of storage time. As shown in Table 2, DM loss level gradually increased but WSC concentration progressively decreased in all silages as ensiling progressed.

Table 1. Dynamics of fermentation profile of silage during storage ($n = 3$).

Item [1]	Treatment [2]	Storage Period (d) [3]			SEM [4]	*p* Value [5]		
		30	60	90		T	S	T × S
pH	Alfalfa	4.61 ^{aC}	4.76 ^{aB}	4.83 ^{aA}	0.07	<0.001	<0.001	<0.001
	M1	4.06 ^{bB}	4.13 ^{bAB}	4.19 ^{bA}				
	M2	3.90 ^{cA}	3.87 ^{cB}	3.86 ^{cB}				
	Corn	3.61 ^{dB}	3.62 ^{dAB}	3.65 ^{dA}				
LA (g/kg DM)	Alfalfa	68.78 ^{bA}	53.28 ^{cB}	47.04 ^{cB}	4.05	<0.001	0.002	<0.001
	M1	111.03 ^{aA}	97.44 ^{bAB}	83.89 ^{aB}				
	M2	120.26 ^{aA}	115.34 ^{aA}	83.32 ^{aB}				
	Corn	78.48 ^{bA}	74.72 ^{bAB}	63.12 ^{bB}				
AA (g/kg DM)	Alfalfa	29.33 ^{aB}	52.65 ^{aA}	56.49 ^{aA}	2.34	<0.001	<0.001	0.101
	M1	17.29 ^{bC}	29.37 ^{bB}	37.22 ^{bA}				
	M2	19.59 ^{bB}	30.81 ^{bA}	37.95 ^{bA}				
	Corn	13.28 ^{bB}	17.56 ^{cB}	23.58 ^{cA}				
PA (g/kg DM)	Alfalfa	23.98 ^{aB}	44.43 ^{aA}	44.71 ^{aA}	2.51	<0.001	<0.001	0.006
	M1	15.83 ^{bC}	33.48 ^{bB}	41.88 ^{aA}				
	M2	12.42 ^{bC}	29.77 ^{bB}	41.93 ^{aA}				
	Corn	3.50 ^{cC}	8.79 ^{cB}	14.01 ^{bA}				
BA (g/kg DM)	Alfalfa	9.77 ^{aB}	12.73 ^{aA}	13.49 ^{aA}	0.89	<0.001	0.001	<0.001
	M1	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}				
	M2	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}				
	Corn	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}				
NH_3-N (g/kg TN)	Alfalfa	99.01 ^{aB}	110.42 ^{aA}	120.16 ^{aA}	4.44	<0.001	<0.001	0.239
	M1	73.64 ^{bB}	76.19 ^{bB}	87.16 ^{bA}				
	M2	50.74 ^{cB}	54.33 ^{cAB}	61.43 ^{cA}				
	Corn	38.68 ^{dB}	42.60 ^{dAB}	48.90 ^{dA}				

[1] LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; DM, dry matter; NH_3-N, ammonia nitrogen; TN, total nitrogen. [2] M1 and M2, alfalfa and corn mixed with ratio of 0.8:0.2 and 0.6:0.4 on a fresh weight basis. [3] Means with different superscripts in the same row $^{A–C}$ or column $^{a–d}$ indicate a significant difference ($p < 0.05$). [4] SEM, standard error of the mean. [5] T, treatment; S, storage period; T × S, interaction of treatment and storage period.

Table 2. Dynamics of chemical composition of silage during storage (n = 3).

Item [1]	Treatment [2]	Storage Period (d) [3]			SEM [4]	*p* Value [5]		
		30	60	90		T	S	T × S
DM (g/kg FW)	Alfalfa	207.04 ^{dA}	201.22 ^{dB}	200.44 ^{dB}	5.07	<0.001	0.024	0.796
	M1	228.33 ^{c}	226.59 ^{c}	224.61 ^{c}				
	M2	241.16 ^{b}	238.14 ^{b}	240.10 ^{b}				
	Corn	286.89 ^{a}	284.69 ^{a}	282.46 ^{a}				
DM loss (g/kg FW)	Alfalfa	19.37 ^{aC}	40.67 ^{aB}	61.94 ^{aA}	2.86	<0.001	<0.001	0.177
	M1	17.50 ^{bC}	38.58 ^{abB}	58.72 ^{abA}				
	M2	17.33 ^{bC}	36.39 ^{bcB}	57.32 ^{bA}				
	Corn	16.15 ^{cC}	35.53 ^{cB}	56.71 ^{bA}				
WSC (g/kg DM)	Alfalfa	14.62 ^{dA}	12.20 ^{dB}	9.49 ^{dC}	3.31	<0.001	<0.001	0.449
	M1	32.16 ^{cA}	24.83 ^{cB}	24.41 ^{cB}				
	M2	42.76 ^{b}	41.13 ^{b}	39.52 ^{b}				
	Corn	68.80 ^{aA}	64.40 ^{aAB}	59.46 ^{aB}				
CP (g/kg DM)	Alfalfa	190.47 ^{aB}	200.08 ^{aA}	202.81 ^{aA}	7.91	<0.001	<0.001	0.002
	M1	164.59 ^{bB}	171.53 ^{bA}	171.12 ^{bA}				
	M2	139.29 ^{c}	138.14 ^{c}	140.77 ^{c}				
	Corn	70.83 ^{d}	72.83 ^{d}	72.64 ^{d}				

[1] DM, dry matter; DM loss, dry matter loss; FM, fresh weight; WSC, water soluble carbohydrates; CP, crude protein. [2] M1 and M2, alfalfa and corn mixed with ratio of 0.8:0.2 and 0.6:0.4 on a fresh weight basis. [3] Means with different superscripts in the same row $^{A–C}$ or column $^{a–d}$ indicate a significant difference ($p < 0.05$). [4] SEM, standard error of the mean. [5] T, treatment; S, storage period; T × S, interaction of treatment and storage period.

3.2. Bacterial Community of Silage during Storage

Bacterial community dynamic changes of silage at the phylum, genus, and species levels are illustrated in Figure 2A–C, respectively. After 30 d of storage, *Firmicutes* relative abundance decreased and *Proteobacteria* abundance increased as corn proportion increased (Figure 2A). With fermentation time, *Firmicutes* abundance was enriched and *Proteobacteria* abundance was reduced in M1, M2, and corn silages. Bacterial community of alfalfa silage stored for 30 d consisted mainly of *Lactobacillus* (72.68%), *Weissella* (14.91%), and *Pediococcus* (7.9%) and was relatively stable thereafter (Figure 2B). In contrast, *Lactobacillus* population increased and *Pediococcus*, *Weissella*, and *Leuconostoc* population decreased in M1, M2, and corn silages during storage. Moreover, *Lactobacillus* population increased and *Pediococcus* and *Weissella* populations decreased as the proportion of corn increased from 20% to 100% in each of these three storage periods. During alfalfa silage storage, *L. curvatus* abundance slightly decreased and *L. brevis* abundance moderately increased (Figure 2C). In contrast, *L. plantarum* was overtaken by *L. acetotolerans* in M1, M2, and corn silages during storage. After 30 d of storage, *L. plantarum*, *L. farciminis*, and *L. acetotolerans* population enriched, whereas *L. curvatus*, *W. cibaria*, and *P. parvulus* population dropped in silage with more corn. In contrast, *L. acetotolerans* accumulated and *L. curvatus*, *L. brevis*, *L. plantarum*, and *W. cibaria* richness reduced in silage stored for 60 or 90 d as corn proportion increased.

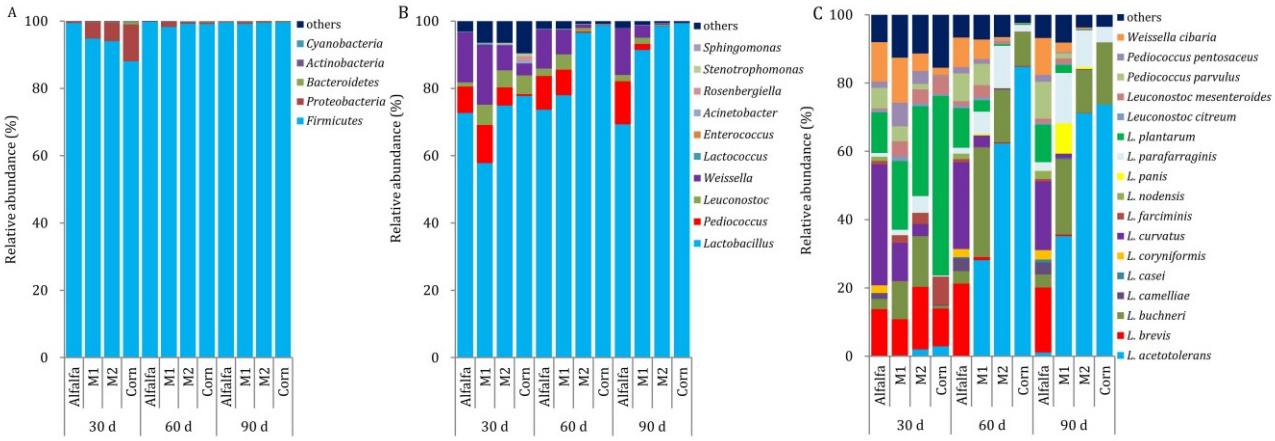

Figure 2. Dynamics of bacterial community of silage during storage at the phylum (**A**), genus (**B**) and species (**C**) levels (n = 3). M1 and M2, alfalfa and corn mixed with ratio of 0.8:0.2 and 0.6:0.4 on a fresh weight basis.

4. Discussion

4.1. Fermentation Profile of Silage during Storage

Few changes occur during storage stage when silage pH is sufficiently low and anaerobic conditions are maintained [30]. A low pH (3.6–4.5) is one of the key characteristics of well-preserved silage [31]. In the current work, alfalfa silage stored for 30 d was poorly fermented, indicated by a moderately high pH (4.61) and high level of AA (29.33 g/kg DM), PA (23.98 g/kg DM), BA (9.77 g/kg DM), and NH_3-N (99.01 g/kg TN). Furthermore, alfalfa silage was anaerobically unstable and fermentation quality progressively dropped during storage. Li et al. (2020) also reported that LA concentration of alfalfa silage decreased and AA, BA, and NH_3-N levels increased during 28–56 d of fermentation [8]. Fresh alfalfa is high in buffering capacity and low in DM and WSC [25,29], which hinders fast initial acidification in the early period of ensiling [1]. As a result, clostridial fermentation developed, accompanied by a high concentration of BA [8,32]. In this work, inclusion of corn improved silage fermentation in a proportion-dependent manner, confirming the results of previous studies [5,25]. Compared to alfalfa silage, M1, M2, and corn silages were better fermented with the pH falling into the range of 3.6–4.5 and more stable during storage.

4.2. Bacterial Community of Silage during Storage

The LAB closely related to silage fermentation are mainly composed of *Pedicoccus*, *Enterococcus*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Weissella*, and *Leuconostoc* [1,2,33]. These bacteria are grouped into homo-fermentative LAB and hetero-fermentative LAB based on fermentation pattern [33]. Both homo-fermentation and hetero-fermentation can occur simultaneously during silage fermentation. In the current work, *L. curvatus* richness in alfalfa silage decreased and *L. brevis* richness increased during storage. These changes were accompanied by an increase in AA production and a reduction in LA production, suggesting the transition from homo-fermentation to hetero-fermentation during storage. These results are similar to those of Guo et al. (2018), who reported that *L. plantarum* population declined and *Weissella* population increased in alfalfa silage during 30–90 d of storage [6]. Based on 1 mole of fructose for the substrate, homo-fermentative LAB can yield 2 moles of LA and hetero-fermentative LAB produce 1/3 mole of LA and 1/3 mole of AA or ethanol [1]. It is usually believed that homo-fermentative lactobacilli, such as *L. curvatus* and *L. plantarum*, predominate in well-fermented silage until the end of fermentation, when they are overtaken by hetero-fermentative LAB, such as *L. buchneri* and *L. brevis* [30]. Beck (1972) explained that these transitions were attributable to the high tolerance of hetero-fermenters to AA [34]. The current work showed that *L. acetotolerans* predominated in the bacterial community of corn silage with AA concentration increasing from 17.56 to 23.58 g/kg DM during 60–90 d of storage. This agrees with the results of Xu et al. (2021) [14].

Some research has shown, however, that *L. acetotolerans*, a homo-fermentative LAB, was detected from fermented vinegar and is tolerant to a high concentration of AA [35,36]. In addition, its physiological characteristics are different from the other homo-fermenters belonging to *Lactobacillus* [35]. In the near future, it is suggested to isolate this species from corn silage and to apply it to alfalfa ensiling for improving silage fermentation quality.

5. Conclusions

In conclusion, storage time significantly lowered the fermentation quality of alfalfa silage, and remarkably decreased the bacterial diversity and optimized the bacterial community structure of well-fermented M1, M2, and corn silages. Alfalfa should be ensiled with at least 20% whole-plant corn to improve the silage fermentation quality and storage stability in practical production. In the following work, it is recommended that the lactic acid bacteria species *L. acetotolerans* should be isolated from corn silage and applied to alfalfa ensiling.

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

Funding: This work was financially supported by the Scientific Research Fund Project of Hainan University (KYQD-ZR22014 and KYQD-ZR22013).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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

References

1. McDonald, P.; Henderson, N.; Heron, S. *The Biochemistry of Silage*; Chalcombe Publications: Shedfield, UK, 1991.
2. Woolford, M.K. *The Silage Fermentation*; Marcel Dekker Incorporation: New City, NY, USA, 1984.
3. Ashbell, G.; Weinberg, Z.G. *Silage Production and Utilization*; Food and Agriculture Organization, FAO Electronic Library: Rome, Italy, 2006.
4. Savoie, P.; Jofriet, J.C. Silage storage. In *Silage Science and Technology*; Buxton, D.R., Muck, R.E., Harrison, J.H., Eds.; Agronomy Monograph 42; American Society of Agronomy: Madison, WI, USA, 2003; pp. 405–467.
5. Wang, M.; Gao, R.; Franco, M.; Hannaway, D.B.; Ke, W.; Ding, Z.; Yu, Z.; Guo, X. Effect of mixing alfalfa with whole-plant corn in different proportions on fermentation characteristics and bacterial community of silage. *Agriculture* **2021**, *11*, 174. [CrossRef]
6. Guo, X.; Ke, W.; Ding, W.; Ding, L.; Xu, D.; Wang, W.; Zhang, P.; Yang, F. Profiling of metabolome and bacterial community dynamics in ensiled *Medicago sativa* inoculated without or with *Lactobacillus plantarum* or *Lactobacillus buchneri*. *Sci. Rep.* **2018**, *8*, 357. [CrossRef] [PubMed]
7. Guo, L.; Yao, D.; Lin, Y.; Bureenok, S.; Ni, K.; Yang, F. Effects of lactic acid bacteria isolated from rumen fluid and feces of dairy cows on fermentation quality, microbial community, and in vitro digestibility of alfalfa silage. *Front. Microbiol.* **2020**, *10*, 2998. [CrossRef] [PubMed]
8. Li, R.; Jiang, D.; Zheng, M.; Tian, P.; Zheng, M.; Xu, C. Microbial community dynamics during alfalfa silage with or without clostridial fermentation. *Sci. Rep.* **2020**, *10*, 1–14. [CrossRef] [PubMed]
9. Liu, F.; Bai, J.; Huang, W.; Li, F.; Ke, W.; Zhang, Y.; Xie, D.; Zhang, B.; Guo, X. Characterization of a novel beta-cypermethrin-degrading strain of *Lactobacillus pentosus* 3-27 and its effects on bioremediation and the bacterial community of contaminated alfalfa silage. *J. Hazard. Mater.* **2022**, *423*, 127101. [CrossRef]
10. Drouin, P.; Tremblay, J.; Chaucheyras-Durand, F. Dynamic succession of microbiota during ensiling of whole plant corn following inoculation with *Lactobacillus buchneri* and *Lactobacillus hilgardii* alone or in combination. *Microorganisms* **2019**, *7*, 595. [CrossRef]
11. Ferrero, F.; Piano, S.; Tabacco, E.; Borreani, G. Effects of conservation period and *Lactobacillus hilgardii* inoculum on the fermentation profile and aerobic stability of whole corn and sorghum silages. *J. Sci. Food Agric.* **2019**, *99*, 2530–2540. [CrossRef]
12. Nazar, M.; Wang, S.; Zhao, J.; Dong, Z.; Li, J.; Kaka, N.A.; Shao, T. The feasibility and effects of exogenous epiphytic microbiota on the fermentation quality and microbial community dynamics of whole crop corn. *Bioresour. Technol.* **2020**, *306*, 123106. [CrossRef]

13. Saylor, B.A.; McCary, C.L.; Diepersloot, E.C.; Heinzen, C.; Pupo, M.R.; Gusmão, J.O.; Ghizzi, L.G.; Sultana, H.; Ferraretto, L.F. Effect of forage processor roll gap width and storage length on fermentation profile, nutrient composition, kernel processing score, and starch disappearance of whole-plant maize silage harvested at three different maturities. *Agriculture* **2021**, *11*, 574. [CrossRef]
14. Xu, D.; Wang, N.; Rinne, M.; Ke, W.; Weinberg, Z.G.; Da, M.; Bai, J.; Zhang, Y.; Li, F.; Guo, X. The bacterial community and metabolome dynamics and their interactions modulate fermentation process of whole crop corn silage prepared with or without inoculants. *Microb. Biotechnol.* **2021**, *14*, 561–576. [CrossRef]
15. Silva, N.C.; Nascimento, C.F.; Campos, V.M.A.; Alves, M.A.P.; Resende, F.D.; Daniel, J.L.P.; Siqueira, G.R. Influence of storage length and inoculation with *Lactobacillus buchneri* on the fermentation, aerobic stability, and ruminal degradability of high-moisture corn and rehydrated corn grain silage. *Anim. Feed Sci. Technol.* **2019**, *251*, 124–133. [CrossRef]
16. Weinberg, Z.G.; Chen, Y. Effects of storage period on the composition of whole crop wheat and corn silages. *Anim. Feed Sci. Technol.* **2013**, *185*, 196–200. [CrossRef]
17. Fernandes, T.; Paula, E.M.; Sultana, H.; Ferraretto, L.F. Influence of sorghum cultivar, ensiling storage length, and microbial inoculation on fermentation profile, N fractions, ruminal in situ starch disappearance and aerobic stability of whole-plant sorghum silage. *Anim. Feed Sci. Technol.* **2020**, *266*, 114535. [CrossRef]
18. Li, X.; Chen, F.; Wang, X.; Sun, L.; Guo, L.; Xiong, Y.; Wang, Y.; Zhou, H.; Jia, S.; Yang, F.; et al. Impacts of low temperature and ensiling period on the bacterial community of oat silage by SMRT. *Microorganisms* **2021**, *9*, 274. [CrossRef] [PubMed]
19. Zhao, J.; Wang, S.; Dong, Z.; Li, J.; Jia, Y.; Shao, T. Effect of storage time and the level of formic acid on fermentation characteristics, epiphytic microflora, carbohydrate components and in vitro digestibility of rice straw silage. *Anim. Biosci.* **2021**, *34*, 1038–1048. [CrossRef]
20. Muraro, G.B.; Carvalho-Estrada, P.A.; Pasetti, M.H.O.; Santos, M.C.; Nussio, L.G. Bacterial dynamics of sugarcane silage in the tropics. *Environ. Microbiol.* **2021**, *23*, 5979–5991. [CrossRef]
21. Ren, F.; He, R.; Zhou, X.; Gu, Q.; Xia, Z.; Liang, M.; Zhou, J.; Zou, C. Dynamic changes in fermentation profiles and bacterial community composition during sugarcane top silage fermentation: A preliminary study. *Bioresour. Technol.* **2019**, *285*, 121315. [CrossRef]
22. Wang, C.; Nishino, N. Effects of storage temperature and ensiling period on fermentation products, aerobic stability and microbial communities of total mixed ration silage. *J. Appl. Microbiol.* **2013**, *114*, 1687–1695. [CrossRef]
23. Chen, L.; Qu, H.; Bai, S.; Yan, L.; You, M.; Gou, W.; Li, P.; Gao, F. Effect of wet sea buckthorn pomace utilized as an additive on silage fermentation profile and bacterial community composition of alfalfa. *Bioresour. Technol.* **2020**, *314*, 123773. [CrossRef]
24. Mu, L.; Wang, Q.; Cao, X.; Zhang, Z. Effects of fatty acid salts on fermentation characteristics, bacterial diversity and aerobic stability of mixed silage prepared with alfalfa, rice straw and wheat bran. *J. Sci. Food Agric.* **2022**, *102*, 1475–1487. [CrossRef]
25. Wang, M.; Franco, M.; Cai, Y.; Yu, Z. Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. *Anim. Feed Sci. Technol.* **2020**, *270*, 114702. [CrossRef]
26. Broderick, G.A.; Kang, J.H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* **1980**, *63*, 64–75. [CrossRef]
27. Murphy, R.P. A method for the extraction of plant samples and the determination of total soluble carbohydrates. *J. Sci. Food Agric.* **1958**, *9*, 714–717. [CrossRef]
28. AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2001.
29. Wang, M.; Wang, L.; Yu, Z. Fermentation dynamics and bacterial diversity of mixed lucerne and sweet corn stalk silage ensiled at six ratios. *Grass Forage Sci.* **2019**, *74*, 264–273. [CrossRef]
30. Dunière, L.; Sindou, J.; Chaucheyras-Durand, F.; Chevallier, I.; Thévenot-Sergentet, D. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Anim. Feed Sci. Technol.* **2013**, *182*, 1–15. [CrossRef]
31. Kung, L.; Robinson, J.R.; Ranjit, N.K.; Chen, J.H.; Golt, C.M.; Pesek, J.D. Microbial populations, fermentation end-products, and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. *J. Dairy Sci.* **2000**, *83*, 1479–1486. [CrossRef]
32. Zheng, M.; Niu, D.; Jiang, D.; Zuo, S.; Xu, C. Dynamics of microbial community during ensiling direct-cut alfalfa with and without LAB inoculant and sugar. *J. Appl. Microbiol.* **2017**, *122*, 1456–1470. [CrossRef]
33. Pahlow, G.; Muck, R.E.; Driehuis, F.; Oude Elferink, S.J.W.H.; Spoelstr, S.F. Microbiology of ensiling. In *Silage Science and Technology*; Buxton, D.R., Muck, R.E., Harrison, J.H., Eds.; American Society of Agronomy: Madison, WI, USA, 2003; pp. 31–94.
34. Beck, T. The quantitative and qualitative composition of the lactic acid bacteria flora of silage. *Landwirt Forsch Sonderheft* **1972**, *27*, 55–63.
35. Entani, E.; Masai, H.; Suzuki, K.I. *Lactobacillus acetotolerans*, a new species from fermented vinegar broth. *Int. J. Syst. Evol. Microbiol.* **1986**, *36*, 544–549. [CrossRef]
36. Haruta, S.; Ueno, S.; Egawa, I.; Hashiguchi, K.; Fujii, A.; Nagano, M. Succession of bacterial and fungal communities during a traditional pot fermentation of rice vinegar assessed by PCR-mediated denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* **2006**, *109*, 79–87. [CrossRef]