

FEEDS

A combination of *Lactobacillus buchneri* and *Pediococcus pentosaceus* extended the aerobic stability of conventional and brown midrib mutants–corn hybrids ensiled at low dry matter concentrations by causing a major shift in their bacterial and fungal community

Juan J. Romero,^{†,1} Jin Park,[‡] Younghoo Joo,[‡] Yuchen Zhao,^{||} Marjorie Killerby,[†] Diana C. Reyes,[†] Francesco Tiezzi,[§] Eduardo Gutierrez-Rodriguez,^{||} and Miguel S. Castillo^{**}

[†]Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine, Orono, ME 04469, [‡]Department of Animal Science (BK21Plus, Institute of Agriculture and Life Science), Gyeongsang National University, Jinju, South Gyeongsang 52828, Republic of Korea, ^{||}Department of Animal Nutrition and Feed Science, China Agricultural University, Beijing, Beijing Municipality 100093, China, [§]Department of Animal Science, North Carolina State University, Raleigh, NC 27695, ^{||}Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523, ^{**}Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695

¹Corresponding author: juan.romero@maine.edu

ORCID numbers: 0000-0002-2545-0003 (J. Romero); 0000-0002-3041-623X (Y. Joo); 0000-0002-9288-9577 (M. Killerby); 0000-0003-2433-0621 (D. Reyes); 0000-0002-4358-9236 (F. Tiezzi); 0000-0003-1066-5906 (M. Castillo).

Abstract

We evaluated the effects of applying a combination inoculant to four corn hybrids harvested at high moisture on their nutritive value and microbial populations. The treatment design was the factorial combination of corn hybrids ensiled with (INO) and without (CON) inoculant. The hybrids were TMF2R737 (MCN), F2F817 (MBR), P2089YHR (PCN), and PI144XR (PBR), ensiled at dry matter (DM) concentrations of 30.5%, 26.3%, 31.1%, and 31.5%, respectively; MBR and PBR were brown midrib mutants (BMR). The inoculant contained *Lactobacillus buchneri* and *Pediococcus pentosaceus* (4×10^5 and 1×10^5 cfu/g of fresh corn). The experiment had a complete randomized design with treatments replicated six times. Corn was treated or not with inoculant, packed into 7.6 L bucket silos, and stored for 100 d. At d 0, the relative abundance (RA, %) of *Enterobacteriaceae* was lower in PBR vs. the other hybrids [51.3 vs. \bar{x} = (average of) 58.4] and in the case of fungi, incertae sedis (i.s.) *Tremellales* and *Mucoraceae* were more and less abundant, respectively, in conventional hybrids vs. BMRs (\bar{x} = 25.8 vs. \bar{x} = 13.9 and \bar{x} = 3.64 vs. \bar{x} = 7.52; $P < 0.04$). After ensiling, INO had higher LAB (9.3 vs. 7.1 log cfu/g of fresh corn) and acetic acid (3.44% vs. 1.32% of DM) and lower yeast (3.1 vs. 4.6) and molds (1.5 vs. 3.0), and also extended the aerobic stability (582 vs. 111 h) but decreased DM recovery (95.6% vs. 97.4%) vs. CON ($P < 0.02$). Inoculation reduced bacterial phylogenetic diversity (6.75 vs. 14.4) but increased fungal observed taxonomical units (46 vs. 20) vs. CON ($P < 0.01$). Also, a higher relative abundance (RA) for *Lactobacillaceae* (99.2% vs. 75.7%) and lower for

Enterobacteriaceae (0.28 vs. 9.93) was observed due to inoculation ($P < 0.001$). For fungi, INO had a lower RA compared to CON for *Monascaceae* (12.6 vs. 44.7) and increased i.s. *Tremellales* (8.0 vs. 1.2) and i.s. *Saccharomycetales* (6.4% vs. 0.3%; $P < 0.006$). Inoculation changed the diverse bacterial community found in the phyllosphere across hybrids to a taxonomically uneven one dominated by *Lactobacillaceae*. In the case of fungi, INO application increased the fungal diversity at d 100 mainly by reducing the dominance of *Monascaceae* vs. CON. In conclusion, the INO treatment overwhelmed the disparate microbial populations found across BMR and conventional hybrids ensiled at low DM concentrations and ensured a significant concentration of acetic acid that modified fungal populations and in turn extended the aerobic stability of all hybrids.

Key words: 16S and ITS sequencing, hybrid, inoculant, silage

Abbreviations

ADF	acid detergent fiber
ADV	additives
CP	crude protein
DM	dry matter
i.s.	incertae sedis
L:A	lactic to acetic acid ratio
LAB	lactic acid bacteria
NDF	neutral detergent fiber
NGS	next generation sequencing
OM	organic matter
OTU	observed taxonomical unit
RA	relative abundance
WSC	water soluble carbohydrates

Introduction

Consistency in silage nutrient preservation is a critical aspect of cattle feeding due to its effects on animal performance (Sniffen et al., 1993; Kertz, 1998; Gerlach et al., 2013), nutrient waste (Tylutki and Fox, 2000), and farm profitability (Rotz et al., 2003). A key source of variability in the preservation of nutrients during silage production is microbial spoilage resulting from less than ideal ensiling conditions (Borreani et al., 2018). Thus, mitigating spoilage processes is one of the most important strategies that can be implemented to preserve the nutritional value of conserved feeds and to prevent the significant dry matter (DM) losses that can occur during the storage and feed-out phases of ensiling (Borreani et al., 2018).

Addition of lactic acid bacteria (LAB) inoculants is an established strategy to mitigate silage nutrient losses (Pitt and Leibensperger, 1987; Muck et al., 2018). In the case of whole-crop corn, storage losses are less of a concern relative to feed-out spoilage due to the high concentration of sugars and low buffering capacity which favors adequate acidification during ensiling (Martinez-Fernandez et al., 2013; Wilkinson and Davies, 2013). However, the acetic acid levels (an antifungal agent) can be often inadequate to ensure optimal aerobic stability of whole-crop corn silage during the feed-out phase (Filya et al., 2006; Basso et al., 2012; Wilkinson and Davies, 2013). To solve this issue, obligate heterofermentative LAB (e.g., *Lactobacillus buchneri*) inoculants were developed to increase the concentration of acetic acid in silages, but it can come at the expense of added DM losses (Blajman et al., 2018). Thus, combination inoculants were designed to include a facultative heterofermentative or homofermentative LAB with the purpose of suppressing spoilage microbes as early as possible during the fermentation phase by an extensive pH drop and in that way mitigate the undesirable secondary effects that can be observed

with inoculants consisting solely of obligate heterofermentative LAB (Blajman et al., 2018; Muck et al., 2018). Even though the benefits of this approach have been reported in the literature, there are several reports where addition of combination inoculants had no positive effect without a clear explanation (Muck et al., 2018).

Due to climate change, the frequency of extreme weather events that can disrupt whole-crop corn harvest has increased in recent years (Rosenzweig et al., 2001; Kung et al., 2015; Wang et al., 2018) forcing producers in some instances to ensile whole-crop corn outside the recommended DM range (32% to 35%) that maximizes economic returns (Allen et al., 2003). Despite low DM (<30%) silages being able to sustain higher fermentation rates, they also typically have higher populations of undesirable microbes such as clostridia and enterobacteria (Queiroz et al., 2018). At high moisture conditions, little is known about how combination inoculants affect the diverse bacterial and fungal communities found across corn hybrids with a diverse genetic background and how this in turn affects the ensiling process and its efficacy to preserve nutrients at both storage and feed-out phases. Our objectives were to assess the effect of a facultative and obligate heterofermentative LAB combination inoculant on the nutritional composition, fermentation profile, aerobic stability, and microbial communities of various corn hybrids ensiled at low DM concentrations. We hypothesized that combo inoculants extend aerobic stability and prevent nutrient losses to different extents across corn hybrids ensiled at low DM concentrations by a dissimilar modulation of spoilage microbial communities across hybrids.

Materials and Methods

Experimental site, design, and treatments

Field plots were located at the Mountain Research Station in Waynesville, NC (35°29' N; 82°58' W). Corn was planted in a clean-tilled seedbed on 3 June 2014 at a rate of 73,143 live seeds/ha in 76-cm row-widths. Based on initial soil test results, liquid N (32%) was applied at a rate of 515 L/ha on 7 May 2014. Four corn hybrids (HYB) were used: TMF2R737 (MCN; Mycogen Seeds, Indianapolis, IN), P2089YHR (PCN; Pioneer Hi-Bred International, Johnston, IA), F2F817 (MBR; Mycogen Seeds, Indianapolis, IN), and P1449XR (PBR; Pioneer Hi-Bred International, Johnston, IA). Hybrids MBR and PBR were brown mid-rib mutants (BMR; loci 3 and 1 mutants, respectively). Corn plots were harvested on 1 October 2014 when DM concentration was ~29% for all hybrids. Plot DM yields were 17.3, 23.9, 16.4, and 18.4 (mg/ha, ±3.55 SD) for MCN, PCN, MBR, and PBR, respectively. Corn was clipped to 18-cm stubble height and chopped to 1.9-cm theoretical

length using a John Deere 3955 forage harvester equipped with a kernel-processor (2.5 mm roll clearance, John Deere, Moline, IL). Two replicated piles (5.9 kg each, fresh weight basis) were obtained from each corn plot (6 per hybrid) for a total of 48 piles.

The two silage additives (ADV) evaluated were sterile double-distilled water (CON) and inoculant (INO; Biotol Buchneri 500, Lallemand Animal Nutrition, Milwaukee, WI). Each ADV treatment (CON or INO) were applied randomly to 1 of the 2 replicated piles at a rate of 1 mL/kg of fresh corn. Inoculation resulted in theoretical final application rates of log 5.6 cfu/g of fresh corn (FW) for *L. buchneri* ATCC number 40,788 and log 5 cfu/g FW for *Pediococcus pentosaceus* plus fibrolytic enzymes from *Trichoderma reesei* (1,103, 3,145, and 50 mg of sugar released/min/g for β -glucanase, xylanase, and galactomannanase activities, respectively; FCC, 2015). Chopped whole-crop corn (5.1 kg fresh basis) was packed into 7.6 L plastic buckets using an A-frame 12-ton hand-press and sealed with a rubber gasket lid and duct tape [\bar{x} = (average of) 202 \pm 15.4 kg DM/m³]. Silos were stored at 23°C (\pm 1°C) for 100 d and weights were recorded individually at d 0 and 100 to determine DM recovery (Arriola et al., 2011).

Sampling procedure

At d 0 and 100, samples (250 g, fresh basis) were taken from each individual replicate to determine nutritive value, fermentation profile, and the bacterial and fungal population via standard plating techniques. At d 0, samples were obtained immediately after treatment application. Additional sample-subsets were collected at d 0 and 100 to determine the composition and structure of the bacterial and fungal communities using next generation sequencing (NGS; 100 g, fresh basis) and also aerobic stability analysis at d 100 (2.5 kg, fresh basis) following the procedures described by Romero et al. (2018).

Laboratory analysis

Chemical, microbial counts, and aerobic stability analyses

Samples from d 0 and 100 were processed for DM determination by drying at 60°C for 48 h in a forced-air oven. Dried samples were ground to pass the 1-mm screen of a Wiley mill (A.H. Thomas, Philadelphia, PA). Water extracts were prepared by mixing 25 g of fresh or ensiled corn with 225 mL of 0.1% sterile peptone water in a 400C Stomacher blender for 3 min (Seward Ltd., Worthing, UK) and the solution was filtered through two layers of sterilized cheesecloth. All nutritional composition, fermentation profile, microbial counts, and aerobic stability analyses were conducted as outlined in Romero et al. (2018).

Microbial and DNA extraction

Samples (100 g, fresh basis) from d 0 and 100 were weighed into 15.2 \times 22.9 cm sterile 0.076-mm filter bags (FILTRA-BAG, VWR Co., Radnor, PA) and processed as described in Romero et al. (2018) to obtain microbial pellets. Extraction of DNA was done using the PowerLyzer-PowerSoil DNA isolation kit (MO BIO Labs Inc., Carlsbad, CA) following the manufacturer recommended procedure. Resulting DNA was quantified and visualized as outlined in Romero et al. (2018). The concentration of DNA for each PCR reaction was standardized for all samples at 5 ng/ μ L.

NGS

Extracted DNA from pellets was analyzed using the Illumina (San Diego, CA) MiSeq platform for pair-end reads and 500 sequencing cycles. Amplification of the V4 hypervariable region of the 16S rRNA was achieved using the primer pair F515

(5'-CACGGTCGKCGGCCATT-3') and R806 (5'-GGACTACHVGGG TWCTAAT-3') and the Internal Transcribe Spacer (ITS-1) region of fungi BITS (5'-NNNNNNNNCTACTGCGGARGGATCA-3') and B58S3 (5'-GAGATCCRTTGYTRAAAGTT-3') as described by Caporaso et al. (2011) and by Bokulich and Mills (2013). The PCR conditions for both the 16S rRNA and ITS-1 amplifications are delineated in Romero et al. (2018). Amplicons were mixed at roughly equivalent ratios based on electrophoretic band intensity and purified using GE Illustra MicroSpin S-200 HR columns (GE Healthcare Biosciences, Piscataway, NJ). Pooled samples were submitted to the University of California Davis Genome Center for library preparation using the Kappa paired-end kit, cluster generation and 250 bp paired-end sequencing on the MiSeq platform.

Sequencing analysis

Raw Illumina fastq files were demultiplexed, quality filtered (Q30), and analyzed using QIIME 1.9.1 and the GreenGenes 13.8, UNITE fungal ITS reference database as outlined in Romero et al. (2018). All samples were rarefied to a depth of 16,000 and 5,000 sequences per sample for 16S rRNA and ITS-1 libraries, respectively, before downstream analysis. The β -diversity (between-samples community dissimilarity) was calculated in QIIME using the weighted UniFrac distances for 16S and the Bray-Curtis distances for ITS-1 (Lozupone and Knight, 2005). The α -diversity was also estimated from rarefied observed taxonomical units (OTU) tables (Lozupone and Knight, 2005; Cole et al., 2009; Caporaso et al., 2011).

Statistical analyses

Data were analyzed separately for d 0 and 100. The experimental design was a completely randomized design replicated six times. The model was:

$$Y_{ijk} = \mu + T_i + C_j + TC_{ij} + E_{ijk}$$

where Y_{ijk} is the response from the k th experimental unit receiving the i th level of corn hybrid and the j th level of ADV, μ is the general mean, T_i is the effect of hybrid i , C_j is the effect of ADV j , TC_{ij} is the effect of the hybrid $i \times$ ADV j interaction, and E_{ijk} is the experimental error.

We used the GLM procedure of SAS v.9.4 (SAS Institute, 2019) for data analysis. When a two-factor interaction effect was present, we used the SLICE option to analyze the simple effects. Microbial data were converted to log₁₀ to conduct statistical analysis and are presented on a fresh basis. Shapiro-Wilk test revealed that data were normally distributed. Mean separation was based on the PDIF procedure of LSMEANS. Treatments were considered different when $P \leq 0.05$.

Further analysis was conducted to understand the overall relationships among the microbiota profile and silage quality variables and microbiota RA were adjusted by the effect of corn hybrid using a linear model. Residuals were considered as adjusted measures and were used in the association analysis. To account for the nonlinearity of the relationship between silage quality and microbiota RA, each bacterial and fungal family RA was classified into three categories: low, medium, and high RA. Categories were allocated based on the 34th and 67th percentiles of the specific family RA. A linear model was used to test the effect of family RA categories on silage quality variables, and pairwise contrasts between the three categories were used to identify trends in the change of silage quality variables as a function of family RA. This analysis was performed in R utilizing

the packages “car” (Fox and Weisberg, 2018) and “lsmeans” (Lenth, 2016), which uses Tukey adjustment for multiple testing.

Results and Discussion

Before ensiling (0 d)

Nutritional composition

All chemical composition response variables were affected by corn hybrid ($P < 0.001$) but not by ADV ($P > 0.18$, Table 1) right after inoculation. Among all hybrids, MBR had the lowest DM concentration (26.3) and it was also the least mature (early dent) compared to the other hybrids for which DM concentration ranged from 30.5–31.5 ($\pm 0.43\%$) and were at $\frac{1}{4}$ milk line at harvest time. Hybrid MBR had the lowest organic matter (OM) concentration (95.5 vs. 96.4–96.8 $\pm 0.08\%$ of DM; $P < 0.001$) and the highest crude protein (CP; 9.63 vs. 7.79–8.33 $\pm 0.146\%$ of DM; $P < 0.001$), $\text{NH}_3\text{-N}$ (0.069 vs. 0.054–0.058 $\pm 0.0021\%$ of DM; $P < 0.001$), and neutral detergent fiber (NDF; 47.8 vs. \bar{x} = 44.0 $\pm 0.88\%$ of DM; $P < 0.001$) when compared to the other hybrids, respectively. The water soluble carbohydrate (WSC) concentration was lower in MBR and PCN (\bar{x} = 4.22) than in MCN and PBR (\bar{x} = 5.63 $\pm 0.324\%$ of DM, respectively; $P \leq 0.01$). Similar maturity effects at d 0 on NDF and CP concentrations have been reported by other studies (Darby and Lauer, 2002; Lewis et al., 2004). Arriola et al. (2012) observed higher CP, NDF, and acid detergent fiber (ADF) and lower OM concentrations across four corn hybrids at \bar{x} = 25.3 vs. \bar{x} = 31.9% DM. Similarly, Windle et al. (2014) observed higher CP and lower WSC concentrations at 30.1% vs. 39.6% DM. In a previous study we conducted, Romero et al., (2018) evaluating the same hybrids treated or not with the same INO but ensiled at a higher DM, we also found that MBR had a much lower DM relative to the other hybrids at d 0 (38.1% vs. 41.3%–44.0%, respectively). Likewise, we also observed that MBR had the highest CP (6.85 vs. 5.46–5.98) and $\text{NH}_3\text{-N}$ concentrations (0.037% vs. 0.030%–0.032% of DM), but NDF was only higher when compared against PCN and PBR (44.7 vs.

\bar{x} = 42.1) and similar to MCN (45.5% of DM, respectively). Conversely, WSC was higher in MBR vs. all the other hybrids (5.49 vs. \bar{x} = 3.49% of DM, respectively; Romero et al., 2018).

Phyllosphere microbial populations

The initial microbial population counts were affected by corn hybrid ($P < 0.001$) but not by ADV ($P > 0.13$, Table 1). A lower LAB population was observed for MBR and MCN (\bar{x} = 7.69) compared to PCN and PBR (\bar{x} = 8.39 ± 0.228 log cfu/g of FW, $P < 0.05$). Hybrid PCN had a higher yeast population (6.79 vs. \bar{x} = 6.0 ± 0.139 log cfu/g of FW; $P < 0.001$) and PBR a lower mold population (4.60 vs. \bar{x} = 5.15 ± 0.147 log cfu/g of FW) compared to the others ($P < 0.001$). Comino et al. (2014) observed no effect of increasing DM from 27.6% to 32.5% on LAB (\bar{x} = 6.51), yeast (\bar{x} = 6.36), and mold (\bar{x} = 6.05; log cfu/g of FW) counts at d 0 in whole-crop corn, respectively, which may indicate that at least within the DM range evaluated in this study maturity effects may not explain the differences in microbial counts. At a higher DM range (38.1% to 44%), Romero et al. (2018) reported that MBR and PBR (\bar{x} = 7.69) had a higher LAB count than MCN and PCN (\bar{x} = 6.90 log cfu/g of FW) and no hybrid effects were observed on yeast and mold counts at d 0 (\bar{x} = 6.67 and \bar{x} = 5.87 log cfu/g of FW, respectively). The current study had comparable yeast and mold counts relative to Romero et al. (2018) but a higher epiphytic LAB count, which may be partly due to the differences in DM, harvest month, and location. Windle et al. (2014) reported no differences in LAB (\bar{x} = 5.97) and yeast (\bar{x} = 5.33 log cfu/g of FW) counts at d 0 in whole-crop corn ensiled at 31% or 40% DM. Conversely, Comino et al. (2014) reported lower LAB (6.44 vs. 7.29), yeast (6.33 vs. 6.80), and mold (6.01 vs. 7.03 log cfu/g of FW) counts between 27.6% and 43.9% DM at d 0, respectively.

All rarefaction curves approached the saturation plateau, indicating that the coverage of bacterial (Supplemental Figure S1) and fungal (Supplemental Figure S2) diversity was sufficient to evaluate the bacterial and fungal community composition of whole-crop corn. At a sequencing depth of 16,000 and 5,000 sequences per sample for bacteria and fungi, respectively, we did not find an INO effect on bacterial and fungal observed OTUs ($P >$

Table 1. Chemical composition and microbial counts of chopped whole corn as a function of hybrid type (HYB) and bacterial inoculation (ADV) at d 0¹

Item	Hybrid				SEM	P-value		
	MCN	PCN	MBR	PBR		HYB	ADV	HYB × ADV
DM, %	30.5 ^b	31.1 ^{ab}	26.3 ^c	31.5 ^a	0.43	<0.001	0.585	0.598
OM, % of DM	96.8 ^a	96.7 ^a	95.5 ^c	96.4 ^b	0.08	<0.001	0.910	0.485
CP, % of DM	7.79 ^c	8.33 ^b	9.63 ^a	7.96 ^c	0.146	<0.001	0.256	0.765
$\text{NH}_3\text{-N}$, % of DM ²	0.054 ^c	0.054 ^c	0.069 ^a	0.058 ^b	0.0021	<0.001	0.539	0.063
NDF, % of DM	44.2 ^b	43.6 ^b	47.8 ^a	44.2 ^b	0.88	<0.001	0.183	0.707
ADF, % of DM	24.0 ^a	22.9 ^b	24.7 ^a	22.8 ^b	0.49	<0.001	0.208	0.664
WSC, % of DM ³	5.35 ^a	4.50 ^b	3.94 ^b	5.90 ^a	0.324	<0.001	0.212	0.425
pH	5.63 ^b	5.59 ^b	5.91 ^a	5.89 ^a	0.083	<0.001	0.972	0.432
Lactic acid bacteria, log cfu/g FW ⁴	7.48 ^b	8.45 ^a	7.89 ^b	8.33 ^a	0.228	<0.001	0.767	0.661
Yeast, log cfu/g FW	6.08 ^b	6.79 ^a	5.98 ^b	5.94 ^b	0.139	<0.001	0.125	0.352
Molds, log cfu/g FW	5.14 ^a	5.20 ^a	5.10 ^a	4.60 ^b	0.147	<0.001	0.180	0.302

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and hybrids PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. Additives were control (water) and inoculant (Biotol Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

² $\text{NH}_3\text{-N}$, ammonia-N.

³WSC, water soluble carbohydrates.

⁴FW, fresh corn.

^{a-c}Means with different superscripts within a row are significantly different ($P \leq 0.05$).

0.20), Heip's evenness index ($P > 0.12$) and bacterial phylogenetic diversity ($P = 0.16$). Bacterial OTUs and phylogenetic diversity were lower ($P < 0.001$) for MCN vs. the other hybrids (500 vs. $\bar{x} = 600$ and 24.0 vs. $\bar{x} = 27.6$, respectively), and for PCN vs. PBR (563 vs. 640 ± 21.3 and 26.2 vs. 28.8 ± 0.80 , respectively). Fungal OTUs were higher for PBR vs. the other hybrids (82.1 vs. $\bar{x} = 73.0$) and for MBR vs. PCN (75.3 vs. 71.3 ± 1.29 ; $P < 0.001$). Also, Heip's evenness index indicates a more even fungal community in PBR vs. other hybrids (0.125 vs. $\bar{x} = 0.096$) and in PCN vs. MCN (0.103 vs. 0.086 ± 0.005 , $P < 0.001$). These results suggest that bacterial and fungal diversity may be higher for BMR compared to conventional hybrids tested in study at d 0, as it was also reported by Romero et al. (2018) for the same hybrids. The implications of these differences may be related to some of the differences in pest resistance between BMR and conventional hybrids (Dowd et al., 2016), further research needs to confirm these observations. The weighted UniFrac (Figure 1) and Bray-Curtis (Figure 2) principal coordinates analysis plots indicated a separation and difference in the structure of the bacterial ($P = 0.001$) and fungal ($P = 0.001$) communities between hybrids at d 0 but no difference between ADV ($P = 0.588$) according to the analysis of similarities test of distance metrics. Similar responses at d 0 were reported by Romero et al. (2018), as expected from samples collected right after ADV application.

The most abundant bacterial families observed at d 0 were *Enterobacteriaceae*, *Pseudomonadaceae*, *Leuconostocaceae*, and *Xanthomonadaceae* ($\bar{x} = 56.7\%$, 9.79% , 5.52% , and 5.32% RA, respectively). The remaining families found had less than 5% RA. No ADV effects were observed on the relative abundance of bacterial families ($P > 0.05$) at d 0 except for *Enterobacteriaceae* ($P = 0.03$; Table 2) which had a lower RA for INO than CON (54.8 vs. $58.5 \pm 1.83\%$). It is unclear how INO could have reduced the

abundance of *Enterobacteriaceae* on d 0 but other studies have reported bacterial inoculant effects on bacterial and fungal populations at d 0 (Queiroz et al., 2013). Corn hybrid effects were observed for *Enterobacteriaceae*, *Sphingobacteriaceae*, *Aeromonadaceae*, *Moraxellaceae*, and *Streptococcaceae* ($P \leq 0.05$). *Enterobacteriaceae* were least abundant in PBR (51.3) compared to the rest of the hybrids ($\bar{x} = 58.4 \pm 1.83\%$; $P \leq 0.01$), while *Sphingobacteriaceae* were higher in PCN vs. MCN and MBR (5.25 vs. $\bar{x} = 3.26 \pm 0.47\%$; $P < 0.001$), and *Aeromonadaceae* were higher in MCN than other hybrids (4.79 vs. $\bar{x} = 3.57 \pm 0.32\%$; $P < 0.05$). Romero et al. (2018) also described *Enterobacteriaceae* as the dominant (61.4%) bacterial family in low-moisture whole-crop fresh corn, followed by *Sphingobacteriaceae* (6.1%), but with no hybrid or ADV effects. Similarly, high abundance of *Enterobacteriaceae*, *Methylobacteriaceae*, and *Sphingomonadaceae* was also identified by Ni et al. (2017) in whole-crop corn by NGS, while Drouin et al. (2019) described that *Enterobacteriaceae* and *Pseudomonadaceae* were the most abundant bacterial families in the silk and tassel of fresh corn and *Sphingobacteriaceae* was mostly found in leaves and tassels. On many instances, beef or dairy pastureland or land and water in close proximity to animal operations presents significant higher populations of *Enterobacteriaceae* and *Pseudomonadaceae* than land and water with little animal pressure (Wilde et al., 2000; Freitas et al., 2003). The most abundant genera were unidentified, *Hafnia* and *Erwinia* for *Enterobacteriaceae*, *Pseudomonas* and unidentified for *Pseudomonadaceae*, and *Sphingobacterium* for *Sphingobacteriaceae*. The genus *Hafnia* has been reported in Italian ryegrass silage (Heron et al., 1993), but few studies describe it as part of the phyllosphere microbiome of fresh corn. Some *Hafnia* species are known to be gut pathogens (Yin et al., 2019). Romero et al. (2018) and Ni et al. (2017) also

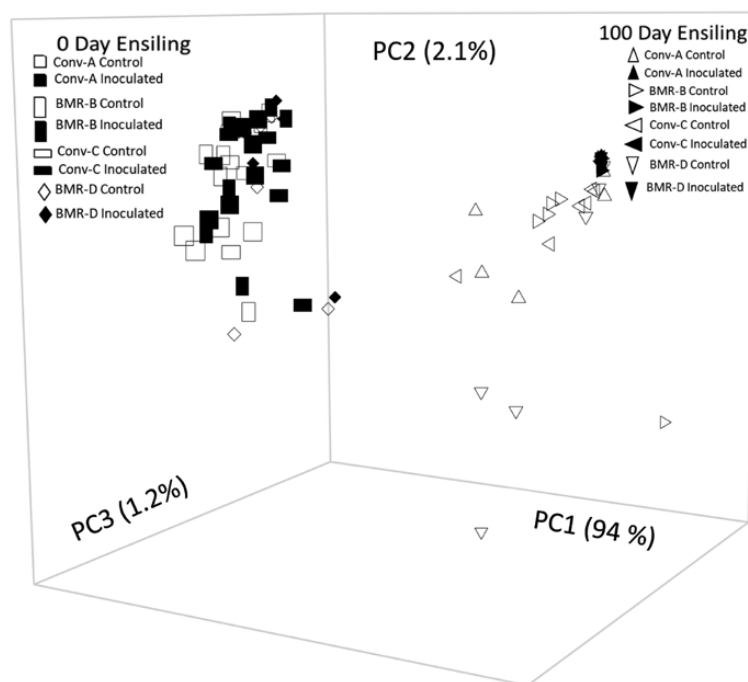


Figure 1. Weighted UniFrac principle coordinate (PC) analysis plots for bacterial OTUs coming from fresh (0 d) and ensiled (100 d) chopped whole-crop corn as a function of hybrid and bacterial inoculation. CON, control (water); INO, inoculant. MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and PCN and PBR of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. Inoculant used was Biotol Buchneri 500 (Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh whole-crop corn, respectively.

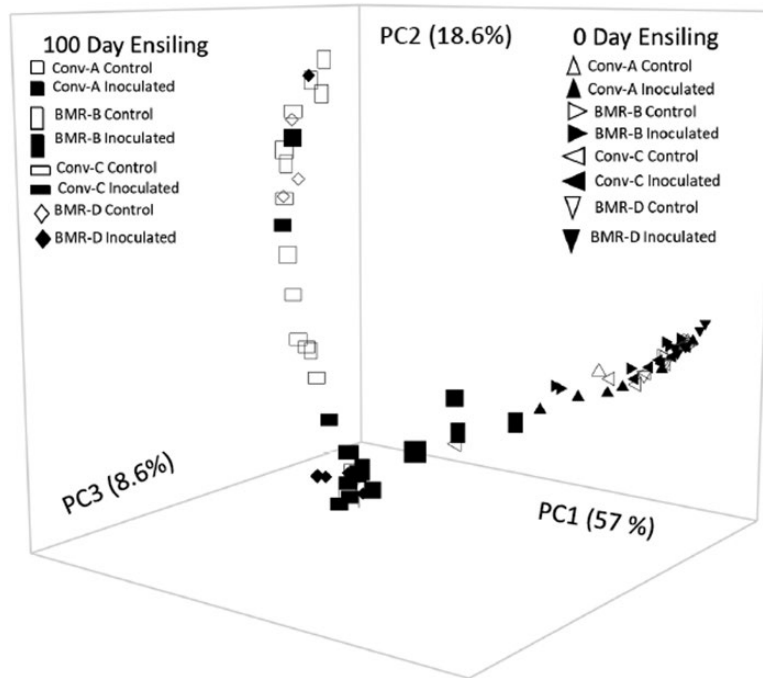


Figure 2. Bray-Curtis principle coordinate analysis plots for fungal OTUs coming from fresh (0 d) and ensiled (100 d) chopped whole-crop corn as a function of hybrid and bacterial inoculation. CON, control (water); INO, inoculant. MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and PCN and PBR of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. Inoculant used was Biotol Buchneri 500 (Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh whole-crop corn, respectively.

Table 2. Relative abundance (%) of bacterial families identified from 16S ribosomal DNA sequences extracted from chopped whole-crop corn as a function of hybrid (HYB) and silage additive (ADV) at d 0¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
<i>Enterobacteriaceae</i>						1.83	0.014	0.034	0.766
CON	–	–	–	–	58.5 ^A				
INO	–	–	–	–	54.8 ^B				
Mean	58.9 ^a	57.8 ^a	58.5 ^a	51.3 ^b					
<i>Pseudomonadaceae</i>	–	–	–	–	9.79	1.25	0.053	0.490	0.441
<i>Leuconostocaceae</i>	–	–	–	–	5.52	2.04	0.700	0.691	0.660
<i>Xanthomonadaceae</i>	–	–	–	–	5.32	0.80	0.151	0.243	0.475
<i>Sphingobacteriaceae</i>	3.29 ^b	5.25 ^a	3.22 ^b	4.44 ^{ab}		0.52	0.009	0.087	0.347
<i>Aeromonadaceae</i>	4.79 ^a	3.42 ^b	3.82 ^b	3.57 ^b		0.35	0.018	0.866	0.560
<i>Moraxellaceae</i>	1.88 ^{ab}	1.35 ^b	2.69 ^a	2.01 ^{ab}		0.25	0.014	0.253	0.663
<i>Streptococcaceae</i>	1.85 ^a	1.09 ^b	0.53 ^c	0.79 ^{bc}		0.23	<0.001	0.873	0.691

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and hybrids PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. CON, control (water); INO, inoculant (Biotol Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

^{A,B} Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-c} Means with different superscripts within a row are significantly different ($P \leq 0.05$).

reported *Erwinia* and *Sphingobacterium* in fresh whole-crop corn, but other studies differ in the main genera identified, including *Acinetobacter*, *Klebsiella*, and *Weissella* (Keshri et al., 2018) and *Agrobacterium*, *Microbacterium*, and *Sphingobacterium* (Xu et al., 2019).

Identification of fungi through ITS-detected sequences at d 0 yielded 240 OTUs belonging predominantly to the phyla *Ascomycota* (28.4%), *Basidiomycota* (21.5%), and *Zygomycota* (5.53%), while 44.6% of the sequences belonged to unidentified fungi.

No ADV effects were observed for the major fungal families ($P > 0.05$; Table 3). Incertae sedis *Tremellales* were more abundant in conventional corn hybrids compared to BMR ($\bar{x} = 25.8$ vs. $\bar{x} = 13.9 \pm 3.82\%$ RA; $P < 0.05$) while *Mucoraceae* were more abundant in BMR compared to conventional hybrids ($\bar{x} = 7.52$ vs. $\bar{x} = 3.64 \pm 1.18$; $P < 0.05$). Unidentified fungi were more abundant in MCN and MBR compared to PCN and PBR ($\bar{x} = 50.7$ vs. $\bar{x} = 38.5 \pm 3.06$; $P \leq 0.02$) while the opposite was observed for the unidentified *Ascomycota* ($\bar{x} = 7.45$ vs. $\bar{x} = 18.1 \pm 2.19$; $P \leq 0.01$). More isolation

and genetic identification work needs to be conducted to expand our knowledge of the fungal phyllosphere. Similar results were reported by Romero et al. (2018), who observed a higher abundance of unidentified fungi in MCN vs. other HYB (58.5 vs. \bar{x} = 40.5% RA), an i.s. (Incertae sedis) *Tremellales* in PCN vs. other HYB (21.4 vs. \bar{x} = 9.55) and unidentified *Ascomycota* in MBR and PBR vs. MCN and PCN (\bar{x} = 15.7 vs. \bar{x} = 6.81). Kong et al. (2020) also observed differences in fungal communities between three corn cultivars from different breeding sources that were cultivated in the same experimental field. Research seems to indicate that host plant genotype only contributes 4% to 8% to the variability in bacterial and fungal communities in leaves and to a lower extent in the rhizosphere depending on sampling depth (Wagner et al., 2020). The most common genera at d 0 in this study were *Hannaella* (15.0% RA) for an i.s. *Tremellales*; and *Mucor* (5.58) for *Mucoraceae*. This differs from the genera reported by Keshri et al. (2018) in fresh corn which were predominantly *Meyerozyma* (53.3%), *Candida* (27.7%), and *Cladosporium* (8.2%), most likely by cultivar, agronomic management, and environmental differences. Romero et al. (2018) also described *Meyerozyma*, *Hannaella*, *Bullera*, and *Bulleromyces* as the most abundant genera at d 0, while May et al. (2001) identified *Aspergillus*, *Acremonium*, *Bullera*, and *Mucor* by DGGE. Yeasts belonging to *Hannaella* genus have been previously described as inhabitants of the corn, rice, and sugarcane phyllosphere (Limtong and Nasanit, 2017; Kaewwichian et al., 2015), while *Mucor* are xerophilic filamentous fungi associated with spoilage that can develop in a yeast-like form that allows them to survive in anaerobic conditions (Lane et al., 2018).

Silo opening (100 d)

Nutritional composition

All nutritional composition variables at opening were affected by corn hybrid ($P \leq 0.005$), except WSC ($P = 0.33$; Table 4). As observed at d 0, MBR had the lowest DM relative to all the other hybrids (25.6 vs. \bar{x} = 30.4 \pm 0.376%; $P < 0.001$). Likewise, MBR had the lowest OM concentration (95.5 vs. 96.6–96.9 \pm 0.08% of DM; $P < 0.001$) and the highest CP (9.31 vs. 7.41–7.88 \pm 0.140% of DM; $P < 0.001$), and NDF (40.2 vs. \bar{x} = 38.2 \pm 0.71% of DM; $P \leq 0.05$) when compared to the other hybrids, respectively. Across

hybrids, MBR and MCN had higher levels of $\text{NH}_3\text{-N}$ relative to PCN and PBR (\bar{x} = 11.3 vs. \bar{x} = 9.9% of total N; $P \leq 0.02$). As discussed previously, these differences are the result of the lower maturity of MBR relative to the other hybrids. Inoculation increased $\text{NH}_3\text{-N}$ (10.9% vs. 10.2% of total N; $P < 0.02$), NDF (39.3 vs. 38.1; $P < 0.05$), ADF (23.1 vs. 22.3; $P \leq 0.02$), and OM (96.5% vs. 96.3% of DM basis; $P < 0.001$). In the case of WSC, inoculation decreased it across all hybrids relative to CON but it did so to a larger extent for MBR (0.92 vs. 2.52), when compared to MCN and PBR (\bar{x} = 1.31 vs. \bar{x} = 2.34% of DM, respectively; Hybrid \times ADV, $P < 0.05$). Romero et al. (2018) also observed an increase in $\text{NH}_3\text{-N}$ (9.7% vs. 8.0% of total N) and a decrease in WSC (0.91% vs. 2.03% of DM) but no changes in CP, NDF, and ADF due to inoculation when compared to untreated, respectively. Bernardi et al. (2019) also reported a decrease in WSC (2.07% vs. 2.57% of DM) but no differences in $\text{NH}_3\text{-N}$ (\bar{x} = 4.47% of total N), CP (\bar{x} = 7.84% of DM), OM (\bar{x} = 94.9% of DM), NDF (\bar{x} = 49.7% of DM), and ADF (\bar{x} = 27.4% of DM) in corn silage treated with combination inoculants like the one used in this study relative to untreated in a meta-analysis of 140 articles. However, the same authors observed that $\text{NH}_3\text{-N}$ increased in combination inoculants relative to untreated when incubation temperatures were below 25°C, like in this study. Other studies have suggested that the increased $\text{NH}_3\text{-N}$ in *L. buchneri*-treated silages is associated with an increased final pH (Driehuis et al., 2001; Table 5).

Fermentation measures

The DM recovery was higher for MCN relative to PBR (97.9 vs. 94.7; $P = 0.004$) but PCN and MBR (\bar{x} = 96.7 \pm 1.05%) were no different than MCN and PBR. Romero et al. (2018) reported that MBR and PBR (93.2 and 93.1) had lower DM recovery than MCN and PCN (95.8% and 95.0%, respectively), which is similar to what we observed in the current study with PBR. To the best of our knowledge, no studies have compared isogenic lines of BMR and conventional corn hybrids in terms of DM recovery, but it may be possible that lignification can have an effect on nutrient losses during storage (Miron et al., 2007). Further research is needed to confirm this observation. Inoculation decreased DM recovery (95.6 vs. 97.4 \pm 1.05; $P = 0.02$) in part due

Table 3. Relative abundance (%) of fungal families identified from internal transcribed spacer 1 (ITS-1) region sequences extracted from chopped whole-crop corn as a function of hybrid (HYB) and silage additives (ADV) at d 0¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB \times ADV
Unidentified fungi	50.7 ^a	37.5 ^b	50.6 ^a	39.4 ^b	3.13	0.003	0.681	0.970	
Incertae sedis <i>Tremellales</i>	26.0 ^a	25.6 ^a	14.6 ^b	13.1 ^b	3.91	0.032	0.359	0.466	
Unidentified <i>Ascomycota</i>	5.80 ^b	18.8 ^a	9.10 ^b	17.3 ^a	2.25	<0.001	0.180	0.779	
<i>Mucoraceae</i>	3.12 ^b	4.16 ^b	7.47 ^a	7.56 ^a	1.18	0.016	0.689	0.256	
<i>Nectriaceae</i>	–	–	–	–	3.32	0.494	0.094	0.232	
Unidentified <i>Pleosporales</i>	2.23 ^c	1.59 ^d	3.00 ^b	4.23 ^a	0.191	<0.001	0.165	0.130	
Incertae sedis <i>Saccharomycetales</i>	0.864 ^c	4.93 ^a	0.440 ^c	3.72 ^b	0.425	<0.001	0.256	0.467	
<i>Pleosporaceae</i>	1.04 ^c	0.611 ^c	3.78 ^a	2.73 ^b	0.240	<0.001	0.326	0.896	
Unidentified <i>Dothideomycetes</i>	0.686 ^b	0.632 ^b	1.80 ^a	1.79 ^a	0.123	<0.001	0.339	0.561	
<i>Phaeosphaeriaceae</i>	0.938 ^c	0.551 ^d	1.32 ^b	1.91 ^a	0.127	<0.001	0.353	0.486	
<i>Pichiaceae</i>	0.084 ^b	0.077 ^b	0.054 ^b	0.218 ^a	0.048	0.025	0.964	0.721	

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and hybrids PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. CON, control (water); INO, inoculant (Biotal Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

^{a-d} Means with different superscripts within a row are significantly different ($P \leq 0.05$).

Table 4. Nutritional composition of chopped whole-crop corn as a function of hybrid (HYB) and silage additives (ADV) at d 100¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
DM, %	30.4 ^a	30.5 ^a	25.6 ^b	30.2 ^a	–	0.376	<0.001	0.112	0.694
OM, % of DM	–	–	–	–	–	0.066	<0.001	<0.001	0.088
CON	–	–	–	–	96.3 ^B				
INO	–	–	–	–	96.5 ^A				
Mean	96.7 ^b	96.9 ^a	95.5 ^c	96.6 ^b					
CP, % of DM	7.41 ^c	7.88 ^b	9.31 ^a	7.63 ^{bc}	–	0.140	<0.001	0.673	0.863
NH ₃ -N, % of DM ²	–	–	–	–	–	0.0050	<0.001	0.029	0.057
CON	–	–	–	–	0.132 ^B				
INO	–	–	–	–	0.140 ^A				
Mean	0.134 ^b	0.122 ^c	0.166 ^a	0.122 ^c					
NH ₃ -N, % of total N	–	–	–	–	–	0.43	<0.001	0.020	0.129
CON	–	–	–	–	10.2 ^B				
INO	–	–	–	–	10.9 ^A				
Mean	11.3 ^a	9.7 ^b	11.2 ^a	10.1 ^b					
WSC, % of DM ³	–	–	–	–	–	0.165	0.329	<0.001	0.038
CON	2.16 ^A	2.06 ^A	2.52 ^A	2.52 ^A	2.31				
INO	1.40 ^{B,a}	1.15 ^{B,ab}	0.92 ^{B,b}	1.22 ^{B,a}	1.16				
NDF, % of DM	–	–	–	–	–	0.71	0.005	0.029	0.333
CON	–	–	–	–	38.1 ^B				
INO	–	–	–	–	39.3 ^A				
Mean	38.8 ^{ab}	37.7 ^b	40.2 ^a	38.0 ^b					
ADF, % of DM	–	–	–	–	–	0.44	<0.001	0.013	0.134
CON	–	–	–	–	22.3 ^B				
INO	–	–	–	–	23.1 ^A				
Mean	23.7 ^a	21.6 ^c	23.1 ^{ab}	22.5 ^b					

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). CON, control (water); INO, inoculant (Biotal Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

²NH₃-N, ammonia-N.

³WSC, Water soluble carbohydrates.

^{A,B}Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-c}Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).

to a large increase in acetic acid production when compared to CON (3.44 vs. 1.32 ± 0.346; $P < 0.001$), respectively. Consequently, INO reduced lactic acid concentration relative to CON in PCN (4.28 vs. 6.35), MBR (2.98 vs. 9.06), and PBR (5.42 vs. 7.52), but not in MCN ($\bar{x} = 4.94 \pm 0.592$, respectively; Hybrid × ADV, $P < 0.001$) and consequently decreased the lactic to acetic acid ratio (L:A) compared to CON (1.51 vs. 5.58, respectively; $P < 0.001$). This is explained by *L. buchneri* conversion of lactic acid into acetic acid and 1,2 propanediol (Oude Elferink et al., 2001). Since acetic acid is a weaker acid relative to lactic acid (4.75 and 3.86 pKa, respectively; Bruice, 2004), *L. buchneri* activity also increased the pH of INO-treated MCN, PCN, and MBR relative to CON (3.97, 3.98, and 4.21 vs. $\bar{x} = 3.85$, respectively) but not of PBR ($\bar{x} = 3.89 \pm 0.032$; Hybrid × ADV, $P < 0.001$). Conversely, ethanol concentration was only reduced by INO when compared to CON in PBR (0.83 vs. 1.25, respectively) but not in the other hybrids ($\bar{x} = 0.62 \pm 0.083\%$ of DM; Hybrid × ADV, $P = 0.009$). This may be because untreated PBR had the highest ethanol levels compared to untreated MCN, PCN, and MBR (1.25% vs. 0.63%, 0.70%, and 0.43% of DM, respectively; Hybrid × ADV, $P = 0.009$). Inoculated silages were the only ones with measurable levels of 1,2-propanediol (detection limit was 0.014% of DM) as a result of the metabolic activity of *L. buchneri* on lactic acid. The concentration was especially high in MBR (1.20), but no different than CON in PBR (0.28 ± 0.110% of DM; Hybrid × ADV, $P < 0.001$). Propionic and butyric acids were not detectable in this study for any treatment (<0.014% of DM).

Across corn hybrids, INO increased acetic acid (3.44 vs. 1.32 ± 0.346) and decreased DM recovery (95.6 vs. 97.4 ± 1.05%) and the L:A ratio (1.51 vs. 5.58; $P < 0.001$). Similarly, Romero et al. (2018) reported an increase in acetic acid (1.69 vs. 0.51) and 1,2-propanediol (0.78 vs. 0.01) and a decrease in lactic acid (2.65% vs. 4.82% of DM) and the L:A ratio (1.9 vs. 9.8) but no change on DM recovery ($\bar{x} = 94.3\%$) due to inoculation relative to CON. In the meta-analysis conducted by Bernardi et al. (2019), it was reported that corn silage treated with combination inoculants reduced DM recovery (94.8% vs. 95.8%), and increased acetic acid (1.75 vs. 1.37) and 1,2-propanediol (1.00% vs. 0.153% of DM) but had no effect on pH ($\bar{x} = 3.82$), lactic acid (4.47), and ethanol (8.78) relative to untreated, respectively. However, an increased pH (3.84 vs. 3.81) and acetic acid (2.72 vs. 1.37) and decreased lactic acid concentrations (2.86% vs. 4.22% of DM) were observed in corn silage treated with obligate heterolactic bacteria inoculants relative to untreated (Bernardi et al., 2019). We believe that our results are similar to the obligate heterolactic bacteria inoculant group in Bernardi et al. (2019) because the acetic acid concentration for INO in our study was even higher (3.44% of DM) and would have released even more CO₂ to the atmosphere.

Microbial population and aerobic stability

We found no corn hybrid effects on LAB ($\bar{x} = 8.18 \pm 0.288$), yeast ($\bar{x} = 3.81 \pm 0.446$), and mold counts ($\bar{x} = 2.24 \pm 0.614$ log cfu/g of FW) at silo opening ($P > 0.09$; Table 6). Addition of INO increased

Table 5. Fermentation measures of chopped whole corn as a function of hybrid (HYB) and silage additives (ADV) at d 100¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
DM recovery, %						1.05	0.035	0.016	0.820
CON	–	–	–	–	97.4 ^A				
INO	–	–	–	–	95.6 ^B				
Mean	97.9 ^a	96.8 ^{ab}	96.5 ^{ab}	94.7 ^b					
pH						0.032	<0.001	<0.001	<0.001
CON	3.86 ^B	3.84 ^B	3.84 ^B	3.85					
INO	3.97 ^{A,b}	3.98 ^{A,b}	4.21 ^{A,a}	3.93 ^b					
Lactic acid (L), % of DM						0.592	0.058	<0.001	<0.001
CON	5.53 ^c	6.35 ^{A,bc}	9.06 ^{A,a}	7.52 ^{A,ab}					
INO	4.35 ^{ab}	4.28 ^{B,ab}	2.98 ^{B,b}	5.42 ^{B,a}					
Acetic acid (A), % of DM						0.346	0.142	<0.001	0.323
CON	–	–	–	–	1.32 ^B				
INO	–	–	–	–	3.44 ^A				
1,2-propanediol, % of DM						0.110	<0.001	<0.001	<0.001
CON	0.00 ^B	0.00 ^B	0.00 ^B	0.00					
INO	0.49 ^{A,b}	0.45 ^{A,b}	1.20 ^{A,a}	0.28 ^b					
Ethanol (E), % of DM						0.083	<0.001	0.338	0.009
CON	0.63 ^b	0.70 ^b	0.43 ^c	1.25 ^{A,a}					
INO	0.65 ^{a,b}	0.79 ^a	0.52 ^b	0.83 ^{B,a}					
L:A ratio ²						0.522	0.972	<0.001	0.115
CON	–	–	–	–	5.58 ^A				
INO	–	–	–	–	1.51 ^B				
L:(A+OH) ratio ³						0.290	0.450	<0.001	<0.001
CON	3.13 ^{A,b}	3.58 ^{A,b}	4.56 ^{A,a}	2.84 ^{A,b}					
INO	1.28 ^B	0.84 ^B	0.55 ^B	1.40 ^B					

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). CON, control (water); INO, inoculant (Biotol Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

²L, lactic acid; A, acetic acid.

³L, lactic acid; A, acetic acid; OH, ethanol plus 1,2-propanediol.

^{A,B}Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-c}Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).

LAB (9.30 vs. 7.05 ± 0.288 log cfu/g of FW) and decreased yeast (3.05 vs. 4.58 ± 0.446 log cfu/g of FW) and mold counts (1.51 vs. 2.96 ± 0.614 log cfu/g of FW) when compared to CON across all hybrids ($P < 0.002$). The reduction in yeasts and molds resulted in an extended aerobic stability across all hybrids when INO was applied relative to CON (582 vs. 111 ± 128 h; $P < 0.001$), because of an increase in acetic acid concentration which acts as an antifungal agent. All INO hybrids were aerobically stable for more than 168 h, which is the minimum recommended time to keep silages aerobically stable (Wilkinson and Davies, 2013). Bernardi et al. (2019) also reported an increase in LAB (6.87 vs. 6.70) and a decrease in yeast counts (2.89 vs. 4.01 log cfu/g of FW) that led to an extended aerobic stability (77.8 vs. 67.7 h) in corn silage treated with combination inoculants relative to untreated. Romero et al. (2018) also reported no effects of hybrid on LAB ($\bar{x} = 6.43$), yeast ($\bar{x} = 4.46$), and mold counts ($\bar{x} = 0.42$ log cfu/g of FW) after 100 d of ensiling and at 36.1% to 42.7% DM. In that study, inoculation also increased LAB (6.59 vs. 6.26) and decreased yeast counts relative to untreated (3.78 vs. 5.13 log cfu/g of FW), respectively, but had no effects on mold counts possibly due to lower production of acetic acid ($\bar{x} = 1.69\%$ of DM) in inoculated silages relative to this study. This decrease in yeast counts resulted also in an extended aerobic stability for all inoculated hybrids compared to untreated (217 vs. 34.7, respectively), except for MBR ($\bar{x} = 49$ h; Romero et al., 2018). Key differences explain the success of INO in preserving

MBR in this study relative to Romero et al. (2018). First, our INO-treated MBR had 3.97% of DM acetic acid compared to the 1.60% of DM found in Romero et al. (2018), which in part is explained by the differences in DM for MBR between our current study and Romero et al. (2018; 25.6% vs. 36.1%, respectively). Concentrations of acetic acid above 3% of DM typically ensure adequate aerobic stability due to antifungal activity (Kung et al., 2018). Second, in spite of both studies having the same ensiling time (100 d) and incubation temperature (23°C), higher residual lactic acid levels were observed in the INO-treated MBR relative to the other INO-treated hybrids (4.63 vs. $\bar{x} = 1.99\%$ of DM, respectively) in Romero et al. (2018), while in this study INO-treated MBR (2.98% of DM) was no different than INO-treated MCN and PCN and even lower than PBR. Third, we speculate that the high abundance of *Leuconostocaceae* in the untreated MBR relative to the other untreated hybrids at d 100 in Romero et al. (28.7 vs. 9.72 RA%; 2018) seems to suggest that *L. buchneri* may have had a much more stringent competition from other bacteria which may have affected its ability to convert lactic acid to acetic acid efficiently. Such difference in the RA of *Leuconostocaceae* was not observed for MBR in this study (Table 7). Furthermore, *Leuconostocaceae* members such as *Leuconostoc mesenteroides* are known to produce bacteriocins that affect the growth of other lactic acid bacteria (Mataragas et al., 2002).

We found that INO reduced bacterial observed OTUs (66 vs. 226 ± 10.7) and phylogenetic diversity (6.75 vs. 14.4 ± 0.60;

Table 6. Microbial counts and aerobic stability of chopped whole corn as a function of hybrid (HYB) and silage additives (ADV) at d 100¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
Lactic acid bacteria, log cfu/g of FW ²						0.288	0.121	<0.001	0.387
CON	–	–	–	–	7.05 ^B				
INO	–	–	–	–	9.30 ^A				
Yeast, log cfu/g of FW						0.446	0.103	<0.001	0.456
CON	–	–	–	–	4.58 ^A				
INO	–	–	–	–	3.05 ^B				
Molds, log cfu/g of FW						0.614	0.088	0.002	0.631
CON	–	–	–	–	2.96 ^A				
INO	–	–	–	–	1.51 ^B				
Aerobic stability, h						128	0.078	<0.001	0.439
CON	–	–	–	–	111 ^B				
INO	–	–	–	–	582 ^A				

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). MBR and PBR are brown midrib hybrids. CON, control (water); INO, inoculant (Biotal Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788, and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

²FW, fresh corn

^{A,B}Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

$P < 0.001$) compared to CON but increased fungal observed OTUs vs. CON (46 vs. 20 ± 2.95 ; $P < 0.01$). These results suggest that INO reduces the bacterial and increases fungal diversity consistently across all ensiled hybrids tested in this study even though richness differences between hybrids were observed at d 0. Likewise, Romero et al. (2018) also observed decreased bacterial and increased fungal OTUs for INO vs. CON. By evaluating Heip's evenness index we observed that fungal OTUs abundance was more evenly distributed in INO compared to CON (0.093 vs. 0.042 ± 0.011 ; $P = 0.003$). Conversely, bacterial OTUs abundance was more evenly distributed in CON vs. INO-treated hybrids ($\bar{x} = 0.030$ vs. $\bar{x} = 0.011 \pm 0.004$). Furthermore, within CON hybrids, PBR was more even relative to MBR (0.044 vs. 0.019; HYB × ADV; $P = 0.009$). Further research should be conducted to understand the effects of inoculants on silage fungal diversity. The weighted UniFrac (Figure 1) and Bray–Curtis (Figure 2) principal coordinates analysis plots indicated a separation and difference in the structure of the bacterial ($P = 0.001$) and fungal ($P = 0.001$) communities between the CON and INO at d 100, and between d 0 and d 100 ($P = 0.001$), according to analysis of similarities test of distance metrics. This same trend was also observed by Romero et al. (2018) for both bacterial and fungal communities. INO treatment increased *Lactobacillaceae* RA compared to CON (99.2 vs. 75.7 ± 2.98 ; $P < 0.001$) and decreased *Enterobacteriaceae* (0.277 vs. 9.93 ± 1.28 ; $P < 0.001$), *Lachnospiraceae* (0.034 vs. 1.71 ± 0.576 ; $P < 0.05$), *Xanthomonadaceae* (0.031 vs. 0.980 ± 0.124 ; $P < 0.001$), *Moraxellaceae* (0.025 vs. 1.01 ± 0.153 ; $P < 0.001$), and *Paenibacillaceae* (0.019 vs. 1.34 ± 0.647 ; $P < 0.05$). INO had a lower RA of *Leuconostocaceae* vs. CON only for PBR (0.21 vs. 14.5) but not for other HYB ($\bar{x} = 0.21$ vs. $\bar{x} = 2.70 \pm 1.87$; HYB × INO, $P = 0.003$). Similarly, Romero et al. (2018) found an increased *Lactobacillaceae* RA in INO-treated silages compared with CON ($\bar{x} = 98.9$ vs. $\bar{x} = 52.7\%$), especially for MBR, which had a lower RA in untreated silages compared with the other untreated hybrids (34.3 vs. $\bar{x} = 58.9$). The RA of *Enterobacteriaceae* was also decreased by the application of INO compared to CON ($\bar{x} = 23.5$ vs. $\bar{x} = 0.614\%$) in that study. The major bacterial genera identified

at d 100, were *Lactobacillus* and *Pediococcus* for *Lactobacillaceae*; unidentified genus and *Cronobacter* for *Enterobacteriaceae*, and unidentified genus and *Leuconostoc* for *Leuconostocaceae*. The high abundance of *Enterobacteriaceae* at d 0 is expectedly replaced by *Lactobacillaceae* in grass silages (Borreani et al., 2018), especially consisting of the *Lactobacillus* genus, as reported by various authors by NGS in treated and untreated whole-crop corn silage (Keshri et al., 2018; Xu et al., 2019; Guan et al., 2020). The selection and proliferation of *Lactobacillaceae* in corn silage can occur as early as 3 h after ensiling (Keshri et al., 2018) and reach a relative abundance of 79.7% to 96.1% after 3 days (Gharechahi et al., 2017). At the genus level, Keshri et al. (2018) described an initial dominance of *Weissella*, *Acinetobacter*, and *Pantoea* until d 1 of ensiling, when dominance is overtaken by *Lactobacillus* in untreated silages and to a larger extent in silages treated with *L. plantarum*. Microbial dynamics during the first days of ensiling are critical for the development of good quality silage because lactic acid bacteria compete with enterobacteria for nutrients (Pahlow et al., 2003). Inoculants combining *P. pentosaceus* and *L. buchneri* have shown rapid pH declines and dominance of the early fermentation stages in silages compared to untreated silages (Muck et al., 2018). These results reinforce the knowledge that LAB inoculants improve the quality and aerobic stability of corn silage by enhancing the microbial composition and limiting the growth of undesirable microorganisms (Blajman et al., 2018; Xu et al., 2019). It is also noticeable that the *Lactobacillaceae* RA of untreated silages in this study is higher and *Enterobacteriaceae* RA is lower than for Romero et al. (2018) at d 100, which could be attributed to the differences in silage DM content, phyllosphere, and agronomic management, between these studies. This was also observed by McEniry et al. (2010) who evaluated microbial counts and reported a higher rate of increase of lactic acid bacteria (4.6 log CFU/g of herbage increase in 14 d) and rate of decrease of enterobacteria (3.04 decrease in 14 d) in low-DM (18.5%) vs. high-DM (40.6%; 1.9 increase and 2.5 decrease in 14 d, respectively) perennial ryegrass silages. The presence of *Clostridiaceae* in silages is undesirable due to

Table 7. Relative abundance (%) of bacterial families identified from 16S ribosomal DNA sequences extracted from chopped whole-crop corn as a function of hybrid (HYB) and silage additives (ADV) at d 100¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
<i>Lactobacillaceae</i>						3.09	0.164	<0.001	0.174
CON	–	–	–	–	75.7 ^B				
INO	–	–	–	–	99.2 ^A				
<i>Enterobacteriaceae</i>						1.32	0.686	<0.001	0.753
CON	–	–	–	–	9.93 ^A				
INO	–	–	–	–	0.277 ^B				
<i>Leuconostocaceae</i>						1.87	0.003	<0.001	0.003
CON	3.37 ^b	3.19 ^b	1.55 ^b	14.52 ^{A,a}					
INO	0.298	0.164	0.159	0.209 ^B					
<i>Lachnospiraceae</i>						0.60	0.315	0.047	0.333
CON	–	–	–	–	1.71 ^A				
INO	–	–	–	–	0.034 ^B				
<i>Xanthomonadaceae</i>						0.128	0.381	<0.001	0.387
CON	–	–	–	–	0.980 ^A				
INO	–	–	–	–	0.031 ^B				
<i>Moraxellaceae</i>						0.158	0.135	<0.001	0.135
CON	–	–	–	–	1.01 ^A				
INO	–	–	–	–	0.025 ^B				
<i>Paenibacillaceae</i>						0.655	0.047	0.049	0.051
CON	–	–	–	–	1.34 ^A				
INO	–	–	–	–	0.019 ^B				
Mean	0.083 ^b	0.141 ^b	0.136 ^b	2.35 ^a					
<i>Clostridiaceae</i>	–	–	–	–	0.252	0.329	0.377	0.196	0.314

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and hybrids PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). CON, control (water); INO, inoculant (Biotol Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

^{A,B}Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-d}Means with different superscripts within a row are significantly different ($P \leq 0.05$).

their capacity to produce butyric acid and promote the growth of spoilage microorganisms (Duniere et al., 2017), nevertheless, minimal abundance of *Clostridiaceae* (0.252% RA) was found in this study most likely due to the low pH typical of whole-crop corn silage.

For fungi, we observed a higher RA of phylum *Ascomycota* (83.5), followed by a reduced presence of *Basidiomycota* (12.2) and *Zygomycota* (1.7%). INO decreased *Monascaceae* vs. CON (12.6 vs. 44.7 ± 8.27 ; $P = 0.006$) as well as an i.s. *Tremellales* (0.076 vs. 0.903 ± 1.311 ; $P = 0.002$), but increased an i.s. *Saccharomycetales* (6.39 vs. 0.250 ± 1.72 ; $P < 0.001$), unidentified fungi (2.04 vs. 0.044 ± 0.60 ; $P < 0.001$), and *Pichiaceae* (0.305 vs. 0.056 ± 0.090 ; $P = 0.005$; Table 8). A significant HYB effect was observed for *Saccharomycetaceae* ($P < 0.02$), due to a lower RA for MBR (9.95) compared to the other corn hybrids ($\bar{x} = 51.1\%$). A HYB × ADV interaction was observed for *Mucoraceae* and unidentified *Pleosporales* because the application of INO increased their RA compared to CON for PCN (3.31 vs. 0.020 and 1.004 vs. 0.011) and MBR (9.75 vs. 0.131 and 5.87 vs. 0.057) but not for MCN ($\bar{x} = 1.03$ and $\bar{x} = 0.362$) and PBR ($\bar{x} = 1.09$ and $\bar{x} = 0.163\%$, respectively; $P < 0.022$). The most abundant fungal genera at d 100 were *Kazachstania* for *Saccharomycetaceae*; *Monascus* for *Monascaceae*; *Hannaella* for an i.s. *Tremellales*; and *Candida* for an i.s. *Saccharomycetales*. The overall results show that INO reduces overall counts of yeasts and molds (Table 6) but increases their diversity. Most molds will not tolerate low pH and low oxygen concentrations, nevertheless, yeasts can remain active due to their tolerance to low pH

and their ability to ferment sugars to ethanol, allowing them to survive the ensiling process and initiate aerobic spoilage when the silos are opened (Ávila and Carvalho, 2020). Among these, *Saccharomycetales* have been reported to dominate over other fungi in oat, triticale, and intercropped silages at d 90, especially genera *Kazachstania* and *Pichia* (Duniere et al., 2017). The fungal community in the current study differed from Romero et al. (2018) who reported *Debaryomycetaceae*, *Pichiaceae*, and an i.s. *Saccharomycetales* as the dominant fungal families in whole-crop low moisture corn silages at d 100, with a significant decrease of *Pichiaceae* and an i.s. *Saccharomycetales*, and an increase of *Debaryomycetaceae* in INO-treated silages compared to CON. The differences in fungal communities at d 100 between these studies may have been influenced by the differences in their respective initial fungal communities, and differences between RA of unknown sequences at d 0. The effects of yeasts present in silages is dependent on their species and metabolism (Pahlow et al., 2003). Keshri et al., (2018) reported a dominance of *Candida* genus in 90 d corn silages, which increased with the application of a homofermentative *L. plantarum* inoculant compared to the control (96.5% vs. 74.1% RA) and was associated with a decrease in aerobic stability. This may be due to the higher levels of lactic acid, which enhances the growth of lactate-assimilating *Candida*. Conversely, inoculation with *L. buchneri* increases the aerobic stability of silages because of the reduced growth of these aerobic spoilage yeasts by the presence of acetic acid (Muck et al., 2018), as observed in this study.

Table 8. Relative abundance (%) of fungal families identified from internal transcribed spacer 1 (ITS-1) region sequences extracted from chopped whole-crop corn as a function of hybrid (HYB) and silage additives (ADV) at d 100¹

Item	Hybrid				Mean	SEM	P-value		
	MCN	PCN	MBR	PBR			HYB	ADV	HYB × ADV
Saccharomycetaceae	53.0 ^a	47.6 ^a	9.95 ^b	52.7 ^a		10.8	0.016	0.228	0.887
Monasaceae						8.27	0.911	0.006	0.369
CON	–	–	–	–	44.7 ^A				
INO	–	–	–	–	12.6 ^B				
Incertae sedis Tremellales						2.32	0.135	0.002	0.907
CON	–	–	–	–	1.19 ^A				
INO	–	–	–	–	8.0 ^B				
Incertae sedis Saccharomycetales						1.72	0.290	<0.001	0.554
CON	–	–	–	–	0.250 ^B				
INO	–	–	–	–	6.39 ^A				
Unidentified fungi						0.60	0.075	<0.001	0.485
CON	–	–	–	–	0.044 ^B				
INO	–	–	–	–	2.04 ^A				
Mucoraceae						1.61	0.018	<0.001	0.022
CON	0.036	0.020 ^B	0.131 ^B	0.032					
INO	2.03 ^b	3.31 ^{A,b}	9.75 ^{A,a}	2.15 ^b					
Unidentified Ascomycota					1.65	2.75	0.349	0.197	0.350
Unidentified Pleosporales						0.41	<0.001	<0.001	<0.001
CON	0.054	0.011 ^B	0.057 ^B	0.015					
INO	0.670 ^b	1.004 ^{A,b}	5.87 ^{A,a}	0.311 ^b					
Pichiaceae						0.090	0.742	0.005	0.459
CON	–	–	–	–	0.056 ^B				
INO	–	–	–	–	0.305 ^A				

¹MCN, PCN, MBR, and PBR corn hybrids are TMF2R737, P2089YHR, F2F817, and P1449XR, respectively. Hybrids MCN and MBR are property of Mycogen Seeds (Indianapolis, IN) and hybrids PCN and PBR are property of Pioneer Hi-Bred International (Johnston, IA). CON, control (water); INO, inoculant (Biotol Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) delivering *L. buchneri* 40788 and *P. pentosaceus* at 400,000 and 100,000 cfu/g of fresh corn, respectively.

^{A,B}Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-d}Means with different superscripts within a row are significantly different ($P \leq 0.05$).

Relationship between microbial taxonomic profiles and silage quality variables at silo opening

The results of the association analysis between microbiota family's RA and silage quality measures at d 100 are presented in Table 9. Only those associations with a P -value ≤ 0.01 are reported. The association analysis was conducted separately for CON and INO samples because these are 2 distinct environments and to allow for the evaluation of the associations between microbiota and silage quality without the overriding the effects of inoculation. We found 17 significant associations for CON and 23 for INO. In CON samples, LAB counts increased at high RA of *Lactobacillaceae* relative to low and medium RA ($P < 0.003$), and at low RA of *Enterobacteriaceae*, *Xanthomonadaceae*, *Leuconostocaceae*, and *Moraxellaceae* relative to their medium and high RA at d 100 ($P < 0.004$). Similarly, the concentration of $\text{NH}_3\text{-N}$ (% of total N) was reduced when the RA of *Lactobacillaceae* was high compared to its low and medium RA, and at a low RA of *Leuconostocaceae* relative to its medium and high RA at d 100 ($P = 0.002$). Lactic acid concentration was increased when *Leuconostocaceae* and *Moraxellaceae* RA at d 100 was high relative to the low category ($P < 0.005$). The most abundant genus for *Moraxellaceae* was *Acinetobacter*, a ubiquitous taxa of Gram-negative nonfermenting coccobacilli that is known to have several species of animal and human pathogens and that can be found in fields with previous manure applications or animal pressure (Al Atrouni et al., 2016) and that has been reported in several silage studies (Liu et al.,

2019). In this study, *Moraxellaceae* RA was correlated with key response variables of CON but its role in silage production is unclear and remains to be elucidated.

For INO samples, LAB counts also increased at a high RA of *Lactobacillaceae* relative to its low RA ($P < 0.007$) and at low RA of *Moraxellaceae* relative to its high RA at d 100 ($P < 0.009$). A high RA of *Clostridiaceae* increased mold counts ($P = 0.004$) and ethanol concentration ($P = 0.01$), and decreased DM recovery ($P = 0.005$) relative to low RA at d 100, which reaffirms the undesirable effects of this bacterial family on silage quality (Kung et al., 2018). High RA of *Pichiaceae* at d 0 and at d 100 were both correlated with increased lactic acid concentration and decreased the pH at d 100 relative to low RA ($P < 0.009$). Moreover, the concentration of WSC increased at high RA of *Pichiaceae* and *Trichosporonaceae* relative to low RA at d 100 ($P < 0.007$). Beneficial effects of *Pichia* sp. on feed preservation under air-tight conditions have been reported (Druevofors et al., 2005; Petersson et al., 1999) and this yeast family is commonly found in silages due to their lactate-assimilating capacity and tolerance to low pH (Ávila and Carvalho, 2020).

Summary and Conclusions

At low DM concentrations (26.3% to 31.5% DM), the combination inoculant tested extended severalfold the aerobic stability of the conventional and BMR whole-crop corn hybrids evaluated

Table 9. Effect of microbiota families relative abundance on quality measures of chopped whole corn at d 100

Item	Family and day	Families relative abundance ¹			P-value
		Low	Medium	High	
CON samples					
Lactic acid bacteria, log cfu/g FW ²	Unidentified <i>Dothideomycetes</i> , 0 d	0.320 ^a	0.666 ^a	-0.655 ^b	0.008
L:A ratio	Unidentified <i>Dothideomycetes</i> , 0 d	-0.679 ^a	-0.838 ^a	1.399 ^b	0.001
L:A ratio	<i>Nectriaceae</i> , 0 d	-0.930 ^a	-0.274 ^{ab}	1.227 ^b	0.006
NH ₃ -N, % of total N	<i>Leuconostocaceae</i> , 0 d	0.003 ^a	-0.007 ^b	0.005 ^a	0.005
Yeast, log cfu/g FW ²	<i>Sphingomonadaceae</i> , 0 d	0.789 ^a	-0.589 ^b	-0.200 ^{ab}	0.008
Lactic acid bacteria, log cfu/g FW ²	<i>Enterobacteriaceae</i> , 100 d	0.855 ^a	-0.372 ^b	-0.540 ^b	0.004
Lactic acid bacteria, log cfu/g FW ²	<i>Lactobacillaceae</i> , 100 d	-0.542 ^a	-0.377 ^a	0.862 ^b	0.003
Lactic acid bacteria, log cfu/g FW ²	<i>Leuconostocaceae</i> , 100 d	0.884 ^a	0.085 ^b	-1.026 ^c	0.000
Lactic acid bacteria, log cfu/g FW ²	<i>Moraxellaceae</i> , 100 d	0.946 ^a	-0.017 ^b	-0.986 ^c	0.000
Lactic acid bacteria, log cfu/g FW ²	Unidentified <i>Ascomycota</i> , 100 d	0.091 ^{ab}	0.628 ^a	-0.717 ^b	0.008
Lactic acid bacteria, log cfu/g FW ²	<i>Xanthomonadaceae</i> , 100 d	0.876 ^a	-0.077 ^b	-0.856 ^b	0.000
Lactic acid (L), % of DM	<i>Leuconostocaceae</i> , 100 d	-0.934 ^a	0.182 ^{ab}	0.992 ^b	0.004
Lactic acid (L), % of DM	<i>Moraxellaceae</i> , 100 d	-0.887 ^a	0.090 ^{ab}	1.037 ^b	0.005
NH ₃ -N, % of total N	<i>Lactobacillaceae</i> , 100 d	0.004 ^a	0.006 ^a	-0.007 ^b	0.001
NH ₃ -N, % of total N	<i>Leuconostocaceae</i> , 100 d	-0.007 ^a	0.004 ^b	0.006 ^b	0.002
pH	<i>Moraxellaceae</i> , 100 d	0.046 ^a	-0.010 ^b	-0.040 ^b	0.002
NDF, % of DM	<i>Moraxellaceae</i> , 100 d	-0.331 ^a	1.071 ^b	-0.969 ^a	0.003
INO samples					
Acetic acid (A), % of DM	<i>Bruceaceae</i> , 0 d	-0.391 ^a	1.193 ^b	-0.344 ^a	0.007
Lactic acid (L), % of DM	<i>Pichiaceae</i> , 0 d	-1.084 ^a	-0.030 ^{ab}	1.114 ^b	0.009
L:A ratio	<i>Bruceaceae</i> , 0 d	0.442 ^a	-0.879 ^b	0.212 ^a	0.008
L:A ratio	<i>Alcaligenaceae</i> , 0 d	0.257 ^a	-0.894 ^b	0.409 ^a	0.007
L:(A+OH) ratio ³	<i>Bruceaceae</i> , 0 d	0.276 ^a	-0.519 ^b	0.125 ^a	0.007
L:(A+OH) ratio ³	<i>Alcaligenaceae</i> , 0 d	0.135 ^a	-0.512 ^b	0.260 ^a	0.009
pH	<i>Pichiaceae</i> , 0 d	0.070 ^a	0.001 ^{ab}	-0.070 ^b	0.004
OM, % of DM	SUP05, 0 d	-0.077 ^a	-0.031 ^a	0.104 ^b	0.004
ADF, % of DM	<i>Comamonadaceae</i> , 0 d	-0.883 ^a	0.608 ^b	0.248 ^b	0.002
DM recovery, %	<i>Clostridiaceae</i> , 100 d	2.063 ^a	-0.522 ^b	-1.541 ^b	0.005
Molds, log cfu/g FW	<i>Clostridiaceae</i> , 100 d	-1.312 ^a	0.398 ^b	0.914 ^b	0.004
Lactic acid bacteria, log cfu/g FW ²	<i>Lactobacillaceae</i> , 100 d	-0.188 ^a	-0.008 ^{ab}	0.196 ^b	0.007
Lactic acid bacteria, log cfu/g FW ²	<i>Moraxellaceae</i> , 100 d	0.209 ^a	-0.049 ^{ab}	-0.160 ^b	0.009
Lactic acid (L), % of DM	<i>Pichiaceae</i> , 100 d	-1.318 ^a	-0.001 ^{ab}	1.350 ^b	0.002
Ethanol, % of DM	<i>Clostridiaceae</i> , 100 d	-0.090 ^a	-0.058 ^a	0.148 ^b	0.010
Ethanol, % of DM	<i>Phaeosphaeriaceae</i> , 100 d	-0.044 ^a	0.152 ^b	-0.108 ^a	0.005
pH	<i>Pichiaceae</i> , 100 d	0.078 ^a	-0.023 ^b	-0.088 ^b	0.000
pH	<i>Trichosporonaceae</i> , 100 d	0.027 ^a	0.057 ^a	-0.094 ^b	0.001
WSC, % of DM ⁴	<i>Pichiaceae</i> , 100 d	-0.157 ^a	0.082 ^{ab}	0.206 ^b	0.007
WSC, % of DM ⁴	<i>Trichosporonaceae</i> , 100 d	-0.082 ^a	-0.104 ^a	0.263 ^b	0.002
DM, %	Unidentified <i>Fungi</i> , 100 d	0.725 ^a	-0.169 ^{ab}	-0.556 ^b	0.005
CP, % of DM	<i>Mucoraceae</i> , 100 d	0.133 ^a	0.129 ^a	-0.321 ^b	0.004
CP, % of DM	<i>Incertae sedis Sporidiobolales</i> , 100 d	-0.033 ^{ab}	0.278 ^a	-0.263 ^b	0.007

¹Microbiota relative abundance was classified into three categories as separated by the 34th and 67th percentiles and tested via least squares analysis with pairwise contrasts. Coefficients with different letters within a row are different, $P \leq 0.05$.

²FW, fresh corn.

³OH, Ethanol plus 1,2-propanediol.

⁴WSC, water soluble carbohydrates.

in laboratory silos by increasing the acetic acid concentration above 3% of DM at the expense of lactic acid. This in turn decreased the yeast and mold counts after 100 d of ensiling but also increased fungal OTU richness and evenness across all hybrids relative to untreated hybrids at d 100. Our study validates that the inoculant evaluated consistently favors certain fungal taxa, including *Mucoraceae*. Simultaneously, an increased dominance of *Lactobacillaceae* at the expense of *Enterobacteriaceae*, among other undesirable bacteria, was observed in INO-treated silages relative to untreated.

Consequently, INO decreased bacterial taxonomic richness and evenness across all hybrids. All these changes due to inoculation occurred in spite of divergent fungal populations across hybrids at d 0, especially for the BMR hybrids. Also, the high production of acetic acid in this study contributed to the decrease in DM recovery across all INO-treated silages. Unveiled correlations with the metadata involving *Pichiaceae*, *Clostridiaceae*, and *Moraxellaceae*, among others implicate novel players and roles that could help explain variable inoculation effects on silage preservation across the literature.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgments

Funding contribution was provided by the North Carolina Dairy Producers Association (Raleigh, NC) and the inoculant was donated by Lallemand Animal Nutrition (Milwaukee, WI). We acknowledge the outstanding work of M. Dalman and A. Heitman (NC State), Carrie Brinton (Official Variety Testing Program at NC State), and the staff from the NCDCA Mountain Research Station (Waynesville, NC). This project was also supported by the USDA National Institute of Food and Agriculture Hatch Project number ME0-21917 through the Maine Agricultural and Forest Experiment Station (Publication number 3823).

Conflict of interest statement

The authors have not stated any conflicts of interest.

References

- Al Atrouni, A., M. L. Joly-Guillou, M. Hamze, and M. Kempf. 2016. Reservoirs of Non-baumannii Acinetobacter Species. *Front. Microbiol.* 7:49. doi:10.3389/fmicb.2016.00049.
- Allen, M. S., J. G. Coors, and G. W. Roth. 2003. *Corn silage, silage science and technology*. Madison (WI): American Society of Agronomy, Crop Science Society; p 547–608.
- Arriola, K. G., S. C. Kim, and A. T. Adesogan. 2011. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. *J. Dairy Sci.* 94(3):1511–1516. doi:10.3168/jds.2010-3807.
- Arriola, K. G., S. C. Kim, C. M. Huisden, and A. T. Adesogan. 2012. Stay-green ranking and maturity of corn hybrids: 1. Effects on dry matter yield, nutritional value, fermentation characteristics, and aerobic stability of silage hybrids in Florida. *J. Dairy Sci.* 95(2):964–974. doi:10.3168/jds.2011-4524.
- Ávila, C. L. S., and B. F. Carvalho. 2020. Silage fermentation-updates focusing on the performance of micro-organisms. *J. Appl. Microbiol.* 128:966–984. doi:10.1111/jam.14450.
- Basso, F. C., T. F. Bernardes, A. P. D. T. P. Roth, B. N. Lodo, T. T. Berchielli, and R. A. Reis. 2012. Fermentation and aerobic stability of corn silage inoculated with *Lactobacillus buchneri*. *Rev. Bras. Zootec.* 41(7):1789–1794. doi:10.1590/S1516-35982012000700032.
- Bernardi, A., C. J. Härter, A. W. L. Silva, R. A. Reis, and C. H. S. Rabelo. 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. *Grass Forage Sci.* 74(4):596–612. doi:10.1111/gfs.12452.
- Blajman, J. E., R. B. Páez, C. G. Vinderola, M. S. Lingua, and M. L. Signorini. 2018. A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage. *J. Appl. Microbiol.* 125(6):1655–1669. doi:10.1111/jam.14084.
- Bokulich, N. A., and D. A. Mills. 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Appl. Environ. Microbiol.* 79(8):2519–2526. doi:10.1128/aem.03870-12.
- Borreani, G., E. Tabacco, R. J. Schmidt, B. J. Holmes, and R. E. Muck. 2018. Silage review: factors affecting dry matter and quality losses in silages. *J. Dairy Sci.* 101:3952–3979. doi:10.3168/jds.2017-13837.
- Bruice, P. Y. 2004. *Organic chemistry*. Upper Saddle River (NJ): Pearson/Prentice Hall.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci.* 108(Supplement 1):4516–4522. doi:10.1073/pnas.100080107.
- Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, and J. M. Tiedje. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* 37(suppl_1):D141–D145. doi:10.1093/nar/gkn879.
- Comino, L., E. Tabacco, F. Righi, A. Revello-Chion, A. Quarantelli, and G. Borreani. 2014. Effects of an inoculant containing a *Lactobacillus buchneri* that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. *Anim. Feed Sci. Technol.* 198:94–106. doi:10.1016/j.anifeedsci.2014.10.001.
- Darby, H. M., and J. G. Lauer. 2002. Harvest date and hybrid influence on corn forage yield, quality, and preservation. *Agron. J.* 94(3):559–566. doi:10.2134/agronj2002.5590.
- Dowd, P. F., D. L. Funnell-Harris, and S. E. Sattler. 2016. Field damage of sorghum (*Sorghum bicolor*) with reduced lignin levels by naturally occurring insect pests and pathogens. *J. Pest Sci.* 89(4):885–895. doi:10.1007/s10340-015-0728-1.
- Driehuis, F., S. J. W. H. Oude Elferink, and P. G. Van Wijkelaar. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass Forage Sci.* 56(4):330–343. doi:10.1046/j.1365-2494.2001.00282.x.
- Drouin, P., J. Tremblay, and F. Chaucheyras-Durand. 2019. Dynamic succession of microbiota during ensiling of whole plant corn following inoculation with *Lactobacillus buchneri* and *Lactobacillus hilgardii* alone or in combination. *Microorganisms* 7(12):595.
- Druvefors, U. A., V. Passoth, and J. Schnürer. 2005. Nutrient effects on biocontrol of *Penicillium roqueforti* by *Pichia anomala* J121 during airtight storage of wheat. *Appl. Environ. Microbiol.* 71:1865–1869. doi:10.1128/AEM.71.4.1865-1869.2005.
- Dunier, L., S. Xu, J. Long, C. Elekwachi, Y. Wang, K. Turkington, R. Forster, and T. A. McAllister. 2017. Bacterial and fungal core microbiomes associated with small grain silages during ensiling and aerobic spoilage. *BMC Microbiol.* 17:50. doi:10.1186/s12866-017-0947-0.
- Filya, I., E. Sucu, and A. Karabulut. 2006. The effect of *Lactobacillus buchneri* on the fermentation, aerobic stability and ruminal degradability of maize silage. *J. Appl. Microbiol.* 101:1216–1223. doi:10.1111/j.1365-2672.2006.03038.x.
- Fox, J., and S. Weisberg. 2018. *An R companion to applied regression*. Thousand Oaks, CA: Sage.
- Freitas, J. R. D., J. J. Schoenau, S. M. Boyetchko, and S. A. Cyrenne. 2003. Soil microbial populations, community composition, and activity as affected by repeated applications of hog and cattle manure in eastern Saskatchewan. *Can. J. Microbiol.* 49(9):538–548. doi:10.1139/w03-069 %M 14608420.
- Gerlach, K., F. Roß, K. Weiß, W. Büscher, and K.-H. Südekum. 2013. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. *Agric. Food Sci.* 22(1):168–181. doi:10.23986/afsci.6739.
- Gharechahi, J., Z. A. Kharazian, S. Sarikhan, G. S. Jouzani, M. Aghdasi, and G. Hosseini Salekdeh. 2017. The dynamics of the bacterial communities developed in maize silage. *Microb. Biotechnol.* 10:1663–1676. doi:10.1111/1751-7915.12751.
- Guan, H., Y. Shuai, Y. Yan, Q. Ran, X. Wang, D. Li, Y. Cai, and X. Zhang. 2020. Microbial community and fermentation dynamics of corn silage prepared with heat-resistant lactic acid bacteria in a hot environment. *Microorganisms* 8(5):719. doi:10.3390/microorganisms8050719.
- Heron, S. J., J. Wilkinson, and C. M. Duffus. 1993. Enterobacteria associated with grass and silages. *J. Appl. Bacteriol.* 75(1):13–17. doi:10.1111/j.1365-2672.1993.tb03401.x

- Kaewwichian, R., S. Jindamorakot, S. Am-In, M. Sipiczki, and S. Limtong. 2015. *Hannaella siamensis* sp. nov. and *Hannaella phetchabunensis* sp. nov., two new anamorphic basidiomycetous yeast species isolated from plants. *Int. J. Syst. Evol. Microbiol.* 65(Pt 4):1297–1303. doi:10.1099/ijs.0.000101.
- Kertz, A. F. 1998. Variability in delivery of nutrients to lactating dairy cows. *J. Dairy Sci.* 81:3075–3084. doi:10.3168/jds.S0022-0302(98)75872-2.
- Keshri, J., Y. Chen, R. Pinto, Y. Kroupitski, Z. G. Weinberg, and S. Sela Saldinger. 2018. Microbiome dynamics during ensiling of corn with and without *Lactobacillus plantarum* inoculant. *Appl. Microbiol. Biotechnol.* 102:4025–4037. doi:10.1007/s00253-018-8903-y.
- Kong, X., Z. Han, X. Tai, D. Jin, S. Ai, X. Zheng, and Z. Bai. 2020. Maize (*Zea mays* L. Sp.) varieties significantly influence bacterial and fungal community in bulk soil, rhizosphere soil and phyllosphere. *FEMS Microbiol. Ecol.* 96(3):1–11. doi:10.1093/femsec/fiaa020.
- Kung, Jr, L., J. M. Lim, D. J. Hudson, J. M. Smith, and R. D. Joerger. 2015. Chemical composition and nutritive value of corn silage harvested in the northeastern United States after Tropical Storm Irene. *J. Dairy Sci.* 98(3):2055–2062. doi:10.3168/jds.2014-8621.
- Kung, L., Jr, R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy Sci.* 101:4020–4033. doi:10.3168/jds.2017-13909.
- Lane, B., S. Sharma, C. Niu, A. W. Maina, J. M. Wagacha, B. H. Bluhm, and C. P. Woloshuk. 2018. Changes in the fungal microbiome of maize during hermetic storage in the United States and Kenya. *Front. Microbiol.* 9:2336. doi:10.3389/fmicb.2018.02336.
- Lenth, R. V. 2016. Using lsmeans. *J. Stat. Softw.* 69:1–33. doi:10.18637/jss.v069.i01.
- Lewis, A. L., W. J. Cox, and J. H. Cherney. 2004. Hybrid, maturity, and cutting height interactions on corn forage yield and quality. *Agron. J.* 96(1):267–274. doi:10.2134/agronj2004.2670.
- Limtong, S., and R. Nasanit. 2017. Phylloplane yeasts in tropical climates. In: Buzzini, P., M.-A. Lachance, and A. Yurkov, editors, *Yeasts in natural ecosystems: diversity*. Cham: Springer International Publishing; p 199–223.
- Liu, B., H. Huan, H. Gu, N. Xu, Q. Shen, and C. Ding. 2019. Dynamics of a microbial community during ensiling and upon aerobic exposure in lactic acid bacteria inoculation-treated and untreated barley silages. *Bioresour. Technol.* 273:212–219. doi:10.1016/j.biortech.2018.10.041.
- Lozupone, C., and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71:8228–8235. doi:10.1128/AEM.71.12.8228-8235.2005.
- Martinez-Fernández, A., A. B. S. Cabezuelo, M. B. de la Roza Delgado, M. G. Arrojo, and A. A. Gutiérrez. 2013. Modelling a quantitative ensilability index adapted to forages from wet temperate areas. *Span. J. Agric. Res.* 2:455–462. doi:10.5424/sjar/2013112-3219.
- Mataragas, M., J. Metaxopoulos, and E. H. Drosinos. 2002. Characterization of two bacteriocins produced by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442, isolated from dry fermented sausages. *World J. Microbiol. Biotechnol.* 18(9):847–856. doi:10.1023/A:1021239008582.
- May, L. A., B. Smiley, and M. G. Schmidt. 2001. Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. *Can. J. Microbiol.* 47:829–841. doi:10.1139/w01-086.
- McEniry, J., P. O’Kiely, N. J. Clipson, P. D. Forristal, and E. M. Doyle. 2010. Assessing the impact of various ensilage factors on the fermentation of grass silage using conventional culture and bacterial community analysis techniques. *J. Appl. Microbiol.* 108:1584–1593. doi:10.1111/j.1365-2672.2009.04557.x.
- Miron, J., E. Zuckerman, G. Adin, R. Solomon, E. Shoshani, M. Nikbachat, E. Yosef, A. Zenou, Z. G. Weinberg, Y. Chen, I. Halachmi, and D. Ben-Ghedalia. 2007. Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. *Anim. Feed Sci. Technol.* 139(1):23–39. doi:10.1016/j.anifeeds.2007.01.011.
- Muck, R. E., E. M. G. Nadeau, T. A. McAllister, F. E. Contreras-Govea, M. C. Santos, and L. Kung, Jr. 2018. Silage review: recent advances and future uses of silage additives. *J. Dairy Sci.* 101:3980–4000. doi:10.3168/jds.2017-13839.
- Ni, K., T. T. Minh, T. T. Tu, T. Tsuruta, H. Pang, and N. Nishino. 2017. Comparative microbiota assessment of wilted Italian ryegrass, whole crop corn, and wilted alfalfa silage using denaturing gradient gel electrophoresis and next-generation sequencing. *Appl. Microbiol. Biotechnol.* 101:1385–1394. doi:10.1007/s00253-016-7900-2.
- Oude Elferink, S. J., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. *Appl. Environ. Microbiol.* 67:125–132. doi:10.1128/AEM.67.1.125-132.2001.
- Pahlow, G., R. E. Muck, F. Driehuis, S. J. W. H. Oude Elferink, and S. F. Spoelstra. 2003. Microbiology of Ensiling. In: Buxton, D. R., R. E. Muck, and J. H. Harrison, editor. *Silage science and technology*. Madison (WI): Agronomy Monographs: ASA, CSSA and SSSA.
- Petersson, S., N. Jonsson, and J. Schnürer. 1999. *Pichia anomala* as a biocontrol agent during storage of high-moisture feed grain under airtight conditions. *Postharvest Biol. Technol.* 15(2):175–184. doi:10.1016/S0925-5214(98)00081-7.
- Pitt, R. E., and R. Y. Leibensperger. 1987. The effectiveness of silage inoculants: a systems approach. *Agric. Syst.* 25(1):27–49. doi:10.1016/0308-521X(87)90097-7.
- Queiroz, O. C. M., K. G. Arriola, J. L. P. Daniel, and A. T. Adesogan. 2013. Effects of 8 chemical and bacterial additives on the quality of corn silage. *J. Dairy Sci.* 96(9):5836–5843. doi:10.3168/jds.2013-6691.
- Queiroz, O. C. M., I. M. Ogunade, Z. Weinberg, and A. T. Adesogan. 2018. Silage review: foodborne pathogens in silage and their mitigation by silage additives. *J. Dairy Sci.* 101:4132–4142. doi:10.3168/jds.2017-13901.
- Romero, J. J., Y. Joo, J. Park, F. Tiezzi, E. Gutierrez-Rodriguez, and M. S. Castillo. 2018. Bacterial and fungal communities, fermentation, and aerobic stability of conventional hybrids and brown midrib hybrids ensiled at low moisture with or without a homo- and heterofermentative inoculant. *J. Dairy Sci.* 101:3057–3076. doi:10.3168/jds.2017-13754.
- Rosenzweig, C., A. Iglesias, X. Yang, P. R. Epstein, and E. Chivian. 2001. Climate change and extreme weather events; implications for food production, plant diseases, and pests. *Glob. Change Human Heal.* 2(2):90–104. doi:10.1023/A:1015086831467.
- Rotz, C. A., S. A. Ford, and D. R. Buckmaster. 2003. Silages in farming systems. In: D. R. Buxton, R. E. Muck and J. H. Harrison, editors, *Silage science and technology* No. 42. ASA, CSS, SSA, Madison, WI. p. 505–546.
- SAS Institute. 2019. SAS/SHARE 9.4 User’s Guide. Cary, NC: SAS Institute Inc.
- Sniffen, C. J., R. W. Beverly, C. S. Mooney, M. B. Roe, A. L. Skidmore, and J. R. Black. 1993. Nutrient requirements versus supply in the dairy cow: strategies to account for variability. *J. Dairy Sci.* 76:3160–3178. doi:10.3168/jds.S0022-0302(93)7655-9.
- Tylutki, T., and D. Fox. 2000. Managing the dairy feeding system to minimize manure nutrients. Proceedings from Managing Nutrients and Pathogens from Animal Agriculture. March 28–30.
- Wagner, M. R., J. H. Roberts, P. Balint-Kurti, and J. B. Holland. 2020. Heterosis of leaf and rhizosphere microbiomes in field-grown maize. *New Phytol.* 228:1055–1069. doi:10.1111/nph.16730.
- Wang, J., S. K. Vanga, R. Saxena, V. Orsat, and V. Raghavan. 2018. Effect of climate change on the yield of cereal crops: a review. *Climate* 6(2):41. doi:10.3390/cli6020041.

- Wilde, F. D., L. J. Britton, C. V. Miller, and D. W. Kolpin. 2000. Effects of animal feeding operations on water resources and the environment. Reston, VA: US Geological Survey.
- Wilkinson, J. M., and D. R. Davies. 2013. The aerobic stability of silage: key findings and recent developments. *Grass Forage Sci.* 68(1):1–19. doi:[10.1111/j.1365-2494.2012.00891.x](https://doi.org/10.1111/j.1365-2494.2012.00891.x).
- Windle, M. C., N. Walker, and L. Kung, Jr. 2014. Effects of an exogenous protease on the fermentation and nutritive value of corn silage harvested at different dry matter contents and ensiled for various lengths of time. *J. Dairy Sci.* 97:3053–3060. doi:[10.3168/jds.2013-7586](https://doi.org/10.3168/jds.2013-7586).
- Xu, D., W. Ding, W. Ke, F. Li, P. Zhang, and X. Guo. 2019. Modulation of metabolome and bacterial community in whole crop corn silage by inoculating homofermentative *Lactobacillus plantarum* and heterofermentative *Lactobacillus buchneri*. *Front. Microbiol.* 9(3299):1–14. doi:[10.3389/fmicb.2018.03299](https://doi.org/10.3389/fmicb.2018.03299).
- Yin, Z., C. Yuan, Y. Du, P. Yang, C. Qian, Y. Wei, S. Zhang, D. Huang, and B. Liu. 2019. Comparative genomic analysis of the *Hafnia* genus reveals an explicit evolutionary relationship between the species *alvei* and *paralvei* and provides insights into pathogenicity. *BMC Genomics* 20:768. doi:[10.1186/s12864-019-6123-1](https://doi.org/10.1186/s12864-019-6123-1).