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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

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ABSTRACT

We evaluated the effects of adding a combination inoculant to 4 corn (*Zea mays* L.) hybrids harvested at low moisture on the nutritive value, fermentation profile, aerobic stability, bacterial and fungal populations, and community structure. The treatment design was the factorial combination of 4 corn hybrids ensiled with (INO) and without (CON) inoculant. The hybrids were TMF2R737 (MCN), F2F817 (MBR), P2089YHR (PCN), and PI144XR (PBR), ensiled at 44.0, 38.1, 42.0, and 41.3% of dry matter, respectively; MBR and PBR were brown midrib mutants. The inoculant contained *Lactobacillus buchneri* and *Pediococcus pentosaceus* (4×10^5 and 1×10^5 cfu/g of fresh corn). The experimental design was a complete randomized design with treatments replicated 6 times. Corn was chopped, treated or not with inoculant, packed into 7.6-L bucket silos, and stored for 100 d. At d 0, we found higher bacterial observed operational taxonomic units in the brown midrib mutants (MBR and PBR) relative to MCN and PCN (654 and 534 vs. 434 and 444 ± 15.5, respectively). The bacterial and fungal families with the highest relative abundance (RA) were *Enterobacteriaceae* (61.4%) and incertae sedis *Tremellales* (12.5%). At silo opening, we observed no effects of INO treatment on dry matter recovery (∼94.3 ± 1.07%), but aerobic stability was extended for all INO-treated hybrids (∼217 vs. ∼34.7 h), except for MBR (∼49 ± 38 h), due to a decreased yeast population (3.78 vs. 5.13 ± 0.440 log cfu/g of fresh corn) and increased acetic acid concentration (1.69 vs. 0.51 ± 0.132%) compared with the control. Furthermore, INO treatment reduced bacterial (61.2 vs. 276 ± 8.70) and increased fungal (59.8 vs. 43.6 ± 2.95) observed operational taxonomic units compared with CON. We observed that INO treatment increased the RA of *Lactobacillaceae* across all hybrids (∼99.1 vs. ∼58.9), and to larger extent MBR (98.3 vs. 34.3 ± 5.29), and decreased *Enterobacteriaceae* (0.614 vs. 23.5 ± 2.825%) among 4 other bacterial families relative to CON. For fungi, INO treatment increased the RA of *Debaryomycetaceae* (63.1 vs. 17.3 ± 8.55) and 5 other fungal families and decreased the RA of *Pichiaceae* (6.47 vs. 47.3 ± 10.95) and incertae sedis *Saccharomycetales* (8.47 vs. 25.9 ± 5.748) compared with CON. The bacterial and fungal community structures changed, due to ensiling, to a distinct and more stable community dominated by *Lactobacillaceae* and *Debaryomycetaceae*, respectively, when INO treatment was applied relative to CON. In conclusion, the INO treatment used in this study improved low-moisture whole-crop corn silage quality because of a shift in the bacterial and fungal community composition during ensiling.

Key words: silage, inoculant, hybrid, next-generation sequencing

INTRODUCTION

The increased frequency of extreme weather events (Rosenzweig et al., 2001) poses challenges for adequate silage production. Adverse weather conditions can affect field productivity of corn (*Zea mays* L.) and limit timely harvest and storage (Rosenzweig et al., 2001; Kung et al., 2015), resulting in corn plants ensiled outside the recommended range of 32 to 35% of DM concentration (Allen et al., 2003). Harvesting below the recommended range of moisture concentration (i.e., dry corn silage) can result in a cascade of negative events that prevent adequate silage preservation due to highly porous silos, lower packing density, and ultimately

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lower DM digestibility of whole-crop corn (Allen et al., 2003). High-porosity silos are more susceptible to spoilage by aerobic microorganisms (Huber and Soejono, 1976; Muck and Kung, 2007), which triggers excessive heating and an undesirable fermentation profile (Huber and Soejono, 1976; Muck and Kung, 2007), ultimately reducing voluntary intake and milk productivity of dairy cattle (Huber and Soejono, 1976).

The microbial community of corn silages produced in suboptimal conditions (i.e., outside the recommended DM concentration) have not been studied in as much detail compared with optimally stored silages (Muck, 2013). Recurrent extreme weather events combined with delayed harvesting pose challenges for consistent high-quality silage production. Consequently, expanding our understanding of silage microbial communities under suboptimal conditions is critical to help in the development of strategies to ensure successful silage production.

Novel culture-independent techniques, such as next-generation sequencing (**NGS**), can help improve our understanding of silage microbial communities. The NGS techniques have been recently used to describe bacterial communities in fresh and ensiled whole-crop corn (Ni et al., 2017) and commercial bunker whole-crop corn silos (Kraut-Cohen et al., 2016). Nevertheless, to the best of our knowledge, an evaluation of silage bacterial and fungal microbiome using NGS to study the effects of bacterial inoculation across different hybrids of fresh and ensiled whole-crop corn has not yet been performed. The objective of the present study was to evaluate the effect of combo inoculant (homolactic and heterolactic bacteria) applied to several corn hybrids harvested at low moisture on the nutritive value, fermentation profile, aerobic stability, bacterial and fungal populations, taxonomic profile, diversity, and community structure. We hypothesized that adding a combo silage inoculant improves nutritive value, preservation, and aerobic stability to different extents by causing a hybrid-dependent shift in the composition and structure of the bacterial and fungal communities compared with untreated control corn silage.

MATERIALS AND METHODS

Experimental Site, Design, and Treatments

The experimental site was located at the Piedmont Research Station in Salisbury, North Carolina (35°41′ N; 80°37′ W). Corn was planted in a clean-tilled seedbed on April 24, 2014, at a rate of 83,980 live seeds/ha. Based on initial soil test results, fertilization followed the recommendations for corn production in North Carolina (Hardy et al., 2014). Treatments were the factorial combination of 4 corn hybrids and 2 inoculations. The 4 corn hybrids were planted in a complete randomized design with plots replicated 6 times. Corn hybrids were TMF2R737 (**MCN**; Mycogen Seeds, Indianapolis, IN), P2089YHR (**PCN**; Pioneer Hi-Bred International, Johnston, IA), F2F817 (**MBR**; Mycogen Seeds), and P1449XR (**PBR**; Pioneer Hi-Bred International). Hybrids MBR and PBR were brown mid-rib (**BMR**) mutants (loci 3 and 1 mutants, respectively). All corn plants were harvested on August 25, 2014, when DM concentration was above 38% for all hybrids. Corn was clipped to 18-cm stubble height and chopped to 1.9-cm theoretical length using a John Deere 3950 forage harvester equipped with a kernel-processor (2.5 mm roll clearance; John Deere, Moline, IL). Two replicated piles (4.3 kg each, fresh basis) were obtained from each corn plot (total of 48 piles). The DM yield was 16.8, 18.7, 17.0, and 20.2 Mg/ha (±2.67 SD) for MCN, PCN, MBR, and PBR, respectively.

The 2 silage additives (**ADV**) evaluated were sterile double-distilled water (control; **CON**) and inoculant (**INO**; Biotal Buchneri 500, Lallemand Animal Nutrition, Milwaukee, WI). Each ADV treatment (CON or INO) was 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 *Lactobacillus buchneri* ATCC number 40788 and log 5 cfu/g of FW for *Pediococcus pentosaceus* plus fibrolytic enzymes from *Trichoderma reesei* (1,103, 3,145, and 50 mg of sugar released/min per gram for β-glucanase, xylanase, and galactomannanase activities, respectively; FCC, 2015). Chopped whole-crop corn (3.5 kg on a 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 (~192 ± 11.4 kg of DM/m^3). Silos were stored at 23°C (±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 on a fresh basis) were taken from each individual replicate to determine nutritive value, fermentation profile, and the bacterial and fungal population via standard plating techniques. In the case of 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 NGS (100 g on a fresh basis) and aerobic stability analysis at d 100 (2.5 kg on a fresh basis).