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Effects of lactic acid bacteria inoculants on fermentation characteristics and microbial community profiles of napier grass (*Pennisetum purpureum*) silage

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Abstract. *Rohmatussolihat, Ridwan R, Fitri A, Sarwono KA, Astuti WD, Firmansyah D, Juanssilfero AB, Fidriyanto R, Ridla M, Mubarik NR, Jayanegara A. 2026. Effects of lactic acid bacteria inoculants on fermentation characteristics and microbial community profiles of napier grass (*Pennisetum purpureum*) silage. Biodiversitas 27 (4): d270438. https://doi.org/10.13057/biodiv/d270438.* Napier grass (*Pennisetum purpureum*) is an important tropical forage; however, its high moisture content and low water-soluble carbohydrates (WSC) often impair silage fermentation. This study evaluated the effects of different lactic acid bacteria (LAB) inoculants on fermentation characteristics, nutrient preservation, and microbial community dynamics of napier grass silage. Four indigenous LAB strains, *Companilactobacillus kimchii* InaCC-B982, *Lactiplantibacillus plantarum* InaCC-B1028, *Limosilactobacillus fermentum* InaCC-B1024, and *Levilactobacillus brevis* InaCC-B1052, were applied individually and as a mixed inoculum and compared with an uninoculated control. Silage was sampled after 7, 14, and 28 days. LAB inoculation significantly improved fermentation quality compared with the control by accelerating acidification, lowering pH (3.69-3.86), increasing lactic acid concentration (2.38-4.90% DM), and suppressing yeasts and coliform bacteria. Among the inoculants, *L. plantarum* InaCC-B1028 showed the most consistent and superior performance, characterized by rapid and stable pH reduction (3.73-3.78), higher lactic acid production, minimal ammonia-N accumulation (≤0.08% DM), and improved crude protein retention (8.7-9.2% DM). In contrast, *L. brevis* and the mixed LAB treatment resulted in higher acetic acid concentrations, suggesting enhanced aerobic stability but less consistent nutrient preservation, whereas *L. fermentum* exhibited intermediate effects. Metagenomic analysis revealed that the bacterial community was dominated by *Lactiplantibacillus* and *Companilactobacillus*, with species such as *L. plantarum*, *L. pentosus*, *L. paraplantarum*, and *C. kimchii* contributing to fermentation dynamics. Genus-level dominance was consistent with species-level composition, indicating robust taxonomic structure across hierarchical levels. Overall, *L. plantarum* InaCC-B1028 is the most effective inoculant for improving fermentation efficiency, nutrient preservation, and microbial stability, highlighting its potential for enhancing silage quality in tropical forage systems.

Keywords: Fermentation characteristics, inoculant, lactic acid bacteria, microbial community dynamics, napier grass silage

INTRODUCTION

Silage is a key strategy for preserving forage biomass and ensuring feed availability in ruminant production systems, particularly in tropical regions where forage supply fluctuates seasonally. Ensiling is a biologically mediated process in which fermentative bacteria convert soluble carbohydrates into organic acids, predominantly lactic acid, leading to a rapid decline in pH and suppression of spoilage microorganisms (Guo et al. 2022). Therefore, successful silage fermentation depends on adequate fermentable substrates and the rapid establishment of beneficial microbial populations under anaerobic conditions.

However, tropical forages present inherent challenges for silage production. Their high structural fiber content, low concentrations of water-soluble carbohydrates, and strong buffering capacity limit lactic acid accumulation and delay acidification, often resulting in elevated pH, excessive proteolysis, and the proliferation of undesirable microorganisms (Sánchez-Guerra et al. 2024). In addition,

morphological characteristics such as thick stems and heterogeneous moisture distribution hinder effective compaction and the establishment of anaerobic conditions, further compromising fermentation quality.

Napier grass (*Pennisetum purpureum* Schumach.) is widely cultivated in tropical regions due to its high biomass yield, adaptability, and moderate nutritional value, containing approximately 8-15% crude protein and essential minerals (Islam et al. 2023). Despite these advantages, its ensiling remains challenging. High moisture content, limited fermentable carbohydrates, and strong buffering capacity frequently slow pH decline, promoting protein degradation and the growth of undesirable microorganisms such as *Clostridium* spp. and members of the Enterobacteriaceae (Chen et al. 2022), ultimately reducing silage quality and stability.

To address these limitations, lactic acid bacteria (LAB) inoculants are widely applied to enhance fermentation efficiency and aerobic stability. Previous studies on tropical forages have shown that LAB supplementation, either alone or combined with cellulase enzymes, accelerates acidification, reduces ammonia nitrogen accumulation, increases lactic acid production, and improves nutrient preservation (Kim et al. 2021; Jaipolsaen et al. 2022). These effects are particularly important for high-fiber tropical forages, where improved substrate availability supports LAB activity.

Beyond fermentation end-products, silage quality is strongly influenced by the structure and succession of microbial communities during ensiling. Fermentation outcomes arise from complex interactions among epiphytic microorganisms and inoculated LAB, which regulate acid production, protein degradation, and aerobic stability (McAllister et al. 2017). Recent advances in next-generation sequencing technologies have enabled high-resolution characterization of silage microbiota, providing new insights into the functional roles of specific microbial taxa.

Different LAB species exhibit distinct metabolic characteristics that influence fermentation outcomes. Homofermentative LAB primarily produce lactic acid, facilitating rapid acidification, whereas heterofermentative LAB generate additional metabolites such as acetic acid that enhance aerobic stability. Under tropical conditions, where high temperature and moisture increase the risk of spoilage, the complementary roles of these LAB groups are likely critical for achieving stable fermentation.

Despite extensive research on LAB inoculation, the interactions between selected LAB strains and indigenous microbial communities in *P. purpureum* silage remain poorly understood. Most studies have focused on fermentation characteristics without integrating microbial community dynamics, limiting mechanistic understanding of how LAB regulate nutrient preservation and suppress undesirable microorganisms under tropical conditions.

Accordingly, this study aimed to evaluate the effects of selected indigenous LAB on fermentation characteristics, nutrient preservation, and microbial community structure of *P. purpureum* silage under tropical conditions. Four strains—*Limosilactobacillus fermentum* InaCC-B1024, *Lactobacillus plantarum* InaCC-B1028, *Levilactobacillus brevis* InaCC-B1052, and *Companilactobacillus kimchii* InaCC-B982—were selected based on their functional traits, including acidification capacity, lactic acid production, acid tolerance, and potential cellulolytic activity.

Based on the known functional differences among homofermentative and heterofermentative lactic acid bacteria (LAB) and the limited understanding of their interactions with indigenous microbial communities in tropical silages, we hypothesize that indigenous LAB strains differentially regulate fermentation efficiency, nutrient preservation, and microbial succession in *P. purpureum* silage. Specifically, we propose that *L. plantarum* will promote rapid acidification and improved protein preservation through enhanced lactic acid production, whereas heterofermentative strains (e.g., *L. fermentum* and *L. brevis*) will contribute to greater aerobic stability via increased acetic acid production. Furthermore, we hypothesize that mixed inoculation will exhibit non-additive effects, resulting in distinct microbial community structures and fermentation outcomes compared to single-strain treatments. Accordingly, this study addresses the following research question: How do selected indigenous LAB strains, individually and in combination, influence fermentation characteristics, nutrient dynamics, and microbial community composition of napier grass silage under tropical conditions?

MATERIALS AND METHODS

Sample preparation

Four LAB selected from 60 LAB isolates from the Indonesian Culture Collection (InaCC-BRIN): *C. kimchii* InaCC-B982, *L. plantarum* InaCC-B1028, *L. fermentum* InaCC-B1024, and *L. brevis* InaCC-B1052 (Rohmatussolihat et al. 2024). The LAB were cultivated in de Man, Rogosa, and Sharpe (MRS) medium (Merck, Germany) and incubated at 37°C for 24 h (Fitriani et al. 2024).

Experimental design and silage preparation

The experiment followed a factorial completely randomized design (CRD) consisting of 6 treatments × 3 fermentation times (7, 14, and 28 days) × 5 replicates, resulting in a total of 90 silos. Treatments included: (i) CK: *C. kimchii* InaCC-B982; (ii) LP: *L. plantarum* InaCC-B1028; (iii) LF: *L. fermentum* InaCC-B1024; (iv) LB: *L. brevis* InaCC-B1052; (v) mixed LAB (1:1:1:1); and (6) uninoculated control.

Napier grass was chopped to 3-5 cm and supplemented with 1% dextrose (fresh weight basis) to ensure adequate fermentable substrate (Dong et al. 2020). LAB inoculants were applied at 1×10^6 CFU g^{-1} fresh material. In the mixed treatment, the total inoculum was equally distributed among the four strains (2.5×10^5 CFU g^{-1} each).

Approximately 200 g of treated forage was packed into plastic silos, vacuum-sealed, and stored at ambient temperature (28-32°C). Silos were opened destructively at each sampling time to maintain anaerobic conditions throughout fermentation.

Microbiology analysis

The microbial populations of total bacteria, LAB, yeasts, and coliforms were assessed from silage extracts diluted 10^ (-3) to 10^ (-7) and cultured on selective agar media, with three replicates at each dilution level. Nutrient agar (NA) was used for total bacteria, De Man Rogosa Sharpe (MRS) agar for LAB, potato dextrose agar (PDA) for yeasts, and Chromocult agar for Coliforms. Incubation conditions were adjusted according to the target microbial groups. Total bacteria and coliforms were incubated aerobically at 35°C for 24 h (Khadse 2025). LAB were incubated under anaerobic conditions at 37°C for 48 h (Peacock et al. 2021; Thompson et al. 2025), and yeasts were incubated aerobically at 30°C for 48 h. After which, visible colonies were counted and results expressed as $\log_{10}$ CFU per gram of silage (Fitriani et al. 2024; Ardiansyah et al. 2025).

Fermentation and chemical analyses

Silage extracts were prepared by homogenizing samples (10 g) with sterile water (1:9 w/v). The extracts obtained were then examined to assess fermentation characteristics and the chemical composition of the silage. Fermentation characteristics were determined by measuring pH, lactic acid, ammonia-N, and organic acids. Lactic acid was quantified spectrophotometrically (Borshchevskaya et al. 2016), and ammonia-N by the phenol-hypochlorite method (Souza et al. 2017). Organic acids and water-soluble carbohydrates (WSC) were analyzed by high-performance liquid chromatography (HPLC) (Juanssilfero et al. 2019), while short-chain fatty acids (SCFA) were determined using gas chromatography-mass spectrometry (GC-MS) (Sarwono et al. 2022).

Chemical composition (dry matter, organic matter, crude protein, ether extract, and crude fiber) was determined according to standard AOAC procedures (AOAC 2005). Acid detergent fiber and neutral detergent fiber were analyzed using an ANKOM Model 200 system (ANKOM Technology, Macedon, NY, USA).

DNA extraction and sequencing

For each treatment, DNA samples from biological replicates were pooled prior to sequencing. Therefore, the sequencing analysis represents a composite sample for each treatment and does not include biological replication. DNA was extracted from silage samples collected on day 28 of fermentation to characterize the microbial community composition. DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol, incorporating modifications as specified by Ridwan et al. (2015) and Adawiyah et al. (2025) to optimize DNA yield and quality from silage material.

Briefly, silage samples were initially treated with RBC lysis buffer to remove non-microbial components, followed by suspension in Inhibitor EX Buffer to reduce the PCR inhibitors commonly present in silage. To enhance microbial cell disruption, lysozyme, RNase (Genomic DNA Mini Kit, Geneaid), and Proteinase K were added during the extraction process before column-based purification. DNA concentration and purity were initially assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and DNA integrity was verified by electrophoresis on a 1.7% TAE agarose gel.

The microbial community was analyzed using next-generation sequencing (NGS). The full-length (V1-V9) regions of the 16S rRNA gene were amplified using universal primers 27F (5′-AGAGTTTGATCCTGGTCCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) (Adawiyah et al. 2025). DNA concentration was further confirmed using both a NanoDrop spectrophotometer and a Qubit fluorometer. Library preparation was performed using Oxford Nanopore Technology (ONT) kits. Sequencing was conducted using MinKNOW software version 23.04.5, and basecalling was carried out using Guppy version 6.5.7 with a high-accuracy model (Wick et al. 2019). The quality of FASTQ files was assessed using NanoPlot, and quality filtering was performed using NanoFilt with default settings (De Coster et al. 2018; Nygaard et al. 2020). Filtered reads were taxonomically classified using the Centrifuge classifier (Kim et al. 2016). Bacterial and archaeal indices were constructed based on the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/). Subsequent data analysis and visualization were performed using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and RStudio with R version 4.2.3 (https://www.R-project.org/).

Microbial community analysis

Alpha-diversity indices, including Shannon, Simpson, and Chao1, were calculated using RStudio with R version 4.2.3 (https://www.R-project.org/), based on normalized read counts. Microbial community structure was visualized by principal coordinates analysis (PCoA). Because DNA was pooled before sequencing, no statistical inference was performed to test differences in microbial community structure among treatments. Diversity indices and ordination plots are presented for descriptive and exploratory purposes only.

Data analysis

The study was carried out using a factorial completely randomized design (CRD) consisting of two factors: inoculum (6 levels) and fermentation time (3 levels), with five replications per treatment. The general linear model (GLM) procedure was applied, and significant differences ($p<0.05$) were further evaluated using Duncan's multiple range test (SPSS version 25, SPSS Inc., USA). Duncan's test is beneficial for assessing the effects of different additives, fermentation conditions, and silage types on fermentation quality and nutritional outcomes (Alhaag et al. 2019).

RESULTS AND DISCUSSION

Nutritional quality of fresh napier grass

The chemical and microbiological characteristics of fresh napier grass are shown in Table 1. The chemical analysis of fresh napier grass showed a relatively high pH (5.82) and low lactic acid content (0.153% DM). Ammonia levels were negligible (0.005% DM). The dry matter (DM) content was

27.57%, which is higher than the average reported for fresh Napier grass (15-20%) in tropical forage studies (Rambau et al. 2022). The crude protein content was 9.01% DM, within the typical range of 8-12% for napier grass (Okukenu et al. 2021). The ether extract content was 5.22% DM, and the ash content was 12.46% DM. The NDF and ADF contents were 49.03% DM and 28.97% DM, respectively, indicating high fiber content, though slightly lower than typical values reported for fresh RG (Okukenu et al. 2021; Islam et al. 2023).

Microbiological analysis showed low LAB populations (2.69±0.164 log_{10} CFU g^{-1}) compared to total bacteria (4.34±0.219 log_{10} CFU g^{-1}). Yeasts (7.79±0.106 log_{10} CFU g^{-1}) and coliforms (7.70±0.227 log_{10} CFU g^{-1}) were present at high levels, indicating that contaminating microorganisms dominated the substrate. Microbiological analysis showed low LAB and high yeast and coliform populations, suggesting suboptimal conditions for ensiling (Jaipolsaen et al. 2022).

Napier grass silage production

As shown in Table 2, the LAB population differed significantly among treatments during fermentation ($p<0.001$). On day 7, the LF treatment had the highest LAB count (9.045 log_{10} CFU g^{-1}), followed by CK (9.001 log_{10} CFU g^{-1}). LAB counts subsequently declined, reaching their lowest levels by day 28 in LP (3.64 log_{10} CFU g^{-1}) and CK (3.75 log_{10} CFU g^{-1}) silage. Total bacterial populations peaked in LF on day 7 (8.271 log_{10} CFU g^{-1}) and decreased by day 28, with the lowest counts observed in CK (4.375 log_{10} CFU g^{-1}) and LP (3.852 log_{10} CFU g^{-1}). Yeasts were initially abundant (7.177-7.866 log_{10} CFU g^{-1}) but declined by day 28, with the lowest levels in LP (3.944 log_{10} CFU g^{-1}) and Mix (3.991 log_{10} CFU g^{-1}) treatments. Coliforms were initially high in LF (6.745 log_{10} CFU g^{-1}) and mixed LAB (6.626 log_{10} CFU g^{-1}) but became undetectable by day 28 in CK, LF, LP, and mixed LAB treatments.

Table 1. Chemical composition and microbial population of napier grass before ensiling

Parameter	Napier grass
Chemical composition	
pH	5.82±0.123
Lactic acid (%DM)	0.153±0.035
NH_3-N (%DM)	0.005±0.005
DM (%)	27.57±0.5
Ash (%DM)	12.46±0.492
OM (%DM)	78.62±0.470
Ether extract (%DM)	5.22±0.001
Crude protein (%DM)	9.01±0.398
Crude fiber (%DM)	24.42±0.909
NDF (%DM)	49.03±0.840
ADF (%DM)	28.98±0.721
Microbial population (log_{10} CFU g^{-1})	
LAB	2.69±0.164
Total bacteria	4.34±0.219
Yeast	7.79±0.106
Coliform	7.70±0.227

Note: DM: dry matter, OM: organic matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, CFU: colony-forming unit

Table 2. Microbial population of napier grass silage added with various lactic acid bacteria (LAB) inoculants

Inoculants	Time (Day)	(log_{10} CFU g^{-1} napier grass silage)			
		LAB	Total Bacteria	Yeast	Coliform
CK	7	9.001±0.041^{k}	7.867±0.257^{f}	7.396±0.407^{ghi}	5.780±0.139^{a}
	14	6.680±0.043^{f}	6.879±0.269^{d}	7.592±0.367^{hij}	5.063±0.282^{b}
	28	3.753±0.455^{a}	4.375±0.993^{b}	3.194±0.703^{a}	ND
LF	7	9.045±0.208^{k}	8.271±0.302^{f}	7.686±0.241^{ij}	6.745±0.373^{f}
	14	7.272±0.260^{hi}	6.975±0.3184^{d}	7.356±0.257^{ghi}	5.498±0.154^{cd}
	28	5.262±0.086^{bc}	3.550±0.313^{a}	3.515±0.179^{ab}	ND
LP	7	8.170±0.399^{j}	7.072±0.356^{d}	6.996±0.305^{fg}	5.982±0.416^{e}
	14	6.912±0.188^{fgh}	6.896±0.205^{d}	7.176±0.457^{fgh}	5.386±0.238^{bc}
	28	3.640±0.737^{a}	3.852±0.532^{a}	3.944±0.105^{bc}	ND
LB	7	7.198±0.412^{ghi}	7.763±0.088^{ef}	7.617±0.456^{hij}	6.601±0.244^{f}
	14	6.800±0.142^{fg}	6.139±0.585^{c}	7.235±0.223^{fghi}	5.890±0.238^{de}
	28	5.616±0.183^{cd}	3.576±0.228^{a}	4.498±0.231^{d}	ND
Mixed LAB	7	8.170±0.111^{j}	7.906±0.0601^{f}	8.001±0.061^{j}	6.626±0.157^{f}
	14	7.521±0.062^{i}	6.835±0.21^{d}	6.816±0.179^{f}	6.143±0.196^{e}
	28	5.026±0.561^{b}	3.771±0.124^{a}	3.991±0.201^{c}	ND
Uninoculated	7	6.142±0.102^{e}	7.311±0.399^{de}	7.573±0.625^{hij}	5.750±0.195^{cde}
	14	5.946±0.172^{de}	7.017±0.247^{d}	5.817±0.098^{e}	5.995±0.123^{e}
	28	3.983±0.628^{a}	4.079±0.091^{ab}	3.645±0.175^{abc}	ND
SEM		0.175	0.18	0.184	0.301
p-value	Inoculum	<0.001	0.002	<0.001	<0.001
	Time	<0.001	<0.001	<0.001	<0.001
	Inoculum x Time	<0.001	<0.001	<0.001	<0.001
	treatment	<0.001	<0.001	<0.001	<0.001

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB, CFU: colony-forming unit, SEM: standard error of the mean, $^{a-k}$Different superscripts in the same column indicate significant differences ($p<0.05$), ND: not detected

These results reflect dynamic shifts in the microbial community during napier grass silage fermentation. LAB and total bacterial counts increased by day 7, especially in single-inoculant treatments, likely due to water-soluble carbohydrates supporting LAB growth (Kim et al. 2021). Counts then declined by day 28 as fermentable substrates were depleted and acidity increased. Similar trends have been reported in napier grass silage, where LAB dominates early fermentation but decreases during storage (You et al. 2021). Yeasts proliferated initially by utilizing residual oxygen and WSC but were suppressed under anaerobic conditions by day 28, consistent with findings that LAB inoculation reduces yeast populations (Ridwan et al. 2023). Coliforms disappeared by day 28, indicating that LAB-acidification effectively inhibited undesirable microbes (Hernández-Aquino et al. 2019).

Chemical composition analysis (Table 3) revealed that LAB inoculation significantly affected ($p<0.05$) most parameters, except ash content ($p>0.05$). Compared with the control, inoculated treatments generally resulted in lower dry matter (DM) values. For instance, DM decreased from 26.31% in the uninoculated silage to 24.27% in CK at day 14. Organic matter (OM) exhibited a similar trend, with the highest value observed in the control (78.55%) and reduced levels in the inoculated treatments (e.g., 74.22% in CK).

Ether extract (EE) content was also affected by inoculation, decreasing from 2.96% in CK to as low as 1.08% in LP. In contrast, crude protein (CP) was better preserved in the inoculated silages. However, the highest value was still observed in the control (11.15%), suggesting a concentration effect rather than true protein preservation. Fiber fractions exhibited treatment-dependent variation. Notably, LP reduced crude fiber to 22.73%, compared with 30.83% in the control, representing a substantial decrease. In contrast, NDF and ADF values were generally higher in the inoculated treatments than in the control treatment, although the differences were modest.

The effects of LAB inoculants on the fermentation characteristics of napier grass silage after 7, 14, and 28 days of incubation are presented in Table 4. LAB inoculation significantly ($p<0.001$) affected all measured parameters, including pH, lactic acid, NH_3, and acetic acid concentrations. Inoculated silage tended to have lower pH values (3.69-3.86) than the uninoculated control (up to 3.82), indicating enhanced acidification. Rapid pH reduction is crucial to inhibit the spoilage of microorganisms, and this finding is consistent with Kim et al. (2021), who reported that LAB accelerated organic acid production and promoted stable acidic conditions.

Lactic acid concentrations ranged from 2.38 to 4.90% DM. The highest concentration was observed in the control on day 14, likely reflecting transient epiphytic LAB activity rather than sustained fermentation efficiency, as indicated by the overall fermentation stability of the inoculated treatments. These indigenous LAB may initiate lactic acid production during the early phase of ensiling; however, their populations are typically insufficient to sustain consistent fermentation compared with inoculated treatments. In contrast, LF and mixed LAB maintained more stable lactic acid levels throughout the incubation period, suggesting a more consistent fermentation process (Wright and Axelsson 2019). This pattern is consistent with the higher and more stable populations of lactic acid bacteria (LAB) observed in Table 2 for the LF and mixed LAB treatments.

NH_3-N content remained low (0.015-0.318% DM), indicating limited protein degradation. Although crude protein (CP) appeared highest in the control, this may reflect a concentration effect associated with dry matter (DM) loss rather than true protein preservation, as indicated by the higher NH_3-N levels. LF exhibited the lowest NH_3-N concentrations, which aligns with Guo et al. (2022), who reported that lower NH_3-N content reflects better protein preservation and more efficient nitrogen utilization in the rumen. Such excessive NH_3-N concentration in the rumen may contribute to a higher nitrogen excretion from ruminants to the environment (Schuba et al. 2017).

Acetic acid concentrations ranged from 12.83 to 43.87 mM, peaking in the control on day 14, likely due to indigenous heterofermentative microorganisms. LB and Mixed LAB also produced relatively high acetic acid levels, which may enhance aerobic stability despite slightly reducing energy digestibility (Kung et al. 2018). The consistently high acetic acid content in the Mixed LAB treatment likely reflects the activity of *L. brevis*.

The addition of 1% dextrose served as a readily fermentable substrate, potentially enhancing lactic acid production and influencing fermentation dynamics. This approach was intentionally applied to ensure uniform substrate availability across treatments and to facilitate a controlled comparison of inoculant effects. However, it should be acknowledged that such supplementation may not fully represent practical silage conditions, where external sugar inputs are typically limited. Consequently, the presence of dextrose may have partially masked or modulated the specific effects of the inoculants. Therefore, further studies without dextrose supplementation are warranted to confirm the robustness and field applicability of these findings. Although environmental conditions were not strictly controlled, all treatments were subjected to identical storage conditions, thereby minimizing experimental variability and allowing valid relative comparisons among treatments. Importantly, the observed fermentation patterns were consistently supported by both microbial dynamics (Table 2) and compositional changes (Table 3), reinforcing the conclusion that LAB inoculation improves fermentation efficiency and overall silage quality.

Table 3. Chemical composition of napier grass silage fermented with various LAB inoculants

Inoculants	Time (Day)	DM (%)	Ash (%DM)	OM (%DM)	Ether extract (%DM)	Crude protein (%DM)	NDF (%DM)	ADF (%DM)	Crude fiber (%DM)
CK	7	24.957±0.391^{a}	10.979±0.815^{a}	75.634 ±0.320^{bcdef}	10.547±2.788^{b}	9.295±0.562^{abc}	44.064±0.763^{a}	27.556±0.684^{abcd}	23.165±1.004^{ab}
	14	24.270±0.521^{ab}	11.305±0.429^{abc}	74.722±0.890^{ab}	2.643±0.316^{a}	8.824±0.389^{abc}	43.537±0.665^{ab}	27.092±0.176^{a}	23.715±2.730^{abcd}
	28	24.482±0.626^{ab}	11.656±0.535^{abc}	75.542±0.893b^{cdef}	2.76±0.749^{a}	8.527±0.320^{a}	43.1±0.918^{ab}	27.452±0.672^{abc}	24.389±3.016^{abcde}
LF	7	25.289±0.650^{ab}	11.936±0.38^{bc}	75.42±0.432^{abcde}	9.02±0.987^{b}	9.221±0.135^{abc}	44.547±0.507^{ab}	27.964±0.375^{abcde}	25.73±1.806^{abcde}
	14	24.509±1.084^{ab}	11.196±0.346^{ab}	75.358±0.579^{abcde}	2.157±0.442^{a}	8.879±0.536^{abc}	44.611±1.050^{ab}	28.094±0.614^{abcdef}	27.837±2.967^{e}
	28	24.647±0.471^{ab}	12.052±0.43^{c}	76.444±0.772^{fg}	2.29±0.369^{a}	9.009±0.534^{abc}	44.635±0.784^{abc}	28.434±0.607^{cdef}	27.662±3.722^{e}
LP	7	24.947±0.697^{ab}	11.923±0.414^{bc}	75.616±0.448^{bcdef}	9.176±0.735^{b}	9.201±0.529^{abc}	46.076±1.085^{abc}	29.035±0.718^{f}	22.786±1.286^{a}
	14	24.667±0.280^{ab}	11.865±0.365^{bc}	75.09±0.652^{abcd}	2.283±0.551^{a}	8.622±0.258a	43.348±0.308^{bc}	27.176±0.132^{ab}	23.788±2.799^{abcd}
	28	25.073±0.193^{abc}	11.873±0.221^{bc}	75.338±0.344^{abcde}	2.567±0.305^{a}	8.768±0.346^{abc}	43.489±1.078^{bcd}	27.723±0.975^{abcde}	23.544±2.291^{abc}
LB	7	25.319±0.781^{abc}	11.583±0.602^{abc}	76.708±0.840^{g}	9.248±0.589^{b}	9.325±0.720^{abc}	46.622±1.518^{bcd}	29.146±0.875^{f}	26.518±1.105^{bcde}
	14	24.509±0.525^{abc}	11.411±0.371^{abc}	76.026±0.956^{defg}	2.6±0.098^{a}	8.873±0.483^{abc}	45.014±0.776^{bcd}	28.553±0.549^{def}	25.193±1.928^{abcde}
	28	24.470±0.474^{abc}	11.861±0.492^{bc}	75.176±0.441^{abcd}	1.979±0.651^{a}	8.966±0.901^{abc}	44.764±0.760^{bcde}	28.648±0.605^{ef}	24.583±3.413^{abcde}
Mixed LAB	7	26.180±0.644^{abcd}	11.904±0.361^{bc}	76.262±0.792^{efg}	9.658±0.259^{ab}	9.700±0.552^{bc}	45.396±1.502^{cde}	27.809±0.967^{abcde}	26.493±1.653^{bcde}
	14	24.364±0.427^{bcde}	11.851±0.468^{bc}	74.786±0.598^{abc}	2.553±0.268^{a}	8.602±0.285^{a}	43.367±0.859^{cde}	27.456±0.897^{abc}	25.358±2.055^{abcde}
	28	24.904±0.701^{bcde}	11.873±0.868^{bc}	75.748±0.545^{cdef}	2.451±0.51^{a}	9.011±0.834^{abc}	44.682±0.939^{cdef}	28.213±0.842^{bcdef}	23.913±0.971^{abcd}
Uninoculated	7	26.031±0.785^{cde}	11.703±0.279^{bc}	75.738±0.502^{cdef}	2.864±0.147^{a}	9.782±0.538^{c}	45.039±1.299^{def}	27.599±0.829^{abcde}	27.344±2.936^{de}
	14	25.226±1.076^{de}	11.545±0.689^{abc}	74.494±0.891^{a}	2.592±0.449^{a}	11.131±1.901^{d}	44.199±0.482^{def}	27.753±0.767^{abcde}	25.575±2.024^{abcde}
	28	25.785±0.323^{e}	11.752±0.319^{ab}	78.552±0.754^{h}	2.415±0.250^{a}	8.678±0.542^{ab}	46±0.994^{f}	29.053±1.002^{f}	27.010±3.381^{cde}
SEM		0.084	0.057	0.114	0.346	0.091	0.138	0.0956	0.284
p-value	Inoculum	<0.001	0.023	0.001	<0.001	0.002	<0.001	<0.001	<0.001
	Time	<0.001	0.245	<0.001	<0.001	0.005	<0.001	0.006	0.969
	Inoculum x Time	<0.001	0.083	<0.001	<0.001	0.001	0.002	0.011	0.512
	Treatment	<0.001	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	0.011

Table 4. Fermentation products of napier grass silage fermented with various LAB inoculants

Inoculants	Time (Day)	pH	Lactic acid (%DM)	NH_3-N (%DM)	Acetic acid (mM)
CK	7	3.734 ± 0.03^{abc}	3.245 ± 0.134^{bcde}	0.156 ± 0.018^{bcde}	15.821 ± 1.864^{abc}
	14	3.86 ± 0.051^{g}	3.634 ± 0.200^{fg}	0.260 ± 0.036^{ef}	40.057 ± 5.963^{g}
	28	3.79 ± 0.062^{cdef}	2.489 ± 0.196^{a}	0.103 ± 0.01^{abc}	12.83 ± 1.771^{a}
LF	7	3.762 ± 0.04^{bcde}	3.083 ± 0.149^{bcd}	0.015 ± 0.001^{a}	19.054 ± 1.504^{cd}
	14	3.762 ± 0.018^{bcde}	3.596 ± 0.169^{efg}	0.026 ± 0.007^{a}	27.701 ± 3.974^{e}
	28	3.796 ± 0.011^{def}	2.593 ± 0.179^{a}	0.019 ± 0.001^{a}	19.450 ± 2.257^{cd}
LP	7	3.744 ± 0.024^{abcd}	3.592 ± 0.159^{efg}	0.076 ± 0.031^{ab}	15.373 ± 1.775^{abc}
	14	3.734 ± 0.013^{abc}	3.925 ± 0.205^{fg}	0.157 ± 0.029^{bcde}	29.480 ± 2.168^{e}
	28	3.782 ± 0.008^{cedf}	2.505 ± 0.272^{a}	0.212 ± 0.046^{cde}	17.109 ± 0.718^{abcd}
LB	7	3.718 ± 0.027^{ab}	3.355 ± 0.149^{cdef}	0.151 ± 0.030^{bcde}	13.998 ± 1.834^{ab}
	14	3.694 ± 0.03^{a}	3.667 ± 0.283^{fg}	0.151 ± 0.027^{bcde}	34.388 ± 2.084^{f}
	28	3.772 ± 0.062^{bcdef}	2.955 ± 0.161^{b}	0.318 ± 0.272^{f}	17.63 ± 1.721^{bcd}
Mixed LAB	7	3.8 ± 0.046^{def}	3.094 ± 0.321^{bcd}	0.199 ± 0.034^{cde}	17.345 ± 3.48^{bcd}
	14	3.774 ± 0.039^{cdef}	3.557 ± 0.409^{efg}	0.232 ± 0.046^{def}	43.493 ± 5.157^{g}
	28	3.764 ± 0.039^{bcdef}	2.384 ± 0.227^{a}	0.237 ± 0.096^{def}	19.786 ± 5.157^{cd}
Uninoculated	7	3.81 ± 0.059^{efg}	3.442 ± 0.157^{def}	0.157 ± 0.026^{bcde}	15.436 ± 2.155^{abc}
	14	3.792 ± 0.018^{def}	4.091 ± 0.314^{h}	0.136 ± 0.028^{bcd}	43.867 ± 5.221^{g}
	28	3.82 ± 0.029^{fg}	3.062 ± 0.603^{bc}	0.21 ± 0.056^{cde}	21.176 ± 3.174^{e}
SEM		0.005	0.058	0.011	1.105
p-value	Inoculum	<0.001	<0.001	<0.001	<0.001
	Time	0.028	<0.001	<0.001	<0.001
	Inoculum x Time	<0.001	0.095	<0.001	<0.001
	Treatment	<0.001	<0.001	<0.001	<0.001

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB, % DM: % dry matter. SEM: standard error of the mean; $^{a-g}$Different superscripts in the same column indicate significant differences ($p<0.05$)

The changes in WSC and WSC-derived alcohols in napier grass silage under different LAB inoculants and fermentation durations are shown in Table 5. Overall, most WSC-related compounds were detected at low concentrations, with distinct patterns depending on the inoculum and fermentation time. Cellobiose was largely undetectable across all treatments, indicating its rapid utilization during ensiling. Xylose appeared only in the uninoculated control at the late fermentation stage, whereas sugar alcohols, such as mannitol, xylitol, and glycerol, generally increased over time, particularly in LAB-inoculated silages. Among these, xylitol reached its highest level in the LF treatment, whereas glycerol accumulation was more pronounced in the control treatment. The concentrations of xylose and sugar alcohols differed significantly among the treatments ($p<0.05$), suggesting that LAB inoculation altered WSC metabolism during ensiling.

To improve analytical clarity, the key fermentation parameters and microbial indicators are consolidated in Table 6 and interpreted alongside the metagenomic profiles (top 20 taxa in Figures 1-4). This integrated presentation allows for a more direct assessment of the relationship between fermentation performance and shifts in the microbial community.

LAB inoculation significantly affected all measured variables ($p<0.001$), with clear interactions between inoculant type and fermentation time. Among the treatments, *L. plantarum* (LP) and *L. fermentum* (LF) consistently showed better fermentation outcomes, as reflected by lower pH values and higher lactic acid concentrations. These patterns align with metagenomic data, in which *Lactobacillus* was the dominant genus, supporting rapid acidification during the early stages of ensiling.

Protein preservation varied across the treatments. The lower NH_3-N concentrations observed in the LF-treated silage suggest reduced proteolysis, which corresponds to a lower abundance of proteolytic bacteria in the microbial profile. In contrast, the increase in NH_3 in the *L. brevis* (LB) treatment at later stages appeared to be associated with a more diverse microbial community and increased deamination activity.

Differences in the fermentation pathways were also evident. Higher acetic acid concentrations in LB-treated silage indicated a shift toward heterofermentative metabolism, consistent with the taxa detected in the metagenomic analysis. In addition, a clear pattern of microbial succession was observed: LAB dominated the early stage (day 7), followed by a gradual decline and a relative increase in other microbial groups by day 28.

Overall, these findings underscore the strong link between microbial community structure and fermentation quality. LP was notably effective in enhancing lactic acid production and facilitating a rapid pH decline, while LF played a more significant role in preserving protein compared to LP. By combining fermentation data with metagenomic insights, we gain a more comprehensive understanding of silage quality, highlighting the importance of selecting suitable inoculants for optimal outcomes.

Table 5. Sugar and sugar alcohol profiles in napier grass silage fermented with various LAB inoculants

Inoculants	Time (Day)	Cellobiose (g L^{-1})	Xylose (g L^{-1})	Mannitol (g L^{-1})	Xylitol (g L^{-1})	Glycerol (g L^{-1})
CK	7	ND	ND	0.013±0.005^{bcd}	0.020±0.002^{a}	0.013±0.012^{ab}
	14	ND	ND	0.014±0.0^{bcde}	0.051±0.022^{ab}	0.040±0.006^{de}
	28	ND	ND	0.015±0.015^{cde}	0.064±0.039^{b}	0.055±0.012^{e}
LF	7	ND	ND	ND	0.030±0.004^{ab}	0.007±0.01^{a}
	14	ND	ND	ND	0.040±0.025^{ab}	0.036±0.011^{cde}
	28	ND	ND	0.005±0.035^{abc}	0.099±0.024^{c}	0.042±0.005^{de}
LP	7	0.03±0.081^{b}	ND	0.020±0.002^{de}	0.028±0.177^{ab}	0.012±0.027^{ab}
	14	ND	ND	0.018±0.0108^{de}	0.043±0.032^{ab}	0.040±0.009^{de}
	28	ND	ND	0.024±0.003^{e}	0.040±0.015^{ab}	0.041±0.002^{de}
LB	7	ND	ND	0.020±0.004^{de}	0.045±0.032^{ab}	0.019±0.022^{abc}
	14	ND	ND	0.018±0.006^{de}	0.037±0.015^{ab}	0.042±0.005^{de}
	28	ND	ND	0.021±0.011^{de}	0.030±0.007^{ab}	0.035±0.006^{cde}
Mixed LAB	7	ND	ND	0.005±0.007^{ab}	0.049±0.044^{ab}	0.037±0.013^{cde}
	14	ND	ND	0.006±0.006^{abc}	0.050±0.034^{ab}	0.050±0.013^{de}
	28	ND	ND	0.008±0.005^{abc}	0.058±0.042^{ab}	0.044±0.006^{de}
Uninoculated	7	ND	ND	0.002±0.004^{a}	0.051±0.036^{ab}	0.040±0.015^{de}
	14	ND	ND	0.005±0.002^{abc}	0.043±0.026^{ab}	0.049±0.007^{de}
	28	0.024±0.035^{ab}	0.52±0.708^{b}	0.002±0.004^{a}	0.052±0.022^{ab}	0.031±0.03^{bcd}
SEM		0.002	0.02	0.001	0.003	0.002
p-value	Inoculum	0.428	0.029	0.000	0.291	0.040
	Time	0.523	0.076	0.296	0.019	0.000
	Inoculum x Time	0.231	0.008	0.971	0.052	0.015
	Treatment	0.326	0.002	<0.001	0.022	<0.001

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB. SEM: standard error of the mean; $^{a-e}$Different superscripts in the same column indicate significant differences ($p<0.05$), ND: not detected

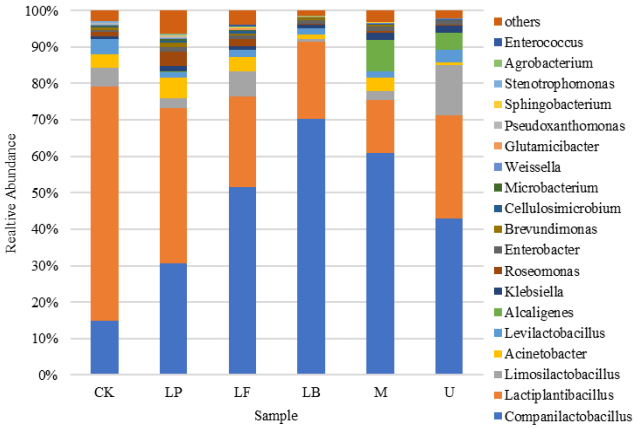

Figure 1. Relative abundance of the top 20 bacterial taxa at the genus level in napier grass silage treated with different LAB inoculants. CK: *C. kimchii* InaCC-B982; LF: *L. fermentum* InaCC-B1024; LP: *L. plantarum* InaCC-B1028; LB: *L. brevis* InaCC-B1052; M: mixed LAB (CK + LF + LP + LB); U: uninoculated control

Microbial community

Abundance by genus

The bacterial genus community in napier grass silage under different treatments (CK, LP, LF, LB, M, and U) is presented in Figure 1, while the complete quantitative data are provided in Table 7. It should be noted that the microbial community analysis is descriptive in nature, as it is based on pooled samples without biological replication; therefore, no statistical analysis was performed on the data. To support the interpretation, key quantitative values, particularly relative abundance percentages, are explicitly reported in the text. Across all treatments, two genera, *Lactiplantibacillus* and *Companilactobacillus*, were dominant, with their relative abundance varying among treatments. In CK, LP, and LF, *Lactiplantibacillus* was the predominant genus, accounting for 64.26%, 42.48%, and 24.84%, respectively. In contrast, *Companilactobacillus* dominated in LB, M, and U, with relative abundances of 70.11%, 60.69%, and 42.91%, respectively (Table 7). The dominance of *Lactiplantibacillus* and *Companilactobacillus* highlights their crucial role in silage fermentation. In CK, LP, and LF, *Lactiplantibacillus* was the predominant genus (>50%), consistent with the known role of *L. plantarum* in driving lactic acid fermentation and creating an acidic environment that inhibits spoilage of organisms (Liu et al. 2024). In contrast, *Companilactobacillus* was more prevalent in LB, M, and U, with LB showing an abundance of over 70%. According to Pang et al. (2024), *Companilactobacillus* contributes to aerobic stability by converting lactic acid into acetic acid, which suppresses yeast and mold growth, resulting in a slightly higher pH but improved long-term storage stability.

Other LAB genera (*Limosilactobacillus*, *Weissella*, *Levilactobacillus*, and *Enterococcus*) and non-LAB genera (*Acinetobacter*, *Agrobacterium*, *Stenotrophomonas* and, *Klebsiella*) were detected at relatively low abundances (<10%). Non-lactic acid bacteria (LAB) genera, including *Acinetobacter*, *Agrobacterium*, *Stenotrophomonas*, and *Klebsiella* were detected in minor proportions. Notably, *Limosilactobacillus* constituted 13.68% in the uninoculated control (U), suggesting a more diverse microbial community in the absence of inoculation.

Table 6. Summary of fermentation characteristics and microbial indicators of napier grass silage across treatments

Inoculants	Time (Day)	pH	Lactic acid (%DM)	NH_3-N (%DM)	Acetic acid (mM)	Crude protein (%DM)	LAB (log CFU/g)
CK	7	3.734 ± 0.03^{abc}	3.245 ± 0.134^{bcde}	0.156 ± 0.018^{bcde}	15.821 ± 1.864^{abc}	9.295 ± 0.562^{abc}	9.001 ± 0.041^{k}
	14	3.86 ± 0.051^{g}	3.634 ± 0.200^{fg}	0.260 ± 0.036^{ef}	40.057 ± 5.963^{g}	8.824 ± 0.389^{abc}	6.680 ± 0.043^{f}
	28	3.79 ± 0.062^{cdef}	2.489 ± 0.196^{a}	0.103 ± 0.01^{abc}	12.83 ± 1.771^{a}	8.527 ± 0.320^{a}	3.753 ± 0.455^{a}
LF	7	3.762 ± 0.04^{bcde}	3.083 ± 0.149^{bcd}	0.015 ± 0.001^{a}	19.054 ± 1.504^{cd}	9.221 ± 0.135^{abc}	9.045 ± 0.208^{k}
	14	3.762 ± 0.018^{bcde}	3.596 ± 0.169^{efg}	0.026 ± 0.007^{a}	27.701 ± 3.974^{e}	8.879 ± 0.536^{abc}	7.272 ± 0.260^{hi}
	28	3.796 ± 0.011^{def}	2.593 ± 0.179^{a}	0.019 ± 0.001^{a}	19.450 ± 2.257^{cd}	9.009 ± 0.534^{abc}	5.262 ± 0.086^{bc}
LP	7	3.744 ± 0.024^{abcd}	3.592 ± 0.159^{efg}	0.076 ± 0.031^{ab}	15.373 ± 1.775^{abc}	9.201 ± 0.529^{abc}	8.17 ± 0.399^{j}
	14	3.734 ± 0.013^{abc}	3.925 ± 0.205^{fg}	0.157 ± 0.029^{bcde}	29.480 ± 2.168^{e}	8.622 ± 0.258a	6.912 ± 0.188^{fgh}
	28	3.782 ± 0.008^{cedf}	2.505 ± 0.272^{a}	0.212 ± 0.046^{cde}	17.109 ± 0.718^{abcd}	8.768 ± 0.346^{abc}	3.64 ± 0.737^{a}
LB	7	3.718 ± 0.027^{ab}	3.355 ± 0.149^{cdef}	0.151 ± 0.030^{bcde}	13.998 ± 1.834^{ab}	9.325 ± 0.720^{abc}	7.198 ± 0.412^{ghi}
	14	3.694 ± 0.03^{a}	3.667 ± 0.283^{fg}	0.151 ± 0.027^{bcde}	34.388 ± 2.084^{f}	8.873 ± 0.483^{abc}	6.8 ± 0.142^{fg}
	28	3.772 ± 0.062^{bcdef}	2.955 ± 0.161^{b}	0.318 ± 0.272^{f}	17.63 ± 1.721^{bcd}	8.966 ± 0.901^{abc}	5.616 ± 0.183^{cd}
Mixed LAB	7	3.8 ± 0.046^{def}	3.094 ± 0.321^{bcd}	0.199 ± 0.034^{cde}	17.345 ± 3.48^{bcd}	9.700 ± 0.552^{bc}	8.17 ± 0.111^{j}
	14	3.774 ± 0.039^{cdef}	3.557 ± 0.409^{efg}	0.232 ± 0.046^{def}	43.493 ± 5.157^{g}	8.602 ± 0.285^{a}	7.521 ± 0.062^{i}
	28	3.764 ± 0.039^{bcdef}	2.384 ± 0.227^{a}	0.237 ± 0.096^{def}	19.786 ± 5.157^{cd}	9.011 ± 0.834^{abc}	5.026 ± 0.561^{b}
Uninoculated	7	3.81 ± 0.059^{efg}	3.442 ± 0.157^{def}	0.157 ± 0.026^{bcde}	15.436 ± 2.155^{abc}	9.782 ± 0.538^{c}	6.142 ± 0.102^{e}
	14	3.792 ± 0.018^{def}	4.091 ± 0.314^{h}	0.136 ± 0.028^{bcd}	43.867 ± 5.221^{g}	11.131 ± 1.901^{d}	5.946 ± 0.172^{de}
	28	3.82 ± 0.029^{fg}	3.062 ± 0.603^{bc}	0.21 ± 0.056^{cde}	21.176 ± 3.174^{e}	8.678 ± 0.542^{ab}	3.983 ± 0.628^{a}
SEM		0.005	0.058	0.011	1.105	0.091	0.175
p-value	Inoculum	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
	Time	0.028	<0.001	<0.001	<0.001	0.005	<0.001
	Inoculum x Time	<0.001	0.095	<0.001	<0.001	0.001	<0.001
	Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB. SEM: standard error of the mean; $^{a\text{-}e}$Different superscripts in the same column indicate significant differences ($p<0.05$)

Table 7. Relative abundance (%) of bacterial taxa at the genus level across treatments

Genus	CK	LB	LF	LP	M	U
Companilactobacillus	14.78	70.11	51.36	30.53	60.69	42.91
Lactiplantibacillus	64.26	21.2	24.84	42.48	14.58	28.26
Limosilactobacillus	5.01	0.68	6.8	2.68	2.44	13.68
Acinetobacter	3.72	1.17	4.14	5.66	3.62	0.8
Levilactobacillus	4.08	1.7	1.76	1.43	1.77	3.48
Alcaligenes	0.1	0.08	0.22	0.37	8.73	4.49
Klebsiella	0.83	0.91	0.91	1.41	1.85	2.1
Roseomonas	1.14	0.36	1.86	3.85	0.42	0.07
Enterobacter	0.6	0.84	0.96	1.23	1.23	1.32
Brevundimonas	0.59	0.5	0.6	1.32	0.71	0.13
Cellulosimicrobium	0.38	0.19	0.68	0.46	0.12	0.03
Microbacterium	0.19	0.05	0.29	0.51	0.07	0.03
Weissella	0.18	0.19	0.1	0.18	0.11	0.25
Glutamicibacter	0.14	0.08	0.36	0.21	0.12	0.01
Pseudoxanthomonas	0.35	0.02	0.11	0.3	0.04	0.01
Sphingobacterium	0.14	0.05	0.19	0.32	0.07	0.04
Stenotrophomonas	0.29	0.07	0.06	0.18	0.07	0.05
Agrobacterium	0.04	0.06	0.17	0.32	0.07	0.02
Enterococcus	0.04	0.09	0.42	0.06	0.06	0.01
Others	2.89	1.42	3.92	6.25	3.03	2.09
Total	99.75	99.77	99.75	99.75	99.8	99.78

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB

Abundance by species

Analysis of the microbial community in napier grass silage (Figure 2; Table 8) revealed a clear predominance of lactic acid bacteria (LAB) with distinct functional roles during fermentation. Homofermentative species, including *L. plantarum*, *L. paraplantarum*, and *L. pentosus*, were primarily associated with early fermentation, rapidly producing lactic acid, reducing pH, and stabilizing the silage environment. In contrast, *Companilactobacillus* species (e.g., *C. kimchii*, *C. pabuli*, and *C. bobalius*) were more active during later stages, converting lactic acid into acetic acid and other metabolites such as 1,2-propanediol, thereby enhancing aerobic stability through the inhibition of yeasts and molds.

Across treatments, LAB dominated the microbial community, although species composition varied depending on inoculation. *C. kimchii* was the most abundant species in LB, LF, and M treatments, whereas *L. plantarum* was more prevalent in the control (CK) and remained present at lower levels across all treatments. Other LAB species, such as *L. pentosus* and *L. paraplantarum*, were more abundant in the control but decreased following inoculation, suggesting competitive exclusion by dominant strains. Minor LAB species, including *L. fermentum* and *L. brevis*, were detected at low abundance, indicating a limited supporting role in fermentation.

Non-LAB species, such as *Acinetobacter* spp. and *Roseomonas gilardii*, were present in minor proportions, particularly in non-inoculated treatments, and likely originated from environmental contamination. Their low abundance suggests that LAB inoculation effectively suppressed undesirable microorganisms. It should also be noted that Figure 2 presents only the top 20 taxa, while additional low-abundance species are grouped as “Others” (Table 8), ensuring consistency between graphical and tabulated data.

Overall, the dominance and succession of LAB species confirm their central role in silage fermentation. The coexistence of homofermentative and heterofermentative LAB contributes to both rapid acidification and improved aerobic stability. These findings are consistent with previous studies (Kim et al. 2021; Guo et al. 2022), which highlight the importance of LAB diversity in maintaining silage quality and minimizing nutrient loss.

Collectively, these LAB species ensure high fermentation quality and maintain silage nutrition for ruminants. A few non-LAB, including *Acinetobacter schindleri*, *Acinetobacter variabilis*, and *Roseomonas gilardii,* were detected in minor quantities. These originate from soil or air and do not contribute to fermentation. If not controlled by LAB, silage quality may degrade. However, the substantial presence of LAB indicated that their addition effectively fostered beneficial microbial community dynamics and suppressed harmful microbes. This aligns with Kim et al. (2021), who advocated using both homofermentative and heterofermentative LAB to enhance silage quality and stability. High-quality silage contains both LAB types. *L. plantarum* excels at rapidly acidifying and stabilizing the environment. This combination results in silage with improved nutrition, stability, and benefits to ruminant health. These findings concur with Guo et al. (2022), who emphasized LAB's crucial role in maintaining feed quality and minimizing nutrient loss during storage.

The heatmap (Figure 3) illustrates the relative abundance of bacterial genera detected in napier grass silage under different inoculant treatments. Color intensity represents bacterial abundance, with darker shades indicating higher abundance and lighter shades indicating lower abundance.

Lactiplantibacillus exhibited the highest abundance across most samples, particularly in the CK and LP treatments, indicating the predominance of *L. plantarum* during fermentation. This finding is consistent with its established role as homofermentative lactic acid producer that effectively reduces pH and stabilizes the anaerobic environment of silage. *Levilactobacillus* and *Limosilactobacillus* were also relatively abundant, especially in CK and LB treatments, suggesting their contribution to heterofermentative activity and the formation of additional organic acids such as acetic acid.

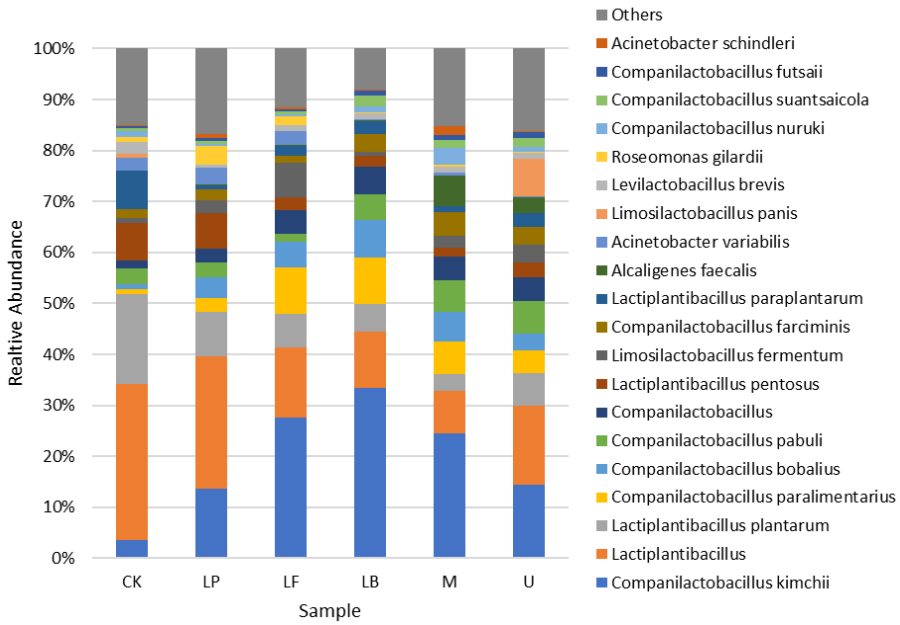

Figure 2. Relative abundance of the top 20 bacterial taxa at the species level in napier grass silage treated with different LAB inoculants. CK: *C.kimchii* InaCC-B982; LF: *L. fermentum* InaCC-B1024; LP: *L. plantarum* InaCC-B1028; LB: *L. brevis* InaCC-B1052; M: mixed LAB (CK + LF + LP + LB); U: uninoculated control

Table 8. Relative abundance (%) of bacterial taxa at the species level across treatments

Species	CK	LP	LF	LB	M	U
Companilactobacillus kimchii	3.54	13.46	27.41	33.26	24.29	14.32
Lactiplantibacillus sp.	30.31	25.9	13.52	10.99	8.32	15.47
Lactiplantibacillus plantarum	17.44	8.51	6.48	5.32	3.27	6.41
Companilactobacillus paralimentarius	0.95	2.69	9.12	9.03	6.31	4.38
Companilactobacillus bobalius	0.96	4.12	4.98	7.33	5.78	3.26
Companilactobacillus pabuli	3.12	2.8	1.54	4.99	6.06	6.3
Companilactobacillus sp.	1.53	2.77	4.67	5.48	4.76	4.63
Lactiplantibacillus pentosus	7.32	6.85	2.42	2.13	1.62	3.01
Limosilactobacillus fermentum	1.02	2.61	6.73	0.67	2.41	3.44
Companilactobacillus farciminis	1.72	2.04	1.4	3.57	4.51	3.52
Lactiplantibacillus paraplantarum	7.34	0.68	1.91	2.47	1.11	2.63
Alcaligenes faecalis	0.06	0.26	0.14	0.05	6.03	3.07
Acinetobacter variabilis	2.54	3.28	2.67	0.32	0.6	0.18
Limosilactobacillus panis	0.82	0.02	0	0	0	7.28
Levilactobacillus brevis	2.15	0.51	1.16	1.05	1.14	1.25
Roseomonas gilardii	1.05	3.66	1.76	0.34	0.39	0.06
Companilactobacillus nuruki	1.06	0.43	0.37	1.06	3.27	1.01
Companilactobacillus suantsaicola	0.61	0.64	0.54	2.06	1.44	1.77
Companilactobacillus futsaii	0.45	0.44	0.33	0.96	1.09	1.12
Acinetobacter schindleri	0.23	0.74	0.36	0.35	1.66	0.2
Others	14.83	16.71	11.52	7.92	15.14	16.15
Total	99.05	99.12	99.03	99.35	99.2	99.46

Note: CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, Mixed LAB: CK+ LF+ LP + LB

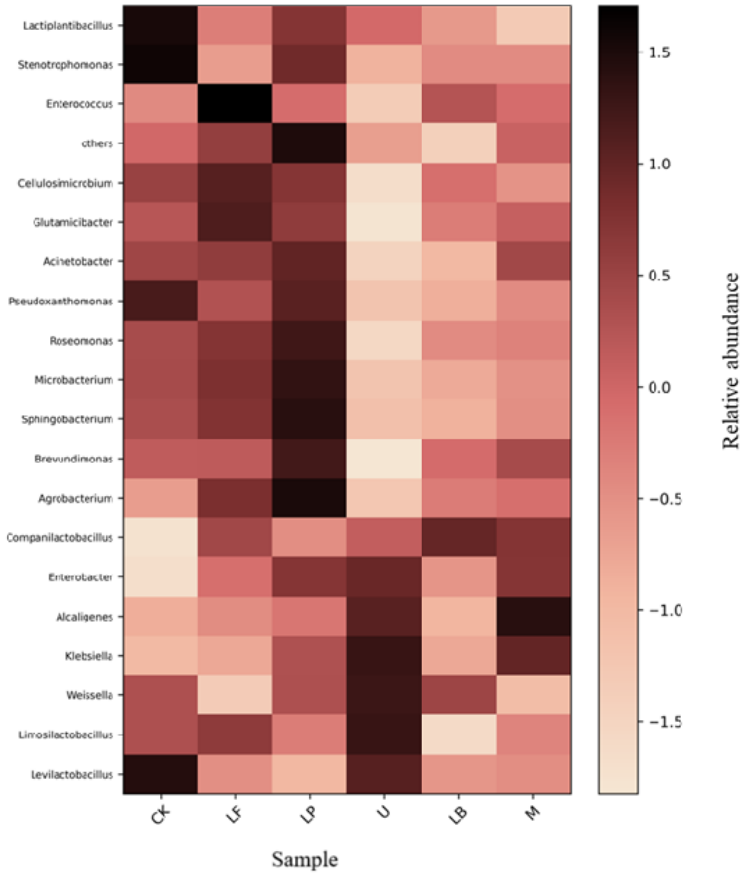

Figure 3. The heatmap of the top 20 distributions of bacterial abundance in the silage of the taxonomic genus. CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, M: Mixed LAB (CK+ LF+ LP + LB), U: Uninoculated

In contrast, non-LAB genera, including *Pseudomonas*, *Acinetobacter*, and *Brevundimonas*, were characterized by darker colors in most treatments, particularly in the M (mixed LAB) and U (uninoculated control) samples, reflecting low abundance. This indicates that inoculation with LAB effectively suppressed the proliferation of undesirable spoilage and aerobic microorganisms.

The heatmap (Figure 4) illustrates the distribution of bacterial species abundance across the different treatments (CK, LF, LP, LB, M, and U). The genera *Lactiplantibacillus*, *Limosilactobacillus*, *Companilactobacillus*, and *Levilactobacillus* were predominant in nearly all treatments, as indicated by their intense red coloration. These genera are LAB that are crucial in the fermentation of silage. They produce lactic acid, which reduces the pH and prevents the proliferation of spoilage microorganisms. In treatments with LAB inoculation (LP and LB), the dominance of *L. plantarum*, *L. paraplantarum*, and *L. pentosus* is more consistent compared to other treatments, suggesting that inoculation promotes efficient homofermentative fermentation through sufficient lactic acid production that establishes acidic conditions unfavorable to contaminants.

A heatmap illustrating the distribution of the relative abundance of bacterial species identified across various silage treatments is presented in Figure 4. The color gradient represents abundance levels, with bright orange to red indicating high abundance and light pink to pale tones indicating low abundance. The microbial composition exhibited distinct variations among treatments. The treatment inoculated with *L. plantarum* (LP) was characterized by a pronounced dominance of this species, as evidenced by the bright red coloration in the *L. plantarum* row. This finding highlights the successful inoculation and the ability of this strain to proliferate during fermentation. In contrast, species such as *Limosilactobacillus panis* and *L. brevis* appeared darker in the LP treatment, suggesting a reduced abundance due to competition with the inoculated strain.

In the uninoculated treatment (U), a generally darker hue was observed for most species, indicating a low total bacterial count and a limited population of lactic acid bacteria (LAB). This condition may be associated with less efficient fermentation due to the absence of dominance by homofermentative LAB species. Other treatments, such as LF− and LB−, displayed intermediate abundance patterns, with several LAB species remaining detectable but not dominant.

Overall, the heatmap demonstrates that the type of inoculant markedly influenced the composition of the microbial community during silage fermentation. Inoculation with *L. plantarum* increased the proportion of specific LAB while suppressing the growth of non-LAB bacteria, potentially contributing to improved fermentation quality and silage stability.

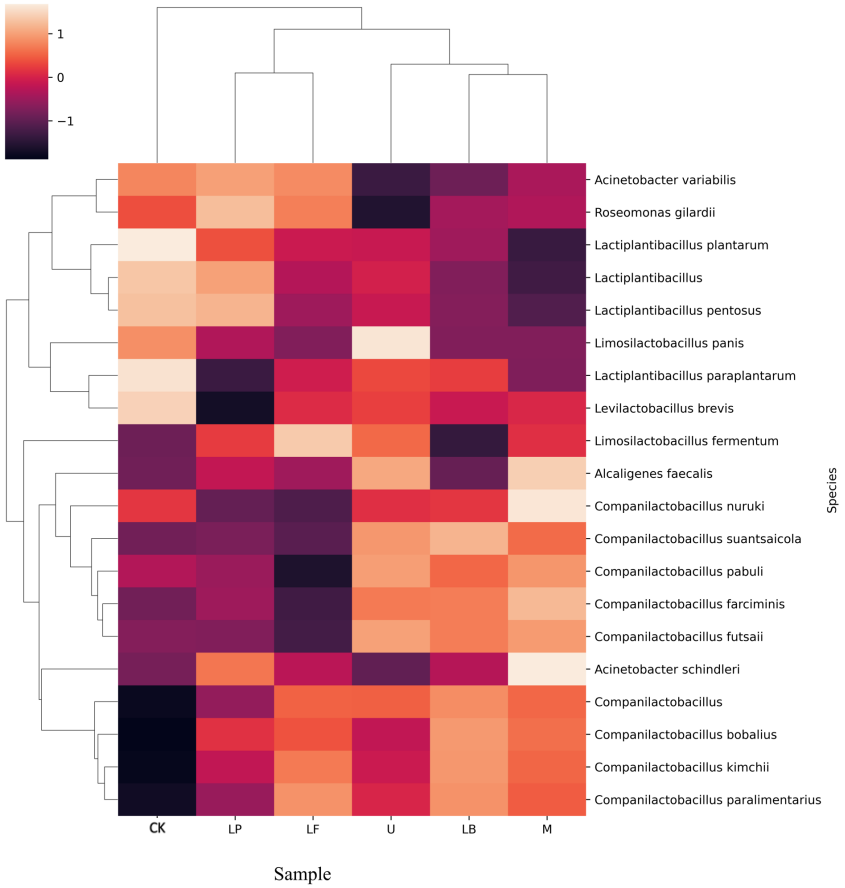

Figure 4. The heatmap of the top 20 distributions of bacterial abundance in the silage of the taxonomic species. CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, M: Mixed LAB (CK+ LF+ LP + LB), U: uninoculated (no LAB inoculated)

The proteolytic activity of these bacteria can negatively affect fermentation quality by causing protein breakdown and the formation of ammonia. The observed variations in microbial distribution across treatments indicated that LAB inoculation not only strengthened the dominance of fermentative bacteria but also suppressed the growth of undesirable species. Moreover, competition among LAB species such as *Lactiplantibacillus* and *Levilactobacillus* may further influence the metabolite profile of silage.

Analysis of alpha diversity indices (Figure 5) revealed clear variations in microbial diversity among the sample groups (CK, LF, LP, LB, M, and U). Richness was indicated by the Observed-graph and Chao1 indices. The Observed and Chao1 indices consistently indicated that the LF and LP groups had the highest richness, reflecting a greater number of microbial species. The LB and CK groups exhibited intermediate richness, whereas the M and U groups had the lowest values. The reduced richness in the U group suggests that a limited number of species are capable of growing, resulting in a less complex microbial community. Diversity and Evenness were indicated by Shannon and Simpson. The Shannon and Simpson indices demonstrated that the LP group had the highest diversity, characterized by a more even distribution of species and the absence of dominance by specific taxa. This indicates that the microbial community in the LP was more balanced than that in the other groups. In contrast, the U group had the lowest Shannon and Simpson values, suggesting dominance by certain species and reduced community evenness. The high richness observed in LF and LP indicates that these treatments support the growth of a large number of microbial species, including minority (rare) taxa. Furthermore, the higher diversity and balance in the LP, as reflected by the Shannon and Simpson indices, suggest that this community is more stable and resilient to disturbance.

Principal coordinate analysis (PCoA) based on Bray-Curtis distances revealed clear differences in microbial community composition among the silage groups (CK, LF, LP, LB, M, and U) (Figure 6). The first principal coordinate (PCoA1) explained the largest proportion of the variation (35.89%), followed by PCoA2 (24.09%) and PCoA3 (17.63%). In the PCoA1 versus PCoA2 plot, the LF and LP groups clustered closely, indicating similar microbial compositions, whereas CK appeared in an intermediate position near LF. The LB and M groups were positioned centrally, with overlapping communities, whereas U was distinctly separated, reflecting substantial structural differences. A similar pattern was observed in the PCoA1 versus PCoA3 plot, with LF and LP clustering together, and U positioned apart.

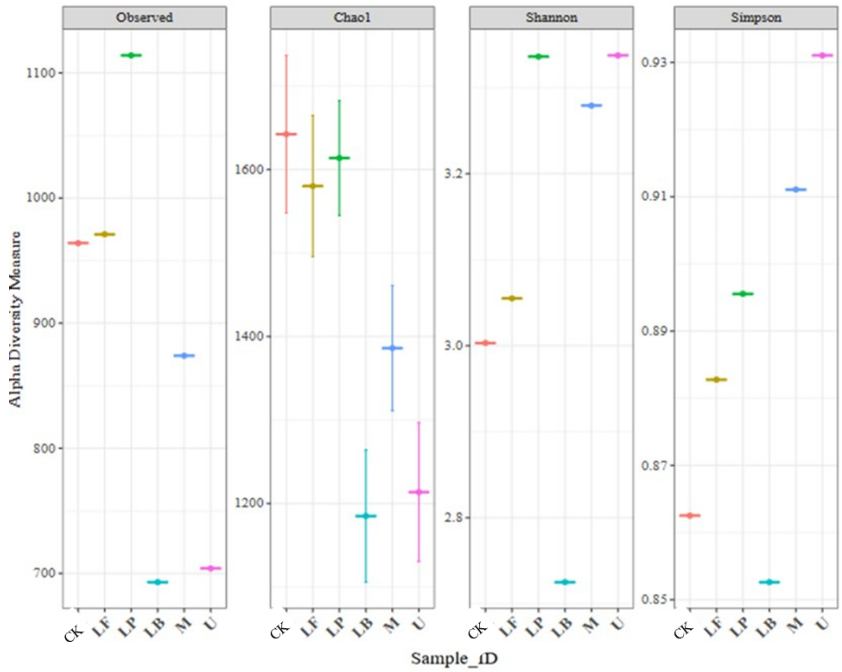

Figure 5. Alpha diversity analysis of microbial communities in silage. CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, M: Mixed LAB (CK+ LF+ LP + LB), U: uninoculated (no LAB inoculated)

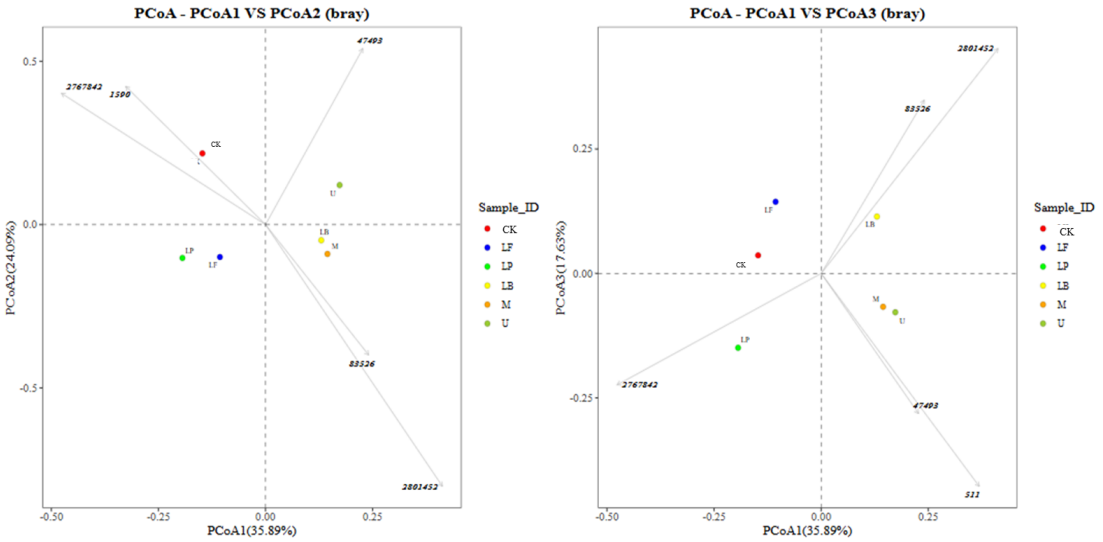

Figure 6. Principal coordinate analysis (PCoA) of the microbial community in silage. CK: *C. kimchii* InaCC-B982, LF: *L. fermentum* InaCC-B1024, LP: *L. plantarum* InaCC-B1028, LB: *L. brevis* InaCC-B1052, M: Mixed LAB (CK+ LF+ LP + LB), U: uninoculated (no LAB inoculation)

The clustering of LF and LP suggests the development of a more homogeneous microbial community dynamics, driven by the stimulation of LAB and other functional microbes. In contrast, the separation of U indicated a community with lower diversity and strong species dominance, implying instability. These observations are consistent with the alpha diversity results, which showed that LF and LP had the highest richness and diversity, whereas U had the lowest values.

Overall, the PCoA results confirmed that fermentation treatments shaped microbial communities, enhancing their balance and stability. This finding aligns with previous studies, which have shown that microbial inoculation can alter community composition (Ni et al. 2017) and that greater diversity contributes to ecosystem stability (Li et al. 2021). Collectively, LF and LP produced more homogeneous and diverse microbial communities. In contrast, U displayed a distinct structure with reduced diversity, demonstrating

how fermentation treatments influence the microbial composition and ecological balance. Changes in the composition and stability of microbial communities offer a mechanistic understanding of the noted enhancements in silage fermentation quality, highlighting the functional benefits of LAB inoculation.

The microbial community of napier grass silage exhibited dynamic changes throughout the fermentation process. Populations of lactic acid bacteria (LAB) and total bacteria increased during the early stage, particularly up to day 7 in single-inoculant treatments, likely driven by the availability of water-soluble carbohydrates (WSC), which supported microbial proliferation (Kim et al. 2021). As fermentation progressed, both populations declined by day 28, coinciding with substrate depletion and increased acidity. Yeasts followed a similar pattern, initially increasing due to residual oxygen and available substrates, but subsequently declining sharply under anaerobic and acidic conditions. The absence of coliforms at day 28 further confirms that LAB-driven acidification effectively suppressed undesirable microorganisms. These patterns are consistent with previous reports describing typical microbial succession in tropical silage systems (Hernández-Aquino et al. 2019; You et al. 2021; Ridwan et al. 2023).

This succession reflects a metabolic shift from homofermentative to heterofermentative pathways. *L. plantarum* likely dominated the early phase by rapidly converting fermentable carbohydrates into lactic acid via glycolysis, leading to a rapid decline in pH and suppression of proteolytic activity. The presence of heterofermentative LAB during the later stages of fermentation may contribute to improved aerobic stability through the production of secondary metabolites, including acetic acid. This sequential dominance highlights the complementary roles of homofermentative and heterofermentative LAB in improving both fermentation efficiency and storage stability.

Changes in microbial dynamics were closely associated with alterations in chemical composition. LAB inoculation significantly influenced dry matter (DM), organic matter (OM), protein fractions, and fiber components, while mineral content remained unaffected. Although control silage retained higher DM and OM, inoculated treatments, particularly those treated with *L. plantarum,* showed improved crude protein preservation and reduced NH_3-N accumulation, indicating more effective inhibition of proteolysis. Variations in fiber fractions further suggest that LAB activity contributed to cell wall modification and enhanced nutrient availability.

Crude protein (CP) was significantly affected by inoculant, fermentation time, and their interaction ($p<0.01$). Although the highest CP was observed in the uninoculated treatment at day 14, this likely reflects a concentration effect due to dry matter loss rather than true protein preservation. In contrast, NH_3-N, a key indicator of proteolysis, was higher in control treatments and markedly lower in LAB-inoculated silages, particularly in LF, indicating better preservation of true protein. These results confirm that LAB inoculation effectively reduces protein degradation during ensiling.

Fermentation characteristics further support these findings. LAB inoculation significantly affected pH, lactic acid, NH_3-N, and acetic acid concentrations ($p<0.001$). Inoculated silages exhibited lower pH values, reflecting more efficient acidification associated with higher LAB activity. Lactic acid concentrations were more stable in LF and mixed-inoculant treatments, indicating improved fermentation consistency. Although the uninoculated control showed transiently high lactic acid levels, this was likely due to the short-term activity of epiphytic LAB that could not sustain stable fermentation. Acetic acid concentrations were higher in treatments involving heterofermentative LAB, contributing to improved aerobic stability, albeit with potential trade-offs in energy efficiency.

From an ecological perspective, the predominance of *Lactiplantibacillus* and *Companilactobacillus* represents a structured microbial succession in which beneficial fermentative bacteria progressively replace aerobic and proteolytic microorganisms. Treatments with higher microbial diversity, such as LF and LP, may support more functionally complementary communities, thereby enhancing overall silage quality (Li et al. 2021). Overall, these results are consistent with previous studies demonstrating that LAB inoculation improves fermentation efficiency, nutrient preservation, and microbial stability in tropical forages (Gulfam et al. 2016; Santos et al. 2016; Ertekin and Kızılşimşe 2019; Sánchez-Guerra et al. 2024).

It should be noted that the microbial community analysis is descriptive in nature due to the use of pooled samples without biological replication; therefore, no statistical comparisons were performed. Nevertheless, the strong consistency between microbial dynamics, fermentation characteristics, and compositional changes supports the biological relevance of the observed patterns. The effectiveness of LAB inoculants may vary depending on environmental conditions, including climate, soil type, and management practices; thus, further validation under diverse field conditions is required. In addition, LAB inoculants should be considered a complementary strategy rather than a replacement for traditional ensiling practices, thereby preserving the diversity of native microbial communities while enhancing fermentation efficiency. Although lactic acid is a key indicator of silage quality, other fermentation products, such as acetic acid and ethanol, also contribute to aerobic stability. Therefore, a more comprehensive evaluation of fermentation metabolites is necessary to achieve a holistic understanding of the silage fermentation process.

Finally, the addition of 1% dextrose provided a readily fermentable substrate that likely enhanced fermentation intensity across treatments. While this approach ensured standardized substrate availability, it may limit direct extrapolation to practical conditions where supplemental sugars are not typically applied. Therefore, further studies under field conditions without dextrose supplementation are warranted.

Limitations

The selection of LAB inoculants should therefore consider intended storage duration and management conditions.

Homofermentative strains such as *L. plantarum* and *L. farraginis* are more suitable for rapid acidification and nutrient preservation, whereas heterofermentative LAB may contribute to improved aerobic stability during prolonged storage. A mixed inoculation strategy combining both functional groups may optimize early fermentation while maintaining aerobic stability. Future studies incorporating biological replication and integrated metagenomic-metabolomic approaches are required to validate these findings and further elucidate inoculant-specific mechanisms.

To strengthen the linkage between microbial succession and fermentation performance, an exploratory multivariate ordination is recommended using the relative abundances of dominant bacterial genera and the corresponding fermentation variables measured on day 28. Because sequencing was conducted on pooled DNA without biological replication, this analysis should be interpreted descriptively rather than inferentially. Nevertheless, such ordination would allow visualization of associations between *Lactiplantibacillus* and favorable fermentation indicators such as higher lactic acid concentration and lower pH, whereas *Companilactobacillus* would be expected to align more closely with acetic acid production and traits related to aerobic stability. This integrative approach would substantially improve the mechanistic interpretation of how microbial community composition relates to silage quality outcomes.

In conclusion, this study demonstrates that lactic acid bacteria (LAB) inoculation markedly improves the fermentation quality, nutrient preservation and microbial stability of napier grass silage under tropical conditions. LAB-treated silages achieved lower pH values (3.69-3.86) compared to less stable conditions in the control, while lactic acid concentrations increased to 2.38-4.90% DM. Protein preservation was enhanced, as indicated by reduced ammonia-N levels (as low as 0.015-0.026% DM in inoculated treatments versus up to 0.260% DM in the control). Among the inoculants evaluated, *L. plantarum* InaCC-B1028 consistently showed superior performance, characterized by stable acidification, improved microbial suppression (yeasts and coliforms reduced to undetectable levels by day 28), and more efficient nutrient retention. Accordingly, *L. plantarum* InaCC-B1028 is recommended for practical silage production under tropical conditions, particularly when rapid fermentation and protein preservation are critical. Microbial analysis further confirmed the dominance of beneficial LAB genera (*Companilactobacillus* and *Lactiplantibacillus*), supporting a structured microbial succession that underpins effective fermentation.

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