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Article

The Effect of Corn Stalks and *Salix psammophila* on the Fermentation Quality and Microbial Community of *Caragana korshinskii* Silage

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Abstract

To expand the utilization of waste from the coppicing of xerophytic shrubs, such as *Caragana korshinskii* (*C. korshinskii*). This study aimed to evaluate the effects of *Salix psammophila* (*S. psammophila*) and corn stalks on *C. korshinskii* silage. Chop the above three experimental materials into segments (2–3 cm). Three treatments were designed: *C. korshinskii* with 10% sugar (CK), *C. korshinskii* mixed with *S. psammophila* in equal amounts and 10% sugar (CS), and *C. korshinskii* mixed with corn stalks in equal amounts (CC). Adjust the moisture content to 60% (fresh weight basis), and anaerobically fermented in sealed 50-L silo buckets for 60 days. After silage is finished, test its nutritional components and microbial communities. After 60 days, three groups showed significant differences in ash (ASH), water-soluble carbohydrates (WSC), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and organic acid ($p < 0.05$). CS had the lowest ASH (3.15%), NDF (72.97%), and the highest ADF (58.12%) and WSC (2.27%). CS had the lowest lactic acid and valeric acid content but the highest isobutyric acid content among the treatment groups. CK had the highest CP content, lactic acid, and lactic acid/ acetic acid ratio among the treatment groups. There were also differences in bacterial and fungal composition among the treatment groups, but the abundance of *Lactobacillaceae* was the highest. These findings confirmed that CK exhibits an optimal lactic acid content and dominance by *Lactobacillaceae*. Corn stalks improved the silage quality of *C. korshinskii* through intensified lactic acid fermentation, ADF and Acid detergent lignin (ADL) reduction, while *S. psammophila* increased isobutyric acid and reduced fermentation quality.

Keywords: *Caragana korshinskii*; *Salix psammophila*; corn stalks; silage

1. Introduction

Feed supply is a key factor in the sustainable development of the livestock industry. With the rapid expansion and intensification of the Chinese livestock sector, the demand for feed has steadily increased [1]. *C. korshinskii* is a drought-resistant leguminous shrub widely cultivated in the northwestern region of China for desertification management [2]. Years of neglecting the coppicing of *C. korshinskii* may lead to an increased risk of branch aging, wilting, and death [3]. Coppicing holds great significance for *C. korshinskii*, but the effective utilization of coppicing waste is still a problem to be solved [4].

The competition for food resources between humans and livestock is becoming increasingly evident, highlighting the importance of developing unconventional feed sources [5]. The *C. korshinskii* branches and leaves contain high CP and trace elements, which are beneficial for animal growth

[6,7]. As a woody plant, *C. korshinskii* can accumulate a large amount of biomass. *C. korshinskii* nutrient content and palatability can be affected by the stage of growth, which causes its harvest time to be seasonally limited. Converting it to hay leads to nutrient loss and increased lignin content. Silage is a convenient method of forage preservation that can alleviate feed shortages in arid regions [8,42]. Silage has become a vital component of livestock feed and is widely adopted in the livestock industry [9,10].

Silage is a complex biochemical process. If the number of *Lactobacillaceae* attached to the forage is insufficient, it will lead to silage failure. The anaerobic environment is conducive to the growth and metabolism of *Lactobacillaceae* [13,14]. *Lactobacillaceae* convert the WSC into lactic acid. When the pH of the silage feed falls below 4, it inhibits the growth and metabolism of most microorganisms, including the *Lactobacillaceae* themselves [11,12]. With the progression of silage, changes in the microbial community have also occurred [15]. In recent years, researchers have utilized molecular techniques to clarify the parameters of silage and the changes in the microbial community [16,17]. Understanding the complex changes in microbial communities and their functional succession during fermentation is crucial for the development of silage [18-20]. High-throughput sequencing has been widely used in the study of silage microbial communities [21,40]. 16S rRNA is a conserved bacterial sequence frequently utilized for microbial identification [37].

The WSC content of *C. korshinskii* is relatively low, and its buffering capacity is high, which leads to a lower success rate of silage. *C. korshinskii* is characterized by a relatively high tannin content, and studies have indicated that tannins can be detrimental to lactic acid fermentation [27]. The co-silage of *C. korshinskii* with other forage crops can effectively reduce the tannin content in the silage system, increase the WSC, and thereby enhance the success rate of silage [48,22].

This study assessed the effects of *S. psammophila* and corn stalks on the nutritional composition and microbial community of *C. korshinskii* silage. The results aim to provide theoretical guidance and technical support for shrub silage production.

2. Materials and Methods

2.1. Experimental Design and Processing

In November 2022, *C. korshinskii*, *S. psammophila*, and corn stalks were collected from Wushen Country, Ordos City, Inner Mongolia (38°36'11.5"N, 108°49'48.8"E). The materials were chopped into a size of 2-3cm and stored in woven bags for subsequent use.

The experiment included three treatment groups:

CK group: 30 kg of *C. korshinskii* supplemented with 3 kg of sugar, with a compacted density of 380 kg/m³.

CS group: 15 kg of *C. korshinskii* mixed with 15 kg of *S. psammophila* and 3 kg of sugar.

CC group: 15 kg of *C. korshinskii* mixed with 15 kg of corn stalks.

After mixing with different materials of *S. psammophila* and corn stalks inoculants respectively, adjusted to approximate 60% moisture content (fresh weight basis), and anaerobically fermented in sealed 100-L silo and vacuum-sealed at room temperature (25–28°C) for 60 days. After measurement and calculation, the final compacted density is about 380 kg/m³. Each treatment was replicated three times. After 60 days, samples were collected using the quartering method for further analysis.

2.2. Fermentation Characteristics and Chemical Composition Analysis

Silage was placed in an air-forced drying oven at 65°C for 72 hours to analyze DM[41]. The dried samples were ground into 1-mm particles using a mill for nutrient analysis[42]. An elemental analyzer determined total nitrogen according to the Dumas method[43]. WSC was analyzed by the anthrone method. NDF and ADF were performed via the method of Van Soest et al, and sodium sulfite and alpha-amylase were added for the NDF producer[44]. The content of ADL in the sample is determined by the gravimetric method[45].

Samples (10 g) were homogenized in distilled water (90 mL) at 4°C for 24 h. Thereafter, the organic acids analysis of the silage extract was made by filtering the mixture through four layers of

cheesecloth and qualitative filter paper. The concentration of organic acids were determined using the Agilent HPLC 1260 (Agilent Technologies, Santa Clara, CA, USA), which was equipped with a 210 nm UV detector (Sciex API 5000; McKinley Scientific, Sparta Township, NJ, USA) and Agilent Hi-Plex H column (Agilent Technologies, USA). The eluent was 5 mM H₂SO₄ with a running rate of 0.7 mL/min at a 55°C column oven temperature.

2.3. Microbial Community Analysis

The original sequencing data is stored on the NovoMagic cloud-based bioinformatics platform (<https://magic.novogene.com>) for subsequent analysis. After quality control and noise reduction through DADA2, high-quality amplicon sequence variants (ASVs) were generated. QIIME2 was used to assess microbial community diversity, including alpha diversity indices (Chao1 richness, Shannon, and Simpson diversity indices) and beta diversity metrics. Multivariate analyses, including principal coordinate analysis (PCoA), were employed to evaluate changes in microbial community composition and structure.

2.4. Statistical Analysis

Data on chemical composition (e.g., ASH, WSC, and CP) and silage quality (e.g., organic acids) were analyzed using Excel and GraphPad Prism 9. Statistical significance was determined using ANOVA, and differences among groups were assessed at $p < 0.05$.

3. Results

3.1. Chemical Composition of Silage

A comparative analysis of nutritional components in the silage samples revealed significant differences in nutrient composition among the experimental groups (Table 1). The ASH content in the CS group (3.15% DM) was significantly lower than that in the CK (4.28% DM) and CC (4.53% DM) groups ($p < 0.01$). The WSC content in the CS group (2.27% DM) was significantly higher than that in the CK group (0.85% DM, $p < 0.0001$). The CP content in the CK group (8.17% DM) was higher than in the CC (6.03% DM) and CS (6.53% DM) groups ($p < 0.001$). The NDF content in the CS group (72.97% DM) was significantly lower than in the CK group (78.35% DM, $p < 0.05$). Significant differences in ADF and ADL content were also observed among the groups ($p < 0.001$).

Table 1. Nutrient composition in different treatment groups after 60 days of silage

Items ¹	Materials ²			SEM ³	P-value ⁴
	CK	CC	CS		
ASH (%)	4.28 ^b	4.48 ^b	3.15 ^a	0.199	**
CP (%)	8.17 ^b	6.03 ^a	6.53 ^a	0.263	***
NDF (%)	78.35 ^b	76.46 ^{ab}	72.97 ^a	0.763	*
ADF (%)	55.33 ^b	49.48 ^a	58.12 ^c	1.063	***
ADL (%)	25.38 ^b	16.50 ^a	25.47 ^b	0.92	***
WSC (%)	0.85 ^a	0.70 ^a	2.27 ^b	0.075	****
NH ₃ -N (g/100g)	0.22 ^a	0.25 ^a	0.17 ^a	0.019	NS

¹ ASH, ash; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; WSC, water-soluble carbohydrate. ² CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*. ADL, acid detergent lignin; WSC, water-soluble carbohydrate. ³ SEM, standard error of the means. ⁴ NS, no significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Means of inoculation treatments within a row with different superscripts differ ($p < 0.05$).

3.2. Fermented Organic Acid Content

A comparative analysis of organic acids in the silage samples revealed significant differences among the groups (Table 2). The CK group had significantly higher concentrations of lactic acid (0.81 g/mL), formic acid (2.58 g/mL), propionic acid (0.36 g/mL), and valeric acid (2.19 g/mL), compared

to both the CC and CS groups ($p < 0.05$). The lactic acid-to-acetic acid ratio in the CK group (0.72) was also higher than in the other groups ($p < 0.05$). The isobutyric acid content in the CS group (2.89 g/mL) was significantly higher than in the CK (0.18 g/mL) and CC (0.12 g/mL) groups ($p < 0.0001$).

Table 2. Comparison of different materials across various items.

Items ¹	Materials ²			SEM ³	P-value ⁴
	CK	CC	CS		
Lactic acid (g/mL)	0.81 ^b	0.34 ^a	0.29 ^a	0.09	*
Acetic acid (g/mL)	1.12 ^a	1.68 ^a	1.44 ^a	0.18	NS
Lactic acid / Acetic acid	0.72 ^b	0.21 ^a	0.21 ^a	0.09	*
Formic acid (g/mL)	2.58 ^b	0.69 ^a	0.71 ^a	0.17	***
Propionic acid (g/mL)	0.36 ^b	0.29 ^a	0.35 ^b	0.05	**
Valeric acid (g/mL)	2.19 ^b	0.12 ^a	0.08 ^a	0.59	****
Butyric acid (g/mL)	0.37 ^b	0.17 ^a	0.20 ^a	0.11	***
Isobutyric acid (g/mL)	0.18 ^a	0.12 ^a	2.89 ^b	0.08	****

3.3. Cluster Analysis of Microorganisms

Microbial profiling of silage samples yielded 1,942 bacterial and 1,593 fungal ASVs (Figure 1). Bacterial ASV (Figure 1A) richness varied by treatment: CS (627) > CK (596) > CC (330). Fungal (Figure 1B) richness showed an inverse trend: CC (510) > CK (480) > CS (260).

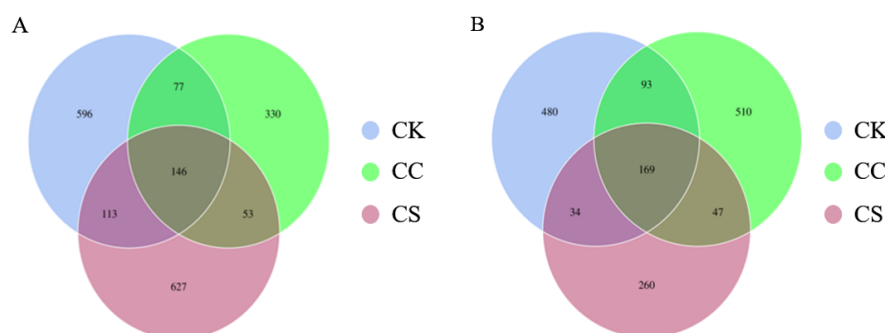


Figure 1. Figure 1. Venn diagrams of bacterial and fungal amplicon sequence variants in different groups. (A) Venn diagram illustrating bacterial ASVs among treatment groups after 60 days of ensiling, based on 16S rRNA gene amplicon sequencing (V3-V4 hypervariable regions). (B) Venn diagram showing shared and unique fungal ASVs among silage treatment groups at 60 days. ASVs were identified by Illumina sequencing of amplified ITS regions using primers ITS1F/ITS2R. CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*.

3.4. Relative Abundance of Microbial Species

At the family taxonomic rank, *Lactobacillaceae* was the dominant bacterial group in all treatment groups, accounting for 72.17% in the CK group, 59.58% in the CS group, and 44.76% in the CC group (Figure 2A). Among fungi, *Didymellaceae* had the highest relative abundance, with 24.68% in the CK group, 37.20% in the CS group, and 9.13% in the CC group (Figure 2B).

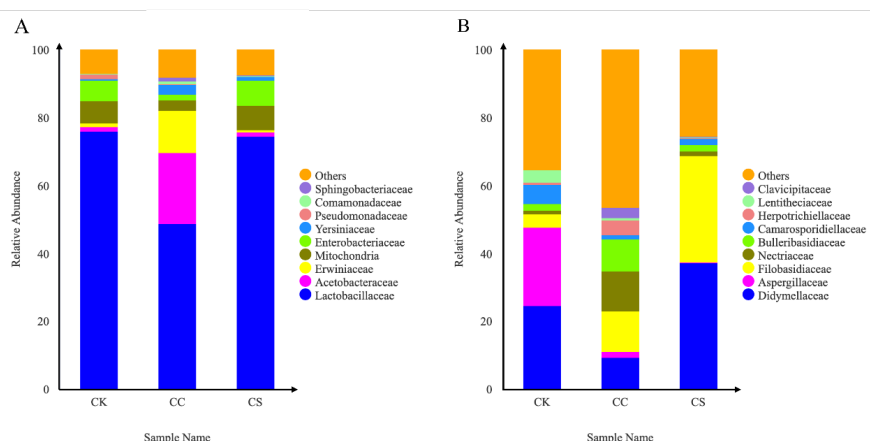


Figure 2. Microbial relative abundance analysis in different groups. (A) Relative abundance of bacterial communities in silage 60 days.(B) Relative abundance of fungal communities in silage 60 days. CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*.

3.5. Alpha Diversity of Silage

The Chao1, Shannon, and Simpson indices were used to evaluate microbial diversity (Tables 3 and 4). The bacterial Chao1 index was relatively high in the CK (410.81) and CS (431.81) groups but lower in the CC group (338.75). The fungal Chao1 index was higher in the CC group (371.08) compared to the CK (340) and CS groups (343.62). The Simpson index indicated lower bacterial diversity in the CK (0.82) and CS (0.79) groups compared to the CC group (0.96).

Table 3. diversity of bacteria in treatment groups

Items ¹	Materials ²			SEM ³	P-value ⁴
	CK	CC	CS		
Chao 1 ³	410.81	338.75	431.81	53.43	0.313
Shannon ⁴	3.83	3.38	3.41	0.21	0.148
Simpson ⁵	0.86	0.77	0.86	0.21	0.549
Coverage	0.99	0.99	0.99	–	–

¹ CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*. ² SEM, standard error of the means. ³ Chao 1, Species richness of the microbial community was assessed based on ASVs (Amplicon Sequence Variants) counts. ⁴ Shannon, Higher index values reflect increased community diversity (rich species composition and even abundance distribution) across treatments. ⁵ Simpson, Higher Simpson index denotes stronger species dominance (reduced diversity).

Table 4. diversity of fungi in three experimental groups.

Items ¹	Materials ²			SEM ³	P-value ⁴
	CK	CC	CS		
Chao 1 ³	340.0	371.08	343.62	35.09	0.473
Shannon ⁴	4.32 ^a	5.6 ^b	3.75 ^{ab}	0.20	0.025
Simpson ⁵	0.82 ^b	0.96 ^c	0.79 ^a	0.02	0.001
Coverage	0.99	0.99	0.99	–	–

¹ CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*. ² SEM, standard error of the means. ³ Chao 1, Species richness of the microbial community was assessed based on ASVs (Amplicon Sequence Variants) counts. ⁴ Shannon, Higher index values reflect increased community diversity (rich species composition and even abundance distribution) across treatments. ⁵ Simpson, Higher Simpson index denotes stronger species dominance (reduced diversity). Means of inoculation treatments within a row with different superscripts differ ($p < 0.05$).

3.6. Principal Coordinates Analysis (PCoA)

Principal Coordinates Analysis (PCoA) utilizing Bray-Curtis dissimilarity metrics demonstrated significant segregation of microbial communities across treatment groups (Figure 3). Figure 3A reveals

the differences in the spatial distribution of bacteria in the three treatment groups, with CS samples predominantly occupying the first quadrant, CC samples distributed across the second and third quadrants, and CK samples clustering in the fourth quadrant. Figure 3B shows the distribution of fungal PCOA in the three groups. The CK group is distributed in the first and second quadrants, the CC treatment group is in the third quadrant, and the CS treatment group is concentrated in the fourth quadrant. This quadrant-specific distribution indicates pronounced structural divergence in microbial community composition among treatments.

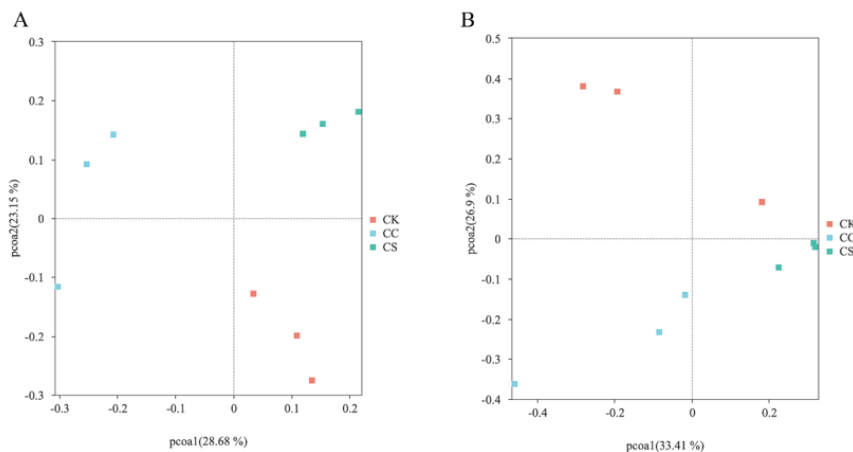
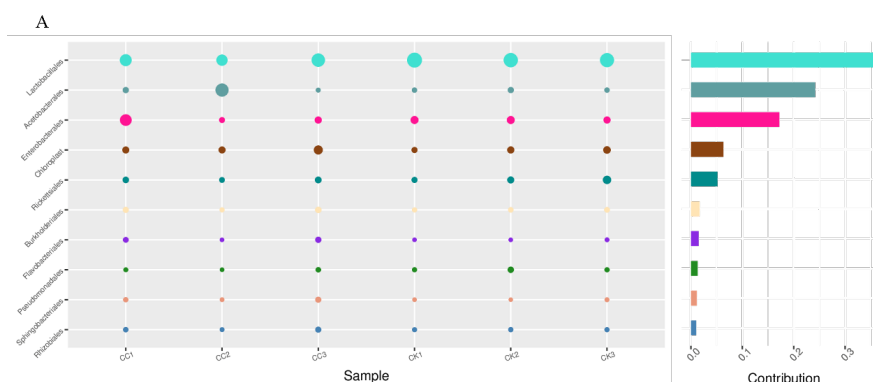


Figure 3. Clustering of microbial communities among different treatment groups. (A) PCoA of bacterial communities in 60-day silage (16S sequencing, Bray-Curtis). (B) PCoA of fungal communities in 60-day silage (ITS sequencing, Bray-Curtis). CK, *C. korshinskii*; CC, *C. korshinskii* + Corn stalks; CS, *C. korshinskii* + *S. psammophila*.

3.7. SIMPER Analysis

SIMPER (Similarity PERcentage) analysis indicates significant differences among the three treatment groups due to the composition of communities (Figure 4). *Lactobacillaceae* were identified as the main bacteria responsible for the differences between the CK and CC groups, accounting for 36% (Figure 4A). At the same time, *Lactobacillaceae* is also the main bacterium responsible for the differences between CK and CS (Figure 4B). The comparison of fungal communities showed that *Pleosporales* and *Eurotiales* caused significant differences between the CK and CC groups (Figure 4C), while *Filobasidiales* and *Eurotiales* were the main reasons for the differences between the CK and CS groups (Figure 4D).



spoilage microorganisms [33,34]. *Lactobacillaceas* have a strong ability to produce lactic acid [38]. *Lactobacillaceas* utilize WSC for growth and metabolism; sufficient WSC is a key factor for the successful fermentation of silage feed [29-31]. The dominance of *Lactobacillaceae* in the CK treatment group further supports this observation. Microbial community analysis shows significant differences in bacterial and fungal diversity among the treatment groups. The relative abundance of *Lactobacillaceae* in the CK group (72.17%) is significantly higher than that in the CS group (58.58%) and the CC group (44.76%), indicating that the supplementation of WSC in the silage system is beneficial for the growth and metabolism of *Lactobacillaceae*.

In contrast, the abundance of *Lactobacillaceas* in the CC and CS groups is relatively low, which may be the reason for their poorer fermentation quality, as insufficient lactic acid production could prevent the pH from reducing to the ideal range, thereby failing to inhibit undesirable microbial activity [35,36].

SIMPER analysis found that *Lactobacillaceae* was the main factor contributing to the differences between treatment groups. In fungi, *Pleosporales* and *Eurotiales* were major contributors to the differences between the CK and CC groups, while the *Filobasidiales* and *Eurotiales* abundance differences led to differences in the CK and CS groups. As shown by the Simpson index, the fungal diversity was higher in the CC and CS treatment groups, indicating that the growth of harmful microorganisms was not inhibited. The CS group had higher ADF and ADL compared to the CC group, indicating that the quality of *S. psammophila* mixed with *C. korshinskii* silage was poor. This is consistent with previous studies that have shown that high ADL content can negatively affect the digestibility and palatability of silage [23,48]. In contrast, the lower ADF and ADL in the CC group suggest that corn stalks may be a more suitable additive to improve the fermentation quality of *C. korshinskii* silage.

This study indicates that the addition of *S. psammophila* and corn stalks significantly alters the nutritional composition and microbial community of *C. korshinskii* silage fermentation. These findings provide empirical evidence for utilizing agricultural by-products to enhance *C. korshinskii* silage production.

5. Conclusions

This study produces silage feed by mixing corn stalks or *S. psammophila* with *C. korshinskii*. The fermentation quality and microbial community were tested, and the results indicated that the silage feed made from the mixture of *S. psammophila* and *C. korshinskii* has relatively poor quality. Corn stalks effectively reduced the content of cellulose and lignin in *C. korshinskii* silage, providing a cost-effective alternative. But its lactic acid content remains lower than that of *C. korshinskii* silage with added sugar. These findings provide valuable insights for optimizing silage practices to enhance feed resource utilization.

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

Abbreviations

The following abbreviations are used in this manuscript:

ASH ash
 WSC water-soluble carbohydrates
 CP crude protein
 ADF acid detergent fiber
 NDF neutral detergent fiber
 ADL Acid Detergent Lignin

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