



Research Article

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Effects of rumen microorganisms on the decomposition of recycled straw residue

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Abstract: Recently, returning straw to the fields has been proved as a direct and effective method to tackle soil nutrient loss and agricultural pollution. Meanwhile, the slow decomposition of straw may harm the growth of the next crop. This study aimed to determine the effects of rumen microorganisms (RMs) on straw decomposition, bacterial microbial community structure, soil properties, and soil enzyme activity. The results showed that RMs significantly enhanced the degradation rate of straw in the soil, reaching 39.52%, which was 41.37% higher than that of the control on the 30th day after straw return. After 30 d, straw degradation showed a significant slower trend in both the control and the experimental groups. According to the soil physicochemical parameters, the application of rumen fluid expedited soil matter transformation and nutrient buildup, and increased the urease, sucrase, and cellulase activity by 10%–20%. The qualitative analysis of straw showed that the hydroxyl functional group structure of cellulose in straw was greatly damaged after the application of rumen fluid. The analysis of soil microbial community structure revealed that the addition of rumen fluid led to the proliferation of Actinobacteria with strong cellulose degradation ability, which was the main reason for the accelerated straw decomposition. Our study highlights that returning rice straw to the fields with rumen fluid inoculation can be used as an effective measure to enhance the biological value of recycled rice straw, proposing a viable solution to the problem of sluggish straw decomposition.

Key words: Rumen microorganisms (RMs); Straw return; Microbial inoculant; Decomposed straw; Soil microorganisms

1 Introduction

In recent years, irrational cultivation and tillage have led to the degradation of soil in China. A variety of methods have been actively applied as soil protection measures, among which returning straw residue to the field has been proved as one of the reliable approaches (Sommer et al., 2011; He et al., 2019). Its benefits include regulating ground temperature, increasing soil organic matter (SOM), elevating soil fertility, modifying soil structure, conserving water, and

reducing fertilizer use (Crecchio et al., 2007; Lenka and Lal, 2013). However, straw decomposes slowly in the field under natural conditions, which increases the risk of rice pests and diseases, and affects the growth of later crops, resulting in lower yields and quality (Khaliq et al., 2011; Su et al., 2016). For instance, a secondary disease, sheath blight, occurs in the winter wheat region of China after the implementation of no-tillage and straw return in farming systems (Li et al., 2011). The decomposition rate of straw in the soil is an important factor determining the utility of incorporation (Dong et al., 2016). The bioconversion of straw by microorganisms in nature is characterized by high degrading efficiency and low energy consumption, which proceeds without pollution and is the safest route (Campanaro et al., 2017). Indeed, the microbial interaction of specific microorganisms with straw may be considered as an appropriate strategy for effective decomposition (Liu et al., 2021). Gai and Nain (2007)

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significantly increased the dehydrogenase and alkaline phosphatase activity, as well as the humus content of soil by adding the fungus *Trichoderma reesei* to the wheat straw returning field. Li et al. (2012) inoculated two cellulose-degrading composite microorganisms (ADS3 and WSD5) into the soil of wheat straw, and achieved higher cellulase activity and organic matter mineralization rate compared to the non-inoculated control group; however, the enhanced degradation effect was not sustained. Due to the consumption of substrates and the buildup of secondary metabolites, the microbial community composition and function will change dynamically, as will the dominating functional bacteria (Sauer and Fischer, 2012; Cobellis et al., 2016). As a result, it can be of significant scientific value to acquire a stable and efficient source that can accommodate wider diversities of cellulose-degrading microorganism strains, which are adaptable to promote straw decay for straw return.

The rumen, as a natural cellulose-degrading ecosystem in ruminant species, is inhabited by complex microbial populations including bacteria, protozoa, fungi, and archaea. Nowadays, rumen microorganisms (RMs) have been universally established as a complex microbial community capable of cooperatively and completely digesting the lignocellulosic biomass (Li et al., 2017; Chung et al., 2019; Seesatat et al., 2021). What is more, its members produce a comprehensive range of enzyme components with high enzyme activity for lignocellulosic biomass degradation, such as a large number of highly active fibrous hydrolases (e.g., cellulase, xylanase, and esterase), which ensure the rapid degradation of the substrate (Seshadri et al., 2018). RMs have been used to digest a diversity of lignocellulosic biomasses, including agricultural leftovers, municipal solid waste organic fractions, and aquatic plants (Barnes and Keller, 2004; Yue et al., 2007). In these systems, inoculated RMs can degrade or convert lignocellulose more effectively compared to the typical digestion method (Hu et al., 2005; Jin et al., 2014). The inoculation of RMs resulted in 96%–97% cellulose/hemicellulose and 42% lignin decomposition after three months (Xing et al., 2020). However, current studies focus primarily on the energy use of RMs for lignocellulose. Williams et al. (2012) has conducted research into the by-products of the sea using rumen microbes to hydrolyze large quantities of seaweed to produce methane and acetate. Li et al. (2017) used

chicken manure and ammoniated straw as substrates and inoculated RMs for dry anaerobic fermentation. The methane content could be maintained at around 60%, with biogas yields of up to 400–420 L/kg total solid (TS). In contrast, the addition of rumen microbes accelerated substrate conversion, and improved the methane production efficiency and dry matter degradation rate. Meanwhile, there has been no study on whether the application of rumen fluid can accelerate the degradation of lignocellulose in a soil environment, or act as an effective means of fertilization.

Based on the rich microbial diversity of RMs, it is useful to explore the mode of utilization in rice straw return. The present investigation was carried out to study the effect of rumen fluid inoculation during the decomposition of rice straw in soil, with reference to the degradation rate of straw under experimental pot conditions. Herein, the morphological and structural changes of straw were unveiled. Furthermore, the varieties of soil biochemical properties before and after inoculation with RMs were explored. The aims of this work were to (1) evaluate the effectiveness of rumen liquid on rice straw return, (2) reveal the mechanisms of accelerated straw degradation, and (3) explore the effects of rumen liquid on soil chemical and biological properties.

2 Materials and methods

2.1 Cattle rumen fluid, soil, and rice straw

Paddy soil and rice straw were collected from a paddy field in Nanchang, China. The properties of the soil samples were determined (SOM 9.16 g/kg, available nitrogen (A-N) 68.03 mg/kg, available phosphorus (A-P) 16.87 mg/kg, available potassium (A-K) 149.26 mg/kg, and pH 5.96). The straw was cleaned and dried before being chopped into stalks measuring 2–3 cm in length. A total of 10.00 g straw was weighed and stored in a 300-mesh net bag. Then, a portion of the straw samples that were not returned to the field (BK) were retained for comparative analysis with the returned straw. Rumen fluids were taken from Jiangxi Agricultural University's beef cattle laboratory, Nanchang, China, filtered through four layers of muslin cloth, and centrifuged at low speed (125g) for 5 min to remove as much nonbacterial debris as possible.

2.2 Experimental design

The pot experiment including a treatment group and a blank control group took place between July to October, 2021. The treatment group (TG) received 40 mL of rumen fluid, while the blank control group (CK) received 40 mL of ultrapure water. Then, at a depth of around 5 cm, the samples were buried in an experimental pot with a mass of 5.00 kg soil. Soil for each treatment was taken in triplicate in a pot adjusted to 70% soil moisture content. Soil and straw samples were taken at Days 10, 20, 30, 40, 60, and 90 for chemical and microbiological analyses.

2.3 Analytical methods

The weight of the straw was calculated by the drying method to test the degradation rate of straw (Huang et al., 2015). The surface morphology of straw samples was characterized using scanning electron microscopy (SEM; Quanta 400 FEG, FEI, USA). An iS50 (Nicolet, USA) was employed for the Fourier transform infrared (FTIR) spectroscopy study. The intensity measurements of X-ray diffraction (XRD; SmartLab 3KW, Rigaku, Japan) were taken at 25 °C in a 2θ range from 5° to 90° (Chen et al., 2013). The crystallinity index (CrI) was calculated according to Segal et al. (1959). The contents of SOM, A-N, A-P, and A-K were referred to the soil analysis method (Lu, 2000). The activity of urease (S-UE), sucrase (S-SC), cellulase (S-CL), and catalase (S-CAT) was measured using the kits from Solarbio Science and Technology Co. (Beijing, China) (Zuo et al., 2022). The soil bacterial community structure was analyzed mainly by bacterial DNA extraction, amplification of V3–V4 hypervariable regions of bacterial 16S ribosomal RNA (rRNA) gene, sequencing on the Illumina MiSeq platform (Illumina, San Diego, USA), and subsequent data processing using the Quantitative Insights Into Microbial Ecology version 2 (QIIME2) pipeline.

2.4 Statistical analysis

Three triplicates were used in all of the experiments in this study. The significance of the experimental results was evaluated using the analysis of variance (least significant difference (LSD) method), where $P < 0.05$ was regarded as statistically significant. SPSS Statistics 26 (IBM Corp., Armonk, NY, USA) was used for data processing and analysis,

and charts were drawn by Origin 2018 (OriginLab, Northampton, MA, USA).

3 Results and discussion

3.1 Variations in straw residual weight

In the experiment, we collected straw samples in TG and CK six times within 90 d of decomposition. Fig. 1 showed the variations in the straw residual weight during the 90-d period. Firstly, all treatments presented a similar change in the degradation rate, indicating the stability of the straw decomposing process. Moreover, both TG and CK demonstrated a pattern of faster early decomposition and slower later decomposition, because rapid decomposition makes it easier to mineralize organic materials, whereas slow decomposition makes it harder to decompose the compounds in straw (lignocellulose, wax, etc.) (Yan et al., 2019). The straw residual weight with rumen fluid treatment during the 90-d period was significantly lower than that in the control under the pot experimental condition. The straw residual weight of the treatment inoculation with RMs at Days 10, 20, and 30 was 9.27, 7.76, and 6.04 g, respectively. According to the analysis, the straw degradation was 7.33%, 22.34%, and 39.52% compared with the control, which increased by 67.94%, 46.31%, and 41.38%, respectively, at Days 10, 20, and 30. During the 40th to 60th days of decomposition, the straw degradation rate for TG increased from 43.56% to 50.32%, and that for CK increased from 35.78% to 43.46%. The increased degradation rates for TG compared to CK on Days 40 and 60 were 21.73% and

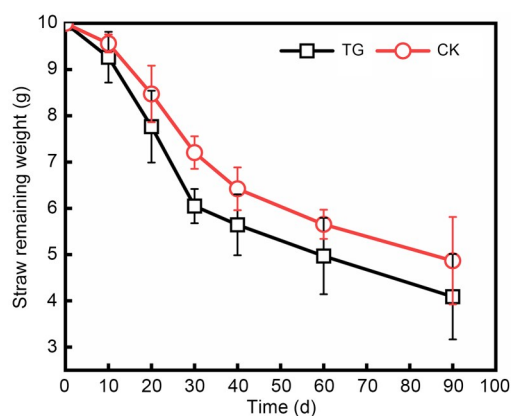


Fig. 1 Variations in straw residual weight. TG: the treatment group received 40 mL of rumen fluid; CK: the blank control group received 40 mL of ultrapure water.

15.79%, respectively. At Day 90, the straw degradation rates of TG and CK were 59.10% and 51.33%, respectively, indicating that the rumen fluid treatment greatly promoted the microbial degradation of straw. This means that early into the experiment, RMs had a stronger effect on promoting decomposition compared to control, and that returning straw to the field together with rumen fluid has a good effect on promoting decomposition in the long run.

3.2 Structure and chemistry characterization of straw materials

The SEM images of the rice straw samples taken from different degradation treatments (BK, CK and TG) were shown in Fig. S1. The morphological photographs of the outer and inner surfaces and cross-sections revealed the structural alterations of the straw caused by RMs. The morphologies of untreated and treated rice straw were obviously different. Meanwhile, it was also indicated that some strains of rumen fluids can remove wax and lignin surface components and improve the accessibility of degradable cellulose and hemicellulose. In contrast, the integral structure of the rice straw in the natural degradation process was slightly destroyed, indicating that its degradation was relatively moderate at this stage.

Fig. S2 presented a comparison of the physical and chemical attributes of straw samples in BK, CK, and TG. The X-ray diffraction pattern in Fig. S2a showed the microcrystalline cellulose in these three samples, which have two main diffraction peaks: one in the 18° non-cellulose crystalline region and the other in the 22° cellulose crystalline region (Pan et al., 2017). Further analysis reveals that the straw samples of BK, CK, and TG have a relative crystallinity of 46.27%, 63.73%, and 58.18%, respectively. The CK and TG exhibit trends of increase in relative crystallinity compared to BK, which are due to the leaching of hemicellulose and lignin—two amorphous zone components. This raised the proportion of cellulose content, resulting in a significant rise in overall crystallinity (Li et al., 2021). Meanwhile, in this case, the relative crystallinity of TG is expected to be higher than that of CK due to the partial removals of lignin and hemicellulose. However, the relative crystallinity of TG was lowered by around 9.54% when compared to CK. The FTIR spectra of untreated and treated rice straw fibers were shown in Fig. S2b. The results indicated that the functional groups in carbohydrates and aliphatic

molecules have been significantly degraded by RMs. It means that a lot of cellulose, hemicelluloses, and lignin were degraded, which agrees with the explanation for the XRD results, showing a decrease in relative crystallinity.

3.3 Variations in soil nutrients

As previously stated, straw return is an effective measure to restore soil nutrient balance and ensure the long-term use of cultivated soil. Fig. 2 showed the change in soil nutrients in this study. The SOM of all groups showed a trend of dynamic increase during the 90-d period of degradation. In addition, the contents of SOM differed significantly among Days 30, 60, and 90. When compared to CK, the SOM contents grew by 15%, 4%, and 8% at Days 30, 60, and 90, respectively, while the content increased by 1.70 g/kg at the 90th day of decomposition, which helped to promote the accumulation of SOM. When compared to soil background values, the A-P increased by 8.60 mg/kg in CK and 13.36 mg/kg in TG. The content of A-P in the soil showed a positive correlation with the time of straw return. Rumen fluid accelerates straw decomposition and also increases the soil A-P content. The differences in soil A-P contents between the early and late decomposition periods were considerable. The variation in the A-K content compared to the soil background was large in the early stages of decomposition, while it was minimal in terms of content change in the later stages. As shown by previous research, the release of A-K is the strongest in the early stages of straw return. These results showed the significant effect on the content of A-K in the soil, which could occur because the rumen fluid decomposed straw quickly in that period.

On the other hand, there were considerable changes in the A-N content during decomposition. In the early stages, the A-N content declined, which might be due to the increase in microbial nitrogen consumed by the gradual growth and metabolism of microorganisms. The immobilized nitrogen was released after 30 d due to the turnover of soil biomass, and was available to the crop (Yao et al., 2013). Therefore, the A-N content was accumulated during the 30th to 60th days of decomposition. Meanwhile, it was reduced in the later stages of decomposition, which is known as the “microbial nitrogen digging effect.” This phenomenon caused a severe change in A-N, and was more significant in TG than the control. To sum up, in the TG,

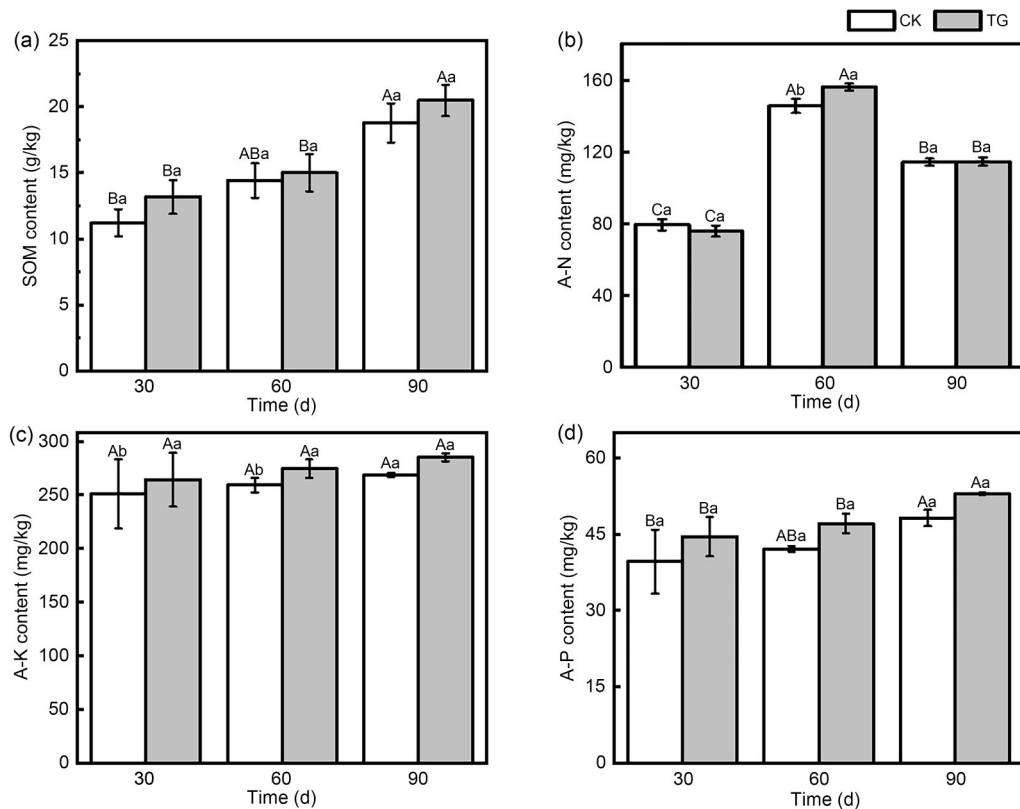


Fig. 2 Changes in soil nutrient content. (a) SOM content; (b) A-N content; (c) A-K content; (d) A-P content. Data are expressed as mean±standard deviation, $n=3$; different uppercase letters indicate significant differences among the different time points in each group ($P<0.05$), and different lowercase letters indicate significant differences between the different groups at Days 30, 60, and 90 ($P<0.05$); and the same as below. TG: the treatment group receiving 40 mL of rumen fluid; CK: the blank control group receiving 40 mL of ultrapure water. SOM: soil organic matter; A-N: available nitrogen; A-K: available potassium; A-P: available phosphorus.

more straw nutrients were released as the bacteria multiplied and reproduced, while they also consumed A-N in the soil.

3.4 Variations in soil enzyme activity

Soil enzyme activity is generally considered as the rate-limiting factor in degradation of lignocellulosic biomass under straw return (Wu et al., 2020). Fig. 3 showed the enzyme activity in the soil samples of TG and CK on the 30th, 60th, and 90th days of decomposition. Straw return gradually improved S-CL and S-SC activity during the 90-d period, and was higher at Day 90 than Day 30. Among them, the S-CL activity for the straw returning with rumen fluid inoculation compared to the control was increased by 22.44%, 5.83%, and 5.70% at 30th, 60th, and 90th days, respectively. S-CL activity increased at a higher rate in the early period, which is attributed to the rapid decomposition of cellulose in straw by RMs at this stage. Similarly, the S-SC activity of TG was raised

by 2.01%, 16.10%, and 18.55%, respectively, compared with the control. This indicated that RMs secrete more S-SC when decomposing straw, which speeds up the acquisition and transformation of soil organic carbon and contributes to the accumulation of SOM.

The S-UE activity of soil were first gradually increased and then decreased by straw return due to the lack of accessible nitrogen for microbial activity in the soil as explained below. However, the injection of rumen fluid still significantly improved the S-UE activity, which was increased by 8.60%, 21.17%, and 13.80% compared with the control at 30th, 60th, and 90th days, respectively. The S-CAT activity were approximately 103.04 U/g during 90 d for TG and CK, which indicated that they were not significantly affected by straw return with rumen fluid inoculation. The S-CAT activity changes with the depth of straw return, which normally occurs at soil depths of more than 20 cm, whereas the depth of the soil in this study was only 5 cm. These results could also be related to

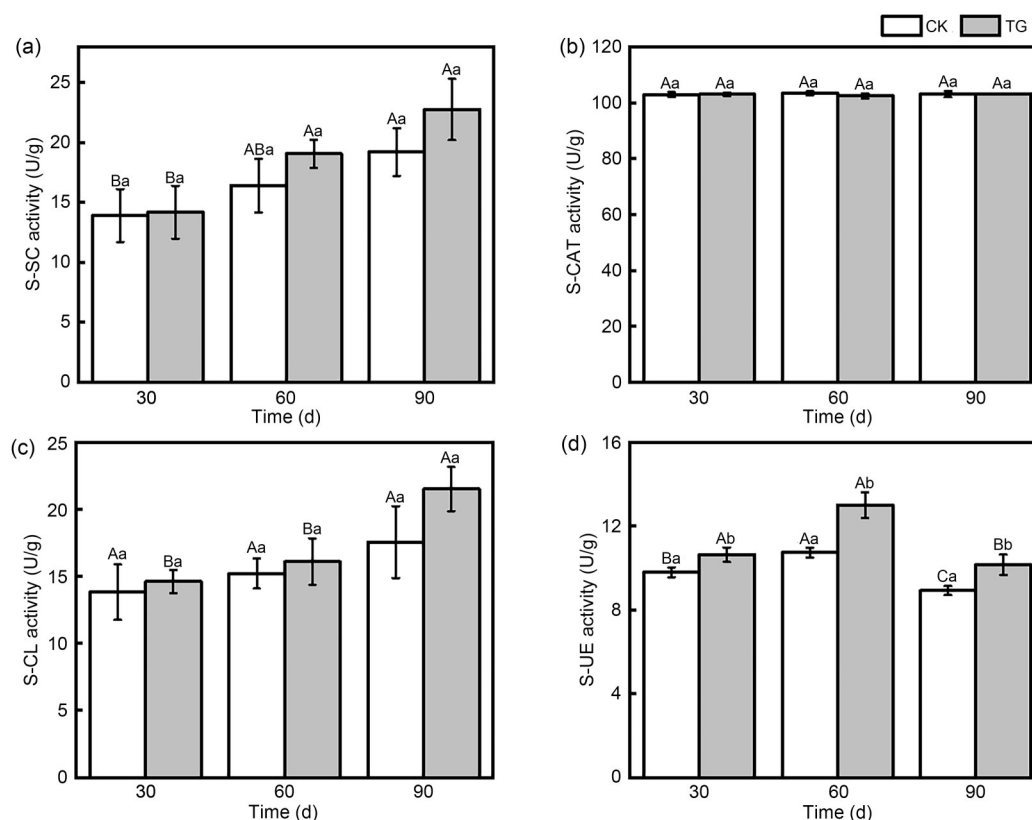


Fig. 3 Variations in soil enzyme activity. (a) S-SC activity; (b) S-CAT activity; (c) S-CL activity; (d) S-UE activity. TG: the treatment group receiving 40 mL of rumen fluid; CK: the blank control group receiving 40 mL of ultrapure water. S-SC: sucrose; S-CAT: catalase; S-CL: cellulase; S-UE: urease.

the fact that no crops were grown in this study, resulting in no change in the physiologic response by the plant roots; the underlying reasons need to be further explored.

3.5 Variations in bacterial microbial community structure

As the main decomposers in soil, bacteria are dominant players in straw degradation. A large body of evidence shows that soil microorganisms are crucial for soil fertility. For example, soil microorganisms can influence the efficiency of straw degradation by modulating microbiome stability and diversity. As the decomposing process progressed to an advanced stage at Day 90, 16S RNA sequencing was performed to estimate the richness and diversity of bacterial communities under different conditions. First, as shown by alpha diversity analysis (Fig. S3), Goods_coverage of TG and CK samples was close to “1,” which indicated that microorganisms were mostly well covered in the samples. Second, both the microbial richness (Chao1, Observed_operational taxonomic units (OTUs)) and

species diversity (Shannon, Simpson) of CK samples were significantly higher than those of TG samples. Obviously, more microorganisms with a degradation ability became dominant, eliminating others with weak competitive ability. Ultimately, this favored the accelerated decay of straw.

Fig. 4 showed the soil bacterial community structure in soil samples of CK and TG in this study. As shown by the analysis of relative abundance greater than 1% at the phylum level, Proteobacteria was the dominating phylum in the community structure of CK. In a study in China, straw return significantly increased the relative abundance of Proteobacteria, while it significantly decreased the relative abundance of Acidobacteria relative to the soil without straw return (Liu et al., 2022). As for the community structure of TG, Actinobacteria became the most predominant phylum with a relative abundance higher than 191.83% of the control. The composition of microbial communities at the genus level was also investigated in this experiment. Streptosporangium was discovered to be the dominant genus in the phylum Actinobacteria of TG,

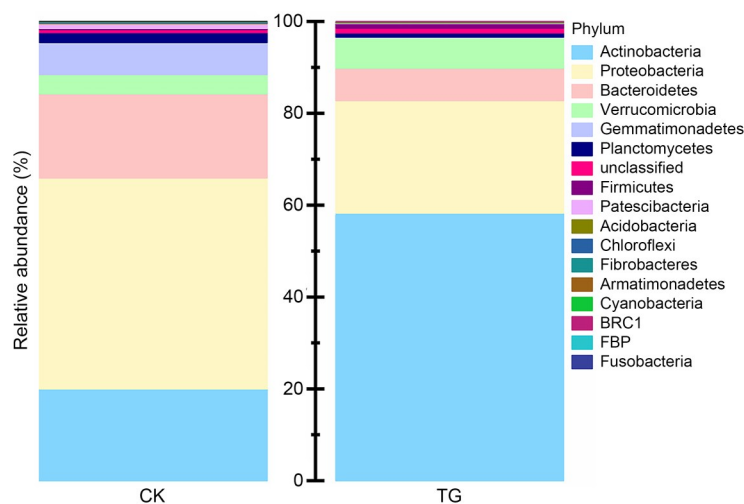


Fig. 4 Variations in the soil microbial community structure (Phylum). TG: the treatment group receiving 40 mL of rumen fluid; CK: the blank control group receiving 40 mL of ultrapure water.

and its relative abundance increased by 57.62% compared to CK (Fig. S4). It has been demonstrated that the particularly strong penetration ability of Actinobacteria increases the water solubility of cellulose while maintaining good breakdown ability, with variations in the reaction substrate (El-Tarabily and Siv-asithamparam, 2006). Chaouch et al. (2016) found that the genus *Streptosporangium* of Actinomycetes is highly active even at high temperatures (Nik Raikhan, 2019), which apparently contributes to straw decay at high temperatures after rice harvest.

Further analysis found that the relative abundance of four phyla in the samples of TG changed significantly (Proteobacteria, Bacteroidetes, Verrucomicrobia, and Gemmatimonadetes). When compared to CK, we observed reduced levels of Proteobacteria, Bacteroidetes, and Gemmatimonadetes (by 46.71%, 61.48%, and 97.69%, respectively) after the return of straw inoculated with rumen fluid, which may be due to competition among dominant bacteria. The relative abundance of Verrucomicrobia increased by 57.62%. In addition, Verrucomicrobia was found to be the dominant phylum of TG (Han et al., 2020). Verrucomicrobia is a glycoside hydrolase pluripotent phylum, and the genes were discovered to be highly abundant in glycoside hydrolase genomes with high glycoside hydrolase activity (Tran et al., 2021). These results indicated that the introduction of foreign microorganisms by RMs altered the relative abundance of other phyla, disrupting the equilibrium of original microbial food

chain and allowing phyla with higher putrefactive potential to gain a competitive edge.

4 Conclusions

In this study, we demonstrated that straw return combined with rumen fluid inoculation accelerated the straw decomposition rate to improve the efficiency of nutrient transformation. RMs were more effective in rupturing the surface structure and reducing the crystallinity of rice straw, as well as secreting considerable contents of S-CL, S-UE, and S-SC to catalyze the degradation reaction. The rumen fluid demonstrated a continuous degradation effect during 90-d of decomposition and subsequently accelerated soil nutrient accumulation. Overall, we showed the great potential of RMs to biotransform and degrade lignocellulose waste, and provided a strong reference for further field experiments. In the future, screening or acclimatizing a cost-effective microbial agent using rumen fluid may be a viable method for effective straw return to field.

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Author contributions

Kailun SONG: validation, formal analysis, investigation, resources, data curation, writing—original draft, writing – reviewing & editing, visualization, supervision, and project administration. Xin YIN: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – reviewing & editing, and visualization. Lichun KANG: conceptualization, methodology, formal analysis, investigation, resources, data curation, and visualization. Chunhuo ZHOU: validation, formal analysis, investigation, and writing – original draft. Zicheng ZHOU, Jinhai LENG, Songwen FANG, and Guorong NI: validation, formal analysis, and investigation. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Kailun SONG, Zicheng ZHOU, Jinhai LENG, Songwen FANG, Chunhuo ZHOU, Guorong NI, Lichun KANG, and Xin YIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Figs. S1–S4