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Effects of Different Soil Moisture Contents on Rumen Fluids in Promoting Straw Decomposition after Straw Returning

Kailun Song [1], Shifei Liu [1], Guorong Ni [1,2], Qinlei Rong [1,2], Huajun Huang [1], Chunhuo Zhou [1,2,*] and Xin Yin [1,2,*]

[1] College of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, China
[2] Key Innovation Center of Agricultural Waste Resource Utilization and Non-Point Source Pollution Prevention and Control of Jiangxi Province, Nanchang 330045, China
* Correspondence: zchh3366@jxau.edu.cn (C.Z.); yinxin1081@163.com (X.Y.); Tel.: +86-791-83813493 (X.Y.); Fax: +86-791-83813493 (X.Y.)

Abstract: Inoculating microbial inoculants to speed up the decomposition of returning straw is currently a hot topic. Meanwhile, the soil moisture content (SMC) could change the diversity, abundance, and metabolism of the soil microbial community structure, which affects the straw degradation rate under the straw returning condition. In this research, rumen microorganisms with strong decomposing abilities in natural systems were used as inoculants to promote straw decomposing and returning to the field. The effects of the SMC on straw decomposition under rumen fluid (RF)-induced returning were investigated. Experiments were conducted for 30 days with typical paddy soil in the south of China under conditions of 30%, 70%, and 100% SMC. With an increase in the SMC within a certain range (30~100%), the decomposition rate of straw showed a trend of first rising and then falling. Treatments of 70% SMC with RF addition generally achieved the maximum rate of straw degradation. The peak value was 49.96%, which was 2.67-fold higher than the treatments of 30% SMC with RF addition (18.74%) and 24.1% higher than those of the control with 70% SMC (40.3%) ($p < 0.05$). Moreover, a straw structural analysis proved that at 70% SMC, microorganisms from RF favored the destruction of functional groups on the straw surface and the degradation of cellulose. Meanwhile, it was shown that RF could promote the decay of straw, leading to increments in enzyme activities and soil nutrients. The higher the soil moisture content, the higher the key soil enzyme activities. This indicates that the diversity and abundance of cellulose-degrading bacteria and fungi in soil microorganisms and rumen microorganisms were changed with different soil moisture contents. The experimental findings suggest an innovative way to further utilize rumen microorganisms.

Keywords: soil moisture content; rumen microorganisms; straw returning; straw decomposition; soil nutrients

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Citation: Song, K.; Liu, S.; Ni, G.; Rong, Q.; Huang, H.; Zhou, C.; Yin, X. Effects of Different Soil Moisture Contents on Rumen Fluids in Promoting Straw Decomposition after Straw Returning. *Agronomy* **2023**, *13*, 839. https://doi.org/10.3390/agronomy13030839

Received: 19 February 2023
Revised: 11 March 2023
Accepted: 12 March 2023
Published: 13 March 2023

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1. Introduction

Straw, as a waste product of agricultural production, is difficult to decompose due to the large amount of lignocellulosic content under natural conditions [1–4]. Currently, returning straw to the soil as a fertilizer is an effective method in agricultural production that could help maintain soil quality and ensure a sustainable environment and biosphere [5]. Returning straw to soils could replenish carbon losses, facilitate the formation of soil aggregates, and improve the soil structure and the soil aeration wastewater-holding cation exchange capacity [6,7]. However, incomplete straw decomposition may hinder the root penetration of the next crop, especially when growing double-cropping rice [8,9]. In addition, it also exacerbates disease and pest infestations and affects later crop growth, resulting in poorer yields and quality [10]. Therefore, it is necessary to find an innovative approach to accelerate the decomposition of straw under the condition of returning to the field.

Inoculation with microbial agents to accelerate straw decay after field return is considered an effective measure [11]. In [12], the authors found that the complete decomposition of straw lignocellulose depends on the joint participation of multiple microorganisms. In [13], the authors screened and isolated complex straw decomposition microbial lines from corn straw. They were applied to straw returning, resulting in an increment of 173.65% in the decomposition rate compared to the control. Obviously, the use of a microbial application can effectively improve the rate of straw decomposition. However, the relationship between the action of the complex bacteria and the metabolites is more complex, and it is crucial to fully consider the functional stability of microorganisms to construct a complex bacterial line [14,15]. In [16], the authors added two cellulose-degrading composite microorganisms (e.g., ADS3 and WSD5) to the soil of wheat straw, which presented higher cellulase activity and a higher organic matter mineralization rate than the non-inoculated control group. However, the enhanced degradation effect could not last for a long time. Evidently, the stability of complex cellulose-degrading bacteria depends on the abundance and diversity of microorganisms. Therefore, it is essential to find a microbial agent with a high diversity and an abundance of microbial species to effectively ensure the prompt decomposition of straw after returning to the field.

The rumen is a natural cellulose-degrading ecosystem in ruminants. Its complex microbial community is able to facilitate the complete digestion of lignocellulosic biomass [17,18]. In addition, it has been demonstrated in numerous studies to play an important role in lignocellulosic resource utilization [19]. Fermentation experiments were conducted on wheat straw by inoculating dairy cows with rumen microbes, and it was found that within three months 96–97% of the cellulose and hemicellulose were broken down and 42% of the lignin was broken down. Nevertheless, the possible utilization of rumen microorganisms to accelerate the decomposition of straw after its return to the field has not been studied. Meanwhile, the decomposition of crop straw and the release of nutrients are closely related to the properties of soil, such as the pH, moisture content, temperature, etc. [9,20]. In particular, the soil water content (SMC) directly affects the community structure and activity of soil microorganisms, playing a decisive role in straw decomposition and carbon conversion [21]. The decomposition of returned straw is a water-demanding process. An inappropriate SMC is not conducive to straw decomposition in the natural environment of the field [22,23]. In [24], the authors found that the closer the SMC was to the field's water-holding capacity, the greater the rate of straw decomposition. Under field conditions, moisture is a key constraint to crop straw decay, and the energy required for straw decay is associated with the SMC. In [25], the authors showed that soil environmental factors, such as moisture, affect the decomposition rates of plant residues, mainly through the growth and reproduction of some soil microorganisms and related enzyme activities, which in turn affect the decomposition of returned crop straw. Therefore, an analysis of the possible effects of the SMC on straw decomposition and soil nutrients can help us understand the process of lignocellulosic degradation with the application of rumen microorganisms and provide an innovative way to advance the return of straw.

The present study aimed to accelerate the decomposition of straw under different SMCs with the addition of rumen microorganisms. It was the first investigation of the variations in straw's physical structure, soil enzyme activity, and nutrient changes with rumen microorganism applications at different SMCs after 30 days of straw returning. Furthermore, for the purpose of elucidating the mechanism of influence, the bacterial and fungal community structures of the soil were further explored.

2. Materials and Methods

2.1. Rumen Fluids, Soil, and Rice Straw Collection

Rice straws and soils were taken from the experimental field of Jiangxi Agricultural University. The experimental field of Jiangxi Agricultural University is located in Xinjian County, north of Nanchang City, Jiangxi Province, located at 115.83° E and 28.76° N. The soil texture included light loam and medium loam with fertile soil. The climate of this

test area is a subtropical monsoonal humid climate type, with abundant annual rainfall, sufficient light in summer and autumn, and concentrated rainfall in winter and spring. On average, this region experiences 1700–1900 annual sunshine hours. The test soil was a Quaternary acidic red loam soil developed into a trapped rice soil. It was located in the distribution area of the typical acidic red loam soil at a low latitude in the subtropical south. The paddy soil was sifted through a 2 mm screen. The properties of the soil samples were determined (soil organic matter (SOM): 11.17 g/kg, available nitrogen (A-N): 75.41 mg/kg, available phosphorus (A-P): 17.78 mg/kg, available potassium (A-K): 51.16 mg/kg, and pH: 5.86). The soil was then placed in pots with a depth of 20 cm and a diameter of 25 cm. The soil bulk density was about 1.03 g/cm^3, the soil porosity reached 57.42%, and the stability of the aggregate composition (>0.25 mm) was about 60%. The straw was cleaned and dried before being chopped into stalks measuring 2–3 cm in length. Then, they were weighed at 10.00 g and packed in a mesh bag of 300. Rumen fluid was placed in a bottle purged with N_2 gas from Jiangxi Agricultural University's beef cattle laboratory. The rumen liquid was first filtered through four layers of muslin cloth before being centrifuged at a low speed (125 × G) for five minutes to remove as much nonbacterial debris as possible. The rumen fluid was kept in the supernatant.

2.2. Experimental Design

The experiment took the form of a potted planting experiment in the greenhouse at 25–30 °C. Two groups of straw were prepared: one group of normal straw (CK) and another group that was pretreated with 40 mL of rumen fluid (RF). In addition, we set three levels of SMC for the straw returning fields (30%, 70%, and 100%) for the two groups. The two groups of straw were buried in plastic pots containing 5.0 kg of paddy soil for 30 days. Soil and straw samples were withdrawn after 30 days for chemical and microbiological analyses. Three replicates were set for each treatment, with consistent management methods.

2.3. Analytical Methods

The straw degradation rate was calculated using the constant weight subtraction method and represented the relative proportion of straw decomposition.

The surface morphology of the straw samples was characterized using scanning electron microscopy (SEM) (QUANTA 400 FEG, FEI, Hillsboro, OR, USA). An iS50 spectrometer was employed for the Fourier transform infrared spectroscopy (FTIR) study (Nicolet, Green Bay, WI, USA). Intensity measurements of X-ray diffraction (XRD, SMARTLAB 3 KW, Rigaku, Japan) were taken at 25 °C in a 2θ range from 5 to 90. The crystallinity index (CrI) was calculated using the method of Segal et al. [26]. The content of SOM was determined using the Walkley–Black acid digestion method, and the A-N content was measured using the semi-micro Kjeldahl procedure. A-P was measured by means of colorimetry after extraction with 0.5 mol L^{-1} $NaHCO_3$ (pH = 8.5) for 30 min. The A-K content was measured using a flame photometer after extraction with 1 mol L1 NH_4Ac (pH = 7.0) for 15 min [27]. The activities of urease (S-UE), sucrase (S-SC), cellulase (S-CL), and catalase (S-CAT) were measured using a kit from Solarbio Science and Technology Co. (Beijing, China) [28]. The soil bacterial community structure was mainly analyzed via bacterial DNA extraction, the amplification of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene, sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA, USA), and subsequent data processing using the QIIME2 pipeline.

2.4. Statistical Analysis

Three parallel samples were used in all experiments in this paper. Data were analyzed by means of a one-way analysis of variance (ANOVA) using version 26.0 of the SPSS software. Differences were considered significant at $p < 0.05$. When the ANOVA results were significant, we compared the means of the main effects using the least significant difference (LSD) test. We calculated Pearson's linear correlation coefficients between the

selected parameters using the SPSS software. A regression analysis was performed using version 2018 of the Origin software.

3. Results and Discussion

3.1. Variations in the Degradation Rate of Straw

The effects on the degradation rate of straw inoculated with RF at different SMCs (30%, 70%, and 100%) were investigated, as shown in Figure 1. When the application volume of rumen microorganisms was 40 mL, the straw degradation rate of the treatment group was significantly higher compared to the control at SMCs of 70% and 100% after returning straw for 30 days. When the contents of soil moisture were 30%, the straw degradation rates of the control and RF addition groups were at the same level, with strawweight loss rates of 18.7–19.0%. In [29], the authors found that a 50% loss of plant material was obtained in wet soil, while only about a 25% loss had occurred in the corresponding dry one, which was similar to our experiment. It could be considered that a 30% SMC would reduce the respiration of the soil microorganisms during decomposition. As shown in Figure 1, the straw degradation rate in RF was 34.84% after 30 days of decomposition, which was increased by 17.60% compared to the 29.63% degradation rate of the control. The above results may be attributed to decreases in solute diffusion and microbial activity due to a decrease in soil pores after irrigation, which is the most limiting factor for supplying substrates and nutrients to decomposers [30]. Fungi, identified by researchers as potential actors of straw decomposition, are less sensitive to changes in water, temperature, and other factors [31,32]. These results demonstrated that the application of a certain volume of RF was beneficial to increase the number and types of fungal microorganisms in the soil, accelerating the degradation rate of straw. With the SMC at 70%, the SMC reached the field water-holding capacity, and the straw exhibited a better decomposition efficiency in straw returning. Conversely, the straw degradation rate when treated with RF at 70% SMC was highest, at 49.96%, which was significantly increased by 24.06% compared to that of the control (40.27%). According to the experimental result, rumen fluid is an efficient straw-decomposing agent that can promote straw decomposition under natural returning conditions. The optimal soil moisture content is 70%.

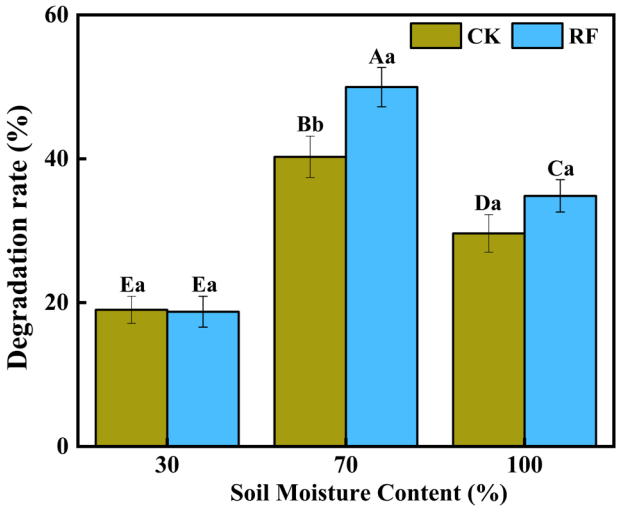


Figure 1. Comparison of the decomposition rate of straw with rumen fluid application at different SMCs (uppercase letters represent the differences between all groups, lowercase letters represent differences between the same groups, and the level of significance was set at $p < 0.05$).

3.2. Structure and Chemistry Characterizations of Straw Materials

Figure 2 shows the variation in the surface structure of the straw after the straw returning on day 30. Generally speaking, the outer surface was flat, and its structure was intact and dense, with neatly arranged waxy crystals, after the rice straw harvesting [33]. Clearly,

the morphologies of the RF-treated and control rice straw samples were very different. As shown in Figure 2A,B, the surfaces of the RF-treated and control samples at 30% SMC had only a small number of crystals that fell off, and no extensive structural damage was found. This was consistent with the results of the straw degradation rate, which was limited by the SMC. However, the straw structure began to break down rapidly when the SMC was sufficient to provide the microorganisms with their metabolic requirements (Figure 2C,E). It was found that the dissolution of the wax–silicon layer resulted in some pores after the degradation of a large amount of granular material on the 30th day of the experiment. In addition, part of the internal tissue was separated from the original straw into fragments. Moreover, after 40 mL of rumen fluid was added, the surface structure of the straw changed significantly compared to the control at 70% and 100% SMC (Figure 2D,F). Due to the large number of microorganisms adhered to the outer surface of the straw, it was evident that the internal structure of the straw was looser, rather than dense as before. These results showed that some strains from the rumen fluid could break down the wax and lignin on the surface of the straw faster, thus promoting further degradation of cellulose and hemicellulose. Similarly, the degree of the structural damage on the surface of the straw at 70% SMC was more dramatic than at 100% SMC.

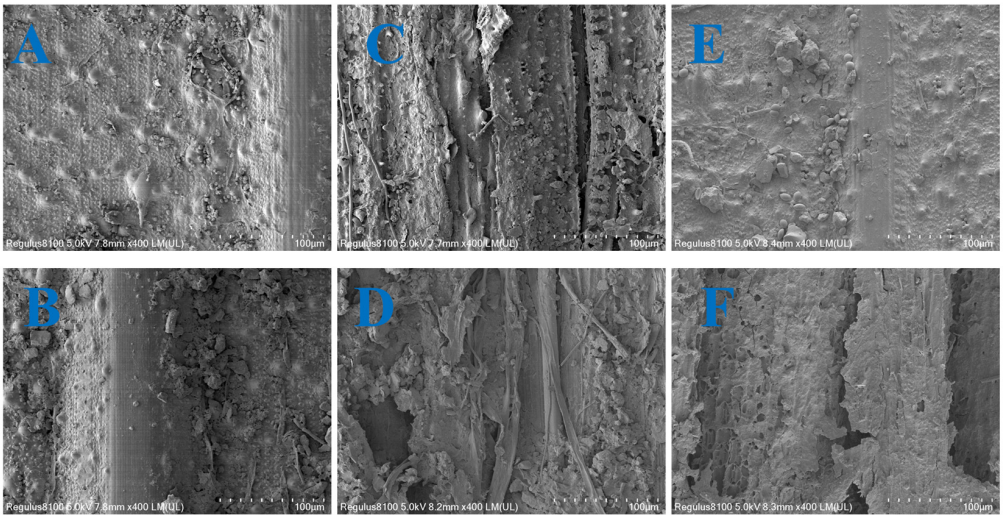


Figure 2. SEM observations of straw materials from control samples and after rumen fluid application at different SMCs ((**A**), CK at 30% SMC; (**B**), RF at 30% SMC; (**C**), CK at 70% SMC; (**D**), RF at 70% SMC; (**E**), CK at 100% SMC; (**F**), RF at 100% SMC) (bar: 0.02 mm).

To further investigate the effect of applying RF on the decay properties of straw under different SMC conditions, the straw samples were analyzed using FTIR. As shown in Figure 3a, the straw was rich in functional groups of straw, but the absorption peaks all showed some degree of attenuation after decay [34]. Among them, the broad 3000–3500 cm^{-1} absorption peaks were from the stretching vibrations of hydroxyl O-H functional groups in the lignocellulose structure. The absorption bands near 900 cm^{-1} represented carbohydrates and aliphatic compounds belonging to the straw's H-C-H and C-H functional groups. The absorption peak at 1630 cm^{-1} represented water, which was weakened by the strong interaction between the cellulose and water molecules, i.e., when cellulose hydrolysis occurred. The absorption peak at 800 cm^{-1} corresponded to the vibrational absorption of Si-O-Si in the straw. The RF-treated and the control absorption peaks fluctuated strongly at 30% SMC when the functional groups of straw were not significantly destroyed. In addition, when the SMC reached 70%, the intensity of the absorption peaks was significantly weakened, the lignocellulosic structure was microbially degraded, and a large number of aliphatic compounds and carbohydrate functional groups were destroyed. The absorption peak of the treated straw was significantly weaker than that of the control straw, which indicates that RF can play a role in the degradation of lignocellulose. When the SMC reached 100%,

the straw was also at a high SMC and was susceptible to hydrolysis. Anaerobic fungi in the RF are apparently better adapted to such a fermentation environment. Compared with the control, the absorption peaks treated with RF almost disappeared, and only the inorganic component Si-O-Si, which is difficult to be degrade with microorganisms, was present.

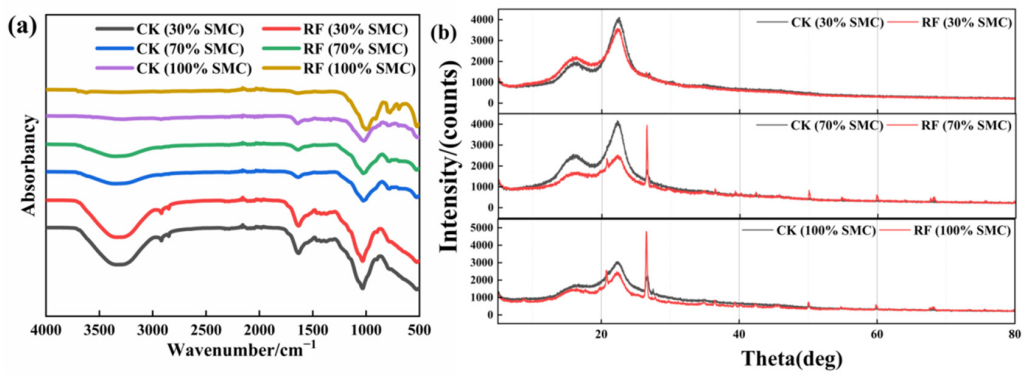


Figure 3. FTIR and XRD observations of straw materials from control samples and after RF application at different SMCs (30%, 70%, and 100%). (**a**) FTIR observations. (**b**) XRD observations.

Figure 3b shows the XRD observations of all experimental samples. All samples had one low diffraction peak and one steeper diffraction peak at θ = 17° and θ = 22° (a non-cellulose crystalline region and a cellulose crystalline region, respectively) [35]. Therefore, there was a typical cellulose type I structure. The process of the microbial decay of straw did not destroy the crystalline structure of cellulose, but its crystallinity was changed. Among them, the intensity of the diffraction peaks treated with RF and the control at 30% SMC changed little, which shows that the structure of the straw was still relatively intact at this time. When the lignocellulose content of the straw decreased, the intensity of the diffraction peaks gradually decreased, the crystallinity of the samples began to decrease, and the degree of decay of the straw gradually increased [36]. The crystallinity values of the treated and control samples at 100% SMC were measured to be 42.21% and 39.19%, respectively, when Segal's (1959) method was adopted for the calculation. The highest weight loss and lowest crystallinity of the straw were achieved when the SMC was 70%. Crystallinity values of 39.93% and 31.96% were obtained for the treated and the control samples, respectively. Apparently, the RF was able to destroy the lignocellulosic structure faster, thus reducing the crystallinity of the straw and eventually speeding up the decomposition of the straw.

3.3. Soil Enzyme Activities

Soil enzymes are an important pathway for soil nutrient transport, while an analysis of soil enzyme activity provides insight into the soil microbial status [37]. Figure 4 analyzes the changes in four key soil enzyme activities in the experimental and control groups under different SMC conditions. First, as in the previous findings, the soil enzyme activity was also relatively low at 30% SMC. There was no significant difference between the RF-treated and control samples for SMC-limited microbial enzyme production. In contrast, at 100% SMC, more water-soluble materials from the straw entered the soil, which led to an increase in soil enzyme activity. In addition, urease, cellulase, and catalase reached their maximum values. As shown in Figure 4b, the maximum urease value was observed in the samples treated with RF at 100% SMC (9.92 U/g), which was 8.22% higher than the control at 100% SMC (9.17 U/g). It could accelerate the conversion of nitrogen, and more nitrogen could be used by microorganisms and accumulated in the soil. The cellulase of the treated samples at 100% SMC also reached a maximum of 17.67 U/g, which was about 20.1% higher than the control (14.72 U/g), and more straw residues were decomposed. The maximum value of 21.64 U/g of sucrase activity occurred in the treated samples at 70% SMC, which was about 12.9% higher than the control (19.17 U/g) at this SMC value. It is clear from the

figures that the activity of soil enzymes can be increased to some extent after RF is applied, which is closely related to the ability of rumen microorganisms to secrete large amounts of enzymes. In [38], the authors found that the addition of the industrial product cellulase to nylon bags of returned straw significantly accelerated the rate of straw decomposition, but such an approach is not sufficiently economically efficient. We believe that RF may be a natural alternative with powerful capabilities compared to industrial enzymes. In addition, the reason for targeting peroxidase activity to reach its maximum at 100% SMC may be due to some environmental stress caused by an anaerobic environment, where more H_2O_2 is produced in the soil.

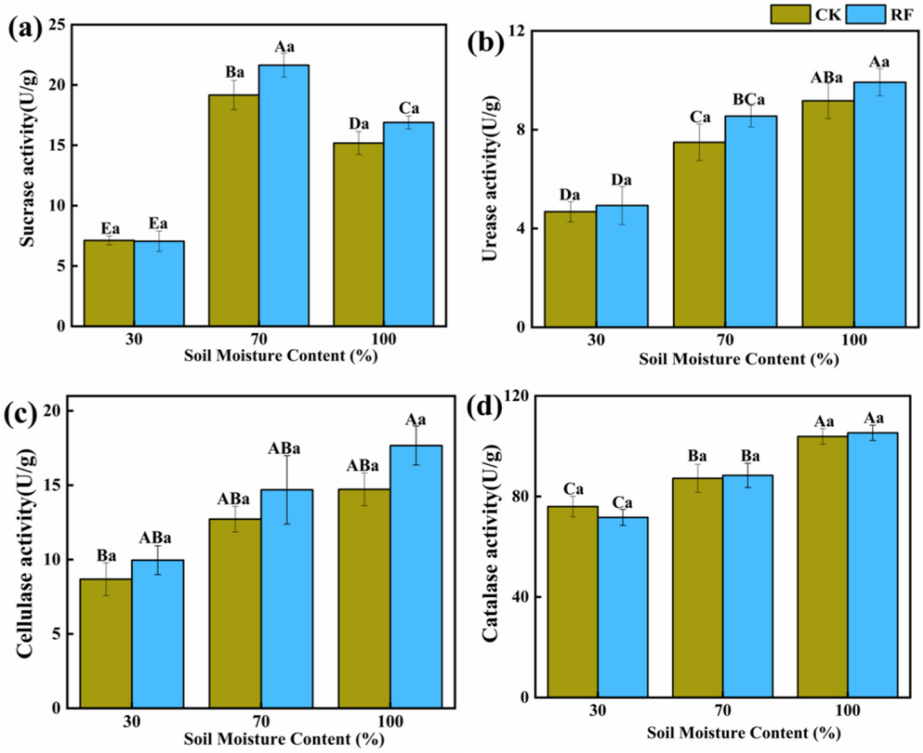


Figure 4. Comparison of soil enzyme activities with RF application at different SMCs: (**a**) sucrase activities; (**b**), urease activities; (**c**), cellulase activities; (**d**), catalase activities (uppercase letters and lowercase letters represent significant differences, as explained in Figure 1).

3.4. Soil Nutrients

Straw return is an effective measure for soil improvement and soil fertility enhancement [39]. Figure 5 analyzes the changes in the contents of the four major soil nutrients after the return of straw. Similar to the changes in soil enzyme activity, the SMC exerted a greater influence on the accumulation of soil nutrients. When the SMC was 100%, the soil had relatively high levels of A-N and A-K. This was due to the faster decomposition of straw at the beginning of flooding and more soluble organic nitrogen and organic potassium being hydrolyzed into the soil. The maximum A-N content was 123.2 mg/kg in soil treated with RF at 100% SMC, which was 14.6% higher than the control (107.5 mg/kg). Moreover, the treatment with RF (at 100% SMC) increased the A-N content by 35.6–0.1% more than the treatments at 30% and 70% SMC. Apparently, after the return of the straw, RF was able to participate in straw decomposition, allowing more nitrogen from the straw to accumulate in the soil. Then, we found that some N was also consumed by rumen microorganisms' growth and reproduction, which was the same as "the microbial nitrogen digestion" suggested in [40]. Therefore, there was no significant difference in the A-N contents between the treated and control samples at 30% to 70% SMC. Moreover, a lower SMC reduced the effectiveness of soil microorganisms on the substrate and N and resulted in a delayed decomposition rate, which was consistent with previous studies [37,41,42].

The A-K content of the soil changed rapidly after straw returning and increased with an increasing SMC. Similarly, the maximum content of A-K was reached in the samples with RF application at 100% SMC (126.4 mg/kg), an increase of 8.85% compared to the control samples at 100% SMC. This value was only 45.6–50.0 mg/kg at 30% SMC. While the SOM and straw decay varied equally, all of the treated soil samples had the highest contents of A-P and SOM at 70% SMC, with values of 36.4 mg/kg and 17.8 g/kg, respectively. After the RF treatment, they were 12.6% and 7.4% higher than the contents of the control samples at 70% SMC (AP, 32.3 mg/kg; SOM, 16.6 g/Kg). In addition, at 30% SMC, the soil nutrients of the treated and control samples did not change significantly from the background soil values, and there were no significant differences between the treated and control samples. This was consistent with the findings of Neve et al. that soil water availability limits microbial activity and affects nutrient accumulation [41,43].

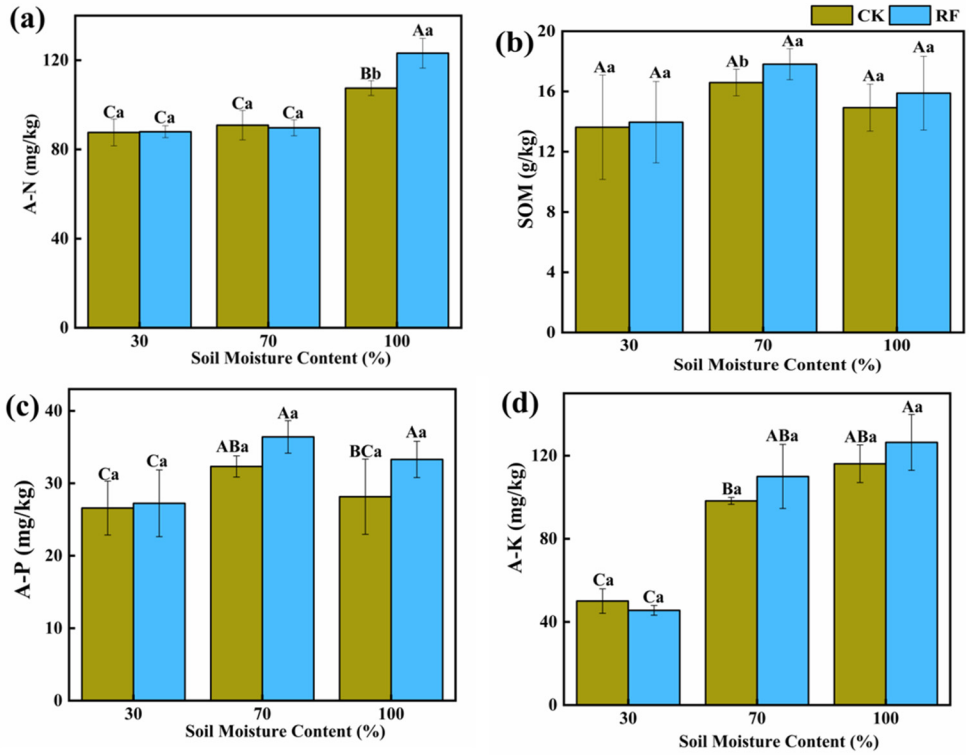


Figure 5. Comparisons of soil nutrients with RF application at different SMCs: (**a**) A-N; (**b**) SOM; (**c**) A-P; (**d**) A-K (uppercase letters and lowercase letters represent significant differences, as explained in Figure 1).

3.5. Soil Microorganisms

We collected samples from the soil 30 days after the straw was returned to the field to analyze changes in the microbial community structures of bacteria and fungi. As Figure 6a shows, more bacteria capable of resisting environmental stresses occupied higher relative abundances when the SMC was low, such as Actinobacteria (7.78–13.20%) and Acidobacteria (20.62–25.41%). Previously, researchers found that Actinobacteria can adapt to conditions of high temperature, high salt, and a lack of moisture. In particular, it still had excellent degradation properties when under different environmental conditions [44,45]. Meanwhile, the analysis found that the relative abundance of Actinobacteria after RF application at 30% SMC was 13.20%, which was significantly higher than that of the control at 30% SMC (7.78%). When the SMC was 100%, the relative abundances of various bacteria tended to balance, and the relative abundance of Firmicutes increased, which was consistent with the results found in flooded paddy soils in [46]. In [47], the authors also found that the relative abundance of Firmicutes gradually increased with the extension

of the flooding time. In [48], the authors suggested that this might be due to their ability to produce budding spores, their high adaptability and reproductive capacity, and their ability to tolerate the environmental stresses generated by the flooding process. At this time, the relative abundance of Firmicutes was highest in the samples treated with RF, reaching 16.65%. In addition, it also reached 10.08% in the control samples. Interestingly, the soil samples treated with RF at 70% SMC, had the highest relative abundance of Proteobacteria (54.87%), and there was also a higher straw degradation rate (70% SMC) than in the other samples. Obviously, this was due to Proteobacteria being an important component of soil bacteria that are mainly heterotrophic and are also the dominant phylum for straw decomposition. In addition, the authors also found that the relative abundance of Proteobacteria increased significantly after straw returning in soil samples with a short-term straw return and that this was the key phylum for straw decomposition [5]. Obviously, this is an important reason for the rapid degradation of straw in the treated samples at 70% SMC. After further analysis, more dominant bacterial phyla or more resistant phyla were present in the soil treated with RF compared to the control soil under different SMC conditions. Apparently, the rich bacterial species in RF provide a strong guarantee for the rapid decomposition of straw back into the field.

Fungi are a potential factor for the decomposition of straw and an important component of rumen microorganisms. An analysis of the structural changes in the fungal community was beneficial to further explore the tolerance of rumen microorganisms under different SMC conditions. Fungi are more sensitive to oxygen conditions, while they are tolerant to water conditions. This is because their mycelium can transfer water from water-filled micropores, while bacteria need water membranes for movement and diffusion [49]. In addition, researchers found that fungi were able to adjust their degradation activity according to the availability of water and nutrients. Therefore, in Figure 6b, we present a comparative analysis of the soil fungal community structures under different SMC conditions. It is clear that Ascomycota is the phylum with the highest relative abundance in the soil fungal community, with a high relative abundance of the control (99.21%) and the soil treated with RF (97.03%) at 70% SMC. Moreover, Ascomycota is mainly saprophytic and parasitic in its nutritional mode and is the main fungal phylum causing the decomposition of returned straw. This likewise explains why the soil treated with RF at 70% SMC had the highest straw weight loss rate. However, when the SMC was low, the relative abundance of some unculturable and unclassified fungi started to increase (13.73–16.06%). The low SMC activated some of the rarer fungi in the soil, including Basidiomycota (2.50–9.86%), Chytridiomycota (0.91–7.14%), and Zygomycota (1.94–4.85%). Previous studies found that some populations of Basidiomycota could decompose lignin with a high carbon-to-nitrogen ratio. Returning straw to the field provided a better growth environment for Basidiomycota to make more use of degraded crop residues, thus promoting their rapid growth. At 100% SMC, the fungal community structure changed significantly, and a large number of unclassified fungi from rumen microorganisms multiplied rapidly in the anaerobic soil environment. Moreover, the relative abundance of unclassified fungi in treated soil at 100% SMC was as high as 62.86%, which increased Ascomycota by 30.02% and was much higher than the relative abundance of unclassified fungi in the control soil at 100% SMC (11.82%). Apparently, this is the reason that the straw degradation rate of soil treated with RF at 100% SMC could be increased by 17.60% compared to the control soil.

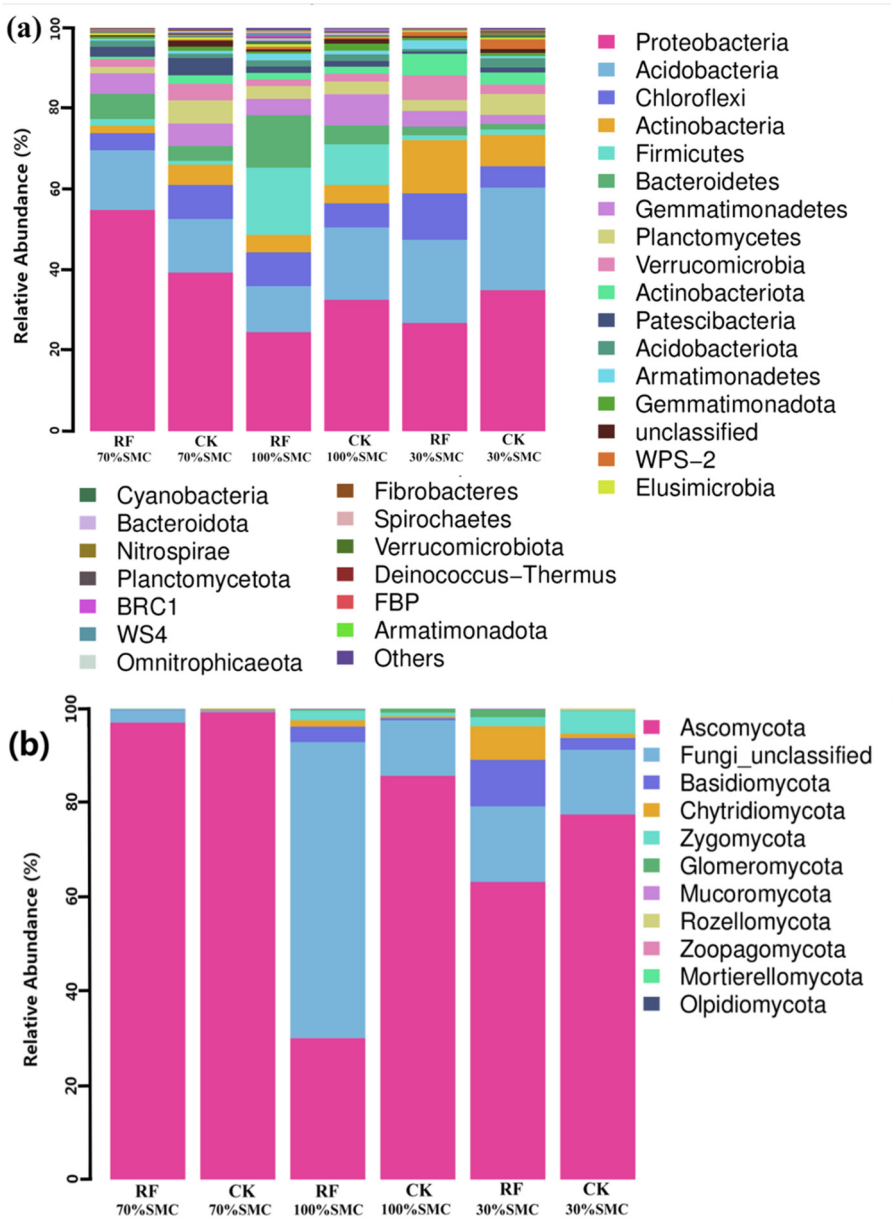


Figure 6. Soil microbial community structure analysis of control samples and after RF application at different SMCs ((**a**) bacteria; (**b**) fungi) (phylum levels).

4. Conclusions

The degradation rate of straw reached 49.96% at 70% SMC when 40 mL of rumen fluid was added after the straw was returned to the field. Compared to 20% and 100% of SMC, there was a significant difference in the straw degradation rate ($p > 0.05$). Obviously, when the SMC was 70%, the rumen fluid was provided a favorable environment for growth and reproduction, and the straw degradation rate was increased by 24.06% compared to the control group with conventional straw return. In a further analysis, we found a high abundance of bacteria and fungi derived from rumen fluids with high cellulose decomposition ability, which entered the soil and became the dominant strains. Moreover, more microorganisms with environmental resistance were involved in the decomposition of straw when there was more or less soil moisture. At the same time, rumen microorganisms hastened the destruction of a large number of organic functional groups such as cellulose, hemicellulose, and lignin in the straw, which led to increases in the rate and efficiency of straw return. A further analysis revealed that the urease, cellulose, and sucrose activities

were enhanced after rumen fluids were applied to the soil, promoting straw decomposition. This also ultimately promoted soil nutrient accumulation. In conclusion, when the soil moisture content was 70%, it was most favorable for microbial agents to accelerate straw degradation. This was a beneficial environment for straw returned with the application of rumen fluid.

Author Contributions: K.S.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft, Writing—review and editing, Visualization, Supervision, Project administration, and Funding acquisition. X.Y.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—review and editing, and Visualization. C.Z.: Validation, Formal analysis, Investigation, and Writing—original draft. G.N.: Validation, Formal analysis, and Investigation. H.H.: Validation, Formal analysis, and Investigation. S.L.: Conceptualization, Methodology, Validation, and Formal analysis. Q.R.: Resources, Software, Validation, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of China (grants 52160002, 21707057, and 31860595), and the APC was funded by Xin Yin.

Acknowledgments: The authors would like to thank Suzhou Deyo Bot Advanced Materials Co., Ltd. (www.dy-test.com) for providing support in material characterization.

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

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