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Bacteria with Different Assemblages in the Soil Profile Drive the Diverse Nutrient Cycles in the Sugarcane Straw Retention Ecosystem

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Abstract: Straw retention, an alternative to artificial fertilization, commonly mitigates soil degradation and positively affects soil fertility. In this study, we investigated the succession of soil bacteria during two sugarcane straw retention treatments (control (CK) and sugarcane straw retention (SR)) and at four depths (0–10, 10–20, 20–30, and 30–40 cm) in fallow soil in a sugarcane cropping system. Using an Illumina MiSeq (16S rRNA) and soil enzyme activity, we explored the SR influence on soil bacterial communities and enzyme activities and its inclusive impact on soil fertility, with an emphasis on topsoil (0–10 cm) and subsoil (10–40 cm). Our results show that SR effectively improved soil fertility indicators (C, N, and P), including enzyme activities (C and N cycling), throughout the soil profile: these soil parameters greatly improved in the topsoil compared to the control. Sugarcane straw retention and soil depth (0–10 cm vs. 10–40 cm) were associated with little variation in bacterial species richness and alpha diversity throughout the soil profile. Subsoil and topsoil bacterial communities differed in composition. Compared to the CK treatment, SR enriched the topsoil with *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi*, and *Nitrospirae*, while the subsoil was depleted in *Nitrospirae* and *Acidobacteria*. Similarly, SR enriched the subsoil with *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Bacteroidetes*, while the topsoil was depleted in *Acidobacteria*, *Gemmatimonadetes*, and *Planctomycetes* compared to the CK. At the genus level, SR enriched the topsoil with *Gp1*, *Gp2*, *Gp5*, *Gp7*, *Gemmatimonas*, *Kofleria*, *Sphingomonas*, and *Gaiella*, which decompose lignocellulose and contribute to nutrient cycling. In summary, SR not only improved soil physicochemical properties and enzyme activities but also enriched bacterial taxa involved in lignocellulosic decomposition and nutrient cycling (C and N) throughout the soil profile. However, these effects were stronger in topsoil than in subsoil, suggesting that SR enhanced fertility more in topsoil than in subsoil in fallow land.

Keywords: soil profile; sugarcane straw retention; soil enzymes; soil fertility; 16S rRNA gene amplicon; bacterial communities

1. Introduction

Soil microbes not only recycle nutrients, degrade pollutants, and decompose organic matter, but also maintain groundwater quality, thereby improving ecosystem functions [1,2]. There is a complex web of microorganisms in soil, and their diversity and composition vary in space [3]. Topsoil (0–10 cm) has great microbial diversity and biomass; however, a large volume of subsoil (below 10 cm) on a depth-weighted basis also leads to substantial microbial diversity and abundance [4,5]. Soil microbial composition varies with increasing soil depth, and microbial diversity commonly declines with depth [5,6]. Advanced intensive agriculture systems have made a considerable contribution to enhancing crop production and meeting the world's food demands [7,8]. Despite the positive impact on crop production, maintaining soil health and biodiversity has become an increasingly severe obstacle to modern agricultural systems [9,10].

Fertilization, an essential agricultural practice based on organic and inorganic fertilizers, is commonly adopted to improve the supply of plant nutrients, causing simultaneous changes in soil properties and microbial communities [11–13]. Though artificial fertilization, particularly nitrogen, positively influences crop yield [14], it also adversely affects the environmental and soil quality via soil acidification, reduced soil microbial activity, enhanced nitrification, and nitrate leaching [10,15–17]. In contrast, low-input agricultural systems based on organic materials appear to be an alternative to inorganic fertilizers in ensuring agricultural sustainability, crop yield, food security, and human health [18–24]. For example, low-input agricultural systems rely on straw retention (SR), particularly 3.75 t ha^{-1} wheat straw returned by deep tillage, as well as inorganic fertilizers, which usually increase microbial biomass, diversity, and activity [25]. In addition, SR maintains or even improves soil fertility, mitigates greenhouse gas emissions, and inhibits soil-borne pathogens, thereby ensuring soil ecosystem sustainability and crop productivity [25–29]. Nutrient addition by SR may directly shift the composition and abundance of topsoil microbial communities [29–33]. These microbial parameters in the subsoil are shifted indirectly through the leakage of soluble nutrients from topsoil [34].

Specific bacterial taxa (phylum and genus level) have shown predictable responses to environmental variables [35]. For example, SR increases the abundance of *Proteobacteria* and *Acidobacteria* in topsoil, whereas the abundance of *Gemmatimonadetes* decreases [32]. However, there is a limited understanding of SR regarding specific soil bacterial populations at low taxonomic levels (e.g., genus and species) in the subsoil. SR appears to select particular microbial groups at low taxonomic levels that commonly survive on organic substrates and multiply significantly, shifting the soil nutrient status and microbial community composition [29,36]. Therefore, particular microbial taxa whose abundance is enhanced by SR should have some degree of relationship with soil nutrients. The predictable response of these distinct microbial populations makes them potentially suitable indicators of soil nutrient status and sustainability [37]. Furthermore, the microbial communities that contain these specific microbial taxa form a complicated interaction in soil ecosystems, and the knowledge of such interactions is essential for exploring the complexity of functional processes [38].

Soil enzymatic activities are identified as a sensitive indicator of soil fertility because they not only catalyze vital biochemical reactions (e.g., nutrient cycling, degradation of xenobiotics and organic nutrients) but also sustain soil fertility [13,22,31,39]. Numerous studies about the effects of SR on soil enzyme activities have shown that SR increases the activities of C- and N-cycling enzymes in the topsoil [40,41]. These observations have focused on surface soil because SR has a direct impact on crop plants and soil fertility [42,43], but spatial variability and the characterization of microbial communities coupled with soil enzymatic activity in deep soil remain scarce. In particular, there is a limited understanding of the interactions between these communities and the soil physiochemical properties in deep soil profiles. To bridge these gaps, this study focused on bacterial communities and their activities in four soil layers (0–10, 10–20, 20–30, and 30–40 cm) in a system with straw retention and investigated the association between soil nutrients and bacterial communities through a soil profile. Our previous research on this clay loamy soil showed that SR increased soil fertility, enzymatic activities, and plant beneficial microbes in a pot experiment [29,31]. Assuming that sugarcane straw retention

affects soil nutrient status and soil physicochemical attributes in a soil profile, we hypothesized that these fluctuations might strongly affect soil bacterial communities and their activities, with a positive impact on soil fertility in the soil profile. Adopting a multidisciplinary approach combining the soil enzymatic activities (C, N, and P cycles) and bacterial communities determined by deep 16S amplicon sequencing, we aimed to compare the influence of SR in improving soil fertility in topsoil and subsoil. The aims of this investigation were (i) to reveal the effect of SR on shifts in bacterial communities in topsoil and subsoil, (ii) to appraise the impact of different bacterial communities on C, N, and P cycle-related enzymes, and (iii) to examine interactions between bacterial community compositions and soil properties in a soil profile.

2. Materials and Methods

2.1. Study Site, Experimental Design, and Soil Sampling

The field experiment was carried out in March 2017 at the Sugarcane Experimental Farm (latitude: 26°05'9.60" N; longitude: 119°14'3.60" E) of Fujian Agriculture and Forestry University, Fuzhou, Fujian, China. The area has a subtropical monsoon climate with an annual average temperature of 20 °C and rainfall of 1369 mm. The raw soil has a clay loam texture: other physicochemical properties of the topsoil and sugarcane straw before the experiment were detailed in previous studies [29]. The sugarcane straw was collected from an experimental sugarcane farm and crushed into small pieces according to a previous study [31]. Two treatments ((i) a control (CK), which was a moldboard plow 40 cm deep in a fallow field without sugarcane straw retention, and (ii) sugarcane straw retention (SR), which was a moldboard plow 40 cm deep in a fallow field with 30 t·ha^{-1} of sugarcane straw retention) were laid out in a split-plot design and replicated three times. After SR, all field plots remained unplanted for one year without any fertilization. For soil sampling from CK and SR plots, different soil depths (0–10, 10–20, 20–30, and 30–40 cm) were selected because soil microbial community composition also varies due to excessive soil volume on a depth-weighted basis [4–6]. In 2018, five sampling locations were randomly selected in each replicate plot and pooled into a composite sample, as used by Gu et al. [6]. Finally, a total of 24 soil samples were collected from the experimental farm. After removing the straw and visible roots and stones, the composite samples were homogenized and separated into two portions. One sample portion was placed in sterile plastic bags, transferred to the laboratory in an icebox, and stored at −80 °C to analyze soil microbial composition and enzyme activity. The other sample portion was sieved through 2-mm mesh and air-dried to analyze the soil physiochemical properties.

2.2. Measurements of Soil Physiochemical Properties

Soil pH was determined by a PB-10 pH meter (Sartorius, Gottingen, Germany) in the suspension (water/dry soil, 2.5:1) [44]. Soil total nitrogen (TN) and total carbon (TC) were determined by using a Flash Smart elemental analyzer (Thermo Scientific™, Waltham, MA, USA). Ammonium acetate and sodium bicarbonate were used for extracting soil available potassium (AK) and phosphorus (AP), and their concentrations were determined using flame photometry and the molybdenum blue method, respectively [45,46].

2.3. Measurements of Soil Enzyme Activities

Soil enzyme activities were determined according to Tayyab et al. [31] and Sun et al. [47]. After the incubation of soil with buffered sodium carboxymethylcellulose solution, cellulase (glucose, mg/g 24 h, 37 °C) activity was calculated colorimetrically by quantifying a decrease in 3,5-dinitrosalicylic acid from reducing sugar. Soil urease activity (NH3-N, mg/g, 24 h, 37 °C) was determined by using improved sodium phenolate and sodium hypochlorite colorimetry. A nitrophenyl phosphate disodium substrate was used to determine acid phosphatase activity (phenol, ug/g, 1 h, 37 °C). After buffering the soil with p-nitrophenyl-β-glucopyranoside, the β-glucosidase activity (p-nitrophenol, ug/g, 1 h, 37 °C) was calculated based on a colorimetric p-nitrophenol assay.

2.4. Soil DNA Extraction

Total genomic DNA was extracted from fresh soil samples using a Fast DNA^{TM} Spin Kit (MP Biomedicals, LLC, Santa Ana, USA) per the manufacturer's specifications. The quantity and quality of extracts were determined by calculating their absorbance (A260 and 280 nm) using a spectrophotometer, and extracts were then preserved at −20 °C until sequencing.

2.5. PCR Amplification and Illumina MiSeq Sequencing

Amplification of the hypervariable V3–V4 region of the 16S rRNA gene was carried out by using bacterial primers 341F and 805R. The PCR reactions were carried out in a 50-μL mixture with 1 mM dNTPs (deoxynucleoside triphosphate), 1 × PCR buffer, 1 U Platinum Taq, 5 μM per primer, and 10 ng of template DNA. The PCR amplification included an initial denaturation at 94 °C for 3 min, denaturation (5 cycles at 94 °C) for 30 s, annealing at 45 °C for 20 s, an extension at 65 °C for 30 s, denaturation (20 cycles at 94 °C) for 20 s, annealing at 55 °C for 20 s, an extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. After purification and quantification, the PCR product of the V3–V4 region of the 16S rRNA gene was determined by an Illumina MiSeq sequencer (Sangon Biotech Shanghai Co., Ltd., Shanghai, China).

2.6. Processing and Analyzing of Sequencing Data

Raw sequences were processed using the QIIME (version 1.17) software package [48] and UPARSE software (version 7.1). Low-quality sequences (quality score <20, length <250 bp) or reads containing ambiguous characters were eliminated. After overlapped sequences longer than 10 bp in size were assembled based on their sequence overlaps, unassembled sequence reads were removed, and sequences with ≥97% similarity were clustered into operational taxonomic units (OTUs), while chimeric sequences were identified and removed through the UCHIME method [49]. For each OTU, representative sequences were selected, and a Ribosomal Database Project (RDP) classifier [50] was used to annotate the taxonomic information for each representative sequence. The species richness (ACE and Chao1 indexes) [51,52], number of observed OTUs, and diversity (Shannon index) [53] were used for estimating the abundance and diversity of the microbial communities in every soil sample using the Mothur pipeline [54]. Nonmetric multidimensional scale (NMDS) analysis was conducted to estimate differences in bacterial community structure across the soil profile [6,55]. Analysis of similarity (ANOSIM) was used to test the dissimilarity in the microbial community structures in topsoil (0–10 cm) and in subsoil (10–40 cm) using Bray–Curtis dissimilarities. Redundancy analysis (RDA) was also carried out for a quantification of the soil factors' influence on bacterial community composition. A Pearson's correlation was calculated separately for the topsoil and subsoil to determine the interaction between soil properties and bacterial taxa (phylum and genus), which was displayed by R-software [56]. The test data were analyzed by ANOVA using DPS software (version 7.05, www.dpssoftware.co.uk), and the differences between the mean values of each treatment were compared by Tukey's procedure at a 5% level [6].

3. Results

3.1. Soil Physiochemical Properties

Compared to the CK treatments, SR significantly increased ($p < 0.05$) soil pH in two soil layers (0–10 and 30–40 cm) (Figure 1A). Moreover, the total soil carbon (TC) in two soil layers (10–20 and 20–30 cm) under SR was significantly higher ($p < 0.05$) than in the CK (Figure 1B). Compared to the CK treatments, SR significantly increased ($p < 0.05$) the total nitrogen (TN) and available potassium (AK) contents in soil layers (10–20 cm and 0–10 cm, respectively) (Figure 1C,E). However, compared to the CK, SR did not considerably alter the TC/TN and available phosphorus (AP) content of the whole soil profile (Figure 1D,F). Compared to the topsoil, the soil TN and TC in the SR and CK treatments were

significantly lower in subsoil (10–40 cm) ($p < 0.05$) (Figure 1A–C). Compared to the topsoil, the soil AP contents in the SR treatments were lower in the subsoil (10–40 cm) (Figure 1F).

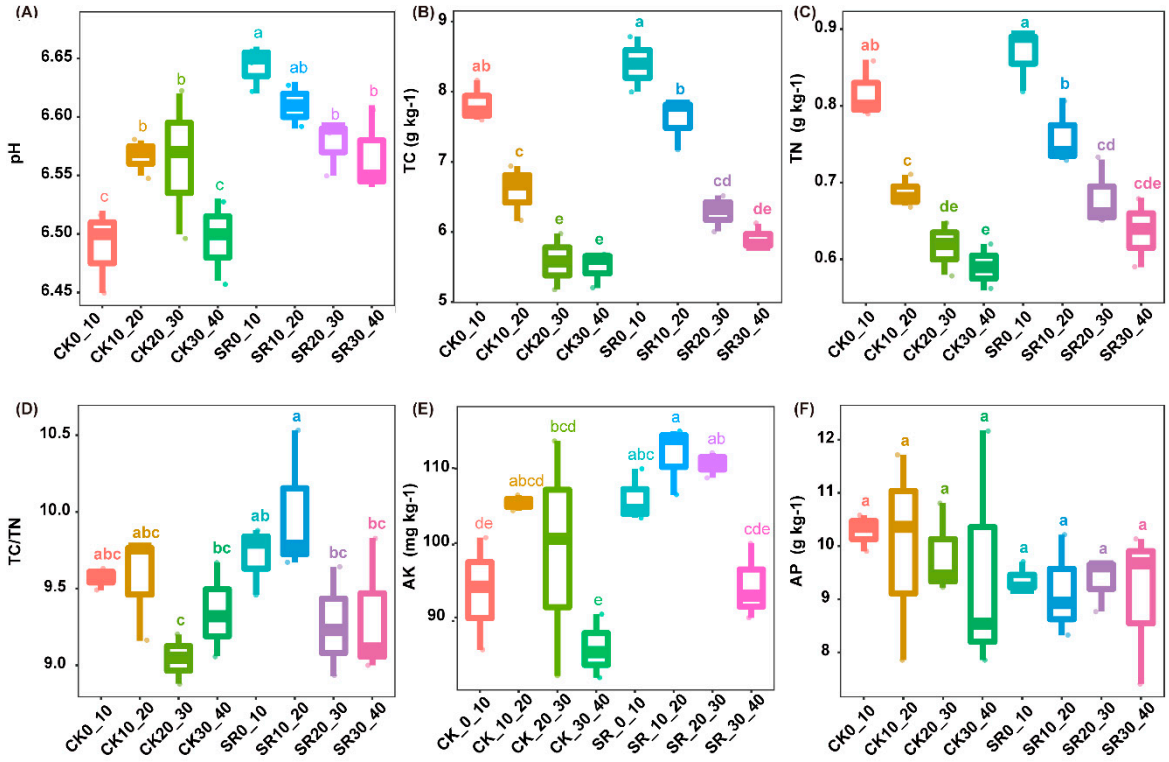

Figure 1. Soil physiochemical properties at 0–40-cm depths in the sugarcane straw retention (SR) treatment compared to the control (CK). Error bars in the boxplots with different lowercase letters show significant differences between treatments (Tukey test, $p < 0.05$). (**A**) pH, potential hydrogen; (**B**) TC, total soil carbon; (**C**) TN, total nitrogen; (**D**) C/N, carbon/nitrogen ratio; (**E**) AK, available potassium; (**F**) AP, available phosphorus.

3.2. Soil Enzyme Activities

Compared to the CK, SR significantly reduced the acid phosphatase activity of the entire soil profile (0–40 cm), while urease activity showed the opposite trend, except for in the soil layer from 20 to 30 cm (Figure 2B,C). Compared to the CK, SR did not increase significantly β-glucosidase and cellulase activity in the whole soil profile (0–40 cm) (Figure 2A,D). All enzyme activities showed a decreasing trend with depth, and compared to the subsoil, the soil enzyme activity was higher in the topsoil in the SR treatments (Figure 2).

3.3. Bacterial Community Structural Diversity and Similarity

A total of 1,032,476 (average of 43,019) bacterial filtered reads were obtained from all the soil samples. The sequencing depth was adequate to capture richness and diversity from all the soil samples (Figure S1; Table S1). Compared to the CK, SR showed little variation not only in the species richness indices (Chao1 and ACE), but also in the alpha diversity index (Shannon) in the soil profile. As soil depth increased, soils in the CK and SR treatments showed little variation in species richness and alpha diversity indices (Table S2).

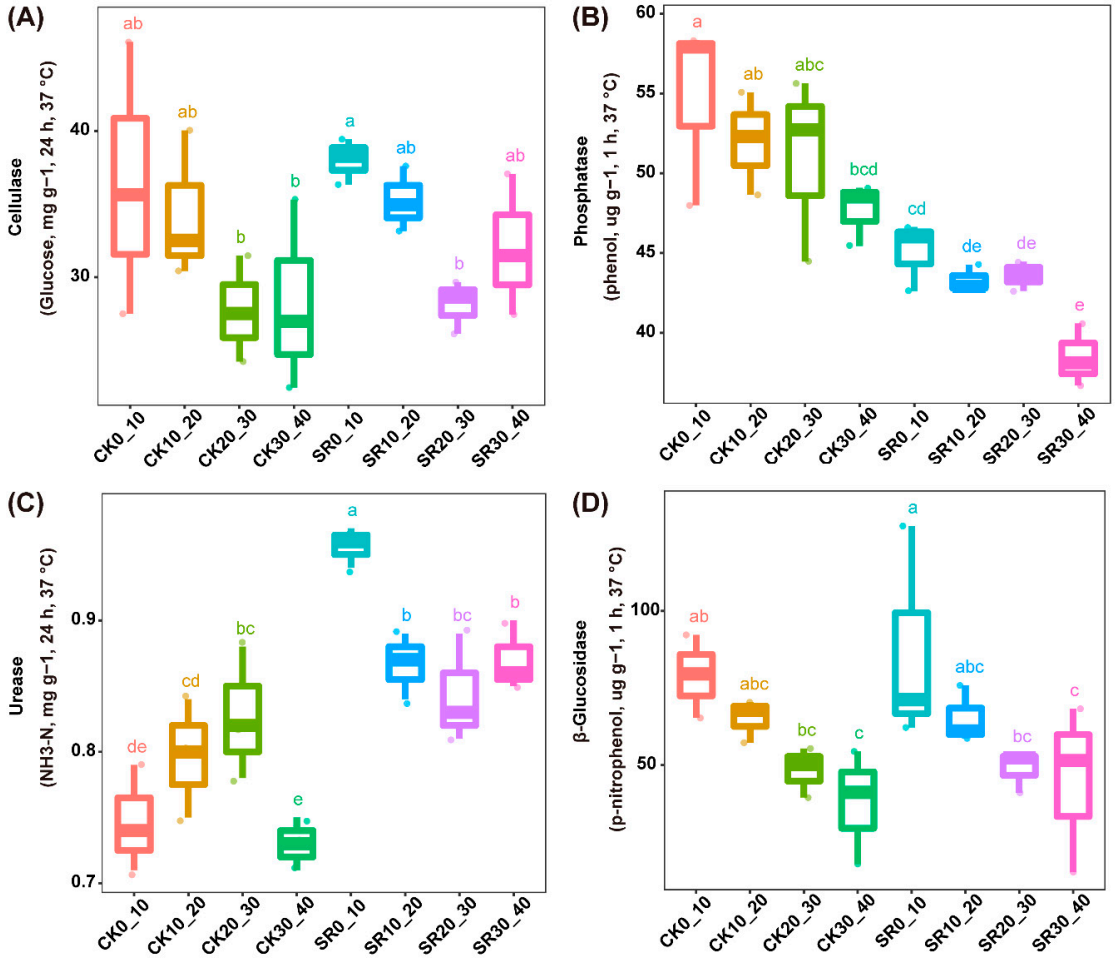

Figure 2. Soil enzymatic activities such as cellulase (**A**), phosphatase (**B**), urease (**C**) and β-glucosidase (**D**) at 0–40-cm depths under sugarcane straw retention (SR) compared to the control (CK). Error bars in boxplots with different lowercase letters show significant differences between treatments (Tukey test, $p < 0.05$).

A nonmetric multidimensional scaling (NMDS) analysis showed that topsoil samples (0–10 cm) were separated from the subsoil samples (10–20, 20–30, and 30–40 cm) (Figure 3). An analysis of similarities (ANOSIM) further confirmed significant differences ($p = 0.26$, $p < 0.05$) between the topsoil and subsoil. On the basis of the NMDS results, a redundancy analysis (RDA) was performed in two sample groups (topsoil (0–10 cm), subsoil (10–40 cm)) to quantify the effects of soil factors on bacterial community composition [6], indicating that pH ($R^2 = 0.82$, $p < 0.05$) and AK ($R^2 = 0.83$, $p < 0.05$) were the main drivers of shifting bacterial community composition in the topsoil (Figure 4A). In the subsoil, pH ($R^2 = 0.44$, $p < 0.05$), AK ($R^2 = 0.42$, $p < 0.05$), and TC ($R^2 = 0.48$, $p < 0.05$) were strongly and significantly correlated with bacterial community composition variation ($p < 0.05$) (Figure 4B).

3.4. Relative Abundance of Dominant Phyla and Genera

The relative abundances of bacteria were examined at the phylum and genus levels for inspecting their responses to SR and different soil depths. *Proteobacteria* (31.5–37.5%) and *Acidobacteria* (25.9–26.7%) were the highly abundant bacterial phyla in topsoil, followed by *Verrucomicrobia* (4.9–12.5%), *Actinobacteria* (3.3–3.5%), *Chloroflexi* (3.3–4.2%), *Gemmatimonadetes* (2.1–3.1%), *Planctomycetes* (3.3–4.0%), *Nitrospirae* (1.5–2.2%), *Bacteroidetes* (1.3–1.7%), and *Latescibacteria* (1.6–1.8%) (Figure 5). The bacterial phyla in the subsoil were altered in terms of relative abundance in both the CK and SR treatments in comparison to topsoil (Figure 5). However, compared to the CK, SR increased the relative

abundance of *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi*, and *Nitrospirae*, but reduced it for *Acidobacteria*, *Gemmatimonadetes*, *Planctomycetes*, and *Bacteroidetes* in the topsoil (Figure 6A). In particular, compared to the CK, SR increased the relative abundance of *Proteobacteria* such as *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* and reduced the relative abundance of *Deltaproteobacteria* in topsoil (Figure 7). In comparison to the CK, SR enriched the subsoil with *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Bacteroidetes*, whereas the abundance of *Nitrospirae* and *Acidobacteria* decreased (Figure 6A). With SR, the *Verrucomicrobia* relative abundance was 2.5-fold higher than in the CK in topsoil and 5.30-fold higher than in the CK in subsoil. At the genus level, compared to the CK, SR enriched the topsoil with *Gp1*, *Gp2*, *Gp5*, *Gp7*, *Gemmatimonas*, *Kofleria*, *Sphingomonas*, and *Gaiella* compared to the subsoil. Compared to the CK, SR enriched the subsoil with *Gp6*, *Gp4*, *Gp7*, *Subdivision3_genera_incertae_sedis*, *Geobacter*, *Anaeromyxobacter*, *Kofleria*, and *Sphingomonas* compared to the topsoil (Table S3; Figure 6B).

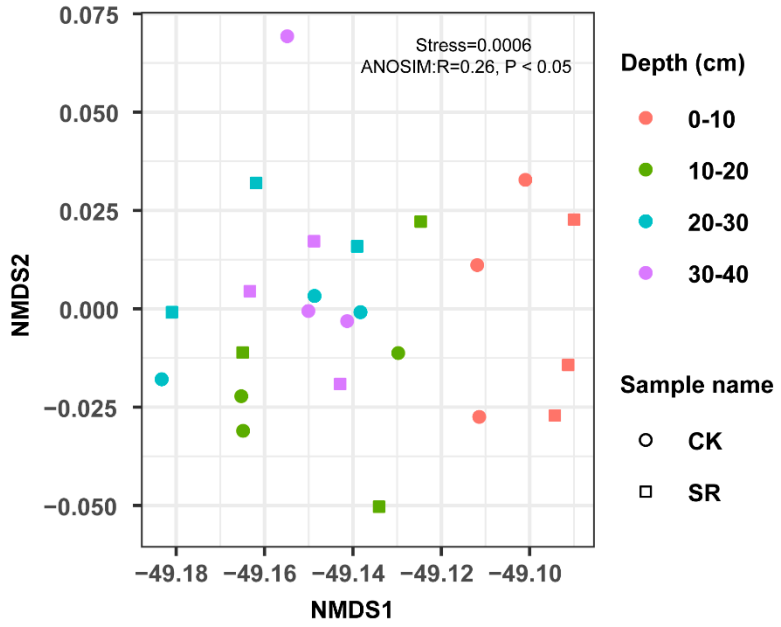

Figure 3. Analysis of nonparametric multidimensional scaling (NMDS) of the bacterial communities in the soil samples at 0–10-cm, 10–20-cm, 20-30-cm, and 30–40-cm depths. CK, control; SR, sugarcane straw retention.

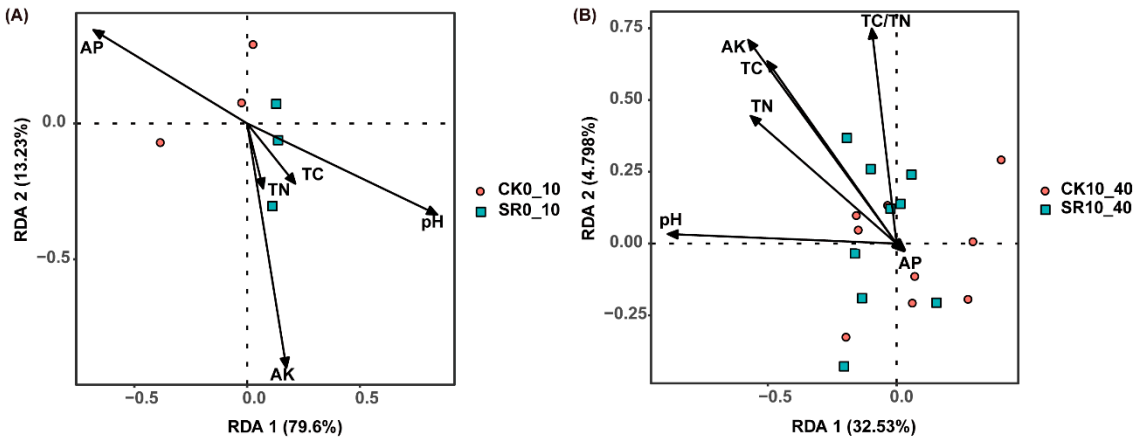

Figure 4. Redundancy analysis (RDA) of bacterial communities and measured soil properties at 0–10-cm (**A**) and 10–40-cm (**B**) depths. CK, control; SR, sugarcane straw retention.

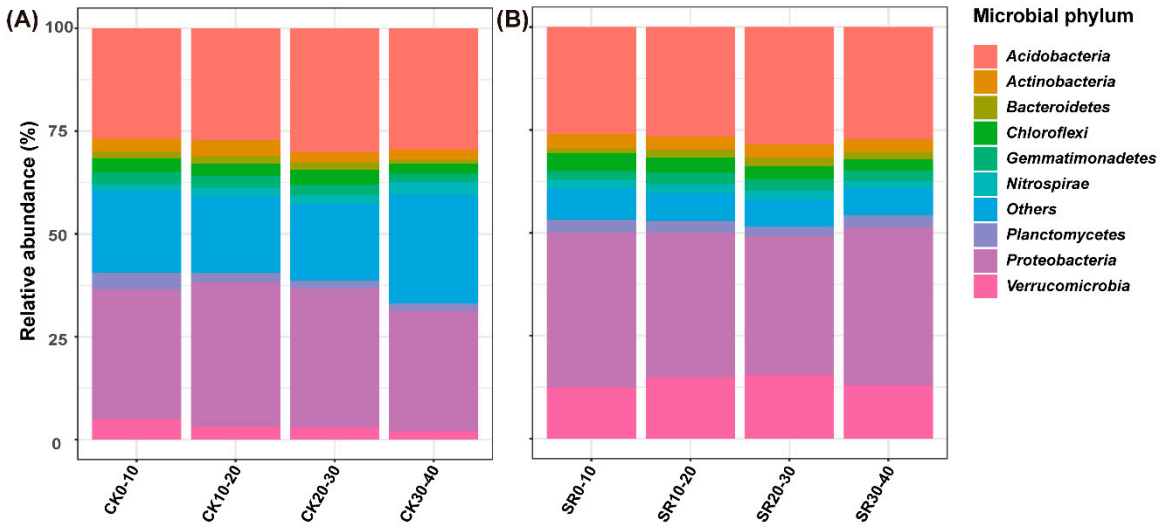

Figure 5. Relative abundances of the top 10 microbial phyla at different depths (**A**) in the sugarcane straw retention (SR) and control (CK) treatments (**B**). "Others" refers to those identified phyla that were beyond the top 10 phyla. CK, control; and SR, sugarcane straw retention.

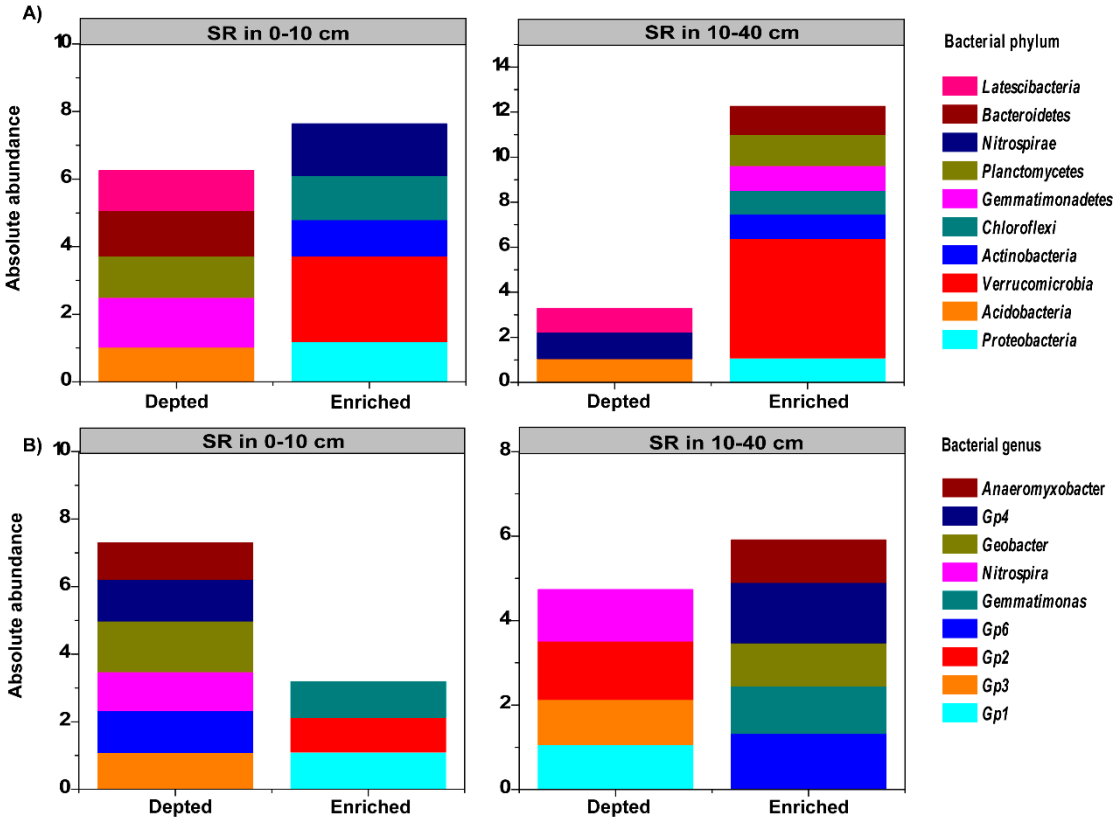

Figure 6. Sugarcane straw retention (SR) depleted or enriched of abundant phyla (**A**) and genera (**B**) at depths of 0–10 and 10–40 cm compared to the control (CK).

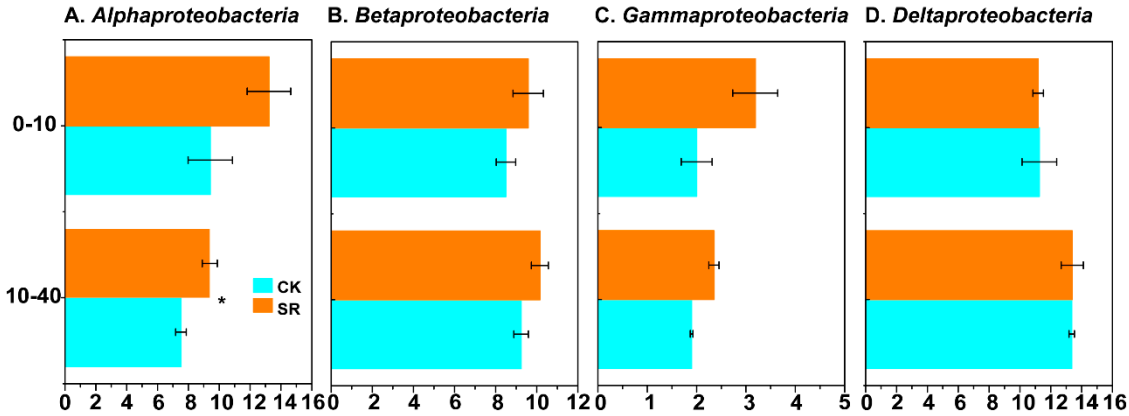

Figure 7. Relative abundances of *Alphaproteobacteria* (**A**), *Betaproteobacteria* (**B**), *Gammaproteobacteria* (**C**), and *Deltaproteobacteria* (**D**) at depths of 0–10 and 10–40 cm in sugarcane straw retention (SR) and control (CK) treatments. The values at depths of 10–40 cm are weighted means. The heat map specified by "*" shows statistical significance: * $p < 0.05$.

3.5. Pearson's Correlation between Soil Attributes and Relative Abundance of Soil Bacterial Taxa

Pearson correlation coefficients were calculated to assess the association between soil physiochemical properties and abundant bacterial taxa (phyla and genera) in topsoil and subsoil. The relationships between these taxa and physiochemical properties were also estimated to explore the potentially important microbial groups in straw decomposition and nutrient cycling. Related heat maps showed that soil physiochemical factors significantly affected the relative abundance of bacterial taxa (phyla and genera) in topsoil and subsoil (Figure 8). At the phylum level, soil pH had a significant and positive correlation with the relative abundance of *Proteobacteria* and *Bacteroidetes* in topsoil and subsoil and *Gemmatimonadetes* in subsoil; however, this correlation was observed to be strongest for *Proteobacteria* in subsoil. In topsoil, TN and TC were significantly and positively correlated with the relative abundance of *Gemmatimonadetes*, and soil pH was significantly and positively associated with this phylum only. In subsoil, *Actinobacteria* and *Verrucomicrobia* were significantly and positively correlated with TN and TC, whereas *Verrucomicrobia* showed a positive correlation with AK. In addition, the relative abundance of *Nitrospirae* was significantly and negatively correlated with soil pH and TN (Figure 8A,B). At the genus level, in topsoil, the relative abundance of *Gemmatimonas* was significantly and positively correlated with soil TN and TC, and in subsoil, it showed a significant and positive correlation with soil pH. The relative abundance of *Sphingomonas* was significantly and positively correlated with soil pH in topsoil, whereas *Latescibacteria_genera_incertae_sedis* was significantly and positively correlated with soil AP in topsoil (Figure 8C). The relative abundance of Gp6 was positively and significantly correlated with soil TC and AK. The relative abundance of *Nitrospira* was significantly and negatively correlated with soil pH, AK, and TN in subsoil (Figure 8D).

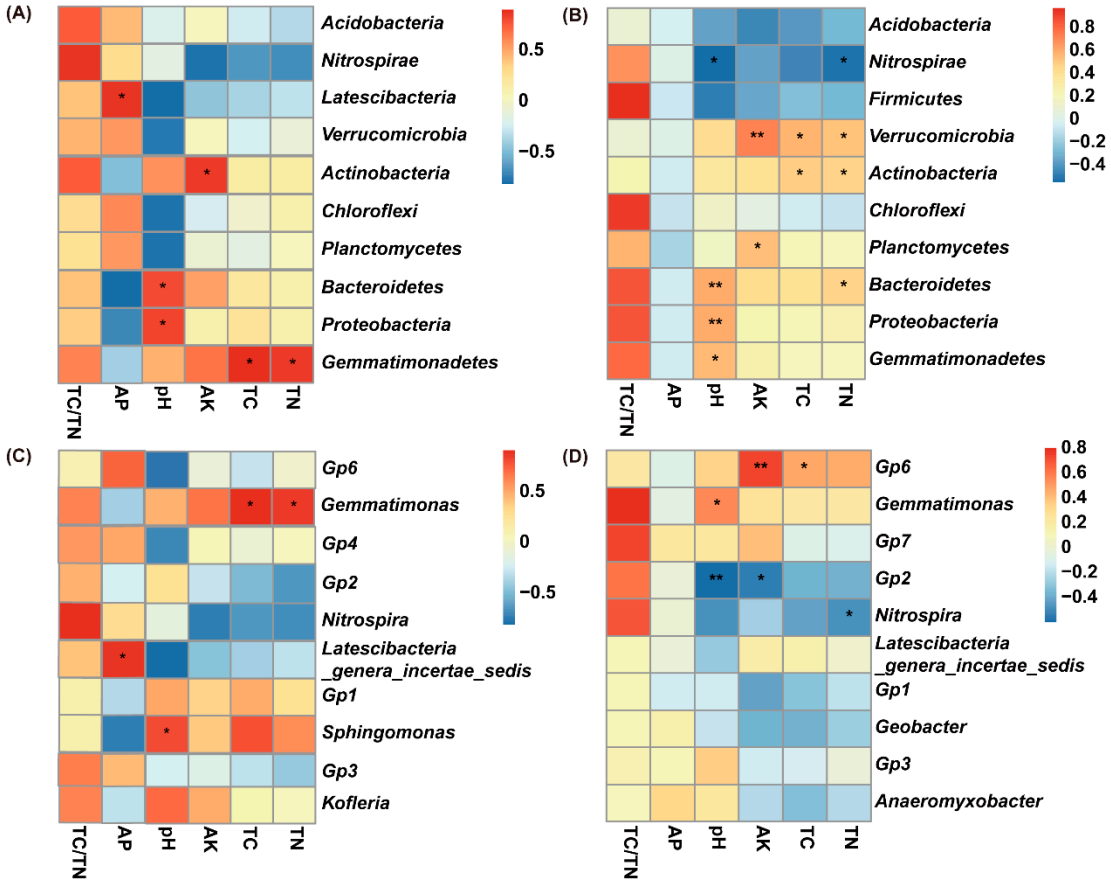

Figure 8. Pearson correlation coefficients for soil physiochemical properties and the most abundant bacterial phyla at 0–10-cm (**A**) and 10–40-cm (**B**) depths. Pearson correlation coefficients for soil physiochemical properties and most abundant bacterial genera at 0–10-cm (**C**) and 10–40-cm (**D**) depths. The heatmap cells marked by "*" or "**" are statistically significant: * $p < 0.05$ and ** $p < 0.01$.

4. Discussion

SR in agricultural systems has been known as an alternative to inorganic fertilization, with the benefits of improving soil fertility and productivity and reducing soil degradation. However, information regarding SR influence on soil microbiota, their diversity, and their effects on soil fertility as they change with soil depth remains scarce. Hence, the current study contributes to understanding the SR impact on soil bacterial communities and enzymatic activities, including the overall effect on soil fertility, with a particular spotlight on deep soil profiles.

This study revealed that SR is a practical approach in terms of improving soil fertility indicators, such as soil pH, TC, TN, and AK, which is consistent with our previous findings [29,31]. Regarding soil depth, soil physiochemical properties are strongly dependent on soil depth [6]. Additionally, reduced-tillage practices, such as sweep and disc plowing, and the application of chemical fertilizers are the main factors in terms of reducing the pH of topsoil [33,57]. Similarly, the study presented here showed that soil pH in the CK treatment was lower in topsoil than in subsoil. On the other hand, SR efficiently enhanced the soil pH across the soil profile, which was significantly more increased in topsoil than in subsoil. The probable explanation is that SR practice leads to nitrate immobilization, which in turn enhances the pH of topsoil [58]. In addition, the increase in subsoil pH can be attributed to a decline in base cation concentrations and the decomposition of organic anions present in dissolved organic carbon [34]. Generally, topsoil has higher TN and TC concentrations than subsoil because crop straw and manure frequently accumulate in topsoil. In the CK treatment, the AP concentration in topsoil was higher than in subsoil, which might have been due to the low solubility of phosphorus in

slightly alkaline soils [59] and the greater accumulation of organic matter on the soil surface [40,60]. In summary, SR effectively increased soil pH and improved soil quality indicators such as TC, TN, and AK in the soil profile compared to the CK, particularly in the topsoil layer.

Soil enzyme activities are identified as sensitive indicators of soil fertility because they not only catalyze vital biochemical reactions (e.g., nutrient cycling, the degradation of xenobiotics, and the decomposition of organic matter) but also sustain soil fertility [39,61]. In the present study, enzyme activities associated with N and C cycles were higher in topsoil than in subsoil, suggesting that topsoil could show a more significant improvement in C and N turnover and fertility than subsoil could. The activity of these enzymes was enhanced due to the SR treatment, increasing the difference in C and N turnover and the fertility between the topsoil and subsoil. The increased substrate availability and C demand by autochthonous microorganisms in the topsoil could have been the reason for the higher activity of C-cycle enzymes in the topsoil compared to the subsoil. It has been reported that in agricultural soils, SR increases the activity of C- and N-cycle enzymes in topsoil [40,41]. Compared to the CK, SR decreased the enzyme activity for P cycling in the soil profile, which was consistent with the findings of Tayyab et al. [31], wherein soil phosphatase activity decreased with SR treatment in pots. In summary, SR enhanced the activities of N- and C-cycle enzymes in the soil profile compared to the CK (most strongly in topsoil). However, soil enzyme activities decreased with increasing soil depth, which could have been due to a decrease in TC and TN [62].

Compared to the CK, SR showed little variation in species richness and alpha diversity in the soil profile. The little variation in bacterial diversity in the subsoil was attributed to the large volume of soil on a depth-weighted basis. Bacterial community structural diversity was not attributed to straw decomposition. However, in the SR and CK soils, the subsoil and topsoil bacterial communities differed in structure and composition, which was consistent with previous findings [5,6].

Compared to subsoil, SR caused the topsoil to be rich in *Proteobacteria*: the members of this phylum have been identified as copiotrophs [63,64]. In this study, SR resulted in a high availability of C and N in the topsoil, which in turn could enrich the topsoil with this phylum. Similarly, in comparison to subsoil, the increased abundance of *Betaproteobacteria* in the topsoil could have been due to a greater availability of C in the SR treatment because it has been documented that this class proliferates in an environment with a high C content [65]. Regarding *Alphaproteobacteria*, most members of this class play a role in N_2 fixation [66]. SR increased the abundance of *Alphaproteobacteria* in the topsoil and therefore could have improved the fixation of N_2 in the topsoil. The obvious enrichment of well-known N_2-fixing genera such as *Sphingomonas* [67] in the topsoil further supported this suggestion. Compared to the topsoil, SR increased the abundance of *Geobacter* in the subsoil (this genus tends to grow in anaerobic environments) [68], which in turn enriched the subsoil with *Deltaproteobacteria*.

Actinobacteria decompose organic matter in soil, such as chitin and cellulose [69], contributing to the N cycle and proliferating in aerobic environments [70]. A greater availability of soil organic matter after straw application and the aerobic environment in topsoil could have been the reasons for the increased abundance of *Actinobacteria* in the topsoil [5]. In addition, *Gaiella* (belonging to *Actinobacteria*) is involved in the N cycle [63,71], and enrichment with this genus in the SR treatment might have had a positive impact on the N cycle in the topsoil.

Nitrospirae play an essential part in regulating the nitrite oxidation process in soil. This result is due to the high affinity of this phylum substrate [64,72,73]. Because this phylum participates in nitrite oxidation [74], the enrichment of this phylum in the topsoil in the SR treatment suggests that this treatment could positively influence nitrification in topsoil. The genus *Nitrospira* has been identified as a mixed-nutrient type and tends to proliferate in deep soils with low C and N [75], indicating that SR reduced *Nitrospira*. SR decreased the relative abundance of *Acidobacteria* in the soil profile in comparison to the CK: this phylum has several oligotrophic members and tends to grow in acidic soil [64,76,77].

At the genus level, compared to topsoil, SR enriched the *Acidobacteria* genera *Gp1*, *Gp2*, *Gp3*, *Gp5*, and *Gp7* in subsoil [6,63,78], likely because they prefer to grow in an environment with limited

nutrients [6]. In contrast, SR increased the abundance of *Gp4*, *Gp5*, *Gp6*, and *Gp7* in topsoil: these genera live at a higher soil pH and play a vital role in disease suppression [79,80], indicating that the topsoil was healthier than the subsoil.

The phyla *Gemmatimonadetes* and *Verrucomicrobia* were found to be more abundant in subsoil in the SR treatment. It is challenging to associate the phylum *Gemmatimonadetes* with its possible functions in ecosystems because it is still difficult to characterize [81]. However, due to its relatively high abundance and a rapid response of this phylum to SR, its ecology, including a role in the environment, must be uncovered. Nearly all *Verrucomicrobia* cultured from soil have been free-living [82], and most are mesophilic, facultatively or obligatory anaerobic [83], and saccharolytic [84]. Under a range of conditions, *Gemmatimonas* regulates carbon and nitrogen intake based on metabolic needs and shows dominance in soils treated with pyrogenic organic material [85,86], indicating that it can decompose polycyclic aromatic hydrocarbons. Moreover, *Gemmatimonas* is considered to be a polyphosphate-accumulating genus [87] whose stimulation can be attributed to organic fertilizers [63]. Our findings also support these results, since *Gemmatimonas* was enriched in the soil profile more by SR than in the CK. *Gemmatimonas* was also involved in straw decomposition and soil nutrient cycling [63,88], indicating that a high abundance of *Gemmatimonas* might contribute to the rapid decay of straw and soil nutrient cycling. In summary, the results of the study presented here show that specific bacterial populations involved in lignocellulosic decomposition and nutrient cycling, especially of C and P, were substantially depleted or enriched in the topsoil and subsoil after the SR treatment.

Soil physiochemical properties have an essential role in shaping microbial community composition and structure [29,31,89]. In the present study, compared to AP and TC/TN, the soil pH and AK were the main driving forces in terms of changing bacterial community compositions in topsoil. Regarding subsoil, the soil pH, AK, and TC were the main factors shaping bacterial communities. On a similar note, soil properties such as TC, TN, and soil pH are known as the main drivers influencing soil microbial communities in response to SR because these soil properties can impact the abundance of some vital bacterial taxa [62]. However, these soil properties were different in the topsoil and subsoil, which may have changed the bacterial groups not only at the highest classification level but also at a low classification level (phylum and genus) in the topsoil and subsoil.

5. Conclusions

SR effectively improved soil enzyme activity (those involved in N and C cycles) and nutrient status (mainly C, N, and P) in the soil profile. SR and the soil depth affected bacterial community composition. In addition, specific bacterial groups involved in the decomposition of organic compounds and soil nutrient cycling (mainly C, N, and P) were significantly enriched or depleted in the topsoil and subsoil by SR. These changes were more obvious in the topsoil, leading to speculation that SR stimulates soil fertility in topsoil more than in subsoil. Metagenomics studies coupled with microbial function-related studies are likely to enhance our understanding of the complexity of soil microbial communities and their interactions with biogeochemical factors.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/11/10/194/s1, Table S1. Distribution of the number of tags across the soil samples; Table S2. Alpha diversity index; Table S3. The relative abundance (%) of selected genera in high-abundance phyla at 0-10 and 10-40 cm depths in the sugarcane straw retention (SR) and the control (CK) treatments; Figure S1. The species accumulation curves presenting the adequacy for sample size.

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