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Effect of Sugarcane Straw and Goat Manure on Soil Nutrient Transformation and Bacterial Communities

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Abstract: Crop residue and animal manure as a soil amendment have been recognized as a feasible agricultural practice owing to its contribution in improving the soil fertility (SF). The primary advantages of this practice are determined by the activities of soil microorganisms. However, goat manure (M), sugarcane straw (S), and goat manure plus straw (MS) amendments influence soil bacteria, their activities, and SF in clay-loam soil remains undefinable. Therefore, this study distinguished the efficacy of M, MS, and S amendment on soil enzyme activities and the availability of nutrients, including various bacterial populations in clay-loamy soil with respect to two different phases (50 and 100 days). In order to analyze the bacterial structure and their activities, we employed high-throughput sequencing (HTS) and soil enzyme activity (SEA) tests. Soil amended with M and MS not only significantly enhanced nutrient availability, including C, P, and N, soil pH, as well as SEA for C and N cycles in both phases. Additionally, the increase in nutrient availability was greater in M- and MS-amended soils in the second phase (100 days) compared to the M- and S-amended soils in the first phase (50 days). Moreover, plant growth promoting and lignocellulose degrading bacterial genera were enhanced under M- and MS-amended soil compared to S-amended soil in both phases. Distance-based redundancy analysis (dbRDA) showed that soil pH, carbon-nitrogen ratio (C:N), and nitrates (NO_3^-) were inducing the fewest changes, while total nitrogen (TN), total carbon (TC), available nitrogen (AN), available phosphorus (AP), total phosphorus (TP), available potassium (AK), and ammonium (NH_4^+) were the main operators in terms of change in bacterial populations. In general, we observed that M and MS are better amendment sources as compared to S amendment in order to enhance the SF in the clay-loamy soil in both phases, but greater fertility was exhibited in the second phase.

Keywords: livestock manure; straw; lignocellulose; soil enzymes; fertility; bacterial dynamics

1. Introduction

Utilization of mineral fertilizers to enhance soil fertility (SF) is a fundamental agricultural practice which is primarily employed to increase the nutrients availability for crops, with response to variations in soil characteristics and microbial communities [1]. In the future, agricultural land will be limited

due to the continuously rising need for food, shelter, and resources. In view of this, ensuring food security under these circumstances is the main concern that cannot be resolved by adopting only mineral fertilizers in the agricultural sector. Soil degradation and acidification are the leading problems triggered by the application of fertilizers, especially nitrogen fertilizers (N), in order to produce high yields which, in turn, lead to a drastic reduction in soil fertility, including crop yields [2–4]. On the contrary, agricultural farming depends on the utilization of organic fertilizers, which is not only highly promising in reducing the application of mineral fertilizers, but also enhances the crop productivity via ensuring ecosystem sustainability [4–8]. In order to increase the SF and productivity, animal manure and crop residues as soil amendments are considered a great agricultural practice [9,10]. Several studies have been carried out to identify the role of organic material (e.g., plant leaves) in the soil. For instance, dry branches and leaves are the main additional resources of organic matter and nutrients as they constitute an imperative operation in the cycling of nitrogen (N) and carbon (C) among plants and soil [11,12]. Additionally, leaves have an enormous quantity of N and C, which are the main nutrient sources used for soil microorganisms' activities [13]. Consequently, decomposed and decayed leaves have a positive influence on soil microorganisms, due to the increase in C content and nutrients in the alternating soil microclimate [14]. Similarly, recently investigators have examined the impacts of plant leaves on soil properties and revealed that leaves were the major sources to add extra carbon and nitrogen to the soil. In the same way, decomposed leaves take part in the release of essential nutrients and these nutrients are important for the growth and development of plants [12,13,15]. More recent evidence has suggested that the addition of litter has a significant effect on the development of soil microbiota leading towards higher nutrient levels in soil and microbial biomass [16].

Soil microbiota have the main role of sustaining soil functions. They have great metabolic functions and are indispensable for almost all biogeochemical processes [4,17]. Using organic fertilizers on agricultural land has a promising effect on bacterial communities in the soil [4]. Fluctuations in bacterial populations in the soil in relation to diverse agricultural practices can help us explain the processes of nutrient cycling in the soil. Recently, researchers have examined microbial ecological changes under the organic waste amendment of the soil [18,19]. For instance, Giacometti et al. [20] and Peruzzi et al. [21] reported that manure amendment shows significant changes in the bacterial communities under S amendment. For instance, Das et al. [9] revealed that cattle manure amendment has developed the nutrient availability, particularly C, P, and N, improving soil microbial biomass, as well as soil pH. Additionally, it enhanced some species that decompose the organic compounds and promote the growth of plants. Similarly, Cesarano et al. [10] compared the mineral fertilizers and various organic amendments of the soil. They revealed that organic amendment not only improves the soil physiochemical features and microbial diversity, but also improves the functions of the microbes.

The traditional techniques for investigating the soil microorganisms are greatly restricted, mainly concerning cultivable microorganisms and a beneficial group of bacteria. With the advancement of molecular biology, HTS has been recognized as an effective method for the evaluation of soil microbial ecology [22]. However, current studies have employed HTS to understand the soil microbial diversity and composition under various organic amendments [9,10,23]. Much work on the potential utilization of organic fertilizers as a promising approach in order to recover soil fertility in agriculture has been carried out [4,24,25]. However, the impact of organic amendments in clay-loam soils in terms of a specific period of time was yet to be explored. Assuming that different organic amendment (goat manure (M), sugarcane straw (S), and sugarcane straw + goat manure (MS)) has diverse effects on the soil physiochemical features, including soil nutrients. Moreover, we assumed that these modifications may greatly alter the soil bacterial population, as well as their functions, which may have an encouraging impact on SF. Adopting a distance-based redundancy approach combined with microbial community characterization by HTS and SEA indicative of C, N, and P cycling, this study was aimed to identify the time and most appropriate M and MS amendment practices (M1, MS1 and M2, MS2) compared to S (S1, S2) to improve SF in clay-loamy soil. The primary objectives

of our experimentation were (a) to examine the differences of soil bacterial populations in M-, S-, and MS-amended-soil; (b) to estimate the significance of different bacterial populations and structures on C, N, and P acquisition; and (c) to investigate the relationships among bacterial composition and soil features in two phases.

2. Material and Methods

2.1. Experimental Design and Soil Sample Preparation

S, M, and MS amendment were examined on soil chemical properties and bacterial dynamics under two phases (50 days and 100 days). Four treatments applying two types of organic matter (M, S, and MS) and without amendment control (CK) soil were designed and conducted from March 2017 to June 2017 at Fujian Agriculture and Forestry, Fuzhou, Fujian Province, China. In this experiment, pots (red PVC) with 180 mm height and 120 mm diameter were filled with clay-loamy soil collected from the a 0–12 cm layer from a university sugarcane cultivation field (latitude: 26°05′9.60″ N; longitude: 119°14′3.60″ E). S was sampled from the university sugarcane garden and chopped into small pieces, air dried for twenty days, and then stored at room temperature. However, M was bought from the local market. Physiochemical properties of soil, sugarcane straw, and goat manure used in this experiment were as follow; (i) soil: total carbon (TC) 0.97%, total phosphorus (TP) 0.07%, total nitrogen (TN) 0.10%, soil mechanical composition was: clay 20.3% silt 43.1% sand 36.6%; (ii) sugarcane straw: TC 44.15%, TN 0.45%, TP 0.04%, total potassium (TK) 0.5%, and C:N ratio 98.11; and (iii) goat manure: TC 17%, TN 0.8%, TP 0.52%, TK 0.41%, and C:N ratio 21.25. For S amendments, the mixture contained 15% S and 85% soil by dry weight. A similar concentration for M amendment had 15% M and 85% soil. However, for MS amendment, the mixture contained 7.5% M, 7.5% S, and 85% soil, respectively [26]. Overall, three replications were considered for each pot. According to Sun et al.'s [16] experiment, mean low and high air temperature (18–30 °C) and 75–80% of relative humidity were maintained throughout the trial. Pots of every treatment were irrigated by one liter of distilled water on a weekly basis. After 50 days (Phase 1) and 100 days (Phase 2), 20 g of soil obtained from each pot was sieved by employing a 2-mm sieve. Furthermore, freshly-sieved soil samples were stored at −80 °C in order to isolate DNA, while some soil samples were air-dried for soil physio-chemical analysis.

2.2. Physiochemical Properties of the Soil

A glass electrode pH meter was used to measure the soil pH. TN and TC were determined by using the Flash Smart elemental analyzer (Thermo Scientific™, Waltham, MA, USA). The Molybdenum Blue method was followed to estimate available phosphorus (AP) via using ammonium fluoride and hydrochloric acid. The alkaline hydrolyzable method was used for calculating the available nitrogen (AN), while ammonium acetate and flame photometry were utilized to extract available potassium (AK) [27]. For the extraction of ammonium (NH_4^+) and nitrate (NO_3^-) contents of soil, 2 M KCl was utilized and, moreover, these contents were measured by a Bran+Luebbe GmbH-AutoAnalyzer 3 (Bran+Luebbe, Norderstedt, Germany).

2.3. Soil Enzymes Activity

Soil urease (UE) and cellulase (CL) activities were determined via protocols utilized by Chen et al. [23] and Sun et al. [26]. Soil cellulase activity (CL) is indicated as the number of milligrams of glucose in 1 g of dry soil at 37 °C for 24 h, which was measured colorimetrically by measuring the 3.5-dinitrosalicylic acid reduction from the reducing sugars leading towards the incubation of soil with a buffered sodium carboxymethylcellulose solution at 37 °C for 24 h. Improved sodium phenate and sodium hypochlorite colorimetry were used to determine the soil urease activity, which has been indicated as the number of μg of NH_3-N in 1 g dry soil at 37 °C for 24 h [28]. Nitrophenyl phosphate disodium was used as a substrate to analyze the neutral phosphatase (NP), which is indicated as the number of nmol of phenol in 1 g of dry soil at 37 °C for 24 h. The activity of β-glucosidase (p-nitrophenol, μmol g^{-1}, 24 h, 37 °C) was estimated

through the release and detection of p nitrophenol (PNP) using p-nitrophenyl-β-glucopyranoside as a substrate in spectrophotometer [29].

2.4. DNA Extraction and Purification

Genomic DNA from all the samples was extracted by employing the Fast DNA™ Spin kit according to the manufacturer's guideline (MP Biomedical, Santa Ana, CA, USA) which is designed for soil DNA isolation. Moreover, all isolated DNA samples were subjected to gel electrophoresis. In addition, DNA purification was completed via DNA purification kits according to manufacturer instructions (Tiangen Biotech Co., Ltd., Beijing, China). Nanodrop was adopted for all DNA samples to quantify prior to storage at −20 °C for further analysis.

2.5. Metagenomics Analysis of the Soil Bacteria

For the measurement of the bacterial community structure, purified DNA samples were transferred to Novo Gene Bioinformatics Technology Co., Ltd. (Beijing, China). Before HTS, amplification of the *16S V4*, *16S* rRNA distinct gene region was amplified via the primer 515F-806R with barcodes [30]. Thirty microliters of total PCR reaction volume was used, containing 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs (Beijing) Ltd., Beijing, China), along with template DNA (~10 ng) and 0.2 M of each primer pair. The PCR conditions involved initial denaturation at 98 °C for 1 min, followed by another denaturation (30 cycles) at the same temperature for 10 s. Then annealing was done at 50 °C for 30 s, followed by elongation at 72 °C for 60 s, and a final extension at 72 °C for 5 min. Afterward, gel electrophoresis was performed via agarose gel solution (2%) for verification of successful DNA amplification and the visualization of bands. Samples showing 400–450 bp bright bands were excised and purified by using a Gene JET Gel Extraction Kit (Qiagen, Hilden, Germany) leading towards their successful Illumina sequencing via generating libraries through specialized NEB Next® Ultra™ DNA Library Prep Kit (New England Biolabs (Beijing) Ltd.) according to the manufacturer's instructions. Index codes were added for each library. An Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA) and the Qubit® 2.0 fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA) was used to check the quality of each library. Finally, 250/300 bp paired-end reads were generated on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

2.6. Statistical and Bioinformatics Analysis

On the basis of specified barcodes given to each sample, all the raw sequences were further classified via using Quantitative Insights Into Microbial Ecology (QIIME) (CO, USA) [30]. FLASH (Baltimore, MD, USA) was employed to merge the paired-end reads (PERs) taken from the original DNA segments leading to assigning of the unique barcodes to each PER [31]. Analysis of each sequence was done through UPARSE-OTU and UPARSE-OUT reference algorithms with the UPARSE pipeline (CA, USA). QIIME (CO, USA) was used to assess the beta and alpha diversity of the samples [30]. Similar operational taxonomic units (OTUs) were assigned to the sequences with 97% in each sample. Each OTU was further represented by a single sequence for annotating the taxonomic information by using the RDP classifier. The OTU table was then rarified for measurement of the alpha diversity (species abundance estimation through Chao1, while Simpson and Shannon's indices were utilized for determination of and the observed species community diversity. Furthermore, for each of these indices, rarefaction curves were developed. From phylum to species to other bacterial taxa, the abundance of each community has been shown via graphs through Krona Chart (V 2.7). On the other hand, QIIME (Version 1.7.0) (CO, USA) was used to measure the beta diversity for both weighted and unweighted UniFrac distances. Principal component analysis (PCA) and principal coordinate analysis (PCoA) were performed and visualized using R (Version 2.15.3) packages stat, WGCNA, and ggplot2 (Elegant graphics for data analysis, New York, NY, USA). db-RDA was used to identify the association between the abundant bacterial phylum [32], further generating the triplots using vegan and ggplot2 packages in R software (R version 3.3.1) (Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Soil Physicochemical Characteristics

Soil physicochemical characteristics have been mentioned in Table 1. Amendment of soil with M, S, and MS (i.e., M1, S1, and MS1) significantly enhanced the soil characteristics, such as pH, TC, TN, AP, AN, AK, and total P, in the first phase (after 50 days). Soil nitrate (NO_3^-) and ammonium (NH_4^+) concentration were high in M1 and MS1, as compared to all other treatments in the first phase, and enhanced more in the M1 and MS1 treatments. A similar trend was observed in all the treatments in the second phase (after 100 days).

3.2. Soil Enzyme Activity (SEA)

Urease (UE) and β-Glucosidase activity (β-GC) was significantly improved in M- and MS (M1 and MS1)-amended soil as compared to CK1 and S1 in the first phase. A similar trend was observed in M2 and MS2, as compared to CK2 in the second phase. Natural phosphatase activity (NP) was significantly decreased in M1 and MS1, as compared to control CK1 and S1 in the first phase. Similarly, natural phosphatase activity (NP) decreases significantly in M2 and MS2, as compared to control CK2 and S2 in the second phase. Cellulase activity (CL) in M1, MS1 increased insignificantly, as compared to CK1 and S1 in the first phase, while increasing significantly in M2 and MS2, as compared to CK2 and S2 in the second phase (Table 2).

Table 1. Physiochemical characteristics of the soil.

Treatments	pH	AP	TP	AK	TN	AN	NH_4^+	NO_3^-	TC	C/N
		g/kg	g/kg	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	g/kg	
CK1	5.64 ± 0.01 f	0.09 ± 0.00 e	0.70 ± 0.07 bc	71.85 ± 3.140 e	1.12 ± 0.07 f	113.05 ± 1.01 cd	2.79 ± 0.13 d	21.85 ± 1.10 b	9.96 ± 0.10 g	8.95 ± 0.660 e
M1	5.72 ± 0.03 e	0.20 ± 0.00 c	0.88 ± 0.08 b	865.92 ± 3.02 c	2.50 ± 0.02 c	307.30 ± 2.02 a	9.49 ± 0.52 a	29.46 ± 0.37 a	32.71 ± 0.36 c	13.09 ± 0.22 a
MS1	7.51 ± 0.01 b	0.25 ± 0.01 b	1.27 ± 0.04 a	1008.41 ± 6.18 b	2.29 ± 0.02 d	207.55 ± 9.09 b	9.79 ± 0.26 a	20.16 ± 0.12 bc	27.32 ± 0.07 d	11.91 ± 0.06 b
S1	6.10 ± 0.02 d	0.10 ± 0.00 d	0.71 ± 0.00 bc	151.46 ± 18.09 d	1.11 ± 0.01 f	113.05 ± 5.05 cd	5.90 ± 0.09 c	19.53 ± 0.49 bc	12.69 ± 0.03 f	11.47 ± 0.09 bc
CK2	5.73 ± 0.02 e	0.09 ± 0.00 de	0.61 ± 0.01 c	65.73 ± 6.28 e	0.93 ± 0.01 g	79.80 ± 4.040 d	2.10 ± 0.05 d	18.89 ± 0.36 bc	10.16 ± 0.06 g	10.89 ± 0.08 cd
M2	6.80 ± 0.02 c	0.39 ± 0.00 a	1.34 ± 0.07 a	1138.21 ± 3.09 a	4.59 ± 0.02 a	327.25 ± 5.46 a	6.90 ± 0.33 bc	32.41 ± 0.12 a	47.50 ± 1.00 a	10.35 ± 0.19 d
MS2	7.61 ± 0.02 a	0.25 ± 0.00 b	1.42 ± 0.11 a	980.61 ± 3.03 b	3.17 ± 0.11 b	222.95 ± 15.16 b	7.71 ± 0.12 b	21.00 ± 0.85 bc	36.70 ± 1.26 b	11.59 ± 0.02 bc
S2	6.15 ± 0.02 d	0.09 ± 0.00 de	0.75 ± 0.04 bc	129.03 ± 6.13 d	1.31 ± 0.01 e	136.50 ± 5.66 c	6.18 ± 1.00 c	18.05 ± 0.12 c	16.67 ± 0.09 e	12.72 ± 0.10 a

For every treatment with three replicates, one-way variance analysis (ANOVA) by LSD test ($p = 0.05$) was performed using DPS 7.5 Statistics. Various letters displaying the significant shift among various treatments. CK1 refers to the control soil without amendment, M1 represents goat manure-amended soil, while MS1 represents goat manure + sugarcane straw-amended soil and S1 sugarcane straw-amended soil of the first phase (after 50 days). Similarly, CK2 refers to the control soil without amendment, M2 represents goat manure-amended soil, while MS2 represents goat manure + sugarcane straw-amended soil, and S2 represents sugarcane straw-amended soil of the second phase (after 100 days).

Table 2. Soil enzymatic activities.

Treatment	Urease (NH_3-N, μg g^{-1}, 24 h, 37 °C)	Neutral Phosphatase (Phenol, nmol g^{-1}, 24 h, 37 °C)	β-Glucosidase (p-Nitrophenol, μmol g^{-1}, 24 h, 37 °C)	Cellulase (Glucose, mg g^{-1}, 24 h, 37 °C)
CK1	14.09 ± 1.09 c	2.71 ± 0.13 b	18.34 ± 0.35 bc	11.65 ± 1.24 bc
M1	31.79 ± 0.08 a	1.58 ± 0.03 d	28.12 ± 1.95 a	11.72 ± 0.68 bc
MS1	25.57 ± 0.08 b	1.09 ± 0.06 e	23.54 ± 2.22 abc	11.48 ± 0.04 bc
S1	12.71 ± 0.50 c	2.50 ± 0.04 bc	20.42 ± 0.49 bc	10.52 ± 0.20 c
CK2	14.60 ± 0.21 c	2.30 ± 0.12 c	17.92 ± 2.57 c	10.58 ± 0.24 c
M2	26.20 ± 0.04 b	1.91 ± 0.01 d	18.06 ± 1.60 c	16.08 ± 2.02 a
MS2	25.61 ± 1.85 b	1.65 ± 0.02 d	24.92 ± 1.11 ab	16.08 ± 0.22 a
S2	13.51 ± 1.35 c	3.56 ± 0.24 a	20.42 ± 3.68 bc	14.15 ± 0.29 ab

For every treatment with three replicates, one-way variance analysis (ANOVA) by LSD test ($p = 0.05$) was performed using DPS 7.5 Statistics. Various letters displaying the significant shift among various treatments. CK1 refers to the control soil without amendment, M1 represents goat manure-amended soil, while MS1 represents goat manure + sugarcane straw-amended soil and S1 sugarcane straw-amended soil of the first phase (after 50 days). Similarly, CK2 refers to the control soil without amendment, M2 represents goat manure-amended soil, while MS2 represents goat manure + sugarcane straw-amended soil, and S2 represents sugarcane straw-amended soil of the second phase (after 100 days).

3.3. Meta-Genomic Analysis Based on 16S rDNA of All Soils

According to analysis of rarefaction, OTUs for the 16S rRNA plateaued after 45,000 sequences through 97% resemblance (Figure 1). This indicated that in order to capture the richness and diversity in organic-amended soil samples, sequencing gravity was enough. The HTS analyzed a total of 1,693,676 (mean: 70,570) number of reads. Bacterial relative abundance was observed by phylum, including the genus level, to inspect the response of S and the M amendment on bacterial composition with different time periods. Thirty-five various phyla were noticed in all soils in two phases (after 50 days and 100 days). However, only *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Firmicutes*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia*, *Actinobacteria*, and *Lagnavibacteriae* were detected in all samples which represent 90–96% of the bacterial sequences (Figure 2a). In the first phase (after 50 days) the composition of the microbial community in the control soil (CK1) without amendment was dominated by *Proteobacteria* (*H16*, *Shingomonas*, *Haliangium*, *Rhizomicrobium*), *Acidobacteria* (*Candidatus koribacter*, *Candidatus soliibacter*, *Bryobacter*), *Bacteroidetes* (*Bacteroids*), *Firmicutes*, *Verrucomicrobia* (*Opitutus*), *Actinobacteria*, *Planctomycetes* (*Candidatus nitrosotalea*), *Chloroflexi*, *Gemmatimonadetes*, and *Ignavibacteriae* with average relative abundances of 39.38%, 24.59%, 10.84%, 4.28%, 4.02%, 2.46%, 1.13%, 2%, 2%, and 0.15%, respectively (Figure 2a,b). The application of M shifted the microbial population in M1 (Figure 2). The dominant phyla in the M1 soil sample were *Proteobacteria* (*Unidentified Xanthomonadales*, *Altererythrobacter*, *Simiduia*, *Luteimonas* and *Azoarcus*), *Acidobacteria*, *Bacteroidetes* (*Chryseolinea*, *Pertrimonas* and *Ruminofilibacter*), *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia*, *Actinobacteria*, and *Ignavibacteriae* with average relative abundances of 55.13%, 3.39%, 24.54%, 3.07%, 0.78%, 0.94%, 0.94%, 2.7%, 1.58%, and 0.09%, respectively. The application of the MS-shifted composition of the microbial community in the MS2 soil sample was dominated by *Proteobacteria* (*Simiduia*, *Luteimonas*, *Azoarcus*, *Pseudomonas*, *Hydrogenophaga* and *Arenimonas*), *Bacteroidetes* (*Proteiniphilum*, *Petrimonas*, and *Ruminofilibacter*), *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia* (*Opitutus*), *Actinobacteria*, and *Ignavibacteriae*, with average relative abundances of 52.41%, 24.50%, 4.53%, 5.17%, 0.92%, 1.46%, 1.46%, 2.3%, 1.6%, and 0.13%, respectively, whereas only S application shifted the microbial community structure in the S1 soil sample, which was dominated by *Proteobacteria* (*Geobacter*, *Anaeromyxobacter*, *Sphingomonas*, *Rhizomicrobium*, *Haliangium* and *H16*), *Acidobacteria* (*Candidatus koribacter*, *Candidatus soliibacter* and *Bryobacter*), *Bacteroidetes* (*Flavisoilbacter*), *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Ignavibacteriae*, *Verrucomicrobia* (*Opitutus*), *Actinobacteria*, and *Gemmatimonadetes* which accounted for average relative abundances of 55.10%, 13.86%, 10.41%, 3.59%, 0.69%, 2.3%,0.51%, 3.18%, 2.5%, and 2.3%, respectively.

Similarly, relative abundances of bacterial taxa were examined in the same soil samples in the second phase (after 100 days); the dominant bacterial communities in control soil (CK2) were *Proteobacteria* (*Rhizomicrobium*, *Dyella*, *Haliangium*, *H16*, *Acidibacter* and *Denitratisoma*), *Acidobacteria* (*Candidatus koribacter*, *Bryobacter*, and *Candidatus solibacter*), *Bacteroidetes* (*Bacteroides*), *Planctomycetes* (*SM1A02*, *Candidatus jettenia*) and *firmicutes* (*Enterococcus*), *Chloroflexi* (*unidentified_SJA−28*), *Ignavibacteriae* (*Ignavibacterium*), *Actinobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia* with average relative abundances of 35%, 22.7%, 8.1%, 5%, 4.4%, 3.7%, 1.9%, 1.3%, 3.7%, and 2.9%, respectively. In the M-containing soil (M2), *Proteobacteria* (*unidentified Xanthomonadales*, *Altererythrobacter*, *Acidibacter*, *Pseudomonas*, *Arenimonas*, *Denitratisoma* and *Haliangium*), *Acidobacteria* (*Paludibaculum*), *Bacteroidetes* (*Chryseolinea*), *Planctomycetes* (*SM1A02* and *Candidatus jettenia*), *Ignavibacteriae* (*Ignavibacterium*), *Chloroflexi* (*unidentified SJA−28*), *firmicutes*, *Verrucomicrobia* (*Opitutus*), *Actinobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia* were dominant with average relative abundances of 50%, 7%, 11%, 6.2%, 1.5%, 3.9%, 2.2%, 2.4%, 2.2%, and 3.9%, respectively (Figure 2a,b).

Application of MS shifted the composition of the microbial community in the MS2 soil sample, which was dominated by *Proteobacteria* (*Acidibacter*, *Denitratisoma*, *unidentified Xanthomonadales*, *Altererythrobacter*, *Pseudomonas*, *Hydrogenophaga*), *Bacteroidetes*, *Acidobacteria* (*Paludibaculum*), *Planctomycetes* (*SM1A02* and *Candidatus_Jettenia*), *Firmicutes*, *Chlorobi* (*unidentified SJA−28*), *Ignavibacteriae*

(*Ignavibacterium*), *Verrucomicrobia* (*Opitutus*), *Actinobacteria*, and *Gemmatimonadetes* with average relative abundances of 51%, 9.6%, 8.5%, 7.6%, 1.7%, 4.3%, 1.8%, 2.7%, 1.3%, and 4.3%, respectively. Likewise, the dominant bacterial communities in S-amended soil (S2) in the second phase were *Proteobacteria* (*Geobacter*, *Anaeromyxobacter*, *Sphingomonas*, *Rhizomicrobium*, *H16*, *Acidibacter*, *Denitratisoma*, *Haliangium* and *Dyella*), *Acidobacteria* (*Candidatus koribacter*, *Bryobacter* and *Candidatus solibacter*), *Bacteroidetes*, *Firmicutes*, *Planctomycetes* (*Candidatus jettenia*), *Ignavibacteriae (Ignavibacterium)* and *Chlorobi* (*unidentified SJA−28*) *Verrucomicrobia*, *Actinobacteria*, and *Gemmatimonadetes* with average relative abundances of 50.2%, 18%, 6.2%, 4.3%, 3.1%, 1.77%, 2.60%, 2.44%, 2.32%, and 2.6%, respectively (Figure 2a,b).

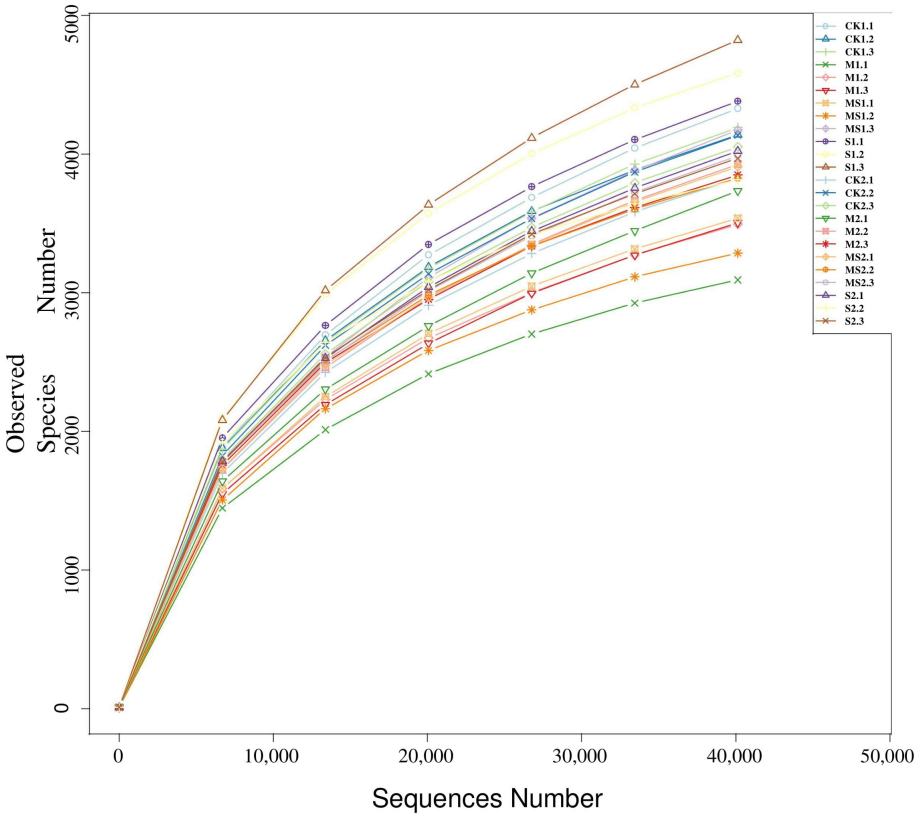
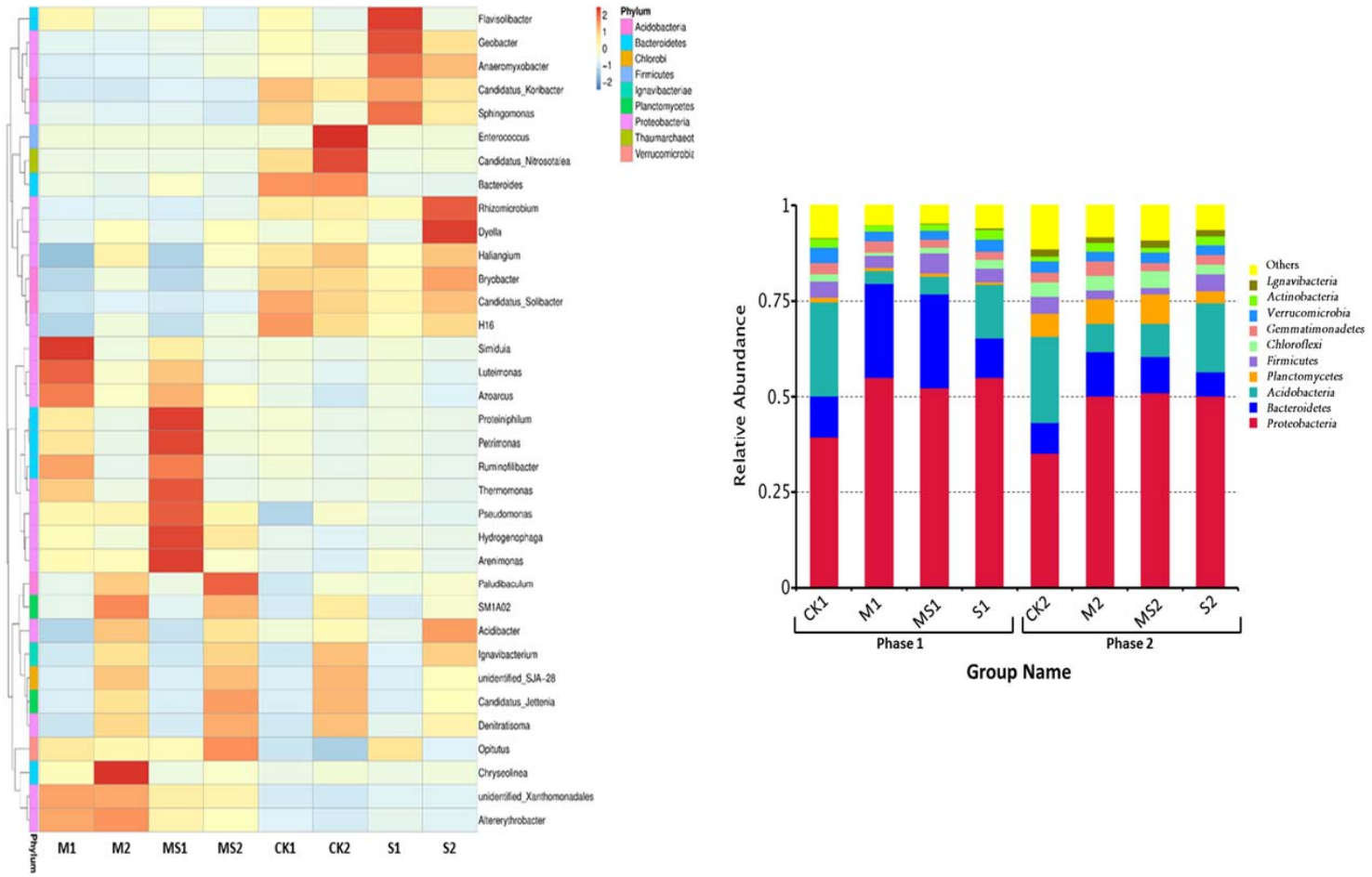


Figure 1. Rarefaction curve established on 97% similarity. CK1.1, CK1.2, and CK1.3 refer to control soil; M1.1, M1.2, and M1.3 represent the goat manure-amended soil; MS1.1, MS1.2, and MS1.3 represent the sugarcane straw and goat manure-amended soil; and S1.1, S1.2, and S1.3 represent the sugarcane straw amended soil in the first phase, respectively. Similarly, CK2.1, CK2.2, and CK2.3 refer to control soil; M2.1, M2.2, and M2.3 represent the goat manure-amended soil; MS2.1, MS2.2, and MS2.3 represent the sugarcane straw and goat manure-amended soil; and S2.1, S2.2, and S2.3 represent the sugarcane straw-amended soil in the first phase, respectively.



(**a**) (**b**)

Figure 2. (**a**) Dispersal of the 35 highly dominant genera in a heat map. (**b**) Dispersal of top 10 highly dominant phyla in control soil (CK1), goat manure-amended soil (M1), goat manure and sugarcane-amended soil (MS1), and sugarcane-amended soil (S1) in the first phase. Similarly, dispersal of highly dominant genera and phyla in control soil (CK2), goat manure-amended soil (M2), goat manure and sugarcane-amended soil (MS2), and sugarcane-amended soil (S2) in the second phase.

3.4. Overlying and Separate Bacterial Groups across All Soil Sample with Different Time

ACE and Chao1 estimation demonstrated that soil amended with M and MS (M1, MS1) showed lower bacterial community richness as compared to control and all other amended soils (CK1, S1), in the first phase, respectively (Figure 3a,b). However, during the second phase, M-amended (M2) soil showed higher bacterial community richness than all other amended and control soils (S2, MS2, and CK2), respectively, while diversity of Simpson including Shannon (SSD) indices declined in S- and M-amended soils (M1 and MS1) as compared to control (CK1) and S-amended soil (S1) during the first phase. Likewise, a significant decrease in the M2 and MS2, as compared to control soil (CK2) and S-amended soil (S2) during the second phase was recorded. From a diversity comparison among all amended soils in both phases, the SSD decreased significantly in M1, M2, MS1 and MS2, however, no significant differences were observed for S1, S2 across all the samples (Figure 3c,d). Both weighted (WT) on the basis of taxa abundance and un-weighted (UWT) based on sensitive to rare taxa UniFrac distance matrices were used to estimate the β-diversity among soil samples. From the WT UniFrac principal coordinate analysis (PCoA) during both phases, soils displayed that bacterial populations in control (CK1, CK2) and (S1, S2) were distinguished from (MS1, MS2) and (M1, M2). Clustering of M1, M2, and MS1 and MS2 was observed along axis-1, while the clustering of S1 and S2 was along axis-2. Furthermore, UWT UniFrac PCoA analysis demonstrated that bacterial populations in the CK1 and S1 were also differentiated from M1 and MS1, and their clustering was observed towards axis-1 in both phases while clustering of M2, S2, and MS2 was along axis-2 (Figure 4a,b).

The difference between the structure of bacterial communities among various samples was shown via the UWT pair group method with arithmetic mean clustering analysis (UPGMA) (Figure 4c). In the first phase, WT and UWT UniFrac distances among CK1, M1, CK1, MS1 CK1, and S1 were 0.424, 0.414, 0.374, 0.430, 0.219, and 0.411, respectively, whereas, in the second phase, distances among CK2, M2, CK2, MS2, CK2, and S2 were 0.279, 0.416, 0.263, 0.408, 0.210, and 0.373, respectively. In comparison between both phases, distances among CK1, CK2 M1, M2, MS1, MS2, S1, and S2 were 0.184, 0.433, 0.305, 0.469, 0.311, 0.487, 0.192 and 0.466 respectively. Comparing CK (CK1, CK2) with all other S-, M-, and MS-amended soils (M1, M2, MS1, MS2, and S1, S2), the WT and UWT UniFrac distances increased in M1, M2 and MS1, MS2 during both phases (Figure 4d).

3.5. Correlation among Bacterial Groups and Soil Variables

Significant correlation was observed between the soil pH, C:N, NO_3^-, TC, TN, AN, TP, AP, AK, and NH_4^+ with soil bacterial communities via Mantel test (MT) analyses (Table 3). The results clarified that the soil TC, TN, AN, TP, AP, AK, and NH_4^+ have a strong influence upon various bacterial communities. Distance-based redundancy analysis (dbRDA) plots showed a distinct grouping of bacterial populations from various treatments. In addition, soil pH, C:N, NO_3^-, TC, TN, AN, TP, AP, AK, and NH_4^+ had a positive and significant relationship with dbRDA axis-1 (Figure 5a,b). Furthermore, *Proteobacteria* displayed positive and significant correlation, whereas *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* demonstrated significant and negative interaction with C:N, pH, NO_3^-, TC, TN, AN, TP, AP, AK, and NH_4^+ (Figure 5a). The genera *Pseudomonas*, *Hydrogenophaga*, and *Arenimonas* had significant and positive interaction, although *Geobacter*, *Sphingomonas*, and *Candidatus soliibacter* had a negative and significant relationship with NO_3^-, pH, C:N, TC, TN, AN, TP, AP, AK, and NH_4^+ (Figure 5b).

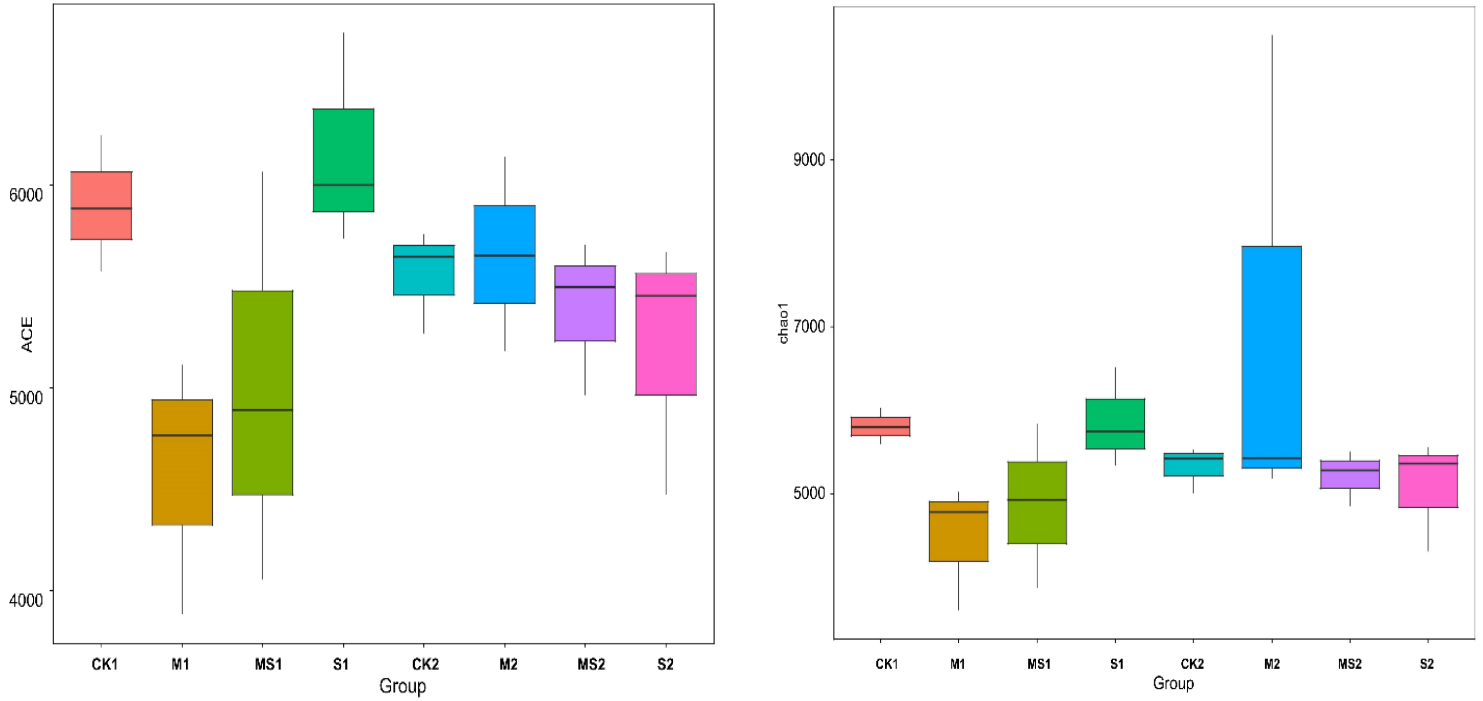


Figure 3. *Cont.*

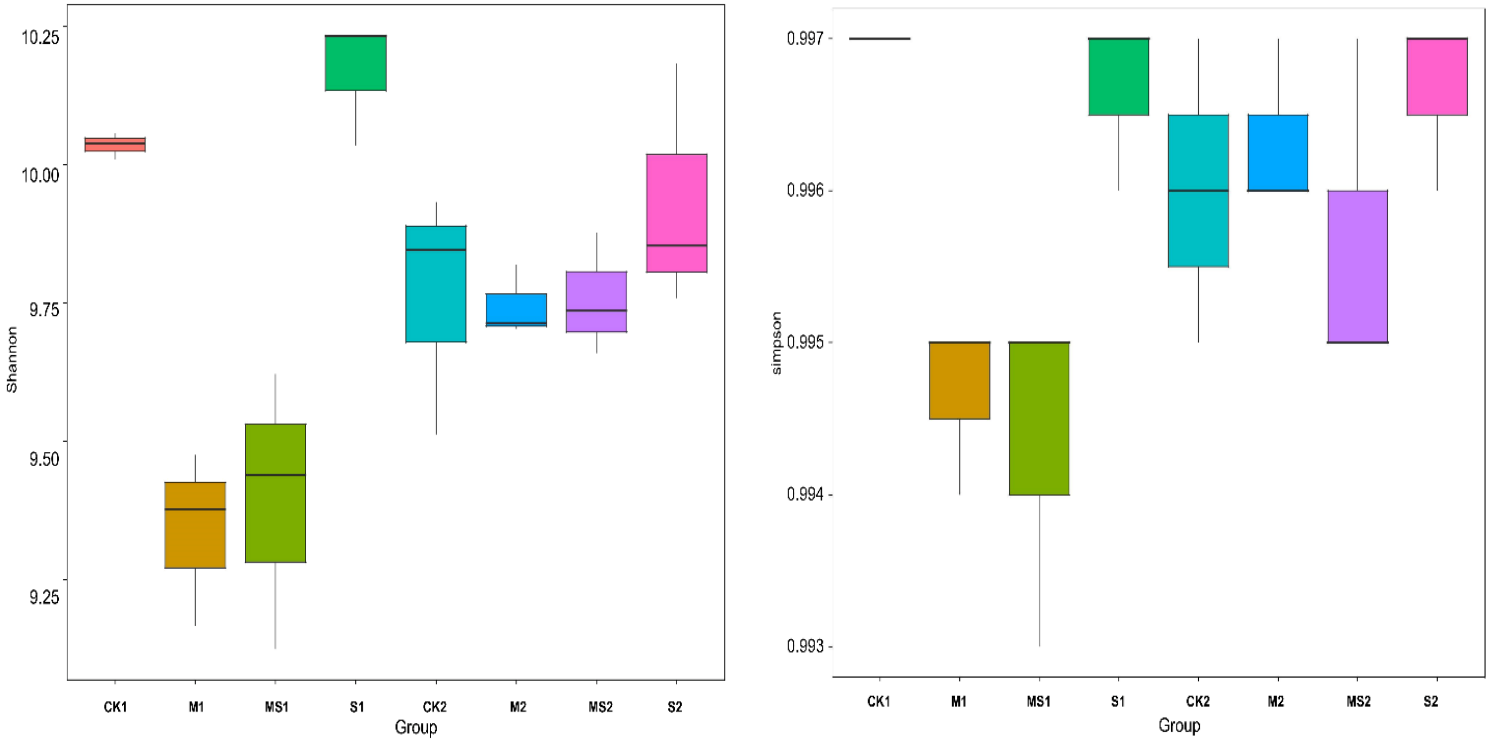


Figure 3. α-Diversity indices containing calculations of (**a**) ACE; (**b**) Chao1; (**c**) Shannon; and (**d**) Simpson in control soil (CK1), goat manure-amended soil (M1), goat manure and sugarcane straw-amended soil (MS1), sugarcane straw-amended soil (S1) in the first phase. Similarly, in control soil (CK2), goat manure-amended soil (M2), goat manure and sugarcan-amended soil (MS2), sugarcane-amended soil (S2) in the second phase.

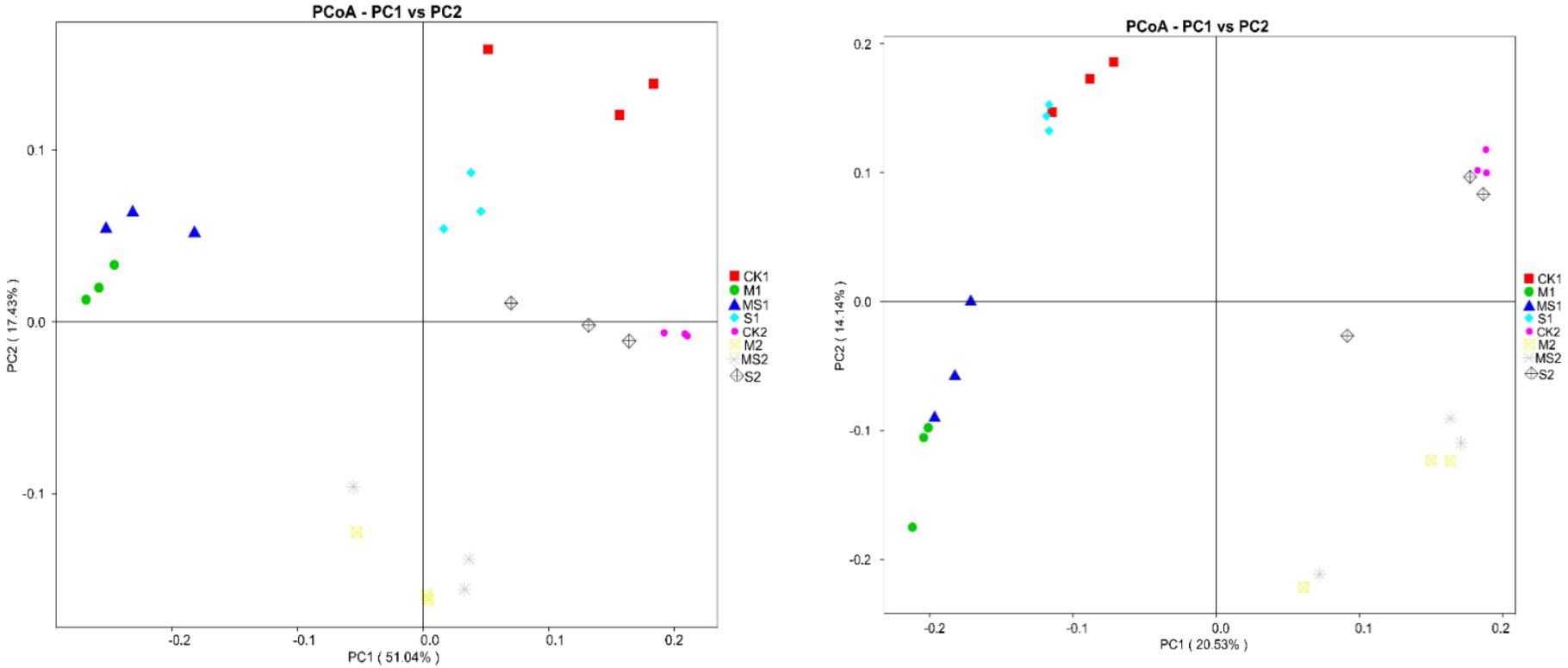


Figure 4. *Cont.*

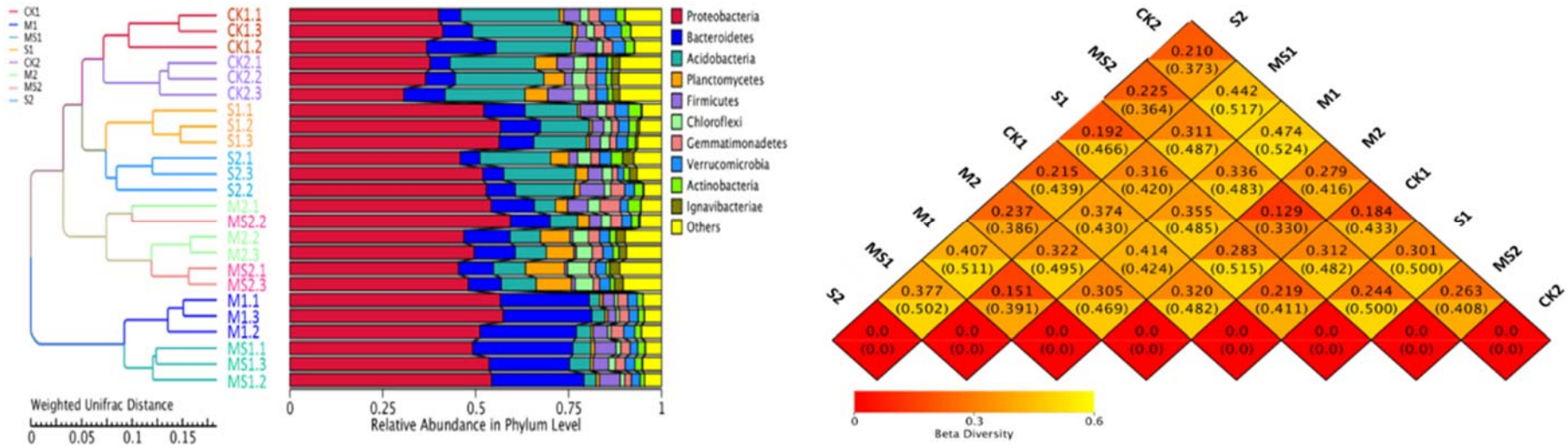
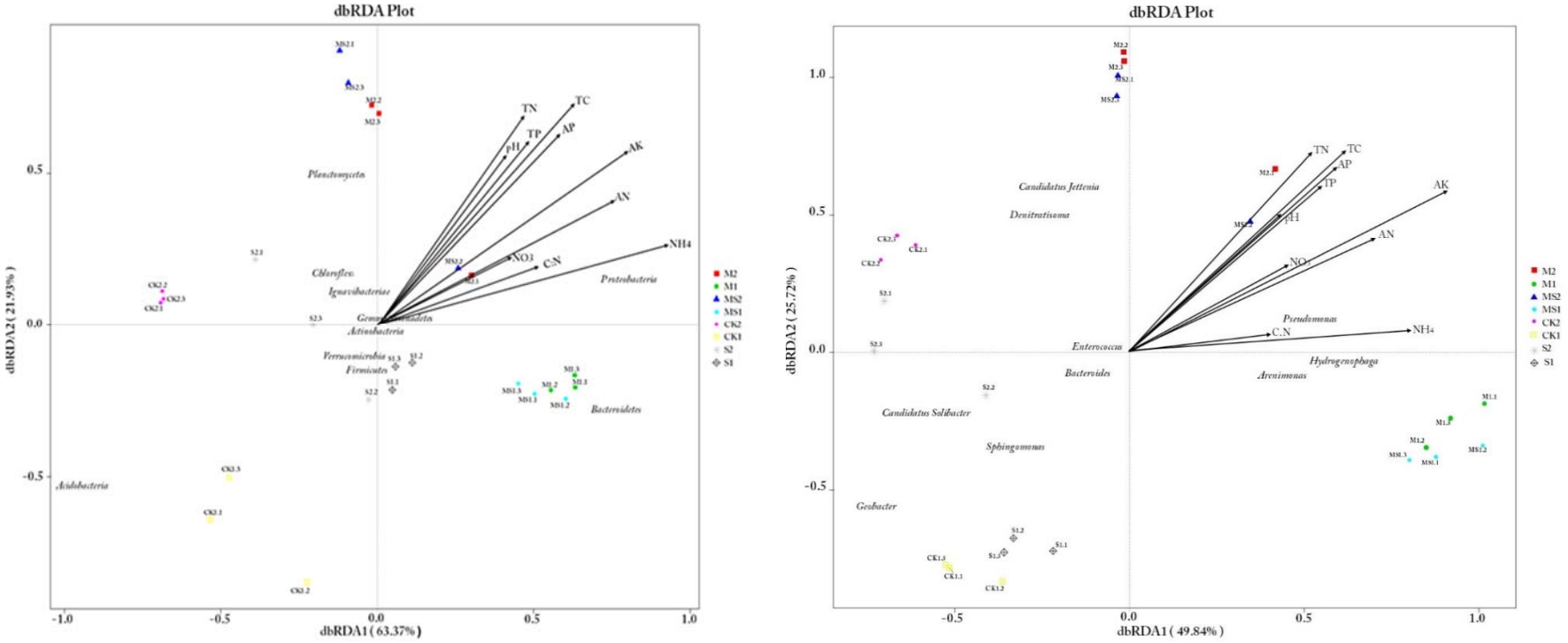


Figure 4. Biplot ordination of (**a**) principal coordinate analysis (PCoA) of weighted Unifrac and (**b**) unweighted UniFrac distances among various samples; (**c**) Based on weighted UniFrac distances UPGMA clustering analysis shows the relative abundance of the utmost abundant bacterial phylum in various amendments at different times; (**d**) Heat map of β-diversity based on unweighted (UUF) UniFrac and weighted (WUF) distances. Lower and upper corner values indicate the UUF and WUF distances.



(**a**) (**b**)

Figure 5. Distance-based redundancy analysis (dbRDA) relating phylum (**a**) and genera (**b**) to selected soil characteristics which are shown by the arrows. The lengths of these arrows shows the relative significance of measured variables, while the angle between the arrows and the axis reflects the degree to which they are correlated.

Table 3. Correlation coefficient (r) among the soil bacterial community and variables revealed by the Mantel test (p-value = 0.01).

Environmental Variable	r-Value
pH + TC	0.5407
pH + C:N	0.2881
pH + NH_4^+	0.5266
pH + TN	0.5005
pH + NO_3^-	0.414
AP + TP + AK	0.5898
NO_3^- + NH_4^+	0.5971
TN + TC	0.6033
NO_3^- + NH_4^+ + TN + AN	0.6564

4. Discussion

Although utilization of organic fertilizers and other agricultural amendments in crop cultivation have been admitted to be an encouraging management method to recover SF, however, there were concerns regarding which organic matter as a soil amendment and time are more effective prior to crop sowing. Therefore, the current research is helpful to distinguish the outcomes of goat manure (M), sugarcane straw (S), or goat manure + sugarcane straw (MS) on SF, with a focus on the soil bacterial communities' structures with different periods. Organic fertilizer application in soil increases or stabilizes the soil pH [4,33,34]. However, the findings of this study indicated that M1, MS1, M2, and MS2 amendments were more effective in increasing the soil pH, which is consistent with the previous results of Han et al. [35] and Das et al. [9]. Adding various organic supplements to the soil enhances the accumulation of basic macronutrients, especially N, K, and P, as well as soil organic matter [16]. Correspondingly, our results suggested an improvement of various soil properties in M1, MS1, M2, and MS2 amendments, such as TC, AP, AK, TN, AN, NH_4^+, NO_3^-, compared to CK1, CK2, and S1, S2 with the same amendment rate in both phases (Table 1). On the other hand, there was a decrease in species richness of M- and S-amended soil (M1, M2, MS1, and MS2) compared to the S-amended (S1, S2) soil in both phases. This may be due to a higher population of oligotrophs (organisms that frequently exist in an environment with low levels of nutrients) in CK and S soils [36]. Furthermore, we believe that the high nutrient availability reduced the species richness in M1-, M2-, MS1-, and MS2-amended soil and a small quantity of nutrients stimulated their growth in CK1, CK2, as well as in S1, S2, in both phases. [9,16]. It is assumed that the increase in beneficial bacterial composition and biomass, including greater availability of C, P, and N in the M1-, M2-, MS1-, and MS2-amended soils, will improve the fertility of the soil.

SEAs are considered sensitive indicators for SF as they are essential to catalyze the key biochemical reactions, such as the degradation of organic nutrients and nutrient cycling [23,37,38]. The evidence presented in this section suggests that M- and MS (M1, M2, MS1, MS2)-amended soil enhanced the N and C cycling enzymes as compared to S-amended soil (S1, S2) in both phases (Table 2). Additionally, a greater turnover amount of C and N in M1-, M2-, MS1-, and MS2-amended soils were recorded as compared to S1- and S2-amended soils. This may be due to the enhanced population of autochthonous microorganisms in M1, MS1, M2, and MS2 soils compared to CK1, CK2, and S1, S2. The improved activities for N, P, and C cycling enzymes have been reported as a consequence of the application of M and S in soils [23,39]. Interestingly, a decline of P-cycling enzyme activity was examined in both M- and MS (M1, MS1, M2, and MS2)-amended soils compared to S-amended soil (S1, S2) and control soils in both phases (Table 2). Previous findings correspond with the reduction in phosphatase enzyme activity via the application of organic manure in agricultural soils. The increase in P demand for the soil microorganisms may enhance the enzymatic acquisition of P in livestock manure-amended soils [9,40].

Researchers have investigated the impact of agriculture management techniques on SEAs, including the microbial composition in soil. Moreover, microbial diversity assessment has become

more authentic in the presence of HTS. Identification of key species that influence ecosystem functions is one of the excellent features of the HTS approach [41]. Our results presented a higher abundance of *Proteobacteria* in all organic amendments compared to CK in both phases, which is in agreement with the previous results [9,10,16]. *Proteobacteria* are known as copiotrophic microorganisms, which exist in an ecosystem with high nutrients. [36,42]. *Bacteroidetes* exhibited much higher abundance under M1, MS1, M2, and MS2 as compared to CK1, CK2, S1, and S2. Notably, in both phases, the relative abundance of *Bacteroidetes* was much lower in CK1-, CK2-, S1-, and S2-amended soils, respectively, which is supported by the previous findings [9]. *Bacteroidetes* was much more abundant in M-amended soil because it is one of the plentiful bacterial phyla of livestock manure [43], recognized as a crop straw decomposer, and survives in the agricultural land [44,45]. *Acidobacteria* exhibited low abundance in M-, MS-, and S-amended soil compared to CK in both phases. By the application of S and M, a decrease in *Acidobacteria* was recorded, which is consistent with the previous results [9,16]. Additionally, HTS-based investigations exposed that the abundance of *Acidobacteria* is reduced significantly by the amendment of organic fertilizer [40,46,47]. *Actinobacteria* decompose the organochemicals [48] and were more abundant in CK1 and MS2 [10], as well as S soil (S1, S2). The abundance of these bacteria was greater in S-amended soil due to their strength to decompose of soil organic matter (e.g., chitin and cellulose) [49]. These findings corroborate the previous results as well [9]. The highest abundance of *Firmicutes* was observed in the MS1- and S2-amended soils. These bacteria thrive in soil with high C availability [50] and are well-known to degrade several complex organic materials [51,52]. Similarly, Wu and He [53] explained that *Firmicutes* can play a role in lignin depolymerization. Moreover, they can take part in rice straw compost [54,55], and decaying wood [56]. The phyla *Verrucomicrobia* and *Gemmatimonadetes* were highly abundant in the S-, compared to the M- and MS-amended soils in the first phase, whereas MS was most dominant, as compared to M- and S-amended soil in the second phase, respectively. It is difficult to conclude their possible function in the ecosystem owing to the absence of cultured representatives of these phyla. *Planctomycetes* are anaerobic ammonium oxidizing bacterium which can contribute to N and C cycling in the soil [57]. A higher abundance of these bacteria in M- and MS-containing soil is due to a large amount of N and C sources than CK- and S-amended soil in the second phase [23].

We found some dominant genera which have a vital role in plant promotion, as well as in lignocellulose degradation. M-amended soil was dominated by *Simiduia*, *Luteimonas*, *Azoarcus*, *Opitutus*, *unidentified Xanthomonadales*, and *Altererythrobacter* genera as compared to CK-, MS-, and S-amended soil in the first phase. Similarly, MS-amended soil was dominated by *Proteiniphilum*, *Petrimonas*, *Ruminofilibacter*, *Thermomonas*, *Pseudomonas*, *Hydrogenophaga*, and *Arenimonas* genera as compared to CK, M, and S in the first phase while, in the second phase, dominant genera (*SM1A02*, *Chryseolinea*, *unidentified Xanthomonadales*, *Altererythrobacter*) were found in the M-amended soil as compared to CK-, MS-, and S-amended soils. Likewise, MS-amended soil was dominated by *Paludibaculum*, *Candidatus jettenia*, and *Opitutus* as compared to CK-, M-, and S-amended soil in the second phase. Evident from multiple studies, most of these dominated genera promote plant growth (Supplementary Table S2) [1,58]

Soil characteristics are the most significant modulators of the bacterial community composition [4,59]. In a comparison of both phases, C:N and NO_3^- were minor, while AK, AN, and NH_4^+ were major factors affecting the bacterial community structure under M- and MS-amended soils in the first phase. In the second phase, soil pH was minor, while TC, TN, TP, and AP were major factors affecting the bacterial community structure under M2- and MS2-amended soil. Overall, our observation revealed that M1, M2, MS1, and MS2 amendment not only increased the major nutrients, especially N, C, and P, and increased soil pH, but also stimulated the positive bacterial genera which have important functions in the plant growth promotion and decomposition of complex organic matters in both phases. The changes can be effective to improve SF under M1, M2, MS1, and MS2 amendment in the clay-loamy soil after 50 days, as well as 100 days. We further disclosed the effect of M and MS amendment, as well as the role of time on SF, soil biochemical properties, and soil microbial diversity as compared to S amendment.

These observations have increased our knowledge of what type and time of organic amendment are suitable before crop sowing.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/10/7/2361/s1, Table S1: Statistics of Operational Taxonomic Units (OUT), Table S2: Dominant genus possible role on soil fertility and plant growth.

Author Contributions: All authors contributed to the intellectual input and provided assistance to this study and manuscript preparation. M.T., W.I., C.Z., and Y.A. designed the research and conducted the experiments. M.T., W.I., Q.P., Y.L., and M.W. analyzed the data. M.T. and W.I. wrote the manuscript. H.Z., W.L., and S.L. reviewed the manuscript. H.Z., supervised the work and approved the manuscript for publication.

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