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Restoration of Long-Term Monoculture Degraded Tea Orchard by Green and Goat Manures Applications System

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Abstract: Tea is an economic shrubby plant in tropical and subtropical regions of the world. To obtain high yield in tea cultivation, chemical fertilizer application rates have generally been used. However, a large quantity of chemical fertilizer application in a long-term continuously ratooned and monoculture tea orchard can inevitably lead to soil acidification and a decline in fertility. Therefore, the restoration of soil fertility and the sustainable development of tea planting by organic ways are critical for the tea industry. In this study, field trials were conducted in the tea orchard that was continuously ratooned and mono-cultured for 20 years. Nitrogen fertilizer (NF), Laredo soybeans green manure (LF), and goat manure (GM) treatments were applied to restore optimum acidity, soil fertility, microbial activity, and the community structure of a long-term continuously monoculture tea orchard. This paper investigated that the pH value was increased from 4.23 to 4.32 in GM and LF, respectively. Similarly, the content of exchangeable acidity (EA) was decreased by 1.21 and 1.46 cmol·kg^{-1} in GM and LF, respectively. Available nutrient results indicated that the content of NH_4^+-N was increased by 3.96, 4.38, NO_3^--N by 1.07, 2.16, AP by 3.46, 6.86, AK by 0.26, 0.3 mg kg^{-1} in GM and LF treatments, respectively. Enzyme analysis revealed that the activity of urease and sucrase was promoted by 7.98 mg·g^{-1}·24 h^{-1} and 6.77 mg·g^{-1}·24 h^{-1}, respectively, in LF treatment. Likewise, the activity of acid phosphatase and polyphenol oxidase was sharply increased by 2.3 mg·g^{-1} h^{-1} and 63.07 mg·g^{-1} h^{-1} in LF treatments. Additionally, the activity of urease, sucrase, acidic phosphatase, polyphenol oxidase, and peroxidase were also significantly increased by applying GM treatments. Meanwhile, LF and GM treatments significantly improved soil microbial biomass as well as low weight organic acid content in degraded tea rhizosphere. Furthermore, high throughput sequence results illustrated that the relative abundance of *Rhizobiaceae* and *Bradyrhizobiaceae* families increased in LF and GM treatments, respectively, which are mostly a kind of nitrogen fixer and plant growth promoting bacteria. Taken together, the physiological traits of the new sprouts and the biochemical components of new tea leaves were also significantly improved by GM and LF treatments. From this study, it is concluded that LF and GM are good agriculture management practices, which promote plant growth, yield, and nutrient availability by maintaining and improving pH, enhancing available nutrients status, improving the secretion of low molecular weight organic acids, and balancing the microbial community structure in the long-term mono-cultured tea orchard.

Keywords: rhizosphere restoration; nitrogen fertilizer; soil microbial community structure; organic farming; monoculture tea plantation

1. Introduction

Camellia sinensis (L.) O. Kuntze is one of the most economic beverage plants in the world. According to 2015 statistics, the total tea cultivated area was 287.7 million hectares and the production reached 227.8 million ton, which makes China the largest producer in the world [1]. The application of nitrogen fertilizer is an effective mean for obtaining high yield in tea cultivation. In modern agriculture, large quantities of chemical fertilizers are applied to tea orchards annually to gain high economic benefits. However, nitrogen fertilizer in the rhizosphere soil of tea plant significantly reduced soil pH, while extractable Al levels grew [2,3]. The pH of long-term tea plantation decreased due to protons released from ammonium fertilizers preferentially for the growth [4]. Moreover, under the tea plantation, acidification took place within a soil depth of 70 cm, with the maximum difference in pH in the upper 17 cm (ΔpH = 2.80) [5]. Furthermore, studies showed that acidification causes leaching of K^+, Na^+, Ca^{2+}, Mg^{2+}, and NH_4^+ in soil [6,7]. Subsequently, the level of available nutrients (NPK) are generally low and total (NPK) concentration were higher [8]. Likewise, most of the inorganic fertilizers were fixed to free iron and aluminum oxide in the soil by weathering and leaching, which cause nutrient sequestration in long-term tea plantations [9,10].

Soil enzymes are responsible for the decomposition of animal, plant and microbial residues, and the biological function of soil fertility formation. It is believed that soil enzyme activity can be used as an important indicator of soil fertility evaluation [11]. The previous study [12] suggested that the increase of the tea mono-cropping period affected the activity of soil enzymes in tea orchards. Additionally, soil microbial biomass is important in the transformation and utilization of soil nutrients and in the degradation of organic matter and pollutants [13,14]. Nioh et al. [15] concluded that excessive application of nitrogen fertilizer in the tea orchard reduced soil microbial biomass, and soil microbial biomass carbon decreased by 83% when the application rate of the nitrogen fertilizer increased from 400 to 1200 kg hm^{-2}. In addition, the activity of soil microbial metabolism and the stability of microbial community structure are disturbed by changing the tea rhizosphere situation [16,17]. Consequently, excessive use of chemical fertilizers for a long time in the tea orchard disturbs absorption and utilization of nutrients in tea rhizosphere, which impact the yield and quality of tea. In an acidic environment, the species and quantity of microorganisms are less, and their activities are reduced, while some of the microbial enzymes are inactivated. The yield of tea was directly affected by the absorption of nutrients from the roots to the aboveground leaves, which resulted in the lower economic efficiency of tea [18].

On the other hand, applying legume straws may ameliorate soil acidity and Al toxicity in acid tea soils by releasing the base cation and increasing the base cation saturation of the soil [19]. The application of organic fertilizers is rich in soil microbial biomass-C and has a significant effect on improving its content in tea plants [20]. Thus, soil microbial biomass carbon, nitrogen, and phosphorus contents increased significantly under the influence of straw mulching and organic fertilizer treatment [21]. The Tayyab et al. [22] study showed that soil amended with goat manure (GM) and goat manure plus straw (MS) not only significantly enhanced nutrient availability, including C, P, and N, soil pH, and soil enzyme activity for C and N cycles. Additionally, the increase in nutrient availability was greater in GM-amended and MS-amended. Similarly, more recent evidence has suggested that the addition of litter has a significant effect on the development of soil microbiota, which leads toward higher nutrient levels in soil and microbial biomass [23]. In view of the above problems, we are developing various comprehensive cultivation techniques for organic farming in degraded tea gardens. It aims to restore the ecology of the degraded tea garden, repair the agricultural habitat and ecological function, reduce the frequency of pests and diseases, and improve the yield and quality of tea. Based on the previous research, green manure of the high quality leguminous

plants were used in a long-term monoculture degraded tea garden. First, legume grass was planted in a monoculture-degraded tea garden in the spring and legume forage planting was done in the winter. The primary objectives of the study were (a) to determine the physio-chemical properties of the long-term monoculture degraded tea orchard in GM and LF amended soil, (b) to examine the change in acidity of a long-term degraded tea orchard, (c) to study the shift of bacterial population after the amendment of GF and LF treatment, and (d) to investigate the relationships among bacterial composition and soil physio-chemical properties.

2. Material and Methods

2.1. Test Area Overview

Field trials were conducted in the experimental station at the Taozhou tea garden (longitude 117°45′, latitude 24°21′) Anxi County Fujian province from 2013–2016. This region lies in a subtropical monsoon climate, with an average annual temperature of 16~18 °C, annual rainfall was about 1800 mm, and the frost-free period was about 260 days. In Anxi County, the soil types were different along with the altitude: yellow soil occur above 880 m, yellow red soil at 700–800 m, brick red soil at 300–700 m, and latosolic red soil below 300 m [24]. The soil type in the test area was brick red soil. The elevation of the test tea field was 700 m and the slope was 50 to 60°. The rhizosphere soil of 20-year-old tea orchard under different fertilization treatments was used as the research object.

2.2. Experimental Design

From April 2013 to May 2016, the tea garden was intercropped with leguminous crops i.e., Egyptian clover, winter pea, Hairy vetch, alfalfa, Laredo soybean in the tea orchard for three consecutive years (Figure S1). The Laredo soybean was selected as green manure for fertility restoration, due to high adaptability, more NPK contents, and highly palatable for goat as compared with other legume plants (Table S1, Figure S1). One part of Laredo soybean was used in the field as green manure (LF) while another part of the Laredo soybean was fed to goats. Later, the goats' manure (GM) were collected, dried and grinded into powder, and were used to determine the relevant nutrient content. In the experimental field, we used four treatments in a monoculture 20-year-old test field CK (without fertilization), NF (nitrogen fertilizer), GM, and LF for three consecutive years. Each treatment area was 16 m^2 (4 m × 4 m). Each row from the sides of the tea tree was 15 cm. Fertilization timing was: 12 December 2013, 15 June 2014, 20 December 2014, 10 June 2015, and 24 December 2015.

2.3. Sample Collection

Soil and fresh leaves sampling was carried out on 10 May 2016. Samples were taken randomly from each treatment area. The soil sampling depth was about 15 to 30 cm. The ice box was used to bring the collected soil and leaf samples back to the laboratory in plastic bags. In order to reduce the error caused by spatial heterogeneity, five random sampling points were used for each replication, and three replications were obtained from each treatment area. Soil samples were sieved from 2 mm to remove the flora and fauna in the soil. After sieving, a part of the soil was stored at −20 °C for soil microbial and enzyme analysis and other parts of the soil were stored for essential nutrient determination.

2.4. Analysis of Essential Nutrients (NPK) and Soil Enzymatic Activities

The total and available essential nutrients (N, P, and K) were measured, according to Reference [25]. Soil urease [EC 3.5.1.5] activity was determined by incubating 5 g soil with 30 mL of extracting solution at 37 °C for 24 h. The formation of ammonium was measured spectrophotometrically at 578 nm [26]. Soil sucrase [EC 3.2.1.26] activity was determined by incubating 5 g soil with 15 mL of 8% sucrose solution at 37 °C for 24 h. The suspension reacted with 3, 5-dinitrosalicylic acid and absorbance was measured at 508 nm [26]. Acidic phosphatase [EC: 3.1.3.2] activity was determined based on a modified

method adopted by Reference [27]. Polyphenol oxidase [EC 1.10.3.1] and peroxidase activity were determined as described by Reference [28].

2.5. Analysis of Acidity and Salt Content of Rhizosphere Soil

Soil pH was determined using a glass electrode pH meter (1:2.5 soils to water suspension). Soil exchangeable acidity (EA) was measured by the KCl exchange-neutralization titration method. The cation exchange capacity (CEC) was determined by 1 mol. L^{-1} ammonium acetate ($CH_3CO_2NH_4$) saturating solution method [29]. The exchangeable aluminum (Al^{3+}) was extracted by 1 mol. L^{-1} KCl solution, 1:10 (v/v) soil/solution ratio, and was determined by titration of 25 mL KCl extract with 25 mmol. L^{-1} NaOH, using 1 g L^{-1} phenolphthalein as an indicator [30]. Soil organic carbon and soil organic matter was determined by the potassium dichromate volumetric method [31].

2.6. Analysis of Low Molecular Weight Organic Acids (LMWOA) in Rhizosphere Soils

Pharmaceutical Reagents

High performance liquid chromatography (HPLC) was used to identify and quantify five low molecular weight organic acid standards (oxalic acid, tartaric acid, malic acid, acetic acid, and citric acid). Standards, chromatographic grade Methanol, the ultra-pure water, excellent grade of pure phosphoric acid, disposable syringes (5 mL), water phase needle filter (aperture 0.45 μm), and 2 mL volume chromatographic bottle were purchased from Cayman Chemical (1180 E. Ellsworth Road, Ann Arbor, MI, USA).

2.7. Chromatographic Conditions

Chromatographic conditions of HPLC consisted of a system controller (Communications Bus Module, CBM-20A, Shimadzu, Japan), a degassing unit (DGU-20A3R), high pressure gradient elution liquid chromatography (UV/VIS Detector, SPD-20A 230V, Shimadzu), a column oven (LC-20 AD, Shimadzu, Japan), an auto sampler (Auto Sampler, SIL-20A 230V) (4.6mm × 150mm, 5 μm), and an Inertsil ODS-3 guard column (4.6mm × 20 mm, 5 μm). The flow rate of the mobile phase A liquid was 0.5% KH_2PO_4 (pH 2.5, about 1150 μl. L^{-1}). The mobile phase B liquid was pure methanol at a flow rate of 0.6 mL. min^{-1} (VA:VB = 98:2). The detection wavelength was 214 nm, the column temperature was 25 °C, the injection volume was 20 μl, and the running time was 50 min.

2.8. Preparation of Standards and their Standard Curve Production

Each standard was accurately weighed at 0.05 g with a small amount of ultra-pure water dissolved, filtered through 0.45 μm pore size filter, transferred to a 50-mL volumetric flask volume, concentrated to 1.0 g L^{-1} stock solution, and stored in a refrigerator at 4 °C. After this, 5 mL of each stock solution was placed in the same 50 mL volumetric flask and set to 50 mL with ultrapure water to make 100 μg mL^{-1} mixed standard solution, diluted 100 μg mL^{-1} solution into 50 μg mL^{-1} mixed standard, and then diluted 50 μg mL^{-1} standard solution to 10 μg mL^{-1} mixed standard, in order to obtain 1, 5, 10, 50, and 100 μg mL^{-1} series of mixed standard solution and the series of standard solution in the same chromatographic conditions (Figure S2). Each stock solution was diluted to 100 μg mL^{-1} standards, and the chromatograms were compared with 100 μg mL^{-1} standard chromatograms in the same chromatographic conditions. Five of the peaks were identified as low molecular weight organic acids (Figure S3).

2.9. Preparation and Determination of Rhizosphere Soil Sample Solution

The soil samples were centrifuged for 30 min at a speed of 14,000 rpm. After centrifugation, the collected centrifugates were filtered through a 0.45 mm filter (Millex-HV, Millipore) and the pH was then determined. Each 1 mL soil solution was filtered to the chromatographic vials for HPLC. The chromatographic results were obtained under the same chromatographic conditions as used for

standards, and the low molecular weight organic acid concentration of the samples was calculated by a standard curve obtained from the different concentration of standard of the respective substances.

2.10. Determination of Soil Microbial Biomass C (SMB-C) and P (SMB-P)

Wu et al. described soil microbial biomass C and P were determined by chloroform ($CHCl_3$) fumigation extraction methods [32]. Extractable C was calculated assuming that 1 mL 66 mM $K_2Cr_2O_7$ is equivalent to 1200 μg C and biomass C from the relationship Biomass C = 2.64 E_c, where E_c is the difference between C extracted from the fumigated and non-fumigated treatments, which are both expressed as μg g^{-1} oven dry soil.

2.11. Soil DNA Extraction

Soil DNA was extracted using the biofast soil genomic DNA extraction kit (Bio Flux, Hangzhou, China). Take 0.5 g soil, according to the kit instructions for DNA extraction. Furthermore, 2 μL of the soil sample DNA was subjected to 1% agarose gel electrophoresis, while DNA concentration and purity were measured with infinite M200PRO Tecan Monochromator. Each purified DNA sample was diluted to 1 ng μL^{-1} using sterile water prior to amplification.

2.12. PCR Amplification

Based on the selection of the sequencing region, the diluted genomic DNA was used as a template, and the specific primers with a barcode were used. A distinct V4 gene region of 16S rRNA was amplified using specific primer 515F-806R with barcodes [33]. All PCR reactions were conducted using 30 μL total reactions volume with 15 μL of Phusion® high-fidelity PCR master mix (New England Bio labs) containing ~10 ng templates DNA and 0.2 μM of each primer pair. The PCR condition was set to denature at 98 °C for 1 min, which was followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s with a final extension at 72 °C for 5 min. Then electrophoresis (using 2% agarose gel solution) was performed to verify the successful DNA amplification mixing PCR products with the same amount of 1X loading buffer (contained SYB green). Samples showed the main strip brightness, which ranged between 400 and 450 bp and was selected for further sequencing. PCR products were purified by using gene JET gel extraction kit (Thermo Scientific) prior to sequencing. Purified PCR products were sent to Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) for high throughput sequencing.

2.13. High Throughput Sequencing and their Bioinformatics Analysis

Sequencing libraries were created in Illumina using specialized NEB Next® Ultra™ DNA library prep kit ((New England Biolabs (Beijing) Ltd., Beijing, China)), according to the manufacturer's instructions, and index codes were added. The quality of the developed sequencing library was checked on both Agilent Bioanalyzer 2100 system and the Qubit @ 2.0 Fluorimeter (Thermo Scientific, Agilent, Santa Clara CA, USA). Then, at last, 250 bp/300 bp paired-end reads were generated on an Illumina MiSeq platform. A sequencing data processing analysis flow diagram is given (Figure S4). Raw sequences were classified, according to the specific barcode assigned to each sample, using Quantitative Insights Into Microbial Ecology (QIIME) (CO, USA) [34]. Paired-end reads were merged from the original DNA segments using FLASH (Baltimore, MD, USA) [35]. Paired-end reads were assigned to each sample, according to the unique barcodes attached with DNA fragments. Sequences were analyzed using UPARSE-OTU and UPARSE-OUT reference algorithms with UPARSE software package (CA, USA). Alpha (within samples) and beta diversity (among samples) were calculated using QIIME (CO, USA). The same Operational Taxonomic Units (OTUs) were assigned to the sequences with $\geq$97% in each sample. One representative sequence was selected for each OTU to annotate the taxonomic information of each representative sequence by using the RDP classifier. To measure the Alpha diversity within the sample, we rarified the Operational Taxonomic Units (OTUs) table and then four diversity matrices were calculated: Chao1 estimates the species abundance, and the

Observed Species, Simpson, and Shannon indices were used to determine the community diversity. Moreover, rarefaction curves were developed for each of these four indices. Abundance of each bacterial taxa, from phylum to species, was shown graphically using a Krona Chart. Beta diversity (among samples) was measured for both weighted and unweighted UniFrac distances using QIIME (Version1.7.0) (CO, USA). Principal Component Analysis (PCA) and Principal Coordinate Analysis (PCoA) were performed and visualized using R (Version 2.15.3) packages known as stat, WGCNA, and ggplot2 (Elegant graphics for data analysis, New York, NY, USA). In order to further excavate the community structure differences among the samples, T-test statistical analysis was used to analyze the species composition and the community structure were significantly different. At the same time, correlation analyses were carried out by using partial-Redundancy analysis (RDA) and triplots were generated using Canoco 5.

2.14. Growth Index and Yield Determination

SPAD-502 Plus was used to calculate the SPAD value of third leaf´s for chlorophyll content averaging 10 leaves in each test plot. The net photosynthetic rate (Pn) of third leaf average (ten 10 leaves) was measured by the American CID-301 portable CO_2 gas analyzer. The air temperature was 18 ± 0.2 °C, the light intensity was 1800 ± 30 μ mol (CO_2) m^{-2} s^{-1}, and the relative humidity was 65%. The concentration of CO_2 in the atmosphere was 330 ± 3 μL L^{-1}. The hundred buds fresh, dry weight and yield was also calculated in g ha^{-1}.

2.15. Determination of Quality Indicators of Tea

The tea leaf samples were picked from each treated area and were dried by using the oven. The total amount of free amino acids in tea leaves was determined by a ninhydrin colorimetric assay [36]. Quality parameters such as theanine (TNN), caffeine (CF) theophylline (TPY), and total polyphenols (TPP) of tea orchards of different treatments were determined by the method described in Reference [37]. Standard curve regression equation of different quality parameters such as TNN, CF, TPY, TPP and amino acids are shown in Table S2.

2.16. Statistical Analysis

ANOVA was implemented to analyze the soil properties and low molecular weight organic acids by using Satistix 8.1 software (2105 Miller Landing Rd Tallahassee FL 32312 USA). All soil enzyme activity and microbial biomass and microbial population abundance were tested for normal distribution. We used Tukey to test the differences between treatments.

3. Results

3.1. Available Nutrient Status in the Tea Rhizosphere Soil under Different Fertilizer Treatments

When applying different fertilizers for three consecutive years to monoculture a degraded tea orchard, the release of effective nutrients in tea rhizosphere was promoted by LF and GM treatments as well as by NF treatment. The content of NH_4^+-N was increased by 3.96, 4.38, NO_3^--N by 1.07, 2.16, AP by 3.46, 6.86, AK by 0.26, 0.3 mg kg^{-1} in GM and LF treatments, respectively. The exchangeable Ca^{2+} was increased by 1.91, 2.48 $cmol_c.kg^{-1}$ and exchangeable Mg^{2+} by 0.19, 0.11 $cmol_c.kg^{-1}$ in NF and GM treatments, respectively (Table 1). It means that legume forage-goat manure applications have the ability to maintain the fertility of tea rhizosphere soil as chemical fertilizers.

Table 1. Available nutrient status in the tea rhizosphere soil under different treatments.

Treatment	(NH_4^+-N)	(NO_3^--N)	(AP)	(AK)	(Soluble-Ca^{2+})	(Soluble-Mg^{2+})
	——————mg·kg^{-1}——————				——cmol$_c$·kg^{-1}——	
CK	1.17 ± 0.00 ^{b}	5.19 ± 0.12 ^{c}	24.67 ± 3.18 ^{c}	0.41 ± 0.14 ^{d}	1.71 ± 0.37 ^{c}	0.3 ± 0.09 ^{b}
NF	4.94 ± 0.87 ^{a}	8.46 ± 0.67 ^{a}	10.35 ± 0.23 ^{d}	0.64 ± 0.20 ^{c}	4.32 ± 0.69 ^{a}	0.49 ± 0.21 ^{a}
GM	5.13 ± 0.13 ^{a}	6.26 ± 1.11^{bc}	28.13 ± 3.32 ^{b}	0.67 ± 0.37 ^{b}	3.62 ± 0.47 ^{b}	0.2 ± 0.08 ^{c}
LF	5.55 ± 0.58 ^{a}	7.35 ± 0.31^{ab}	31.53 ± 1.43 ^{a}	0.71 ± 0.17 ^{a}	4.19 ± 0.9 ^{a}	0.41 ± 0.16 ^{a}

Note: A 20-year monoculture degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). The data mean ± standard deviation, different letters within the same column denoted a significant difference at ($p > 0.05$). Ammonium nitrate ion (NH_4+-N). Nitrate ions (NO_3-N). Available Phosphorus (AP). Available Potassium (AK). Soluble Calcium ions (Soluble Ca^{2+}). Soluble Magnesium ions (Soluble Mg^{2+}).

3.2. Acidity Status of Tea Rhizosphere Soil under Different Treatments

The acidity of tea rhizosphere soil for different treatments is shown in Table 2. Compared with the rhizosphere soil of a tea plant without any fertilization (CK), the acidity and aluminum toxicity of tea rhizosphere soil was decreased by LF and GM applications as compared to NF. The pH value was increased from 4.23 to 4.32 in GM and LF, respectively. Meanwhile, the content of exchange acid (EA) was decreased by 1.21, 1.46 cmol·kg^{-1}. It was exchangeable H^+ by 0.94 and 1.14 and exchangeable Al^{3+} was decreased by 0.28, 0.31 in GM and LF, respectively. However, NF treatment decreased pH while it increased the exchange Al^{3+} to 2.88 cmol$_c$.kg^{-1}. Non-exchangeable acid (NEA) was significantly decreased in the order of NF > GM > LF, respectively, at ($P < 0.05$), as shown in Table 2.

Table 2. Acidity status of tea rhizosphere soil under different treatments.

Treatment	Active Acid pH	Exchangeable Aluminum (Al^{3+})	Exchangeable Hydrogen (H^+)	Exchange Acidity (EA)	Non-Exchangeable Acidity (NEA)
CK	4.23 ± 0.02 ^{b}	2.72 ± 0.04 ^{a}	1.42 ± 0.19 ^{a}	4.14 ± 0.21 ^{a}	3.15 ± 0.21 ^{a}
NF	4.20 ± 0.03 ^{b}	2.88 ± 0.06 ^{a}	0.33 ± 0.04 ^{bc}	3.21 ± 0.09 ^{b}	2.67 ± 0.79 ^{ab}
GM	4.32 ± 0.01 ^{a}	2.46 ± 0.31 ^{a}	0.48 ± 0.03 ^{b}	2.93 ± 0.31 ^{b}	2 ± 0.5 ^{b}
LF	4.32 ± 0.02 ^{a}	2.41 ± 0.47 ^{a}	0.28 ± 0.04 ^{c}	2.68 ± 0.51 ^{b}	2.04 ± 0.28 ^{b}

Note: 20-year degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). The data mean ± standard deviation, different letters within the same column denoted a significant difference at ($p > 0.05$).

3.3. Analysis of Five Low Molecular Weight Organic Acids in Rhizosphere Soil of Tea Treated with Different Fertilization Treatments

Table 3 shows the results of five common low molecular weight organic acids (LMW-OA) in rhizosphere soil of the tea plant under different fertilizer treatments as compared to CK. The content of tartaric acid (TA), Acetic acid (AA), and citric acid (CA) in tea rhizosphere increased significantly under GM and LF treatments, respectively. However, Malic acid (MA) content was significantly improved after application of GM, while LF treatment increased Oxalic Acid (OA) (Table 3).

Table 3. The content of LMW-OC in tea rhizosphere soil under different treatments.

Treatment	(Oxalic Acid)	(Tartaric Acid)	(Malic Acid)	(Acetic Acid)	(Citric Acid)	Low Molecular Weight Organic (LMW-OC)
	——————————mg·kg^{-1}——————————					
CK	229.49 ± 8.60 ^{c}	3.32 ± 0.96 ^{b}	12.96 ± 1.48 ^{b}	280.94 ± 85.20 ^{c}	5.47 ± 0.32 ^{c}	532.19 ± 93.24 ^{c}
NF	368.55 ± 0.89 ^{a}	1.76 ± 0.09 ^{b}	8.43 ± 0.38 ^{c}	220.27 ± 16.65 ^{c}	6.4 ± 0.86 ^{c}	605.41 ± 17.97 ^{bc}
GM	219.44 ± 3.89 ^{c}	6.28 ± 1.60 ^{a}	17.3 ± 1.77 ^{a}	407.19 ± 11.95 ^{b}	10.31 ± 1.91 ^{b}	660.52 ± 15.92 ^{b}
LF	352.03 ± 8.31 ^{b}	6.64 ± 0.29 ^{a}	11.8 ± 2.20 ^{b}	818.74 ± 38.19 ^{a}	15.31 ± 2.09 ^{a}	1204.53 ± 49.78 ^{a}

Note: A 20-year degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). Values are means ± standard deviations, different letters within the same column denoted significant difference at ($p > 0.05$).

3.4. Analysis of Soil Enzyme Activities in Rhizosphere of Tea under Different Fertilizer Treatments

Soil enzyme activity can be used as an important indicator of soil fertility evaluation. All fertilizer treatments can effectively improve the enzyme activity of tea rhizosphere soil after 24 h of incubation. The activity of urease and sucrase was promoted by 7.98 $mg{\cdot}g^{-1}{\cdot}24\ h^{-1}$ and 6.77 $mg{\cdot}g^{-1}{\cdot}24\ h^{-1}$, respectively, in LF treatment. Likewise, the activity of acid phosphatase and polyphenol oxidase was sharply increased by 2.3 $mg{\cdot}g^{-1}\ h^{-1}$ and 63.07 $mg{\cdot}g^{-1}\ 2\ h^{-1}$ in LF treatments. Furthermore, applying GM treatment significantly increased the activity of urease, sucrase, acidic phosphatase, polyphenol oxidase, and peroxidase. However, applying NF decreased the activity of acid phosphatase (Table 4).

Table 4. Enzyme activities of tea rhizosphere soil under different treatments.

Treatment	Urease	Sucrase	Acidic Phosphatase	Polyphenol Oxidase	Peroxidase
	——$mg{\cdot}g^{-1}{\cdot}24\ h^{-1}$——		$mg{\cdot}g^{-1}{\cdot}1\ h^{-1}$	——$mg{\cdot}g^{-1}{\cdot}2\ h^{-1}$——	
CK	11.06 ± 0.08^{c}	1.98 ± 0.0^{d}	6.71 ± 0.20^{b}	122.42 ± 4.28^{c}	22.03 ± 0.52^{c}
NF	17.56 ± 0.39^{b}	2.14 ± 0.1^{c}	2.01 ± 0.15^{c}	139.30 ± 0.52^{b}	24.58 ± 0.1^{b}
GM	17.38 ± 0.63^{b}	6.54 ± 0.1^{b}	8.37 ± 0.57^{a}	140.68 ± 10.83^{b}	24.26 ± 0.36^{b}
LF	19.04 ± 0.55^{a}	8.75 ± 0.26^{a}	9.01 ± 0.24^{a}	185.49 ± 4.56^{a}	25.97 ± 0.22^{a}

Note: A 20-year degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). Values are means ± standard deviations and different letters within the same column denoted a significant difference at $p > 0.05$.

3.5. Soil Microbial Biomass Carbon and Phosphorus in Tea Rhizosphere Soil under Different Fertilizer Treatments

Soil microbial biomass carbon (SMB-C) and soil microbial biomass phosphorus (SMB-P) were significantly improved in the tea rhizosphere in the order of LF > GM > CK, respectively. However, NF treatment did not improve SMB-C and SMB-P in the rhizosphere of tea but inhibited it to some extent (Figure 1a,b). The utilization rate of organic matter (UOM) = (SMB-C/OM) and total phosphorus (UTP) = (SMB-P/TP) ratio (calculated at 1000 times the original value) of rhizosphere microbes in LF and GM treatments were significantly increased as compared to the sole mono-cropping tea orchard (CK) (Figure 1c,d).

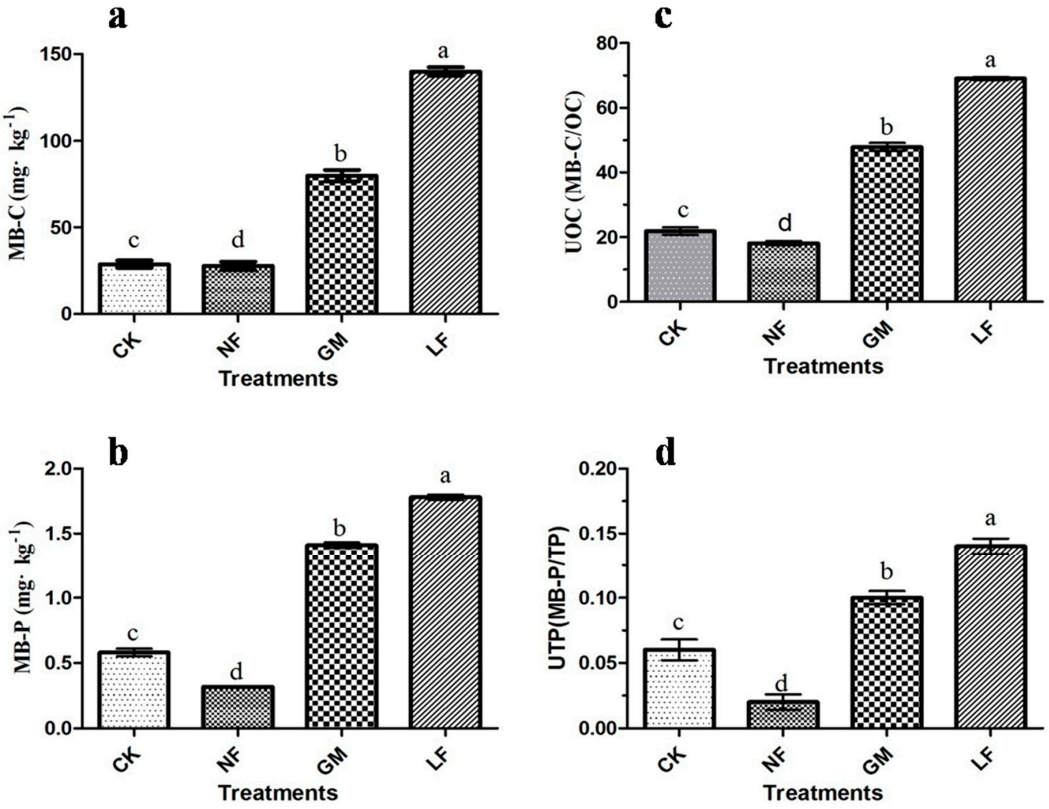

Figure 1. (**a**) Content of microbial biomass C in tea rhizosphere soil by applying different treatments. (**b**) Content of microbial biomass P in tea rhizosphere soil by applying different treatments. (**c**) Utilization rate of organic carbon. (**d**) Utilization rate of phosphorus in tea rhizosphere by applying different treatments.

3.6. Alpha-Diversity and Richness Indices of Soil Bacterial Community Based under Different Treatments

The richness indices (Chao1 and ACE) and diversity index (Shannon) were significantly increased by applying GM and LF treatments, respectively. It was, however, noted that the nitrogen fertilizer application had no significant effect on diversity and richness indexes of tea rhizosphere soil bacteria (Table 5).

Table 5. Alpha-diversity and richness indices of the soil bacterial community based on OTUs under different treatments.

		Diversity Index (Community Index)		Abundance Index (Community Richness)	
Treatments	**Observed Species**	**Shannon**	**Simpson**	**Chao1**	**ACE**
CK	2068 ^{c}	8.58 ^{b}	0.992 ^{a}	2337.66 ^{c}	2409.00 ^{c}
NF	2051 ^{c}	8.51 ^{b}	0.991 ^{a}	2327.94 ^{c}	2421.41 ^{c}
GM	2388 ^{a}	8.79 ^{a}	0.988 ^{a}	3676.65 ^{a}	3169.94 ^{a}
LF	2209 ^{b}	8.79 ^{a}	0.993 ^{a}	2713.86 ^{b}	2696.22 ^{b}

Note: A 20-year degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). Values are means ± standard deviations and different letters within the same column denoted a significant difference at $p > 0.05$.

3.7. Shift in Bacterial Community Structure and Composition under Different Fertilization Treatments

High throughput sequence analysis results showed that *Proteobacteria* (35.91%), *Acidobacteria* (23.22%), *Chloroflexi* (12.27%), and *Actinobacteria* (8.23%) were major bacterial phylum in tea rhizosphere soil (Figure 2a). The relative abundance of bacterial communities in the top three families is shown in Figure 2.

Proteobacteria contained *Xanthomonadaceae* (7.34%), *Burkholderiaceae* (2.69%), and *Rhodobacterace* (2.42%) families in tea rhizosphere soil. Most of the bacteria were types of plant pathogens in the *Xanthomonadaceae*. These results showed that the relative abundance of the *Xanthomonadaceae* family in the rhizosphere of 20-year monoculture degraded tea garden was the highest followed by GM treatments (Figure 2b). *Burkholderiaceae* and *Rhodobacteraceae* (*DA111*) showed an increasing trend under NF and GM treatments, respectively. In the *Burkholderiaceae*, most of the bacteria could break down aromatic compounds. The relative abundance of *Burkholderiaceae* was 2.17% and 3.92% under NF and GM treatments, respectively (Figure 2b). Furthermore, the relative abundance of *Rhizobiaceae* and *Bradyrhizobiaceae* increased in LF and GM treatments, respectively, which are mostly a kind of nitrogen fixer and plant growth promoting bacteria. The study identified *Rhizobium* as a kind of nitrogen-fixing bacteria, and the relative abundance of *Rhizobium* was increased by 0.88% under LF treatment, and there was a downward trend in N fertilizer. The relative abundance of *Rhizobiaceae* and *Bradyrhizobiaceae* was 2.22% and 3.0%, respectively (Figure 2b). *Acidothermaceae* is the main family of *Actinobacteria* (Figure 2c) and its relative abundance in LF and GM was in the range of 1.63% to 3.21%. Compared with untreated soil (CK), the relative abundance of *Acidothermaceae* was increased in the order of NF > LF > GM > CK, respectively. The relative abundance of *Acitinospicaceae* and *Micrococaceae* were increased in the order of GM > LF > NF > CK, respectively (Figure 2c). *Acidobacteriaaceae*, Unidentified_*Acidobacteria*, and *Holophagaceae* were the main populations of *Acidobacteria* in the rhizosphere soil of tea (Figure 2d).The relative abundance of *Acidobacteriaaceae* was improved in the order of LF > GM > CK > NF, respectively (Figure 2d). The relative abundance of Unidentified_*Acidobacteria* in GM treatment was significantly higher than that of CK, LF, and NF treatments (Figure 2d).

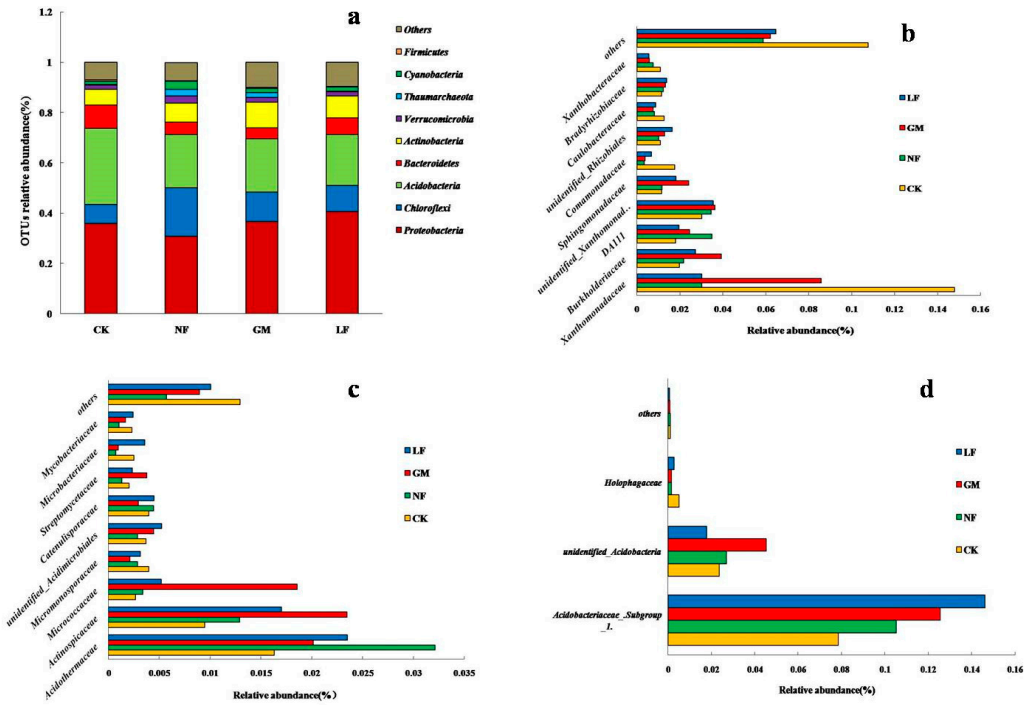

Figure 2. (**a**) Relative abundance of phylum in tea rhizosphere bacteria under different treatments. (**b**) Relative abundance of the dominant soil bacteria phyla in *Proteobacteria* phylum at the level of family in tea rhizosphere soil. (**c**) Relative abundance of the dominant soil bacteria phyla in *Acidobacteria* phylum at the level of family in tea rhizosphere soil. (**d**) Relative abundance of the dominant soil bacteria phyla in *Actinobacteria* phylum at the level of family in tea rhizosphere soil.

3.8. Effects of Different Fertilization Treatments on the Growth, Net Photosynthetic Rate, and Third Leaf´s Chlorophyll Content

Application of LF and GM into tea rhizosphere significantly improved the growth, development, physiology, and yield of tea plants. New tea sprout´s length, third leaf´s chlorophyll content, and the net photosynthetic rate (Pn) were improved by 44.91%, 8.54%, and 6.78%, respectively, by applying LF treatments. Moreover, in GM treatment, the sprout´s length was improved by 42.06%, the content of the third leaf's chlorophyll was increased by 4.42%, and the Pn of the leaf was increased by 5.96% (Figure 3a–c). These results showed that the tea-Legume–goat model could effectively improve the physiological growth of tea shoots in the rhizosphere soil of the tea plant without applying nitrogen fertilizer in organic tea gardens.

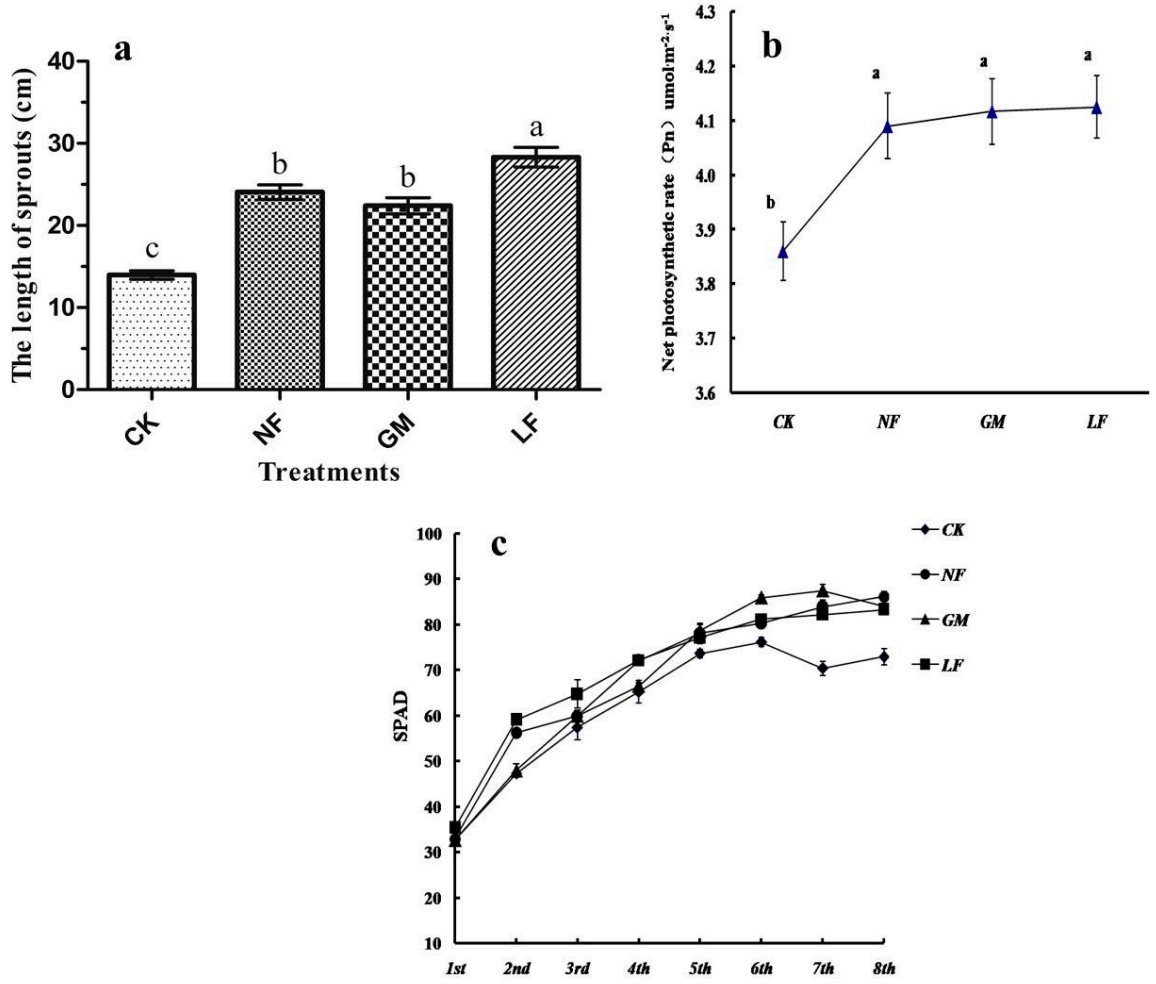

Figure 3. Effects of Different Fertilization Treatments on the growth, developments, and physiological characteristics of tea leaves. (**a**) Length of new tea sprouts under different treatments. (**b**) Photosynthetic rate (Pn) of the third tea leaf in young sprouts under different treatments. (**c**) Content of the leaf chlorophyll from the top to the base of the tea plant under different treatments. Note: Different letters i.e., a, b and c showed a significant difference from each other at $p > 0.05$.

3.9. Hundred-Bud Weight and Yield of Tea Leaves under Different Fertilizer Treatments

The hundred-bud fresh weight and yield of tea leaves in GM treatment was significantly increased by 37.33% and 10.78%, respectively. Likewise, in LF treatment, it was increased by 72.38% and 9.43%, respectively. However, in NF treatment, this increase was 27.97% and 10.05%. Furthermore, the hundred-bud dry weight and yield in GM was significantly increased by 42.93% and 15.81%,

respectively. Likewise, in LF treatment, it was increased by 75.75% and 17.95%, respectively, while, in NF treatment, this increase was 30.27% and 13.15%, respectively (Table 6).

Table 6. Hundred-bud weight and yield of tea leaves under different treatments.

	(Hundred-Bud Weight)		(Yield per Unit)	
Treatment	**(Fresh Weight)**	**(Dry Weight)**	**(Fresh Weight)**	**(Dry Weight)**
	———-g————		——-kg·ha^{-1}——	
CK	70.77 ± 4.31 ^{c}	18.1 ± 1.71 ^{c}	1337.02 ± 7.28 ^{b}	340.51 ± 11.41 ^{c}
NF	90.57 ± 1.96 ^{b}	23.58 ± 0.57 ^{b}	1471.42 ± 11.85 ^{a}	385.28 ± 2.14 ^{b}
GM	97.19 ± 9.5 ^{b}	25.87 ± 2.5 ^{b}	1481.17 ± 9.85 ^{a}	394.36 ± 3.73 ^{b}
LF	121.99 ± 3.72 ^{a}	31.81 ± 1.12 ^{a}	1463.14 ± 11.26 ^{a}	401.62 ± 4.57 ^{a}

Note: A 20-year degraded tea field without any fertilization (CK). Litrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). Values are means ± standard deviations and different letters within the same column denoted a significant difference at $p > 0.05$.

3.10. Biochemical Components of Tea Leaves under Different Treatments

Further study investigated the quality parameters in the new shoots of tea plants under different fertilizer treatments. The content of tea polyphenols in new leaves were decreased by 13.71% with N fertilizer. However, it was remarkably improved by 20.12% and 44.36% in GM and LF treatments, respectively. Caffeine is an important alkaloid in tea. It has an important impact on the improvement of tea quality. From this study, it was noted that caffeine was also increased by 14.07% and 15.45% by applying GM and LF fertilization while 27.89% by NF. Furthermore, Theanine is also an important constituent in tea quality, which was increased by 0.19 and 0.18 g·kg^{-1} with GM and LF treatments, respectively, while theanine increased by 0.29 g·kg^{-1} by applying N fertilizer. Free amino acid content in new tea leaves was significantly increased by 8.29 and 11.73 g·kg^{-1} in GM and LF fertilizers, respectively, while tea leaves increased by 9.52 g·kg^{-1} by applying NF (Table 7).

Table 7. Biochemical component analysis of tea leaves by different treatments.

Treatment	**Polyphenol**	**Caffeine**	**Theanine**	**Free Amino Acid**	**(TP/AA)**
	——————————g·kg^{-1}——————————				%
CK	76.39 ± 12.46 ^{bc}	18.97 ± 0.39 ^{c}	0.31 ± 0.02 ^{c}	3.24 ± 0.25 ^{d}	23.58 ± 6.35 ^{c}
NF	65.92 ± 4.04 ^{c}	24.26 ± 1.96 ^{a}	0.6 ± 0.02 ^{a}	12.76 ± 1.29 ^{b}	5.17 ± 2.66 ^{a}
GM	91.76 ± 15.38 ^{ab}	21.64 ± 0.88 ^{b}	0.5 ± 0.04 ^{b}	11.53 ± 0.67 ^{b}	7.96 ± 8.02 ^{a}
LF	110.28 ± 9.32 ^{a}	21.9 ± 0.41 ^{b}	0.49 ± 0.02 ^{b}	14.97 ± 0.7 ^{a}	7.37 ± 5.01 ^{a}

Note: A 20-year degraded tea field without any fertilization (CK). Nitrogen fertilizer (NF). Goat manure (GM). Leguminous green manure (LF). Values are means ± standard deviations and different letters within the same column denoted significant difference at $p > 0.05$.

3.11. Relationship of Acidity, Soil Enzymes, and Available Nutrients with Abundance of Bacterial Phyla in Rhizosphere Soil under Different Fertilizer Treatments

Redundancy analysis (RDA) ordination between acidity parameters and bacterial families' abundance illustrated that the abundance of most bacterial families such as *Xanthomonadaceae*, *Comamonadaceae*, *Caulobacteraceae*, *Xanthobacteraceae*, and *Holophagae* have show strong positive correlation with exchangeable Al^{3+}. Meanwhile, *Sphingomonadaceae*, *Streptomycetaceae*, *Microbacteriaceae*, *Burkholderiaceae*, and *Actinospicaceae Acidimicrobiaceae* have a positive correlation with pH. On the other hand, *Rhizobiaceae*, *Acidobacteriaceae_.Subgroup_1*, *Bradyrhizobiaceae*, *Rhodospirillum* (*DA111*), and *Acidothermaceae* have a positive correlation with exchangeable salts ions (Figure 4).

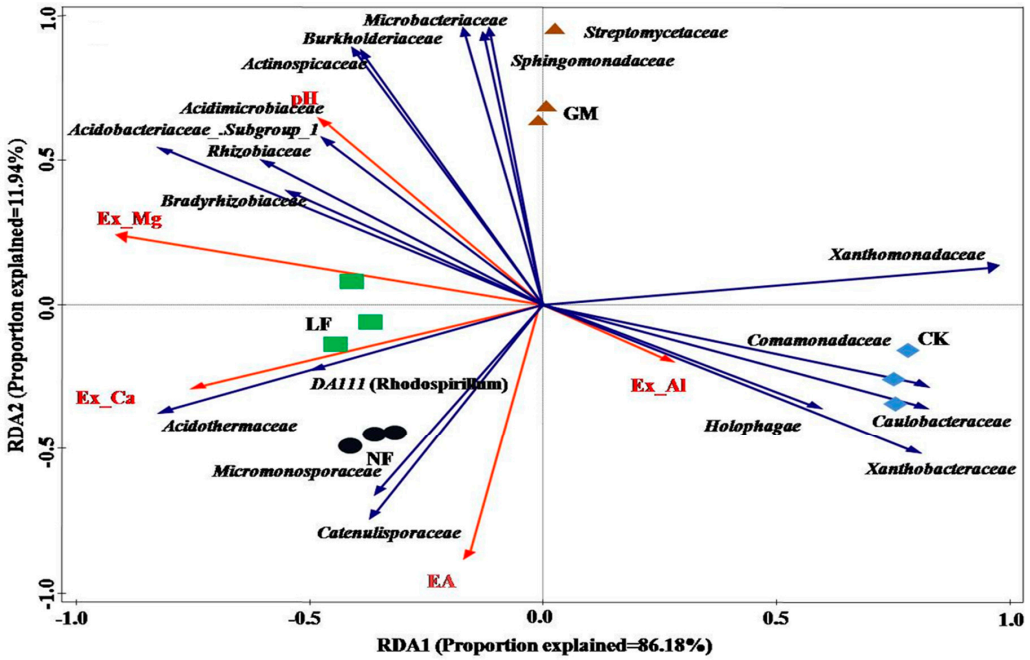

Figure 4. RDA triplot and exchangeable acidic ion interactions with major bacteria families of tea rhizosphere soil under different treatments.

Redundancy analysis (RDA) ordination among enzymes, SMB-C, SMB-P, and most dominant families showed relationships with each other. All the enzymes such as *sucrase*, *Urease*, *acidic phosphatase*, *polyphenole oxidase*, *peroxidase*, SMB-C, and SMB-P have strong negative correlations with *Xanthomonadaceae*, *Comamonadaceae*, *Caulobacteraceae*, *Xanthobacteraceae*, and *Holophagae*. However, *Sphingomonadaceae*, *Streptomycetaceae*, *Microbacteriaceae*, *Burkholderiaceae*, *Actinospicaceae Acidimicrobiaceae*, *Rhizobiaceae*, *Acidobacteriaceae_ Subgroup_1*, and *Bradyrhizobiaceae* have a strong positive correlation with SMB-C, SMB-P, and with all enzymes (Figure 5).

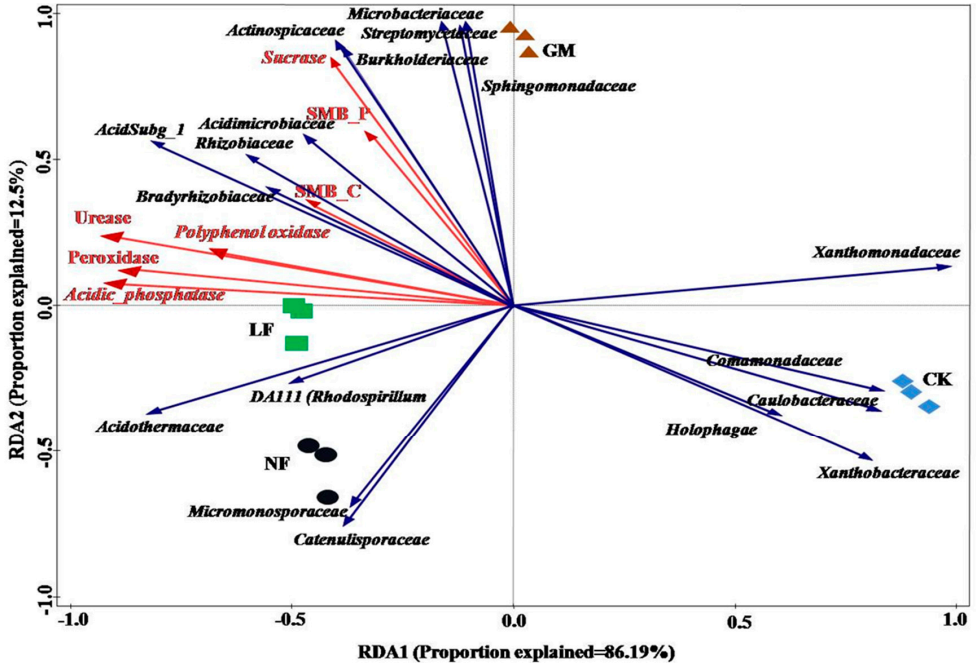

Figure 5. RDA triplot. Enzymatic and microbial biomass carbon (SMB_C) and phosphorus (SMB_P) interactions with major bacteria families of tea rhizosphere soil under different treatments.

RDA was also performed to study the relationships of most dominant bacterial families with available nutrients. Strong positive associations were found between AN and *Sphingomonadaceae*, *Streptomycetaceae*, *Micrococaceae*, *Actinospicaceae*, and *Burkholderiaceae* while AP, AK, ACa, AMg, OM, and nitrate have strong positive associations with *Acidimicrobiaceae*, *Rhizobiaceae*, *Acidobacteriaceae_.Subgroup_1*, *Bradyrhizobiaceae*, *Acidothermaceae*, *Micromonosporaceae*, and *Catenulisporaceae* (Figure 6). Most of the members of these families are involved in the biogeochemical cycle.

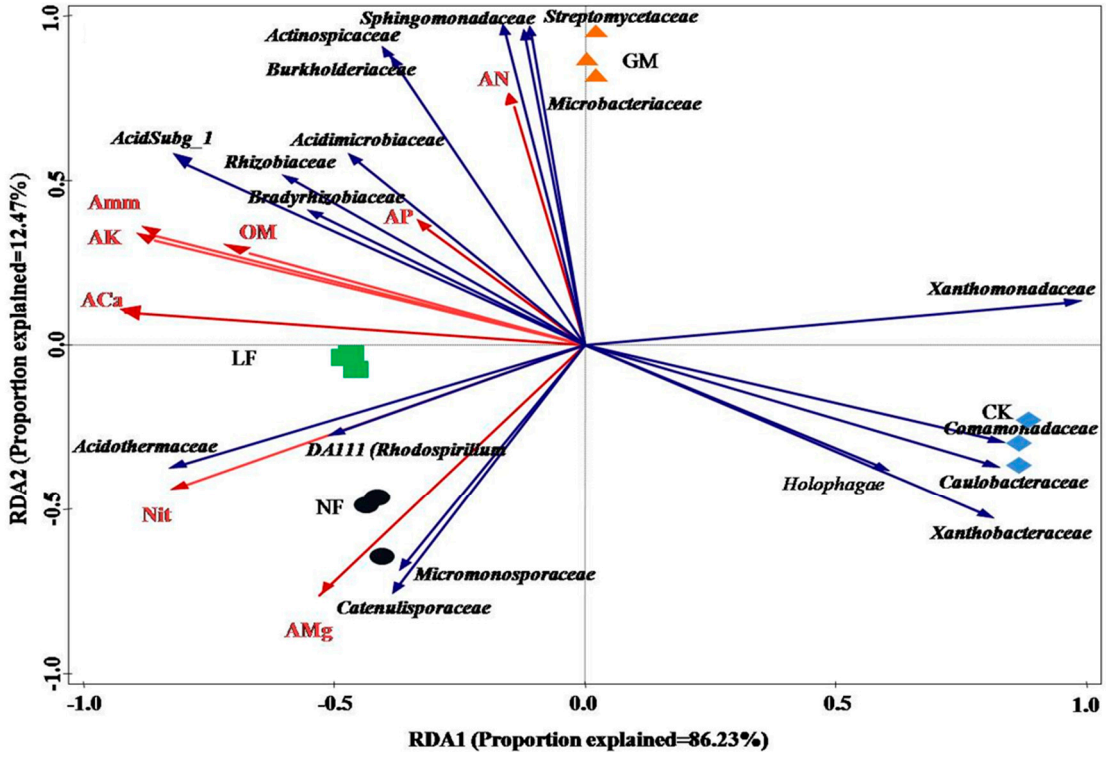

Figure 6. RDA triplot. Available nutrient interactions with major bacteria families of tea rhizosphere soil under different treatments.

4. Discussion

In recent years, legumes intercropping, green manure, and goat manure has been widely used in red soil acidity and fertility improvement, which has gradually become a hot spot [38–41]. Most of the previous studies have shown that legumes intercropping and manure are beneficial for improving soil fertility and increasing yield [42–45]. In this study, chlorophyll contents, sprouts lengths, biochemical components, and the weight of hundreds 20-Y tea plantation improved with the integrated use of green and goat manure. These profitable effects may be due to the maintenance and improvements of pH [46–48], available nutrients status [40,49], Aluminum detoxification [45,50], stimulation of low molecular weight organic acid [51], and the improvement of microbial activity and community structure [52]. Leguminous green manure has nitrogen fixation ability, plays an important role in the soil nitrogen balance, and can increase the effectiveness of phosphate and some trace elements.

It is familiar that the growth of plants depends mostly on soil fertility, and soil fertility is closely associated to soil enzymatic activities and soil microorganisms [53]. Soil enzymes are derived from soil bacteria, fungi, and plant roots. Plant and animal residues and their activities can reflect the strength of biochemical processes in the soil. The activation of *urease*, *sucrase*, *acid phosphatase*, *polyphenole oxidase*, and *peroxidase* activities by applying green forage manure and goat manure fertilizer in the degraded 20-year tea garden was similar to some previous results [54–56]. Soil *urease* and *phosphatase* activities, which are responsible for nitrogen and phosphorus mineralization, respectively, could be

indicators of soil health for nutrient availability to some extent in different cropping systems and environments [57]. Our results showed the highest activities of urease and phosphatase in LF and GM treatments, respectively (Table 4), which may enhance the availability of nitrogen and phosphate to tea plants in the form of NH_4^+-N, NO_3-N, and AP, respectively (Table 1). *Sucrase* catalyzes the breakdown of water soluble plant material in the soil [58]. *Sucrase* activity is influenced by the application of nitrogen-based fertilizer, the green legume fertilizer, and goat manure. These results are similar to previous results in which *sucrase* activity is influenced by manure application and application of chemical fertilizers [21]. Meanwhile, this paper results showed that acid phosphatase activity was enhanced by applying all forms of fertilizers that were applied. However, the most pronounced effect was in green manure and goat manure applications, respectively. Acid phosphatase activity was associated with organic phosphate mobilization and available phosphorus is an important element necessary for plant growth and development [59].

Microbial biomass indicates the size of the soil microbial community and is believed to be an indicator of microbiological properties and soil fertility [11]. In these results, green manure and goat manure application enhanced microbial biomass carbon and phosphorus significantly in a 20-year monoculture degraded tea garden (Figure 1), which may improve growth, quality, and yield of the tea orchard. These results was similar to those of Reference [60], in which long-term organic manure fertilizers greatly increased soil microbial biomass C and dehydrogenase activity as well as the promoting effect of the compost on the growth of an indigenous growth promoting bacteria (*Bacillus* sp.) in the soil. Meanwhile, the better growth of tea orchards in green and goat application improved the secretion of low molecular weight organic acids such as malic acid (MA), tartaric acid (TA), acetic acid (AA), and citric acid (CA) to tea rhizosphere soil (Table 3), which recruits beneficial microbes in rhizosphere and ensues benefits to microbial growth [61,62]. In this study, most of the bacterial groups were similar among treatments in 20-year monoculture degraded soil, but we observed shifts in the relative abundance of major families of bacteria in GM and LF treatments (Figure 2). These results indicated that GM and LF applications can stimulates the growth of beneficial bacterial families such as *Rhizobiaceae*, *Bradyrhizobiaceae*, and *Burkholderiaceae*, which are mostly involved in biogeochemical cycles of N, P, and C and that this practice might recover the nutrient uptake and, in turn, the yield and quality of the tea plant. Peacock et al. (2001) [63] results showed that the abundance of soil bacteria had a significant positive correlation with microbial biomass carbon by applying dairy manure application. Similarly, Chen et al. (2012) [64] results indicated that the application of chemical fertilizers to tea trees can significantly increase the number of rhizosphere fungi, but significantly reduce the number of nitrogen-fixing bacteria while, intercropping can significantly reduce the number of rhizosphere fungi and increase the number of nitrogen-fixing bacteria. Most of the studies have shown that the fertilization could affect the number of soil bacteria in long-term crop plantations [65–67]. Studies have also shown that chemical fertilizers applied to the tea soil altered their bacterial community structure [68]. Furthermore, this paper illustrated that some of the flora that have significant benefits to soil fertility, including nitrogen-fixing bacteria, nitrobacteria, fiber decomposition bacteria, and other functional bacteria were increased by using green manure and goat manure. This study also found that tea root rhizosphere produces a high amount of simple amino acids as energy for bacteria. The rhizosphere soil microbial community is a complex and diverse biological community. With its specific physiological activity, functional diversity, and other characteristics, it can promote the plant root nutrient absorption. The application of leguminous green manure in the tea garden has the ability to promote the degradation of plant litter, decomposition, and mineralization of organic matter [45]. Leguminous green manure has a positive effect on soil phosphorus uptake. Brookes and McGrath (1984) [38] suggested that leguminous green fertilizers, on one hand, could provide the energy and nutrient phosphorus required for microbial growth. On the other hand, it was able to saturate soil phosphorus fixation sites, which improve the effective utilization of phosphate fertilizer [69]. Leguminous forage green has a positive significance on the growth of tea and tea quality. The results showed that the content of soil organic matter, available nitrogen and phosphorus increased, and the

microbial biomass carbon and phosphorus were also improved. It was also found that increasing the content of soil organic matter, available N, available P, and available potassium could increase soil pH by 0.17 units with the role of alleviating soil acidification. This study also found that the addition of goat manure can promote the spring tea shoots germination and autumn tea buds than the control. These results showed that the application of green and goat manure in acidic 20-year monoculture degraded tea garden could improve soil exchangeable salt ion content, and could effectively alleviate the main problems such as phosphorus deficiency and aluminum toxicity in soil, and provide a good living environment. At the same time, the green manure could improve the soil humus content and soil structure of the tea garden. Pandey and Palni (1996) [70] showed that the same tea quality and higher tea yield can be achieved by applying organic bio-fertilizers to tea trees at the level of application of lower chemical fertilizers. Soil microorganisms are important in soil nitrogen fixation, phosphorus and potassium release, and soil moisture retention. It can regulate the micro-climate of the tea garden and promote the germination of tea buds and its special metabolism [71]. Organic fertilizer is rich in organic matter, nitrogen, phosphorus, potassium, and other nutrients. Therefore, long-term application to the soil not only improved the soil nutrients and organic matter content, but also the formation of the soil aggregate structure and improve the soil physical and chemical properties.

5. Conclusions

From this study, we concluded that green and goat manure applications are good agriculture management practices, which may promotes plant growth and yield, and increase nutrient availability in long-term continuously monocultured tea plantation by maintenance and improvement of pH. The green and goat manure applications enhance the available nutrient status, improve the secretion of low molecular weight organic acids, and balances the community structure. From this study, it is recommended that green and goat manure application systems can used in long-term continuous monoculture degraded tea orchards for fertility restoration in order to meet the growing market demands. Future studies should be done that show why the pH of the tea garden decreases with increasing plantation age.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/4/1011/s1, Figure S1: Green and goat manure application system, Figure S2: Five low molecular organic acid chromatograms, Figure S3: Standard curve for five kinds of low molecule weight organic acids, Figure S4: Sequencing Data Processing Analysis Flow, Table S1: Nutrient content in the green manure (Leguminous crops) and goat manure, Table S2: Standard curve regression equation of different components.

Author Contributions: W.L., Y.J., and Y.A., conceived the study. Y.A., Y.J., and W.L., wrote the paper. Y.J., and Y.A., performed field sampling and lab experiments. Y.A., LA., and Y.J., performed the statistical analyses. All authors discussed the results and commented on the manuscript.

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