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Effects of Compound Fertilizer Decrement and Water-Soluble Humic Acid Fertilizer Application on Soil Properties, Bacterial Community Structure, and Shoot Yield in Lei Bamboo (*Phyllostachys praecox*) Plantations in Subtropical China

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Abstract: Lei bamboo (*Phyllostachys praecox*) is an economically viable bamboo species with rich nutrition, a good taste, and a high yield. However, heavy fertilization and covering cultivation are used to produce off-season bamboo shoots, resulting in soil degradation and a decline in site productivity. This study investigated how compound fertilizer decrement and water-soluble humic acid fertilizer application affects soil properties and shoot yield in Lei bamboo plantations of subtropical China. The soil nutrients, enzyme activities, and shoot yield were examined, the bacterial community structure was determined using the high-throughput sequencing method, and their relationships were evaluated under different fertilization treatments: single compound fertilizer and compound fertilizer decrement with water-soluble humic acid fertilizer applications. Compared with those after single compound fertilizer treatments (CF1, CF2), water-soluble humic acid fertilizer addition (CF2HA1, CF2HA2) increased soil organic carbon (SOC), available phosphorus (AP), microbial biomass nitrogen (MBN) contents, the ratio of SOC to total nitrogen (C/N), and sucrase and acid phosphatase (Acp) activities, and decreased alkali hydrolyzed nitrogen (AN) and microbial biomass carbon (MBC) contents. The bacterial community phyla comprised 83.62%–86.16% Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi. Water-soluble humic acid fertilizer application also significantly increased yields by over 30%. AP and MBN were important drivers affecting soil bacterial communities, whereas SOC, MBN, and Chloroflexi affected Lei bamboo shoots. Overall, compound fertilizer decrement and water-soluble humic acid fertilizer application shifted the available soil nutrients, sucrase and Acp activity, bacterial community diversity, and shoot yield. An improved understanding of humic acid and the application of humic acid water-soluble fertilizer are of great significance for soil improvement, ecological restoration, and the sustainable management of bamboo forests in the future.

Keywords: humic acid water-soluble fertilizer; soil properties; bacterial community; yield

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1. Introduction

Lei bamboo (*Phyllostachys praecox*) is an economically effective bamboo species for bamboo shoots, characterized by its easy cultivation, fresh taste, and high yield [1,2]. It is a fast-growing forest resource which can reproduce asexually through its rhizome with many buds. These buds can develop into bamboo in the spring and summer, or shoots are harvested when edible in the spring. It is widely cultivated in the southern region of the Changjiang River in China [3]. Fertilization is an important factor in the production and cultivation management of Lei bamboo, and the type, amount, time, and method of fertilization play decisive roles in yield and quality [4].To achieve higher economic benefits, in the past 20 years, the technology used for the early emergence of bamboo shoots in Lei bamboo forest land, characterized by heavy fertilization in winter and the use of organic material cover (such as bamboo leaves, deciduous broad-leaved trees, rice husks, etc.) for heating and warming, has been widely promoted and applied in the Lei bamboo main production areas, such as Lin'an City and Yuhang District in Zhejiang Province [5]. This technology can promote the early emergence of bamboo shoots and increase yield, thereby significantly improving the associated economic benefits. However, the long-term application of chemical fertilizers can easily lead to the degradation of the bamboo forest ecosystem, resulting in declining fertility, soil erosion, a reduction in microbial community diversity, and the eutrophication of water systems, seriously hindering the sustainable development of bamboo forest populations [1,6–8]. Therefore, it is necessary to investigate the effects of reduced chemical fertilizer application on the productivity of Lei bamboo.

Humic acid is a type of organic matter that accumulates through the decomposition and transformation of plant and animal remains by microorganisms and a series of chemical processes [9–12]. It has physical, chemical, and biological effects and can promote the formation of soil aggregates, reduce soil bulk density, increase cation exchange, regulate soil pH, and help improve soil water, fertilizer, insulation, and ventilation capacity [9,13,14]. Humic acid fertilizers are made from natural resources containing humic acid (such as peat, brown coal, weathered coal, etc.) combined with substances containing various nutrients (such as potassium, sodium, phosphorus, calcium, magnesium, etc.). Fertilizers containing humic acid also have numerous functions, such as nitrogen control and slow-release, phosphorus solubilization and efficiency enhancement, potassium leaching prevention, and the activation of trace nutrients, making it an excellent fertilizer enhancer [15–17]. Humic acid is derived from the soil, and various fertilizers comprising humic acid as a raw material are green fertilizers [18]. Many researchers and producers have studied the application of humic acid compound fertilizers on various crops. Research on crops, such as corn, rice, cotton, peanuts, Chinese cabbage, cucumber, tomato, oil peaches, and apples, have shown that compound fertilizers containing humic acid can have a positive effect on improving crop yield or quality. Meanwhile, humic acids also play an important role in soil remediation and improvement [9,19,20].

Soil enzyme activity is considered an important indicator of soil fertility and can reflect the rate of nutrient transformation and material cycling in the soil. Simultaneously, soil enzymes can cycle and transform plant nutrients and organic matter [3]. Fertilization also provides abundant available resources for the reproduction and physiological activities of microorganisms, thus increasing the diversity of soil microbial communities [21,22]. Soil microorganisms directly participate in important biochemical processes, such as soil nutrient cycling, and soil microbial biomass carbon and nitrogen (MBN and MBC) are important parameters in the study of soil carbon and nitrogen nutrient transformation and cycling, which reflect the status of soil microorganisms and soil fertility [23,24]. Soil enzymes are biocatalysts for organic matter decomposition and nutrient cycling in agricultural ecosystems, and soil enzyme activity is typically used to characterize the intensity of soil microbial activity [25,26]. Urease is closely related to soil nitrogen cycling, and its activity can reflect soil nitrogen supply capacity. Sucrase is related to the hydrolysis of sugars and can catalyze the hydrolysis of sucrose into glucose and fructose. Acid phosphatase is involved in the cycle of phosphorus and is related to the hydrolysis of organic phosphorus and the activation of inorganic phosphorus. Soil enzymes are secreted by microorganisms, which can utilize humic acid to alter enzyme secretion.

To explore the effect of humic acid compound fertilizer application on the production of Lei bamboo shoots, we applied water-soluble humic acid fertilizers during the growing season of Lei bamboo shoots and explored their impact on the main nutrient indicators of soil, the microbial community, and bamboo shoot yield. This study aimed to (1) elucidate the effects of compound fertilizer decrement and water-soluble humic acid fertilizer application on soil nutrients, enzyme activities, and bacterial community structures; (2) determine the changes in shoot yield after compound fertilizer decrement and water-soluble humic acid fertilizer application; and (3) examine the relationships among soil properties, the bacterial community, and shoot yield. Overall, our study data provide support for partially replacing chemical fertilizers and reducing their use. Moreover, it provides a theoretical and practical basis for rational fertilization in bamboo shoot cultivation.

2. Materials and Methods

2.1. Field Site

The study site was located in the village of Minjin (30°73′ N, 119°97′ E), Deqing County, Huzhou City (Zhejiang, China). It experiences a typical subtropical monsoon climate, with an average annual temperature of 15.6 °C, an annual rainfall of 1379 mm, a frost-free period of about 220–236 days, and an altitude of 54 m. The experimental forest comprises two years of mulching management of Lei bamboo forest, with a standing bamboo density of 14,850–16,500 stems/ha and an average diameter at breast height of 4.24–4.58 cm. Red soil originates from granitic rock, with a soil layer thickness greater than 50 cm. The soil bulk density and pH were 0.89–0.96 g/cm^3 and 4.79–5.23, respectively. The soil composition consisted of the following: soil organic carbon (SOC), 95.6–109 g/kg; total nitrogen (TN), 4.48–4.67 g/kg; total phosphorus (TP), 2.51–2.77 g/kg; total potassium (TK), 11.1–11.9 g/kg; alkali-hydrolyzed nitrogen (AN), 355–374 mg/kg; available phosphorus (AP), 446–470 mg/kg; and available potassium (AK), 402–468 mg/kg.

2.2. Experimental Design

In March 2021, four sites were selected for investigation based on their similarities in altitude, slope, and aspect. Twelve plots were divided into four different fertilization treatments, with three plots for each treatment used as three replicates, separated by an interval of 4 m between each plot. Fertilization was carried out in late April, early August, and late October in 2021 at a ratio of 2:5:3 for the water-soluble humic acid fertilizer and 2:2:3 for the compound fertilizer. The water-soluble humic acid fertilizer (pH = 7; produced by Zhejiang Dongjie Biotechnology Co., Ltd., Jiaxing, China) was diluted 500× before being evenly sprayed on the Lei bamboo forest soil. The water-soluble humic acid fertilizer contains 20 g/kg organic matter, 112.18 g/kg nitrogen, 1.805 g/kg phosphorus, 30.95 g/kg potassium, 4.03% fat, 23.77 g/kg humic acid, and 18.85 g/kg amino acids. After applying the water-soluble humic acid fertilizer for 3–5 days, a compound fertilizer ($N:P_2O_5:K_2O$ = 17:7:17; total nutrients $\geq$ 45%) was applied, and the soil reclaimed at 15–20 cm. Uncooked chicken manure was applied to all treated plots at a rate of 52.5 t/ha before being covered with 30 cm thick rice husk in November 2021. The fertilization time, type, and amount used in the experimental forests are shown in Table 1.

Table 1. Fertilization time, type, and amount used in the experimental forests.

		CF1 (CF)	**CF2 (0.7CF)**	**CF2HA1 (0.7CF + HA)**	**CF2HA2 (0.7CF + 2HA)**
Fertilization Time	**Fertilization Type**	**Fertilization Amount (kg/ha)**			
April 2021	Compound fertilizer	643	450	450	450
	Water-soluble humic acid fertilizer	-	-	30	60
July 2021	Compound fertilizer	643	450	450	450
	Water-soluble humic acid fertilizer	-	-	75	150
October 2021	Compound fertilizer	964	675	675	675
	Water-soluble humic acid fertilizer	-	-	45	90
Total	Compound fertilizer	2250	1575	1575	1575
	Water-soluble humic acid fertilizer	-	-	150	300

CF1 and CF2 = compound fertilizers; CF2HA1 and CF2HA2 = compound fertilizers + water-soluble humic acid fertilizers.

2.3. Soil Sampling and Properties Tests

Soil samples were collected from five points in each plot in March 2022. Five soil cores were collected from each plot and mixed to form a composite soil sample. All fresh soil samples were passed through a 2 mm sieve. One part was stored at −80 °C for molecular analysis, and another collected in a nylon mesh bag and air-dried for measurements. Soil pH was measured using the potentimetric method using a glass combination electrode [27]. SOC was measured using a total organic carbon (TOC) analyzer (Multi N/C 3100; Analytik, Jena, Germany). TN was measured using the micro-Kjeldahl method. TP was determined via $HClO_4$-H_2SO_4 digestion and the Al antimony colorimetric method. AN was determined using the alkaline hydrolysis diffusion method. AP was extracted using a hydrochloric acid–ammonium fluoride solution and analyzed using a visible-range spectrophotometer. TK was measured using flame photometry, and AK extracted with an acetic acid–ammonium solution and quantified using a flame photometer [28].

2.4. Soil Microbial Biomass Carbon and Nitrogen

MBC and MBN were determined using the chloroform fumigation extraction method, and the conversion coefficient of the calculated values was 0.45 [29]. The soil samples after and without fumigation treatment were leached with 0.5 mol·L^{-1} K_2SO_4 solution, and the extracts were then measured directly using a total organic carbon analyzer. The calculation formula is as follows: MBC/MBN = Ec/En/0.45, where the Ec/En is the ratio between the organic C and N (g·kg^{-1}) measured in the samples after and without fumigation treatment, and 0.45 is the ratio of the C in microorganisms killed after chloroform fumigation and the N being leached out.

2.5. Soil Extracellular Enzyme Activities

Soil urease, sucrase, and acid phosphatase (Acp) activities were determined using test kits. Urease activity was measured using sodium phenol–sodium hypochlorite colorimetry, and was expressed as the amount of $NH_4{}^+$-N per gram of soil after 24 h. Sucrase activity using 3,5-dinitrosalicylic acid colorimetry, and was expressed as the amount of sodium thiosulfate (0.1 mol·L^{-1}) of soil after 24 h. Acp activity was determined using phenyl disodium phosphate [30].

2.6. DNA Extraction and Sequencing

Soil DNA was extracted from a 0.5 g soil sample using a soil DNA Extraction Kit (DP812; Tiangen, Beijing, China), according to the manufacturer's protocol. The V3 and V4 regions were amplified using the following primers: 5′-CCTACGGGNGGCWGCAG-3′ (forward) and 5′-GACTACHVGGGTATCTAATCC-3′ (reverse) [31]. A 0.5 g sample was added into a 2 mL centrifuge tube with 500 µL buffer SA, 100 µL buffer SC, and 0.25 g grinding beads. The sample was mixed well with a vortex mixer for 15 min or by using a TGrinder H24 tissue grinding homogenizer (OSE-TH-01) (shaken at a speed of 6 m·s^{-1} for 30 s, with an interval of 30 s, for a total of two cycles). The samples were then centrifuged at 12,000 rpm for 1 min, whereafter the supernatant (approximately 500 µL) was transferred to a new 2 mL centrifuge tube. A Synergy HTX reader was used to detect the concentration of extracted nucleic acids, which were then amplified via PCR. After amplification, the PCR products were detected using electrophoresis with a 1.8% agarose gel. The library was subjected to quality inspection using the Qsep-400 method, and the constructed library was sequenced using Illumina Novaseq 6000 (Beijing Biomarker Technology Co., Ltd., Beijing, China).

2.7. Statistical Analysis

All statistical analyses were performed using the SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA) and Excel 2016 (Microsoft Corp., Redmond, WA, USA). One–way analysis of variance, followed by Duncan's multiple range test, were used to detect significant differences ($p < 0.05$) between the treatment means. Based on Bray–Curtis distance, non–metric multidimensional scaling (NMDS) and redundancy analysis (RDA) were performed in the R 'Vegan' package (v3.1.1., R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Effects of Water-Soluble Humic Acid Fertilizer on Soil Properties

The soil physicochemical indexes varied significantly among the four treatments (Table 2). The pH values of CF2HA2 were significantly lower than those of CF1, CF2, and CF2HA1. Compared with CF1, the SOC content of CF2HA1 and CF2HA2 significantly increased by 11.04% and 17.56%, respectively. CF2HA2 treatment significantly increased the AP content compared with that of CF1, CF2, and CF2HA1. CF1 had the highest TN and AN contents, and CF2HA2 exhibited the highest C/N ratio.

Table 2. Basic soil characteristics of Lei bamboo forests under four fertilizer treatments.

Treatments	pH	SOC (g/kg)	TN (g/kg)	TP (g/kg)	AN (mg/kg)	AP (mg/kg)	C/N
CF1	5.68 ± 0.55^{a}	110.67 ± 1.15^{c}	5.90 ± 0.11^{a}	3.37 ± 0.09^{b}	502.33 ± 21.96^{a}	469.00 ± 42.44^{b}	18.76 ± 0.54^{c}
CF2	5.40 ± 0.28^{a}	105.33 ± 2.52^{c}	5.31 ± 0.11^{c}	2.63 ± 0.18^{c}	418.67 ± 70.53^{d}	457.33 ± 41.14^{b}	19.82 ± 0.09^{c}
CF2HA1	5.33 ± 0.06^{a}	116.97 ± 4.05^{b}	5.42 ± 0.16^{c}	3.12 ± 0.18^{b}	442.00 ± 15.62^{c}	473.00 ± 18.36^{b}	21.59 ± 0.50^{b}
CF2HA2	4.96 ± 0.19^{b}	123.83 ± 1.61^{a}	5.62 ± 0.51^{b}	3.59 ± 0.19^{a}	476.33 ± 47.65^{b}	506.67 ± 27.06^{a}	22.14 ± 0.81^{a}

Different lowercase letters indicate significant differences ($p < 0.05$) between fertilizer treatments. CF1 and CF2 = compound fertilizers; CF2HA1 and CF2HA2 = compound fertilizers + water-soluble humic acid fertilizers. SOC, total soil organic carbon; TN, total nitrogen; TP, total phosphorus; AN, alkali-hydrolyzed nitrogen; AP, available phosphorus; C/N, SOC to TN ratio.

As shown in Figure 1, the MBC and MBN contents were significantly affected by the application of the water-soluble humic acid fertilizer, and the MBC content of the CF2HA1 and CF2HA2 treatments significantly decreased by 42.56% and 31.50%, respectively, compared with that of CF2 ($p < 0.05$). In contrast, the MBN content of the CF2HA1 and CF2HA2 treatments significantly increased by 40.98% and 110.66%, respectively, compared with that of CF2. The MBC/MBN ratios of CF2HA1 and CF2HA2 were significantly lower than those of CF1 and CF2. However, no significant differences of MBC/MBN ratios were observed between CF1 and CF2.

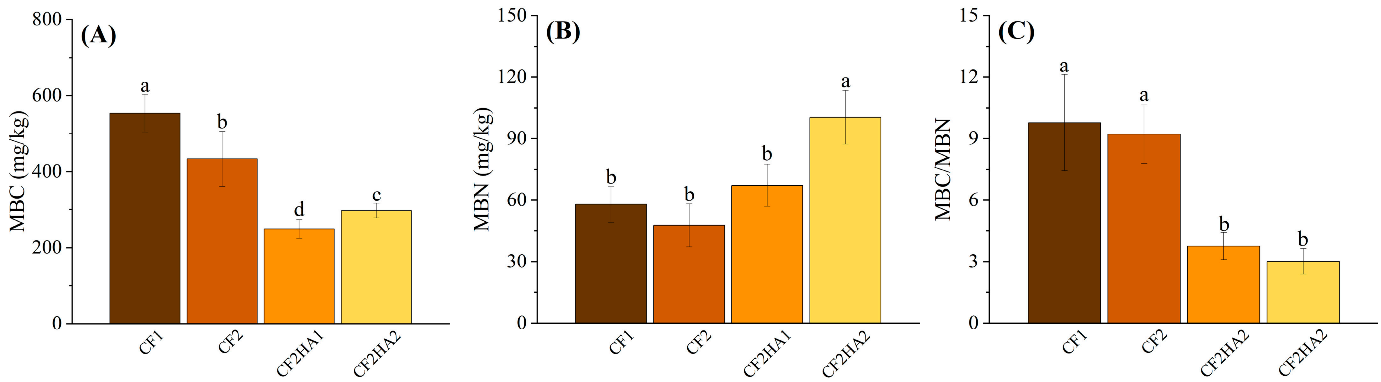

Figure 1. Effects of water-soluble humic acid fertilizer on (**A**) soil microbial biomass carbon (MBC), (**B**) microbial biomass nitrogen (MBN), and (**C**) the MBC to MBN ratio (MBC/MBN). Different lowercase letters indicate significant differences ($p < 0.05$) between treatments. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

3.2. Effects of Water-Soluble Humic Acid Fertilizer on Soil Enzyme Activities

Soil urease, sucrase, and Acp activities are shown in Figure 2. There were no significant differences in urease activity among the four fertilizer treatments. CF2HA2 showed significantly increased sucrase activity compared with that of CF1 and CF2, whereas CF2HA1 showed no significant difference. CF2HA1 and CF2HA2 showed significantly increased Acp activity compared with that of CF2.

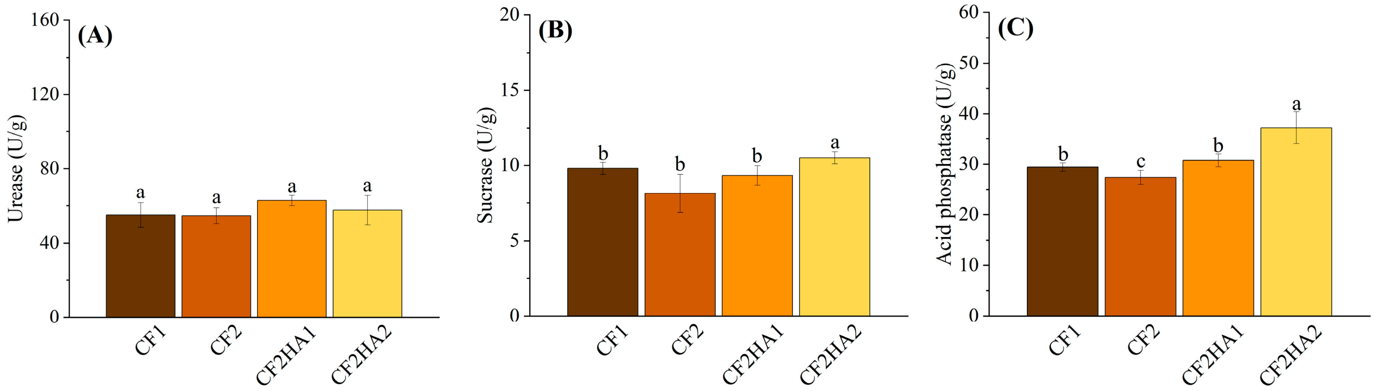

Figure 2. Effects of water-soluble humic acid fertilizer on soil (**A**) urease, (**B**) sucrase, and (**C**) acid phosphatase activities. Lowercase letters in each column indicate significant differences ($p < 0.05$) between different fertilizer treatments. CF1 and CF2 = compound fertilizer; CF1HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

3.3. Effects of Water-Soluble Humic Acid Fertilizer on Soil Bacterial Community

According to the statistical analysis of the high-throughput sequencing results, a total of 7375 OTUs (ranging from 1817 to 1870 per sample) assigned with a similarity level of ≥97% were found across the 12 soil samples tested. A good coverage of the observed OTUs (99%) was observed in all soil samples, indicating that the libraries covered most of the bacterial species present (Table 3). The alpha diversity indices of the community richness index (ACE and Chao1) were not significantly different between the four treatments. The alpha diversity indices of community diversity (Shannon diversity index) in the soil varied among the four fertilizer treatments. The Shannon diversity indices in the soil of CF1 and CF2 were significantly higher than those in CF2HA1 and CF2HA2.

Table 3. Sequence statistics and alpha diversity indices of soil bacteria in Lei bamboo forests.

Treatments	OTUs	ACE	Chao1	Shannon	Coverage
CF1	1870 ± 7 [a]	1917 ± 9 [a]	1945 ± 7 [a]	9.22 ± 0.08 [a]	0.99 ± 0.01
CF2	1848 ± 41 [b]	1905 ± 32 [a]	1930 ± 41 [a]	9.29 ± 0.09 [a]	0.99 ± 0.01
CF2HA1	1840 ± 38 [b]	1891 ± 36 [a]	1912 ± 38 [a]	8.91 ± 0.35 [b]	0.99 ± 0.01
CF2HA2	1817 ± 56 [b]	1889 ± 44 [a]	1907 ± 44 [a]	9.04 ± 0.11 [b]	0.99 ± 0.01

Different lowercase letters indicate significant differences ($p < 0.05$) between fertilizer treatments. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

The species composition of soil bacteria at the phylum level is shown in Figure 3. The dominant bacterial phyla included Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes, Verrucomicrobia, Patescibacteria, and WPS-2. The top five phyla of the bacterial communities were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi, accounting for 40.27%–42.97%, 24.69%–26.25%, 4.58%–13.52%, 3.74%–5.61%, and 3.48%–5.38%, respectively. CF2 and CF2HA2 treatment significantly decreased the relative abundance of Bacteroidetes compared with that in CF1 and CF2HA1. Based on the Bray–Curtis distance, nonmetric multidimensional scaling analysis revealed significant differences in bacterial community composition at the OTU level (Figure 4).

3.4. Effects of Water-Soluble Humic Acid Fertilizer on Lei Bamboo Shoot Yield

Figure 5 shows the Lei bamboo shoot yields under different fertilizer treatments. Compared with that of CF1 and CF2, CF2HA1 and CF2HA2 treatment significantly increased bamboo shoot yield by 32.33%–40.37% and 41.63%–50.23%, respectively. Additionally, there was no significant difference between CF1 and CF2 ($p > 0.05$).

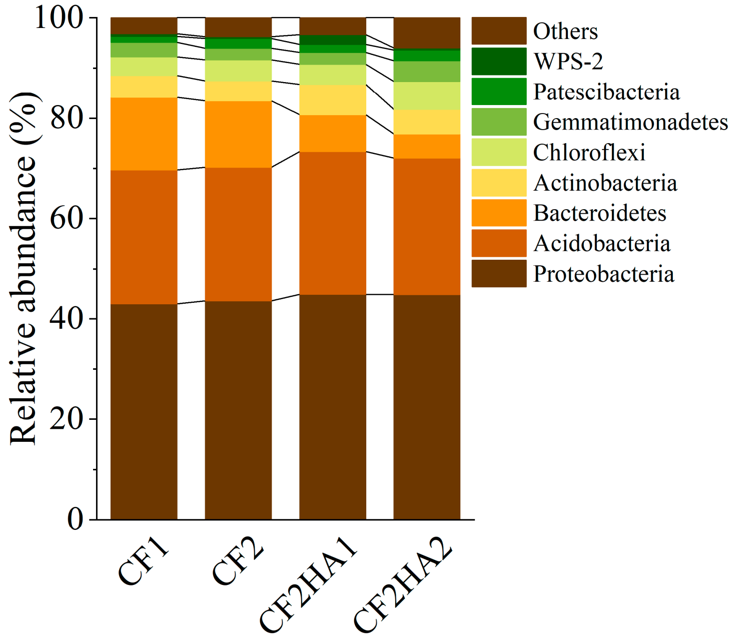

Figure 3. Species composition of soil bacteria at the phylum level under different fertilizer treatments in Lei bamboo forests. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

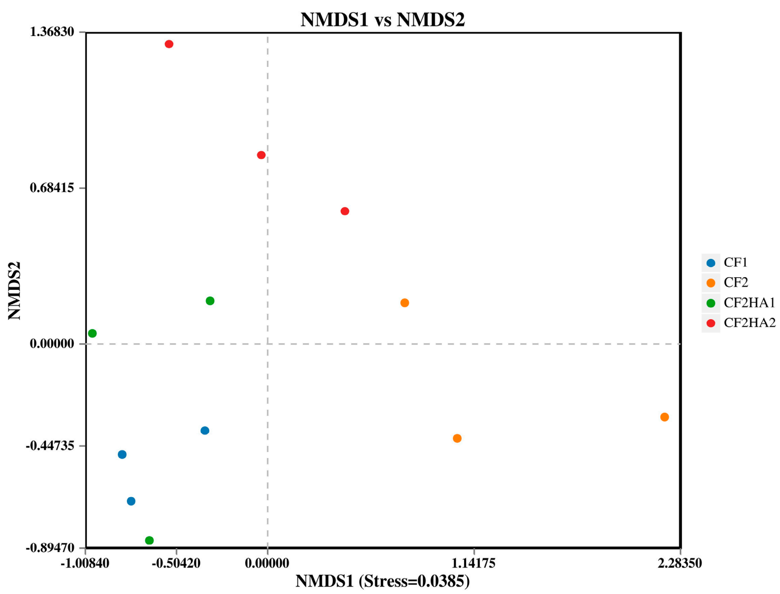

Figure 4. Changes in soil bacterial beta diversity in Lei bamboo forests between different fertilizer treatments. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

3.5. Relationships among Soil Properties, Bacterial Community Structures, and Bamboo Shoot Yields

The correlation heatmap (Figure 6) shows the relationships between the soil bacterial community composition (phylum level) and the main soil characteristics. It can be seen that the SOC and yield were negatively correlated with Firmicutes and WPS-2, but positively correlated with Chloroflexi. Acp activity was negatively correlated with Acidobacteria, whereas sucrase activity positively correlated with Bacteroidetes. TN positively correlated with WPS-2 and negatively correlated with Chloroflexi. AP was positively correlated with Actinobacteria and WPS-2. In the redundancy analysis (RDA), the first two axes explained 20.44% and 18.26% of the variation observed in the microbial communities (Figure 7). AP ($p = 0.015$) and MBN ($p = 0.004$) had significant effects on the soil bacterial communities (Table 4).

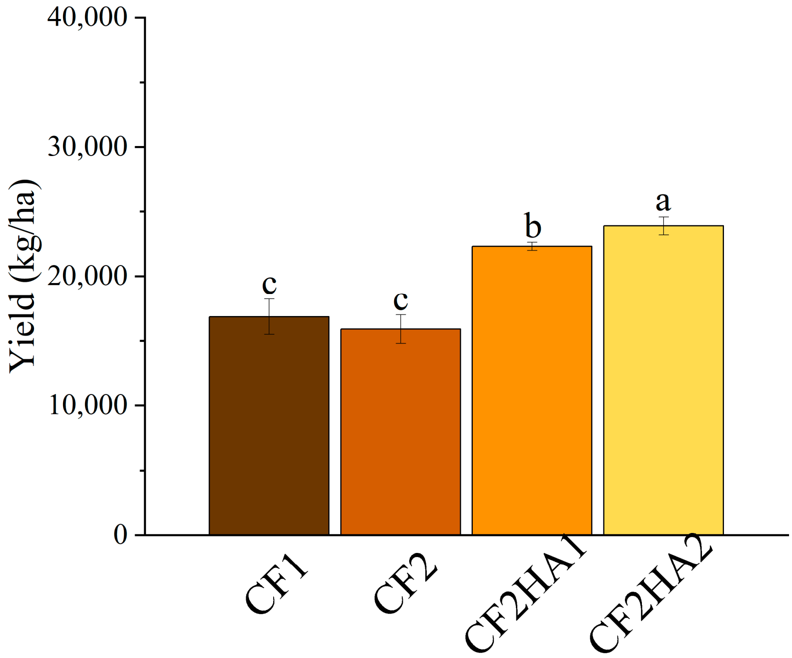

Figure 5. Effect of water-soluble humic acid fertilizer on Lei bamboo shoot yield. Lowercase letters in each column indicate significant differences ($p < 0.05$) between different fertilizer treatments. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

Table 4. Environmental factors affecting soil bacterial communities in Lei bamboo forests.

Envfit	RDA1	RDA2	R^2	p-Value
pH	−0.28	0.96	0.05	0.805
SOC	−0.21	0.98	0.14	0.514
TN	0.55	−0.83	0.21	0.327
TP	0.78	−0.63	0.16	0.449
AN	0.95	−0.32	0.11	0.558
AP	0.34	−0.94	0.65	0.015 *
MBC	−0.12	0.99	0.30	0.190
MBN	−0.06	−1.00	0.70	0.004 **
Acp	0.87	0.49	0.31	0.202
Urease	−0.83	0.55	0.25	0.265
Sucrase	−0.57	0.82	0.23	0.331

Acp, acid phosphatase; AN, alkali-hydrolyzed nitrogen; AP, available phosphorous; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; RDA, redundancy analysis; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorous. * $p < 0.05$; ** $p < 0.01$.

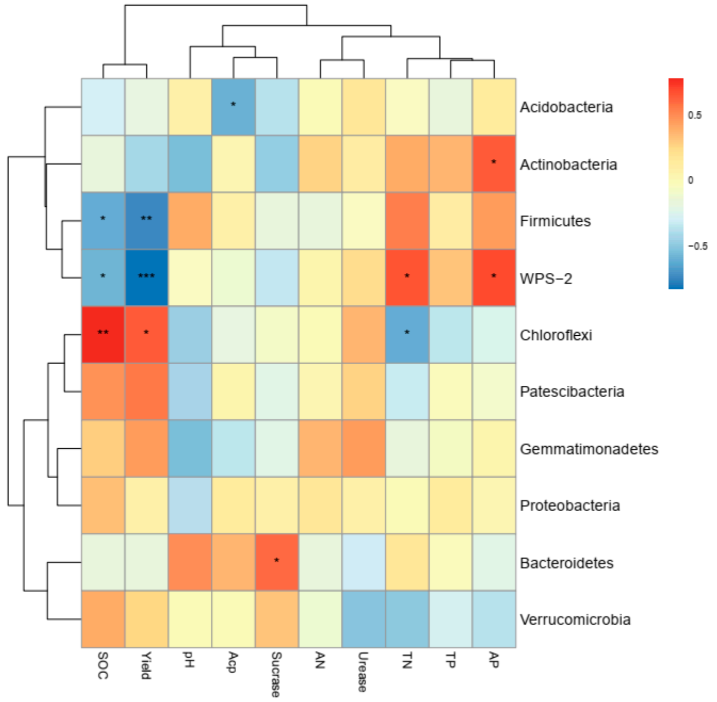

Figure 6. Correlation heatmap of soil bacterial community compositions (phylum level) and soil characteristics under different fertilizer treatments in Lei bamboo forests. Acp, acid phosphatase; AN, alkali-hydrolyzed nitrogen; AP, available phosphorous; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorous. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

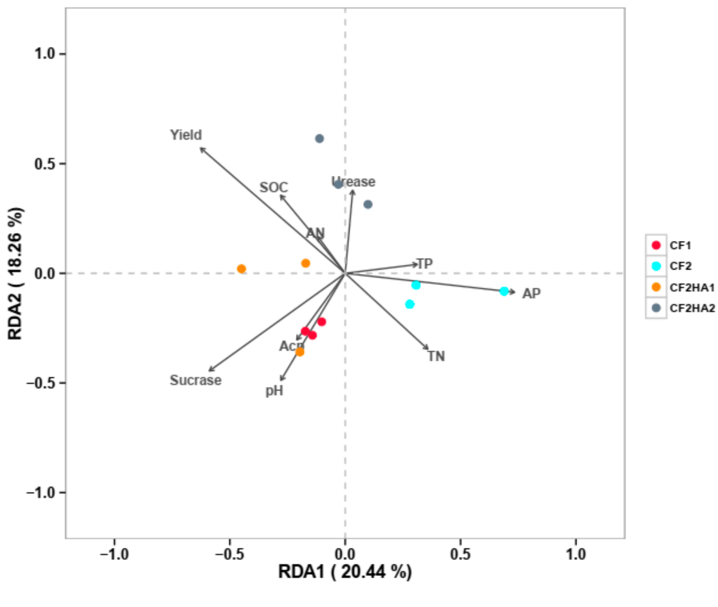

Figure 7. Redundancy analysis (RDA) of soil bacterial community (OTU level) and soil characteristics for individual samples in Lei bamboo forests. CF1 and CF2 = compound fertilizer; CF2HA1 and CF2HA2 = compound fertilizer + water-soluble humic acid fertilizer.

The linear relationships between soil nutrients, enzyme activities, and shoot yield are shown in Figure 8. SOC, TP, and MBN contents were significantly positively correlated with shoot yield. In addition, soil urease, sucrase, and Acp activities were significantly positively correlated with shoot yield. SOC content was strongly positively correlated with MBN but negatively correlated with MBC.

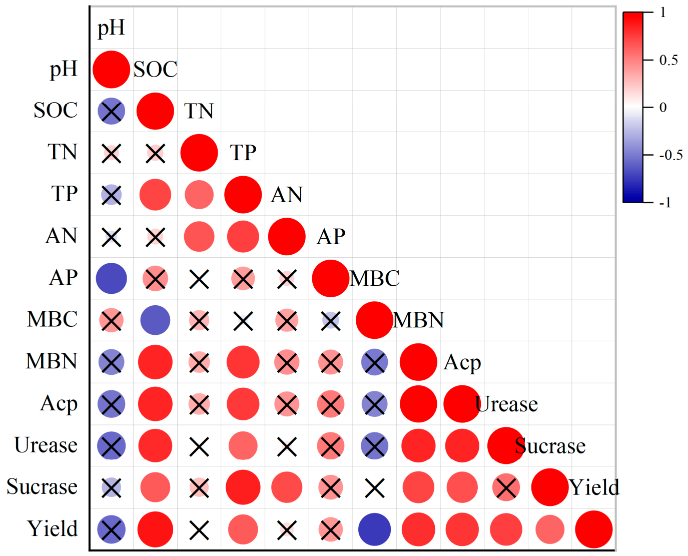

Figure 8. Pearson's correlation for testing the association among soil properties, bacterial diversity indices, and bamboo shoot yield. Red and blue indicate positive and negative correlations, respectively, and × inside the squares indicates insignificant correlations; $p < 0.05$. Acp, acid phosphatase; AN, alkali-hydrolyzed nitrogen; AP, available phosphorous; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorous.

4. Discussion

4.1. Effects of Water-Soluble Humic Acid Fertilizer on Soil Properties and Enzyme Activities

Humic acid changes the soil particle structure through flocculation and uses its strong solubility to control nitrogen, release phosphorus, promote potassium, and reduce soil salt content through its chemical structure in the soil [9,11]. A previous study found that humic acid can decrease soil pH, as well as affect nutrient availability and the absorption of soil nutrients via crop root zones [32,33], which is in agreement with our results. This finding could be explained by the fact that humic acid increases the ability to release and exchange H^+ [34].

In this study, the addition of humic acid fertilizer increased the SOC content and decreased the MBC content. This is likely because humic acid contains organic matter, and nondegradable carbon reduces SOC mineralization by updating the SOC pool, thereby reducing soil CO_2 emissions [35,36]. The decrease in microbial quotient (the proportion of MBC to total SOC) indicates that, with the addition of humic acid, the increase in soil MBC was slower than that of SOC, and the utilization efficiency of carbon sources by microorganisms was reduced, resulting in a decrease in the decomposition rate of organic carbon. In addition, water-soluble humic acid fertilizers contain a large amount of nitrogen, and a high nitrogen input can reduce the carbon utilization efficiency of microorganisms [37]. Humic acid leads to a decrease in the soil water conservation capacity, making it easier for active organic carbon in the soil to be lost [9]. The increased soil AN content observed in our study suggests that humic acid improved the soil nitrogen supply capacity. This is likely because humic acid can form humic acid–urea complexes by reacting its carboxyl and phenolic hydroxyl groups with the amide group of urea, which is highly stable and can inhibit urea decomposition, improve nitrogen utilization efficiency, and achieve the slow long-term release of urea [16]. Meanwhile, humic acid can fix ammonium nitrogen in fertilizers through nonbiological processes and form chemical substances and binding forms represented by indole, pyrrole, amide peptide structures, etc. [38]. After nitrogen is added to the soil, it is quickly utilized by microorganisms, leading to an increase in nitrogen use and MBN. Furthermore, an increase in the microbial stoichiometry of MBC/MBN also demonstrates that humic acid application may exacerbate microbial nitrogen limitation [39]. The soil C/N ratio increased with increasing humic acid content, explicating that the accumulation of SOC was stronger than that of soil N.

Humic acid is an activator and synergist of phosphorus and phosphorus compound fertilizers. The main effect of humic acid substances on phosphorus fertilizers is improving the compound form of phosphorus, that is, activating phosphorus and greatly enhancing its effectiveness in the soil. Our results suggest that humic acid increases soil AP content, which is consistent with the results of previous studies [17,40]. Li et al. [41] showed that the AP content in the soil significantly increased after the application of humic acid and phosphorus fertilizer, and the soil phosphorus fixation rate was reduced by 7.32% compared with when ordinary phosphorus fertilizer was used. The mechanism is as follows: (a) Al^{3+}, Ca^{2+}, and Mg^{2+} are chelated in the soil, inhibiting their binding with phosphorus, decreasing phosphorus fixation, increasing the content of AP in the soil, and slowing down the transformation process of AP to delayed and ineffective phosphorus [42]; (b) the movement of phosphorus in the soil is increased, promoting crop root absorption; and (c) by activating insoluble phosphorus, the effectiveness of TP in the soil can be improved [9].

The cycling and transformation of soil nitrogen, carbon, and phosphorus require the participation of a series of hydrolytic enzymes, such as soil urease, sucrase, and Acp [28]. Urease catalyzes the hydrolysis of nonprotein nitrogen-containing compounds in soil to produce ammonia and carbon dioxide. It can only hydrolyze urea, and its activity reflects the efficiency of soil nitrogen fertilizer utilization. In the present study, there was no significant difference in urease activity among the four fertilizer treatments. This may be related to the sampling time. Research suggests that humic acid can inhibit soil urease activity in the early stages of addition, reduce the rate of urea hydrolysis, and thus reduce the volatilization of ammonia hydrolysis products. However, adding urea at a later stage can stabilize urease activity, allowing urea to continue to be converted into ammonia at a relatively stable rate for plant growth [43,44]. We found that the sucrase and Acp activities were higher in CF2HA2 than in CF1 and CF2. Soil sucrase can catalyze the conversion of sugars into glucose and fructose, and participate in the mineralization of organic carbon, and its activity is closely related to the decomposition and transformation of SOC. The increase in soil Acp activity was closely related to the AP content in the soil. Peng et al. showed that, by applying humic acid compound fertilizer to Chao brown soil where rapeseed was planted, the soil alkaline phosphatase and catalase activities could be improved more than when ordinary compound fertilizer was used, thereby activating phosphorus in the soil and increasing the effective phosphorus content [45]. In addition, Liu et al. confirmed through community experiments that humic acid increases soil Acp and sucrase activities [46].

4.2. Effects of Water-Soluble Humic Acid Fertilizer on Soil Bacterial Community Structure

Soil microorganisms participate in the entire process of soil material cycling and energy conversion, and drastic changes in the physical and chemical properties of soil cause significant changes in microbial quantity, biomass, and activity [47,48]. In our study, water-soluble humic acid fertilizer application changed the bacterial communities by altering the microenvironment, similar to the results in other previous studies [49,50]. Humic acid input resulted in a decline in bacterial diversity (Shannon diversity index), which may be related to the application rate or duration of fertilizer use [51]. Tian et al. reported that the continuous application of organic fertilizer in the short term significantly increased the richness of soil bacterial communities while decreasing their diversity [52]. Under experimental conditions, the application of organic fertilizer stimulated the proliferation of certain bacterial groups with specific resistance, resulting in changes in soil ecosystem stability and a decrease in bacterial diversity.

Previous studies have shown that the dominant bacterial phyla in the soil are Actinobacteria, Bacteroidetes, and Proteobacteria [36]. Meanwhile, our study identified Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria as the dominant bacterial phyla, which agrees with previous results. Fertilization can change the structure and composition of the microbial community. The relative abundance of Proteobacteria, Acidobacteria, and Actinobacteria increased strongly, whereas that of Bacteroidetes decreased strongly after humic acid addition. Proteobacteria and Acidobacteria are important groups that participate in soil C cycling [53]. Proteobacteria tend to grow in nutrient-rich environments, and the application of water-soluble humic acid fertilizers provides sufficient carbon sources for heterotrophic Proteobacteria, promoting their growth and reproduction. The extracellular polysaccharides secreted by Acidobacteria are precursors to the formation of humic substances, indirectly indicating that Acidobacteria are related to the transformation of humic substances, such as SOC [54]. In our previous study, the enrichment of Proteobacteria with humic acid application was mostly attributed to an increase in N fixation and plant improvement [30]. Bacteroidetes is an important group participating in soil P mineralization. Some of its bacterial genera secrete organic acids and phosphatases to convert insoluble phosphorus into two forms of phosphorus, namely $H_2PO_4^-$ and HPO_4^{2-}, which can be absorbed and used by plants [55]. Actinobacteria were enriched in the soil after organic fertilizer addition, contrary to the findings of Yang et al. [56]. This may be because Actinobacteria can rapidly decompose organic matter and convert it into available nutrients (N, P, and K) that plants can use, thereby providing nutrients for plant growth. Therefore, they exhibit good growth and reproduction in environments with high humic acid content.

4.3. Relationships among Soil Properties, Bacterial Communities, and Bamboo Shoot Yields

Humic acid has physical, chemical, and biological properties, such as adsorption, complexation, and oxidation–reduction capacities, which can increase crop yield. Some studies reported that humic acid can serve as a plant growth regulator and promote crop growth [57]. Our findings suggest that humic acid increases SOC and shoot yield. Previous studies have demonstrated that SOC is an important driver of changes in crop yield [58]. Data from 13,662 control field trials were processed in 66,593 treatments across a wide range of soil, climate, and management practices, and crop yield was found to increase with increasing SOC [58]. In the current study, SOC, TP, and MBN contents were found to be significantly positively correlated with shoot yield (Figure 8), indicating that they likely promote shoot growth.

Environmental factors (soil physicochemical properties and enzymatic activities) have a significant impact on the abundance, diversity, and function of soil microorganisms and are important influencing factors in the composition of soil microbial communities. The RDA results showed that AP and MBN were strongly correlated with bacterial communities (Figure 7). Chloroflexi is a phylum of bacteria that produce energy through photosynthesis and can decompose polysaccharides in the soil into organic acids and hydrogen, promoting the degradation of organic matter and cellulose [59]. A correlation heatmap (Figure 6) revealed that the SOC and shoot yield were negatively correlated with Firmicutes and WPS-2, but positively correlated with Chloroflexi. The positive links between SOC, shoot yield, and Chloroflexi demonstrate that Chloroflexi are closely related to C cycling and bamboo forest productivity.

5. Conclusions

Compound fertilizer decrement and water-soluble humic acid fertilizer application altered soil nutrients, enzyme activities, and shoot yield. Humic acid addition increased the contents of SOC, AP, and MBN and the activities of sucrase and Acp, but decreased the contents of AN and MBC. The soil C/N ratio increased with increasing humic acid content, indicating that the accumulation of SOC was likely higher than that of soil N. The top five phyla of the bacterial communities observed in the soil were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi, accounting for 83.62%–86.16%. Furthermore, the addition of humic acid decreased the Shannon diversity indices. Compared with that of compound fertilizer application (CF1 and CF2), compound fertilizer decrement and water-soluble humic acid fertilizer application (CF2HA1 and CF2HA2) significantly increased bamboo shoot yield by 32.33%–40.37% and 41.63%–50.23%, respectively. AP and MBN were important drivers affecting soil bacterial communities, whereas SOC, MBN, and Chloroflexi affected Lei bamboo shoots. Therefore, the addition of humic acid shifted the available N and P soil nutrients, the activities of sucrase and Acp, and the diversity of the bacterial community structure, as well as facilitating shoot yield. Overall, our results provide new insights into how compound fertilizer decrement and water-soluble humic acid fertilizer application could potentially improve soil nutrient availability and shoot yield. The application of humic acid water-soluble fertilizer is of great significance for soil improvement, ecological restoration, and the sustainable management of bamboo forests in the future.

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