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# Production of Bamboo Source Microbial Fertilizer and Evaluate Its Effect on Soil Organic Carbon Fractions in Moso Bamboo Plantations in South China

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**Abstract:** Bamboo shoot processing wastewater (BBPW) is rich in organic matter and organic acids and can be used as a nutrient source for microbial growth and biofertilization. In this study, *Pseudomonas* K22-D and *Terribacillus goriness* CS3 were isolated from bamboo forest soil with plant growth-promoting properties. Biofertilizers were prepared by inoculating bacteria into BBPW, and the effects of their application in a bamboo forest were evaluated. The chemical oxygen demand, TOC, TN, and $NH_4$-N contents decreased after inoculation, indicating that the bacteria were able to degrade macromolecules in BBPW. The BBPW biofertilizer produced by mixed bacteria (CS3 + K22-D) significantly improved the soil organic carbon and mineral-associated organic carbon content and reduced the pH, alkali-hydrolysable nitrogen, available phosphorus, and available potassium content of the soils in the bamboo forest, which might be attributed to the high C:N ratio and microbial synergism in the biofertilizer and the fast growth period of bamboo shoots. Notably, the CS3 biofertilizer significantly increased soil-available phosphorus (90.25%), and the K22-D biofertilizer significantly decreased soil-available phosphorus (70.33%) compared with CK, suggesting that the presence of inorganic phosphorus-solubilizing bacteria can promote soil P. We believe that the return of inoculated bamboo shoot processing wastewater to bamboo plantations can be an eco-friendly, sustainable practice for bamboo forest management.

**Keywords:** *Terribacillus goriness*; *Pseudomonas*; bamboo shoot wastewater; microbial biofertilizer; soil fertility; soil carbon

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## 1. Introduction

Bamboo is widely distributed worldwide, and bamboo forests are “the world’s second-largest forest” type [1]. Notably, there are many bamboo and bamboo shoot processing factories, and they significantly boost local economies. China is the largest producer of bamboo shoots, with approximately 5–6 million tons of bamboo shoots annually [2,3].

The scale of bamboo shoot processing has expanded, and economic benefits have increased, but there are environmental risks [4]. In particular, the water demand for cleaning, boiling, and rinsing in bamboo shoot processing is high, and the seasonality of wastewater discharge is strong. Approximately 2 t of wastewater is discharged per 1 t of boiled bamboo shoots [5]. In China, bamboo shoot processing wastewater (BBPW) is discharged at a rate of approximately 10–12 million tons per year. Direct discharge of untreated bamboo shoot processing wastewater can cause severe water and soil pollution [6,7].

However, unlike industrial wastewater, BBPW contains almost no toxic or harmful substances. It is enriched with organic matter, lignin, cellulose, pectin, fructose, sodium sulfite, or salt [6]. Thus, BBPW resource recovery has substantial potential for sustainability.

Microbial fertilizer that provide nutrient-sourced wastewater have become an important initiative for sustainable development and agricultural production [8]. Microbial fertilizer is a new type of biological fertilizer that contains live beneficial microorganisms (e.g., nitrogen-fixing bacteria, phosphate-solubilizing bacteria, heavy metal ion-absorbing bacteria, and organic matter-decomposing bacteria), which have the advantage of improving soil fertility by maintaining the physical properties of the soil, encouraging the antagonism of plant pathogenic bacteria, and promoting plant growth [9–11]. Another study found that food wastewater can be used as a phosphate-solubilizing bacteria (PSB) liquid fertilizer to promote plant growth and increase the soil phosphorus and catalase activity [10]. This approach of using wastewater as a source of microbial nutrients not only reduces the workload of organic matter degradation in wastewater treatment factories but also provides a new pathway for the resourceful use of organic wastewater [8,11]. However, there is a shortage of germplasm resources and a single-bacterial function of the known biotrophic bacteria, which can only alleviate a certain fertility deficiency in the soil.

Plant growth-promoting bacteria (PGPB) help plants grow by supplying nitrogen, phosphorus, and potassium, and hormones [12]. Screening of PGPB from natural environments has been conducted in many fields [12–14]. Bamboo forests growth-promoting bacteria have received less attention than PGPB. Moreover, many PGPB have enzymatic properties (e.g., proteases, cellulases, lipases, amylases, and extracellular polysaccharides), essential for the degradation of complex organic matter and harmful substances [15–18]. However, according to our review of the literature, no studies have investigated the combination of BBPW and PGPB prepared as microbial fertilizers and applied in the field.

The main objectives of this study were to: (1) isolate PGPB from bamboo plantations and find the suitable conditions for the inoculation of the PGPB in the wastewater of bamboo shoot processing (liquid biofertilizer), and (2) apply the prepared liquid biofertilizers in the bamboo plantation and evaluate their effect on soil fertility. We hypothesized that (1) bacteria isolated from soil can survive in bamboo shoot processing wastewater and can degrade high concentrations of organic matter, and that (2) BBPW-biofertilizer affects soil fertility, with carbon being the primary sensitive element in Moso bamboo plantations soils. This study aims to provide a feasible solution to the pollution problem caused by bamboo shoot processing wastewater and open up a new method of wastewater resource utilization.

## 2. Materials and Methods

### 2.1. Isolation and Identification of PGPB

The soil was diluted to $10^{-2}$, $10^{-3}$, and $10^{-4}$ and incubated for 24–48 h at 30 °C, and 100 μL of the diluent was spread on Luria-Bertani (LB) medium. Microbial colonies obtained from the agar plates were screened for phosphorus solubilization, nitrogen fixation, iron carrier secretion, indole acetic acid, and enzyme function using bacterial functional media (organic phosphorus medium, inorganic phosphorus medium, chrome azurol S assay, Ashby's medium, skimmed milk agar, soluble starch medium, and tryptophan medium). Reference to Supplementary Materials for medium composition. Strains with more than four functions were purified based on the appearance of a clear water ring or a chromogenic reaction on the functional medium; they were designated K22-D and CS3, respectively, and stored at −80 °C (broth to glycerol ratio 7:3, *v*/*v*).

We further extracted and amplified the genomes of the two strains using universal primers (27F: AGAGTTTGATCMTGGCTCAG and 1492R: GGTTACCTTGTTACGACTT) and performed biochemical tests for bacterial identification. The 16S rRNA sequencing of the two strains was performed by Sangon Biotech (Shanghai, China). The obtained 16S RNA sequences were compared with those from NCBI, and the neighbor-joining method was used to construct an evolutionary tree. The strains were conserved at the China General Microbiological Culture Collection Center (CGMCC No. 26735; CGMCC No. 26119).

### 2.2. Wastewater Collection and BBPW-Fertilizer Preparation

Wastewater samples were collected from a bamboo shoot processing factory in Anji County, Zhejiang Province, China (May 2022). The basic physical and chemical characteristics of the bamboo shoot processing wastewater are shown in Table 1. Wastewater samples were stored in a refrigerator at 4 °C for subsequent testing. To ensure homogeneity of the wastewater samples, the wastewater samples were collected from each of the five drums in the anaerobic reactor and mixed into one sample (Supplementary Materials).

The pH of wastewater samples was adjusted to 6.5–7 (2 $mol \cdot L^{-1}$ NaOH); the samples were centrifuged (4000 rpm, 4 °C, 5 min) and filtered through a glass fiber membrane (pore size 0.45 μm) [19]. The supernatant was sterilized in an autoclave (121 °C, 20 min), cooled, and used as a microbial culture for testing, with distilled water as control [20]. Then, the two isolated strains, CS3 and K22-D, were inoculated in sterile wastewater and cultured for 6 days, respectively. Meanwhile, the fermentation products were taken every 24 h under aseptic conditions and filtered through a 0.45 μm filter.

All experiments were performed with five biological replicates. The following parameters were determined: The physicochemical characteristics of the wastewater (Ammonium nitrogen ($NH_4$-N), total phosphorus (TP), and chemical oxygen demand (COD), and turbidity) were determined using a multiparameter water quality meter 5B-6C by Lianhua YongXing Science and Technology Development Co., Ltd. (Beijing, China) (SEPA, 2002). The TN content was determined using a TOC analyzer (Multi N/C 3100, Analytik Jena, Germany). The pH and EC of each sample were measured using a benchtop pH meter (PHS-3E, Shanghai, China) and conductivity meter (Mettler Toledo S30, Shanghai, China), respectively.

**Table 1.** Properties of bamboo shoot processing wastewater.

| | **$COD_{Cr}$ (mg/L)** | **pH** | **EC μs/cm** | **TN (mg/L)** | **$NH_4$-N (mg/L)** | **TP (mg/L)** | **Turbidity (mg/L)** |
|---|---|---|---|---|---|---|---|
| Wastewater | 43,486 | 4.13 | 4630 | 95.96 | 207.65 | 87 | 14.70 |

Total phosphorus (TP), total nitrogen (TN), ammonium nitrogen ($NH_4$-N), chemical oxygen demand ($COD_{Cr}$), electrical conductivity (EC).

### 2.3. Optimization of Various Growth Parameters

The PGPB isolates were grown in LB broth to study the various optimization conditions [21]. One-factor-at-a-time methods [22] with a carbon source (Carboxymethyl Cellulose-Na (CMC-Na), glucose, sucrose, maltose, and $Na_2CO_3$), nitrogen source (Peptone, pancreatic Peptone, urea, and $(NH_4)_2SO_4$), pH (3~9), and inoculation rate (2%, 4%, 6%, 8%, and 10%) were used to determine the optimal environmental conditions for the growth of the CS3 and K22-D strains. Their growth was measured using a fully automated bacterial growth curve meter (Bioscreen C PRO, Helsinki, Finland) at the regular time interval of 24 h at 600 nm.

### 2.4. Field Experiment and Soil Sample Preparation

This study was conducted in Tianhuanping Town, Anji County, Zhejiang Province, China (30°29′ N, 119°42 E). This region has a subtropical monsoon climate, a mean annual temperature of 16.2 °C and an annual precipitation of 1300 mm. The plant types were dog's spine fern, hare's umbrella, lox, white septoria, baoduo grass, and bilberry, and the soil type was *Ferralsols* according to the FAO classification system [4]. Four 10 m × 10 m sampling plots with the same slope, slope surface, and elevation were established for the Moso bamboo plantation (August 2022). Before use, the liquid BBPW-fertilizer was diluted with tap water at a dilution ratio of 15:1 (*v*/*v*). The liquid BBPW-fertilizer was automatically sprayed on the soil surface (thrice at two-week intervals, 450 L/hm$^2$); tap water was the control (no-fertilizer).

In each sampling plot, soil was collected using a 5-point sampling method (April, 2023). To remove visible roots, plant residues, and stones, the fresh soil samples were sieved through a 2 mm sieve. A total of 20 fresh soil samples were collected transported to the laboratory as soon as possible in holding boxes, partly air dried for testing, and partly frozen at −80 °C. Five replicates collected from each group, and five points were collected from each replicate.

### 2.5. Soil Fractions and Soil Sample Analyses

Soil bulk density was determined using the ring knife method. Soil pH was recorded from a soil suspension (with a soil: water ratio of 1:2.5) using a pH meter (PHS-3E; REX, Shanghai, China). Alkali-hydrolysable nitrogen (AN) content was determined using the 1 M NaOH alkali solution diffusion method. Available phosphorus (AP) content was determined using 0.03 M $HCl\text{-}NH_4F$. Available potassium (AK) content was determined using the 1 M $CH_3COONH_4$ solution-flame photometer method. Total soil organic C (SOC) was analyzed using a total organic carbon analyzer (Multi N/C 3100; Analytik, Jena, Germany). SOC fractions were measured using physical fractionation methods. Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) were measured using 5% $Na(PO_3)_6$ dispersion method and sieved over a 53 μm mesh sieve [23]. The aggregate fraction > 53 μm (POC) was dried at 60 °C and weighted, and their carbon content was determined. The second fraction < 53 μm (MAOC) was the difference between the SOC and POC. Soil samples were sieved through a 150 μm sieve, and the total soil organic C (SOC) was analyzed using a total organic carbon analyzer (Multi N/C 3100; Analytik Jena, Germany).

### 2.6. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software (version 26.0). One-way ANOVA and post-hoc Tukey's tests were used to analyze the significance of the variations in wastewater properties and soil properties among the microbial fertilizers. Different letters in all the figures and tables indicate significant differences ($p < 0.05$). Graphs were plotted using the Origin (version 2023b) and R programming language (version 4.1.3). Principal component analysis (PCA) was conducted to analyze the relationships between basic soil properties and soil carbon fractions.

## 3. Results

### 3.1. Isolation and Screening of PGPB

Two bacterial strains with promoting functions were screened from bamboo plantation soil using seven special media, and the size of the clear water circle represented the ability to promote plant growth. The CS3 strain could fix nitrogen, solubilize inorganic phosphorus, and produce proteases, while the K22-D strain exhibited organic phosphorus solubilization, indole acetic acid, iron carrier, and amylase (Figure 1). Phylogenetic analysis and biochemical test revealed that the strains K22-D and CS3 were >99% identical to *Pseudomonas* ($10^{12}$ CFU·mL$^{-1}$) and *Terribacillus goriness* ($10^8$ CFU·mL$^{-1}$) (Table 2, Figure 2A). On the basis of this information, K22-D and CS3 were identified as the species of *Pseudomonas* and *Terribacillus goriness*, respectively.

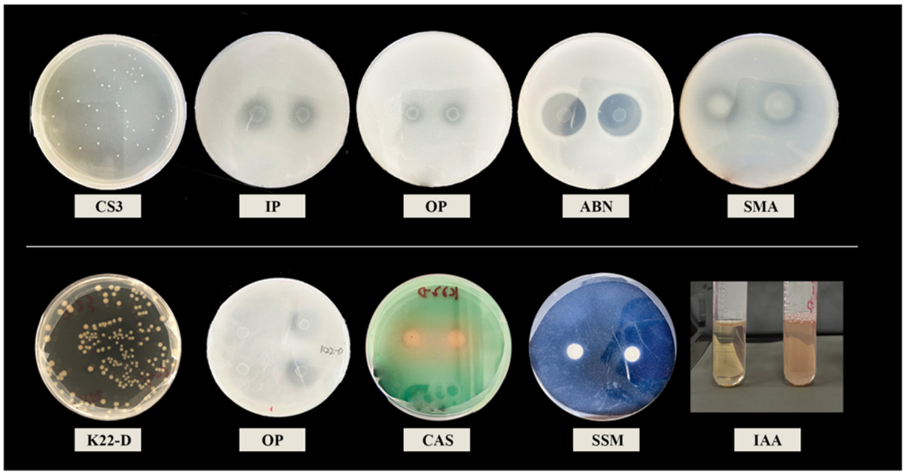
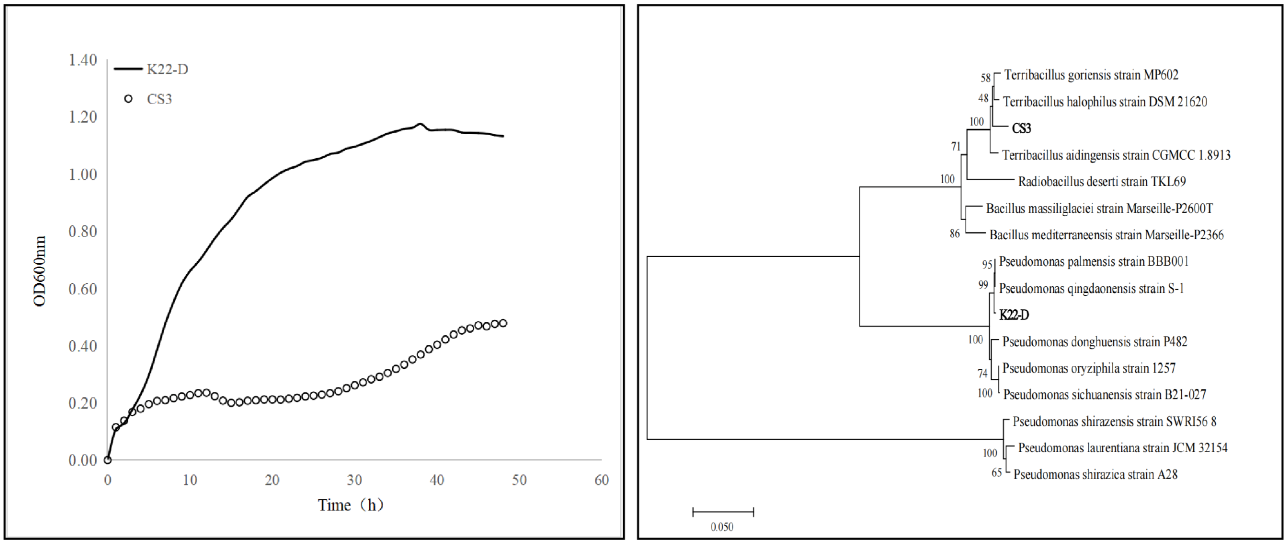


**Figure 1.** The morphology and function of strain K22-D and CS3. Note: IP (inorganic phosphorus medium), OP (organic phosphorus medium), CAS (chrome azurol S assay), ABN (Ashby's medium), SMA (skimmed milk agar), SSM (soluble starch medium), IAA (indole acetic acid).



(A) (B)

**Figure 2.** Phylogenetic tree and growth curves of strain K22-D and CS3. Growth curves OD600 (**A**); Phylogenetic tree (**B**).

**Table 2.** Biochemical test results of K22-D and CS3.

| Characteristics | CS3 | K22-D |
|---|---|---|
| V-P | + | − |
| Citrate | + | + |
| Propionate | + | − |
| Nitrate reduction | − | − |
| D-xylose | − | + |
| L-arabinose | + | + |
| D-Mannitol | + | − |
| Lactose | − | − |
| Glucose | + | + |
| Sucrose | − | − |

| | | |
|---|---|---|
| Gelatine | − | + |
| NaCl > 7% | − | + |
| Gram | + | − |

+ positive; − negative.

### *3.2. Optimization of CS3 and K22-D Culture Conditions*

The liquid fertilizer preparation from culturing the CS3 and K22-D in BBPW, the pH, carbon and nitrogen sources, and the inoculum levels of the two bacteria were investigated in the culture conditions (Figure 3). It displays that CS3 strain had the highest OD600 value utilizing sucrose and peptone as carbon and nitrogen sources and the highest OD600 of CS3 strain at pH 5 (Figures 3A–F). Whereas the K22-D strain had the highest OD600 value utilizing glucose and peptone as carbon and nitrogen sources and adapted to neutral and alkaline environments. The sucrose and peptone were conducive to the growth of bacteria by providing sufficient nutrient (Figures 3G–L).

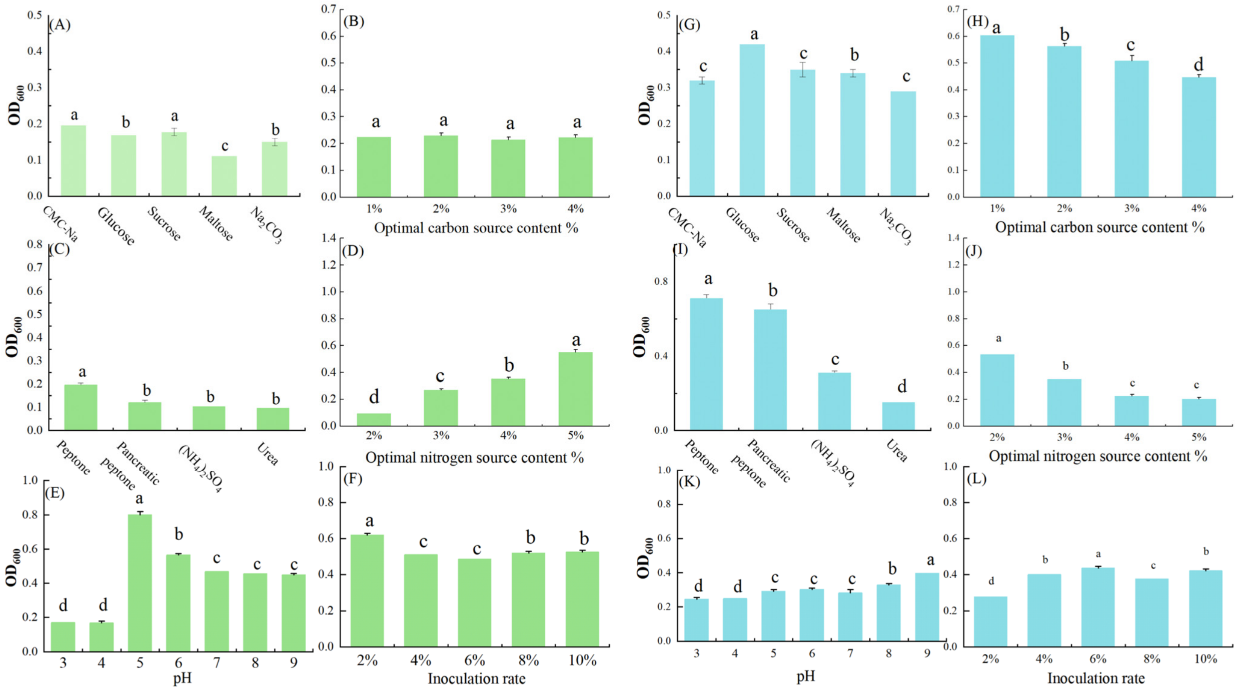


**Figure 3.** Optimization results of strain CS3 (**A**–**F**) and K22-D (**G**–**L**). Note: different lowercase letters indicate significant differences between treatments at $p < 0.05$.

### *3.3. Biological Treatment of the Bamboo Shoot Wastewater*

The growth rate of the K22-D strain in BBPW was significantly higher than that of the CS3 strain, and the mixture of the two bacteria entered the logarithmic growth phase at 40 h (Figure 4A). This finding indicates that the CS3 and K22-D strains could grow gradually in BBPW without any extra nutrients, and the OD600 value increased with time. The concentration of COD was 40.90%–79.40% less than the initial concentration for the single-bacteria and mixed-bacteria groups cultured in BBPW. Moreover, the COD concentration of mixed bacteria was lower than that of single-bacteria groups and gradually decreased to be the lowest (8.47 mg$\cdot^{L-1}$) (Figure 4B). Compared with CK, single-bacterial CS3 and K22-D groups had increased TN concentrations (the maximum values were 3.51 g$\cdot$L$^{-1}$ and 2.45 g$\cdot$L$^{-1}$, respectively), and mixed-bacterial groups were insignificant (Figure 4D). Compared with CK, $NH_4$-N concentration increased initially and then decreased in the single-bacterial CS3 and K22-D group (the maximum values were 1.34

g·L⁻¹ and 1.06 g·L⁻¹, respectively), whereas the mixed group exhibited a continuous decrease with time (Figure 4E). The TP concentrations of the mixed-bacterial cultures in BBPW increased, reaching a maximum at 144 h (from 17.35 to 22.85 mg·L⁻¹), and no significant change was observed in single-bacteria groups (Figure 4F).

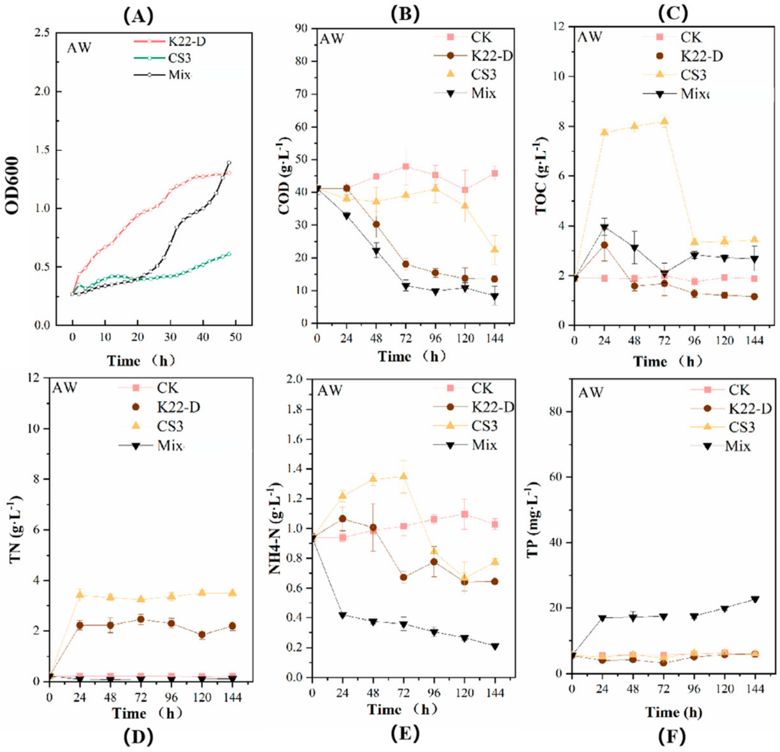


**Figure 4.** Effect of different microorganisms on wastewater properties. CK, no inoculation; CS3: CS3 fertilizer; K22-D: K22-D fertilizer; Mix: K22-D + CS3 fertilizer. OD600 (**A**); COD (**B**); TOC concentrations (**C**); TN concentrations (**D**); $NH_4$-N concentration (**E**); TP concentrations (**F**).

### *3.4. Effect of Different Microbial Fertilizers on Soil Properties and Carbon Fractions*

Soil AN and AK content decreased significantly with application of the three fertilizers compared to no fertilizer. Notably, the mixed fertilizer had the lowest values, decreasing by 6 4.42% and 17.62%, respectively (Table 3). Soil pH decreased significantly with the application of mix bacterial fertilizers, while single-bacterial fertilizers were not significant ($p > 0.05$). Compared with that of no-fertilizer treatment, the AP of the CS3 fertilizer increased by 90.25%, and K22-D and the mixed-bacterial fertilizers decreased by 70.33% and 30.93%, respectively (Table 3).

**Table 3.** Soil fertility in Moso bamboo plantations.

| Treatment | No-Fertiliter | K22-D | CS3 | Mix |
|---|---|---|---|---|
| BD | 1.27 ± 0.23 a | 1.06 ± 0.28 a | 1.37 ± 0.24 a | 1.37 ± 0.12 a |
| pH | 4.89 ± 0.01 a | 4.98 + 0.02 a | 4.87 ± 0.02 a | 4.5 ± 0.11 b |
| SOC (g·kg⁻¹) | 19.12 ± 1.45 b | 15.96 ± 1.16 c | 16.44 ± 0.86 c | 21.71 ± 0.71 a |
| AN (mg·kg⁻¹) | 168 ± 18.91 a | 127.4 ± 7.98 c | 147 ± 10.61 b | 59.78 ± 8.86 d |
| AP (mg·kg⁻¹) | 2.36 ± 0.52 b | 0.70 ± 0.04 d | 4.49 ± 0.81 a | 1.63 ± 0.28 c |
| AK (mg·kg⁻¹) | 94.54 ± 4.31 a | 85.56 ± 4.68 bc | 87.68 ± 9.45 ab | 77.88 ± 14.93 c |

BD: bulk density; SOC: total organic carbon; AP: available phosphorus; AN: available nitrogen. AK: available potassium. Values within the same row followed by the different letters indicate significant difference ($p < 0.05$).

In the mixed-bacterial fertilizers, the SOC content was significantly higher (by 13.55%) than that of no-fertilizer group and lower in the K22-D and CS3 groups than in no-fertilizer group. The MAOC content in the mixed-bacterial fertilizers was higher than that in no-fertilizer, and that in the single-bacterial fertilizers was lower than that in no-fertilizer. The POC content did not change significantly in the microbial fertilizers ($p > 0.05$) (Figure 5).

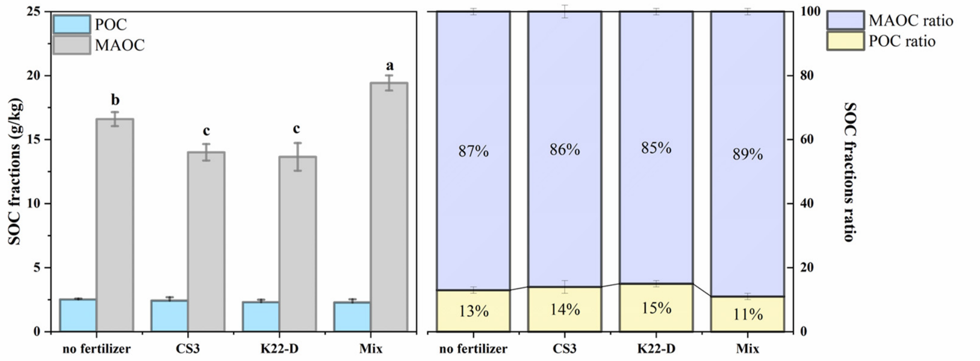
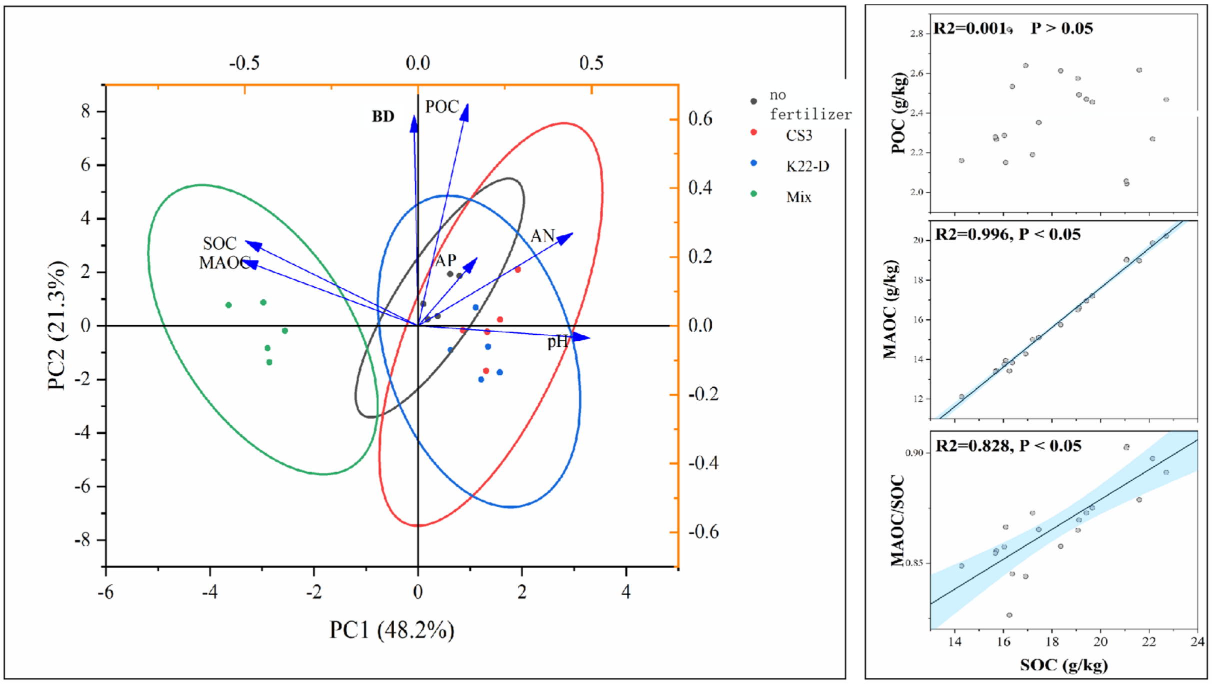


**Figure 5.** Effect of different biofertilizer on soil carbon fractions. Values with different lowercase letters indicate significant differences from carbon content ($p < 0.05$).

### 3.5. Effects of Soil Properties on Soil Carbon Fractions

PCA analysis revealed that pH was significantly negatively correlated with SOC and MAOC in the soils (Figure 6A), and the MAOC content was highly and positively related to SOC (R2 = 0.996, $p < 0.05$) (Figure 6B).



(**A**) (**B**)

**Figure 6.** (**A**,**B**) Principal component analysis (PCA) of soil properties on soil carbon fractions.

## 4. Discussion

### 4.1. Isolation and Screening of Functions Bacteria

We screened two bacterial strains with promoting functions from bamboo plantation soil. The CS3 strain fixed nitrogen, solubilized inorganic phosphorus, and produced proteases. The K22-D strain exhibited organic phosphorus solubilization, IAA, iron carrier, and amylase (30 °C). These findings support those in the literature [24,25]. Phosphate-solubilizing and nitrogen-fixing bacteria can produce IAA, which partially explains why phosphate-solubilizing bacteria can improve the P and N uptake of plants [26,27]. IAA and iron carrier production by bacteria can stimulate plants to grow more root hairs; thus, root secretions are, in turn, directly and indirectly involved in the activation of ion, which promotes the uptake of additional nutrients and alleviates salt stress in plants [26,28]. Phylogenetic analysis revealed (Figure 2A) that the strains K22-D and CS3 were >99% identical to *Pseudomonas* ($10^{12}$ CFU·mL$^{-1}$) and *Terribacillus goriness* ($10^8$ CFU·mL$^{-1}$). K22-D and CS3 function bacteria were further biologically characterized (Table 2, Figure 2B). Based on this information, K22-D and CS3 were identified as the species of *Pseudomonas* and *Terribacillus goriness*, respectively. Although studies have shown that *Pseudomonas* sp. and *Terribacillus* sp. can promote plant growth and degrade polybutylene succinate [29,30], there has been limited research on their use in combination with waste to prepare microbial fertilizers has been limited.

### 4.2. Biological Treatment of the Bamboo Shoot Wastewater

Using BBPW as a biofertilizer can result in renewable resources and an “energy saving” model. As shown in Figure 4A, strains CS3 and K22-D grew gradually in BBPW without extra nutrients, and both bacteria showed synergistic growth promotion. Wastewater is treated at high temperatures, increasing the solubility and liquefaction of organic matter [11]. Macromolecule organic matter is hydrolyzed into small molecules such as disaccharides, monosaccharides, amino acids, and fatty acids [31], which are sufficient to provide nutrients for microorganisms. High-temperature fermentation reduces the infectious activity of harmful bacteria or viruses in wastewater and facilitates more hygiene practical applications [32]. This might explain the survival of strains CS3 and K22-D strains in BBPW.

In the early stages of the single-bacteria and mixed-bacteria cultures in BBPW, the concentration of COD decreased by 40.90%–79.40% compared with the initial concentration. Mixed bacteria showed lower COD concentration than single bacteria, which gradually decreased with time to the lowest value (8.47 mg·L$^{-1}$) (Figure 4B). These results are consistent with the finding of Wang et al. [10], who reported that *Kosakonia cowanii* was inoculated into food wastewater to prepare a phosphate-solubilizing bacteria (PSB) liquid fertilizer. Single and mixed bacteria were conducive to degrading the organic matter in BBPW without additional nutrient sources; thus, it is convenient for microbial utilization [33].

The concentration of $NH_4$-N in single-bacterial groups was higher than that of the initial concentration, and then the $NH_4$-N decreased sharply. These results are consistent with those of Wang et al. [10], who demonstrated that the increase in $NH_4$-N content to microbial mineralization degrades free amino acids and large peptides and observed a decrease to microbial nitrification. However, the $NH_4$-N concentration of the mixed bacteria decreased dramatically over time, suggesting that the interaction of the two bacteria (CS3 and K22-D) releases more out of $NH_3$, $N_2O$, $N_2$ and NOx [34].

The TP concentration of mixed-bacterial cultures in BBPW increased with time and reached a maximum value of 22.85 mg·L$^{-1}$ at 144 h, and single bacteria showed no significant change in BBPW (Figure 3F). Our microbial fertilizer had a higher phosphorus content than the biofertilizer made from a mushroom substrate (13.0 mg) of Zhu et al. [35]. This indicates that mixed bacteria can solubilize organophosphorus in BBPW, and their interaction accelerates the decomposition of organophosphorus compounds, increasing the ease with which elemental phosphorus is released. Studies have shown that microbial growth produces large amounts of organic acids (oxalic, malic, lactic, acetic, citric, and succinic acids) and enzymes (phytase and phosphatase) that play key roles in the release of phosphorus ions [36]. By 2070, 70% of the world's energy is expected to originate from renewable sources. Bioenergy technologies focus on the recovery of bioenergy through the action of microorganisms and their enzymes, as well as the treatment of organic pollutants [37]. Therefore, the microbial fertilizers prepared using BBPW can reduce the operational costs of local bamboo shoot processing wastewater management and achieve promising environmental sustainability.

### 4.3. Effect of Different Microbial Fertilizers on Soil Properties

In our study, the soil pH, AN, and AK decreased significantly with the application of mixed-bacterial fertilizers, but there were no significant changes with single-bacterial fertilizers (Table 3). These findings are consistent with those of Roohi et al. [38], Ben et al. [39], and Mojid et al. [40], which may be the result of acidification due to microbial interactions that decompose organic matter and producing free hydrogen ions. However, other studies have reached the contrasting conclusions. For instance, Kaushik et al. [41] found that irrigation with distillery wastewater caused soil pH to increase, which they attributed to the $K^+$ exchange between wastewater and soil cations, which led to an alkaline hydrolysis reaction and consequent deterioration of the soil structure. Our study discovered that the biofertilizer treatment had no effect on the soil bulk density. In contrast with the findings of Mojid et al. [40], agricultural wastewater with a high salt concentration hindered the dispersion of fine soil particles and lowered soil bulk density. Gou et al. [26] and Romero et al. [42] have found that the application of *E. cloacae* and *Rhizobium chroococcum* fertilizers increased soil TN content, which differs from our findings. On the one hand, we collected the during the explosive growth period of bamboo shoots when the nutrient transportation system was opened, leading to the rapid uptake of soil available nutrients by the bamboo. On the other hand, various nitrogen-fixing bacteria may have completely different Nif A-Nif L systems that control the gene expression of nitrogen-fixing enzymes. *Terribacillus goriness* CS3 was the first autotrophic N-fixation bacterium discovered in bamboo forest soil, and its N fixation regulatory system has not yet been studied. It may differ from other autotrophic nitrogen-fixing bacteria (*Azospirillam brasilense*, *Herbaspirillum seropedicae*, and *Azotobacter vinelandii*) [43]. Thus, the explosive growth period of bamboo shoots leads to the uptake of N and K fixed by microbial fertilizers into the vegetation.

Notably, compared to no-fertilizer, the AP of CS3 fertilizer application increased by 90.25%, whereas the AP of K22-D and mixed fertilizer application decreased by 70.33% and 30.93%, respectively (Table 3), indicating that differences exist in the adaptation of inorganic and organic phosphorus-solubilizing bacteria to the soil and influence the efficiency of phosphorus solubilization. Two reasons can be explained as follows: (1) *Terribacillus goriness* CS3 strain has more function of solubilizing inorganic phosphorus than K22-D strains (Figure 1), which is more favorable for P release. Zhu et al. [35] also applied inorganic phosphorus solubilizing bacterium *P. farinose* FL7 in soybean soil and found that soil AP increased above 200 mg/L, which was similar to the results of the CS3 fertilizer. (2) The optimum pH of CS3 was 5 (Figure 3E). Moso bamboo soil is acidic (pH 4.98), benefiting for phosphorus release by bacteria [25], which may be another reason for the reduced AP in the soil.

### 4.4. Effect of Different Microbial Fertilizers on Carbon Fractions

Moso bamboo planted with mixed microbial fertilizers increased the SOC and MAOC content, with no significant change in POC (Figure 5, Table 2). A possible reason for this result is the biochemical properties of the organic fertilizers. As Samson et al. [44] observed, liquid organic fertilizer favored the accumulation of SOC, especially MAOC. High C: N ratios in liquid swine manure resulted in the gradual formation of MAOC fractions through soil clay particles and microbial decomposition. In the prior subsection, we mentioned that the mixed microbial fertilizer prepared using BBPW had a higher C:N ratio (24) of than liquid swine manure, which was more favorable for the formation of the MAOC fraction (Figure 3). Moreover, the ability of the mixed bacteria to remove COD and $NH_4$-N concentrations from the wastewater was greater (Figure 3E). This mixed-bacterial biofertilizer stimulates microbial activity and promote soil carbon mineralization and soil dehydrogenase activity; thus, synergistic effects among microorganisms should considered [38]. This may also be one of the reasons why the MAOC and SOC contents of single-bacterial fertilizers were lower than those without fertilizers. Furthermore, the metal oxides controlling the change in MAOC are poorly crystalline (Alo) and free (Ald) aluminum oxides, and Al co-precipitates with organic matter, thereby adsorbing organic molecules and isolating them from Moso bamboo soil surroundings [45]. These favorable conditions promote the synthesis and continuous accumulation of MAOC caused by the application of liquid-mixed biofertilizers in Moso bamboo plantations. PCA analysis revealed that pH was significantly negatively correlated with SOC and MAOC in the soils (Figure 6A), and the MAOC content was highly and positively related to SOC (R2 = 0.996, $p < 0.05$) (Figure 6B). These findings support those of Cotrufo et al. [23], who confirmed that soil edaphic factors (pH) control carbon by affecting MAOC. This may help to explain how mixed-bacterial fertilizers can increase the organic carbon content of Moso bamboo plantation soils as the MAOC fractions were most responsive. As a result, our study provides a completely new concept for carbon sequestration in bamboo plantations and demonstrates the potential of microbial fertilizers to replace chemical fertilizers. This minimizes the cost of chemical fertilizers and reduces harm to the environment.

## 5. Conclusions

To develop an environmentally friendly biofertilizer to increase soil fertility, two new strains of phosphorus-solubilizing, nitrogen-fixing, and enzyme-producing bacteria, *Pseudomonas* K22-D and *Terribacillus goriness* CS3, were isolated from bamboo plantations soil. The single and mixed bacteria were inoculated in BBPW, and the mixed bacteria favored the reduction of COD and $NH_4$-N concentrations from wastewater more than the CK, and the CS3 bacteria were able to release more TOC and TN than the CK. Mixed microbial fertilizers could effectively increase the MAOC content and SOC of the soil in the Moso bamboo plantation and soil pH-controlled carbon by affecting the MAOC and higher C:N ratio of microbial fertilizers. Remarkably, the mixed-bacterial fertilizer reduced the soil nutrients due to the fact that available nutrients were absorbed and utilized by bamboo shoots during the explosive growth period. Therefore, microorganisms isolated from bamboo forests can not only degrade of degrading bamboo shoot processing wastewater but can also be prepared as microbial fertilizers and effectively sequester carbon for resource utilization.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15030455/s1. S1. Bacterial culture media; S2. Wastewater Sample Preparation; Figure S1. Anaerobic fermenter.

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