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Effects of Biochar and Plant Growth-Promoting Rhizobacteria on Plant Performance and Soil Environmental Stability

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Abstract: (1) Background: Biochar and plant growth-promoting rhizobacteria (PGPR) are widely used as amendments to increase the availability of nutrients and the diversity of the bacterial community within soil. (2) Methods: In this study, we investigated the effects of biochar and PGPR amendments on plant performance, soil physicochemical property, and soil microbial diversity, as well as their relationship in a Eucalyptus (clone DH32−29) plantation in Guangxi, China. We determined the microbial *AWCD*, Simpson, Shannon, and McIntosh indices, and soil inorganic nitrogen (NH_4^+, NO_3^-), total phosphorus (TP), total potassium (TK), total nitrogen (TN), and plant growth and nutrient concentrations; (3) Results: Biochar-only had a significant impact on soil microbial community function, although the effects on plant performance were limited. PGPR plus biochar was found to significantly increase the diversity indices of soil microbes, as well as soil TK and TP. Besides, soil microbes displayed a preference for carbohydrates rather than other carbon sources. (4) Conclusion: Soil microbial functional diversity responded to changes in plant performance and, therefore, it could indicate soil ecological stability and ecosystem productivity. These findings may suggest that biochar and PGPR could potentially maintain ecological sustainability in the soil and improve plant performance through altering soil physicochemical properties in a eucalyptus plantation.

Keywords: rhizobacteria; eucalyptus; microbial diversity; carbon use; *Bacillus megaterium*; plant growth

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1. Introduction

Eucalyptus species belonging to the family Myrtaceae are mainly distributed in the subtropical region of China following their introduction from Australia in the 1970s [1]. Eucalyptus trees are widely planted in Southern China because of their rapid growth, which can provide prominent pulp and raw wood materials of substantial economic benefits [2]. However, because of its fast growth, short logging cycle, and strong ability to take up soil nutrients (e.g., N, P, K) and water, eucalyptus can rapidly decrease the competitiveness of understory plants and soil quality, further suppressing soil microbial activity and crop productivity [3]. Inorganic fertilizer has been widely applied to meet the heavy demand for soil nutrient input required for eucalyptus growth. However, soil acidification, underground water contamination, nitrate accumulation, and other negative impacts on the soil environment may also arise as a consequence of excessive use of inorganic fertilizer [4]. One potential approach to solving this, meeting the nutrient supply, and maintaining a healthy ecosystem within the soil is the utilization of biological fertilizers composed of beneficial microorganisms and biochar [5–8].

Plant growth-promoting rhizobacteria (PGPR) are beneficial microbes isolated from plant root nodules and rhizosphere soil [9]. These microbes can potentially improve the environment of the soil and growth of the plant by promoting the cycling of nutrients between the plant and soil [10–13]. It is well known that PGPR can improve soil fertility

and quality through direct and indirect mechanisms, such as the fixation of biological N, dissolution of phosphate (P) and potassium (K), and decomposition of agricultural and forestry production residues [14]. Welbaum et al. [15] has reported that when PGPR isolated from plant nodules was applied on crops, it could improve the agricultural soil environment, plant resistance to biotic and abiotic stress, and plant growth rate. Studies on the diverse symbiotic rhizobacteria in leguminous plant nodules have been widely reported [16]. However, the study of N-fixing bacteria in rhizosphere soil is also essential for regulating the plant-soil ecosystem and the growth and development of non-leguminous plants [17]. For instance, Pseudomonas stutzeri A15 is commonly isolated from rhizosphere soil of the family Poaceae, and its application to paddy rice can lead to increases in seedling growth and yield, consequently lowering the costs of agricultural management [18].

Biochar has been widely reported as a potential soil amendment [19] for improving soil quality while increasing soil water and nutrient retention, with the potential to change the composition of the soil microbial community [20,21]. Biochar is beneficial to soil fertility, soil carbon sequestration, as well as soil microbial community diversity [19,22] because of its high porosity, specific surface area (SSA), and cation exchange capacity (CEC) [23,24]. It can also influence soil microbial activity and microbial biomass, which may further change the soil from "fungal based" to "bacterial based" by altering specific communities and functions of the soil microbes [20,25]. The enhancement of the microbial community is mainly ascribed to the shift in abiotic factors following biochar amendment, consequently providing a more favorable environment for the soil microorganisms. These types of effects illustrate that biochar potentially affects the soil environment indirectly rather than directly as a soil conditioner [26]. For instance, the reproduction and metabolism of soil microbes may be partially influenced by nutrients (N, P, K) directly supplied by biochar.

The diversity, composition, and function of the microbial community reflect the changes in the plant-soil ecosystem after disturbance, which is an essential index of soil biological fertility and plays an indicative role in soil ecology and management. Research that evaluates the contents of soil nutrient has shown promise as an indicator of soil-environment response to soil amendments and may provide essential and meaningful information for evaluating the stability of soil micro-environment [20]. Soil microbes have also been reported to have preferences for the type of carbon source, and this pattern of carbon-usage capacity can potentially reflect the functional diversity of the soil microbes [27]. To our understanding, pyrolysis biochar [28] can be used as a potential soil amendment to improve the physicochemical properties of the soil and the yield of the crop [29], and PGPR application may increase the diversity of the soil microbes and the uptake of nutrient by the plant. However, data on the effects of PGPR application on plant performance or yield remains relatively limited on crops [10,11,14], and few studies have applied PGPR and biochar as biofertilizers to amend the micro-environment of the soil in forestry or agriculture ecosystems in the short term. In this study, we evaluated the effects of biochar and PGPR on the stability of the soil micro-environment by directly affecting soil-nutrient supply for microbial growth and activity. Specifically, we studied (1) soil physicochemical property and soil microbial functional diversity; (2) plant performance and nutrient content; (3) the potential relationship between plant and soil following biochar and PGPR treatments in a eucalyptus plantation. We hypothesized that the application of biochar and PGPR would improve the physicochemical property and microbial diversity in the soil, as well as the growth of the plant.

2. Materials and Methods

2.1. Study Site

Our study was conducted in January 2018 at the Guangxi University Tree Nursery in Nanning, Guangxi, China (107°45′ 108°51′ E, 22°13′ 23°32′ N). Mean seasonal temperatures at the study site ranged from −2.4 °C in winter to 40.4 °C in the summer from the year 2005 to 2015 [20]. The average seasonal rainfall is approximately 1304 mm, and the mean annual humidity is 79%. The soil is classified as acidic and metabolic red soil, with a pH in

the range of 4.5–5.5. The soil nutrient content prior to cultivation was SOM from 2% to 3%, TN 0.73 mg g^{-1}, TK 1.33 mg g^{-1}, and TP 0.62 mg g^{-1}.

Eucalyptus was the crop of focus because Guangxi is the most prominent producer of eucalyptus species for the pulp and wood industries, and it contributes about 1/3 of China's timber production annually. We used pyrolysis biochar and PGPR as soil amendments. The biochar was provided by Tairan Organic Fertilizer Company in Henan, China. It was made from waste wheat straw, which was carbonized at 600 °C for 3 h. Biochar was used as a potential soil amendment because the transformation of crop straws (e.g., wheat, paddy rice, sorghum straws) into biochar could reduce agricultural waste in Northern China, especially in Guangxi Province. The target biochar application rate was set at 20.0 t hm^{-2}. The biochar amendment rate and application process were based on our previous study [20]. The basic properties of the biochar are shown in Table 1. At the time of application, the biochar had approximately 0% water content.

Table 1. Basic properties of pyrolysis biochar in our study (C: carbon; EC: electronic conductivity; CEC: cation exchange capacity).

Fixed C (mg g^{-1})	Bulk Density (g cm^{-3})	pH	EC (mS cm^{-1})	CEC (cmol kg^{-1})
650	0.19	10.24	4.68	60.80

PGPR (Strain DU07) was isolated from eucalyptus rhizosphere in solid lysogeny and stored in the Environment Microbial Laboratory in Forestry College, Guangxi University, China, in 2010. It was genotyped as *Bacillus megaterium* (Record number on NCBI: MK391000). The stored bacterial strain DU07 was activated and cultured in liquid lysogeny broth (LB) with shaking (120 r min^{-1}) for six days and then diluted to 5×10^{10} CFU L^{-1} with sterile water After planting, each seedling was irrigated with 2 mL of the logarithmic-phase liquid culture of strain DU07. Sprinkling irrigation was provided during the early establishment stage to prevent mortality resulting from moisture deficiency.

To determine the effects of biochar and PGPR on the contents of soil nutrient and the carbon-usage capacity of the soil microbes, three different treatments were applied to the eucalyptus seedlings on the same day that they were planted. These treatments were PGPR-only, biochar-only, and co-application of PGPR and biochar. Thus, the following amounts of PGPR and biochar were applied: (I) 5×10^{10} CFU L^{-1} PGPR, (hereafter, referred to as MB0); (II) 20.0 t hm^{-2} biochar (B20); (III) 5×10^{10} CFU L^{-1} PGPR plus 20.0 t hm^{-2} biochar (MB20). In addition, a control was also included in which the soil was not subjected to any treatment (M0B0).

The research site was plowed with a cultivator in July 2018 and then divided into three experimental units of 46 m × 10 m each (Figure S1). Each experimental unit was further divided into four blocks of 10 m × 10 m, separated by 2-m buffer strips and one block was kept as the control while the other three were treated with biochar and PGPR. Each block consisted of 25 plots, each measuring 2 m × 2 m. To ensure no contamination in the blocks, a minimum distance of 2 m was kept between any two blocks. The amounts and manners of biochar and PGPR applied were based on our previous research [16]. Bare-root eucalyptus seedlings (mean height of 25 cm) were obtained from Guangxi Dongmen Forestry Center and planted at the study site after all treatments were conducted properly in July 2018. We dug a hole (20 cm × 20 cm × 20 cm) for each seedling after biochar being spread, then refilled the holes with the compound of soil and biochar for planting.

2.2. Field Sampling and Lab Measurements

The top 20-cm soil samples (3 replicates) were randomly collected from each block six months after the planting of the eucalyptus seedlings. Each sample was collected from a different plot within the unit following the method of quadrate sampling (Figure S1). The soil samples were analyzed for nutrient content and microbial functional diversity. Half of the fresh sampled soil was then stored at 4 °C, and the remaining was air-dried for further

analysis. The seedling diameter and height were measured using a band tape and length rod on the same day we collected soil samples.

The carbon-usage capacity and diversity indices of the soil microbes were measured with a MicroStation (Biolog, Biolog MicroStation III, Hayward, CA, USA) and Biolog-Eco plates, respectively [30]. The microbes were cultured in a specified carbon source, and their ability to utilize the carbon source would lead to respiration, growth, and metabolization, eventually resulting in a color change of the tetrazoles (TV) solution from achromatous to violet through oxidation-reduction reaction (Figure S2). Carbon sources in 31 Biolog Eco-plate wells could be divided into six types, including carbohydrate (12 types), amino acid (6 types), carboxylic acids (5 types), multipolymer (4 types), phenolic acids (2 types), and amines (2 types).

A sample (5 g) of fresh soil was added to 45 mL normal saline (0.9%) in a 250 mL moist-heat, sterilized, conical flask, and the mixture was diluted with normal saline to give a final soil concentration of 0.01 g mL^{-1}. After the bacterial suspension was cultured under shaking at 200 r min^{-1} for 30 min and rested for 10 min, 1 mL supernatant liquid was extracted and added to 9 mL sterilized, normal saline for determining. The mixture was incubated at 25 °C for 30 min with shaking at 200 r min^{-1}. It was allowed to stand for 10 min, and 1 mL of the clear liquid was taken and added to 9 mL of sterile normal saline. An aliquot (150 mL) of this diluted sample was added to the Biolog-Eco plate. The inoculated Biolog-Eco plates were incubated at 27 °C, and the absorbance value of the plate was recorded at 590 nm and 750 nm wavelength at 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h after incubation. The expression of soil microbial community metabolism was indicated by *AWCD* (Average well color development) (Formula S1). The absorbance value of each well at 120 h was used to calculate the soil microbial community diversity indices (Formulas S1–S3).

The soil pH and electronic conductivity (EC) were determined with a pH meter (PB−10, Sapeen, Shanghai, China) and a conductivity meter (HI 8733, HANNA Instruments, Kehl am Rhein, Germany). A flow-injection auto-analyzer (Technicon, AA3, Hamburg, Germany) was used to determine the content of inorganic nitrogen (NH_4^+ & NO_3^-) in the soil following digestion with 0.01 mol L^{-1} $CaCl_2$ extraction [31]. Soil and plant foliage total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were also measured. TN was determined via the flow-injection auto-analyzer (Technicon, AA3, Germany) following digestion with H_2SO_4 and $CuSO_4$. TP was colorimetrically measured at 700 nm on a Biotek Synergy H1 microplate reader (Winooski, VT, USA). TK was measured on the flame photometer (Shuangxu, FP6430, Shanghai, China) following digestion with H_2SO_4 and HNO_3.

2.3. Statistical Approach

The effects of biochar and PGPR on the microbial diversity indices, soil-nutrient contents, and the microbial utilization efficiency were evaluated by the one-way analysis of variance (ANOVA) and least significant difference (LSD) in R 3.4.2 [32]. The assumptions of normality of residuals and homogeneity of variances were assessed for all treatments, and data transformation was applied when appropriate to meet the assumptions in soil-nutrient status analysis. The soil microbial diversity indices were calculated according to Formulas S1–S4. The package ggbiplot generated principal component analysis (PCA) in R 3.4.2 after standardizing all data to determine the carbon-source utilization under biochar and PGPR amendment regimens. Potential relationships between microbial carbon use and soil nutrient contents in biochar and PGPR treated soil were analyzed using canonical redundancy analysis (RDA) and Monte-Carlo permutation tests with Canoco 5.0 (https://www.canoco5.com/, accessed on 24 July 2021).

3. Results and Discussion

3.1. Soil Nutrient Contents

This study investigated the effects of biochar and PGPR on the physicochemical properties and microbial functional diversity of the soil and plant growth, all of which are important for the plant-soil ecosystem stability in the first growing season after amendment.

The significant differences in soil TN, TP, TK, and NO_3^- concentrations occurred in biochar and PGPR treated soil at $\alpha = 0.05$ and $\alpha = 0.01$. For soil TN level, a significant ($p < 0.01$) decrease was evident in B20 (0.84 mg g^{-1}) and MB0 (0.90 mg g^{-1}) relative to the control (1.63 mg g^{-1}), whereas no significant difference was observed between MB0 and MB20. Soil TP was significantly ($p < 0.05$) increased in MB20 (0.60 mg g^{-1}) relative to the control (0.24 mg g^{-1}), but no significant differences were observed among MB0, B20, and M0B0. A significant increase in soil TK concentrations was observed for the MB20 treatment (2.71 mg g^{-1}) compared with the control (1.71 mg g^{-1}). For soil NO_3^-, a significant decrease was observed for MB0 (0.01 mg g^{-1}), B20 (0.0094 mg g^{-1}) and MB20 (0.016 mg g^{-1}) relative to M0B0 (0.028 mg g^{-1}). In general, MB20 treatment produced significantly higher soil TP and TK concentrations than the control, indicating an improvement in the accumulation of P and K in the soil following the co-application of biochar and PGPR in the short term (Figure 1a).

The significant decrease in NO_3^- observed for all treatments relative to the control soil was consistent with other studies [33], as NO_3^- was the preferred N form used by both the extrinsic rhizobacteria and eucalyptus seedlings in the early stage for reproduction and growth, respectively. Biochar amendments are known to influence soil N availability, plant N uptake, and/or soil microbial N utilization [34]. NH_4^+ was found to drive the overall effect of PGPR and biochar on the total nitrate and nitrite contents in the soil (Figure 1a), consistent with the fact that the N in NO_3^- rather than in NH_4^+, is the preferred inorganic N for the growing eucalyptus seedlings. The lack of specific adsorption of nitrate by the plants potentially leads to the loss of NO_3^- through diffusing and leaching in soils. The increase in soil TP and TK we observed was consistent with other studies on biochar and soil mixtures [35], as biochar could directly increase the soil nutrient conditions through releasing the nutrient ions from the pores on its surface. PGPR has the potential to solubilize P and K as well as decrease the nutrient competition between microbes and plants in the short term. It is well-known that the content of K in a biochar depends mainly on its raw material, and it is kept at a relatively high level because K is very stable even under high pyrolysis temperature, and a higher TK content is found in charcoal produced from plants than other biological matters. However, the decreased soil TK displayed by the biochar-only treatment might have resulted from the potential microbial consumption of K, and this was supported by the soil microbial metabolism results.

3.2. Diversity Indices of Soil Microbial Carbon Use

No clear effects of PGPR and biochar amendments on microbial Simpson and Shannon indices were observed. There were significant ($p < 0.01$) effects of the co-application and sole application of biochar (B20 & MB20) on *AWCD* and McIntosh indices at 120 h, whereas no significant effect was observed between PGPR only and the control. Besides, the effects of the co-application of biochar and PGPR on *AWCD* and McIntosh were significantly greater than that of the sole application of biochar. The result suggested a positive response by the metabolism rate and evenness of the soil microbial community to the biochar plus PGPR treatment (Figure 1b).

The observed increases in the *AWCD* index over incubation time agreed with the other studies as the volatile concentrates of our high-temperature (600 °C) pyrolysis biochars supplied the potential carbon source (e.g., amino acid) from its mineral ash on the surface for microbial decomposition [36], and PGPR are known to accelerate the metabolism and reproduction of soil microbial communities through increasing soil-nutrient availability [37].

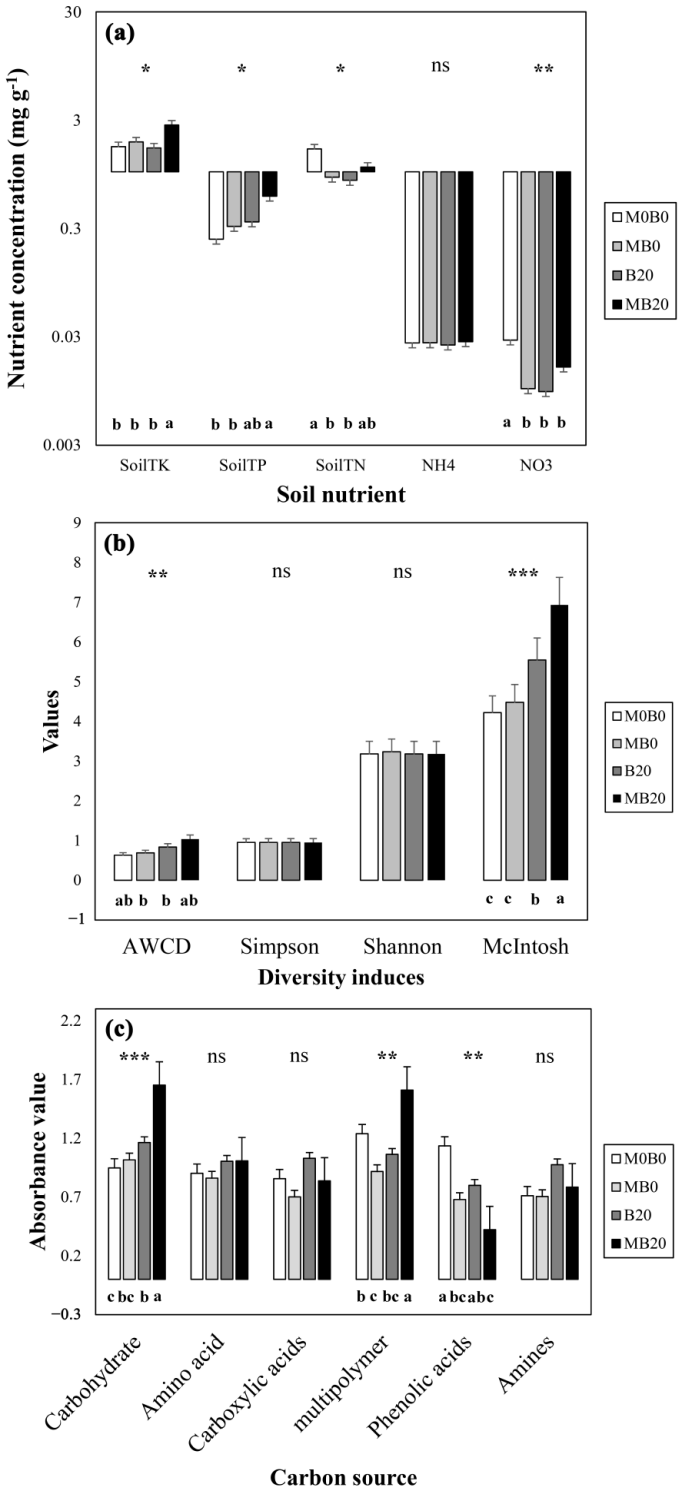

Figure 1. Soil nutrient concentrations, soil microbial diversity indices, and carbon utilization capacity of soil microbes measured in biochar and PGPR treated soils in July 2018 at the Guangxi University Tree Nursery in Nanning, Guangxi, China (treatment N = 3). *, **, *** indicate statistically significant differences among treatments and the control at $\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$, "ns" indicates "no significance". Lowercase letters within panel indicate statistically significant differences among treatments and the control. (**a**): mean (±standard errors) soil nutrient concentrations in PGPR and biochar treated soil, including soil total potassium (soilTK), soil total phosphorus (soilTP), soil total nitrogen (soilTN), soil ammonium nitrogen (NH4), soil nitrate nitrogen (NO3); (**b**): mean (±standard errors) soil microbial diversity indices following biochar and PGPR treatments; (**c**): mean (±standard errors) carbon utilization capacity of soil microbes in PGPR and biochar treated soil.

In general, soil microbial functional diversity increase relative to the control was observed for the biochar-only rather than for the PGPR-only treatment, conflicting with the widespread assumption that PGPR can increase soil microbial diversity. The lack of statistically significant effect of PGPR on soil *AWCD* and McIntosh indices suggested that rhizobacteria may require time to interact with the soil matrix as the mechanism of PGPR affecting the soil environment has been attributed to a time-released manner [38]. It is possible that biochar amendment may produce a more significant effect than PGPR on the carbon-usage efficiency of the soil microbes. This could be due to the biochar used in this study belonging to the type of "amino acid charcoal", which can contribute most to the carbon source supply in the short term [29]. It is also possible that the absence of a significant effect of PGPR may occur outside the study period. This is an important avenue for future study.

Biochar has been reported to improve the soil environment by supplying the organic carbon, accelerating nutrient (N, P, K, etc.) cycling, and by providing more surfaces for microbial attachments [39]. The significant increase in the evenness of the soil microbial community in the co-application of PGPR and biochar treatment suggested that increased soil-nutrient availability may be contributing to the reduction in the competition of nutrient acquisition between the microorganisms and plants in the short term [40]. This is in accordance with the result of soil microbial diversity positively responding to PGPR plus biochar relative to PGPR-alone and biochar-alone.

3.3. Differs in Soil Microbial Carbon Source Utilization

The effects of biochar and PGPR on carbon sources are shown in Figure 1c. For pairwise comparisons, there was no significant ($p > 0.05$) difference between treatments and the control in the cases of amino acids, carboxylic acids, and amines, whereas all treatments significantly influenced the utilization of carbohydrate, multipolymer, and phenolic acids by the soil microorganisms. For carbohydrates, the effects of MB20 and B20 were significant ($p < 0.001$), yielding an increase of 73.68% and 22.1% when compared with the control, while there were no differences among any other treatment comparisons. For multipolymer, the effect of MB20 was significantly ($p < 0.01$) increased relative to the control, whereas a significant decrease was observed in MB0. There was a trend towards a decreased utilization of phenolic acids brought by all treatments, but only a significant difference was observed between MB0, MB20, and the control.

The lack of significant effect of PGPR on carbohydrate utilization observed was consistent with the results reported by other studies [41], since PGPR has the potential to promote plant-root branching, root-hair development, as well as root exudates, which can increase carbohydrates' content in soils, thereby leading to the inhibition of carbohydrate-usage in the microplate. The statistically significant effect of biochar-only and biochar plus PGPR on carbohydrates observed also suggested that biochar application may improve the soil environment for microbial growth through changing the soil active carbon pools after interacting with the soil matrix, leading to the increased growth of autochthonous Carbohydrate-related microorganisms (e.g., Gram-positive bacteria and fungus) [42,43]. Eucalyptus is well-known for its allelopathy to weeds, and one of the main typical and allelochemical organic compounds in the volatile matter is phenolic acids [44]. PGPR treatment with or without biochar tended to show decreased utilization of phenolic acid, suggesting that increased soil pH and decreased soil water retention capacity may also contribute to the neutralization and insolubilization of phenolic acids, and thereby decreasing the microbial utilization of phenolic acids by reducing the relevant substrate. N fixation and P solubilization in non-legumes are closely related to the exopolysaccharides (EPS) in bacteria, especially in plant growth-promoting bacteria [45]. This supports the view that application of PGPR will contribute to decreased microbial use of multipolymer. The increasing biofilms on biochar may contribute to the increased utilization of multipolymer by microbial metabolism and reproduction [46], which is in accordance with the strong response of multipolymer observed for the biochar plus PGPR treatment.

3.4. Specific Carbon Source Utilization

Principal component analysis (PCA) was used to examine the changes in soil microbial function brought about by the biochar and PGPR amendments (Figure 2 & Table 2). In the biplot, all of the variances could be attributed to two PCs, PC1 & PC2, which explained 30.20% and 16.7% of the variation, respectively. According to the PCA result, the first PCA axis (PC1) was mainly negatively correlated with D-mannitol, D, L-a-glycerol, and D-glactonicacid γ lactone. The second PCA axis (PC2) was mainly positively correlated with L-asparagine and r-hydroxybutyric acid while negatively correlated with L-phenylalanine. In general, soil microbes have preference for carbohydrate (PC1(D-mannitol) = −0.30, PC1(D, L-a-glycerol) = −0.29, PC1(D-glactonicacid γ lactone) = −0.29) as a carbon source. Sample separation between B20, MB20, and the control were marked by PC1 & PC2, which occurred in the different quadrants of the biplot, indicating that the sole application and co-application of biochar had a significant impact on soil microbial community function. However, a clear separation between MB0 and the control was not observed, indicating that the PGPR-only treatment did not affect soil microbial functional diversity in the short term.

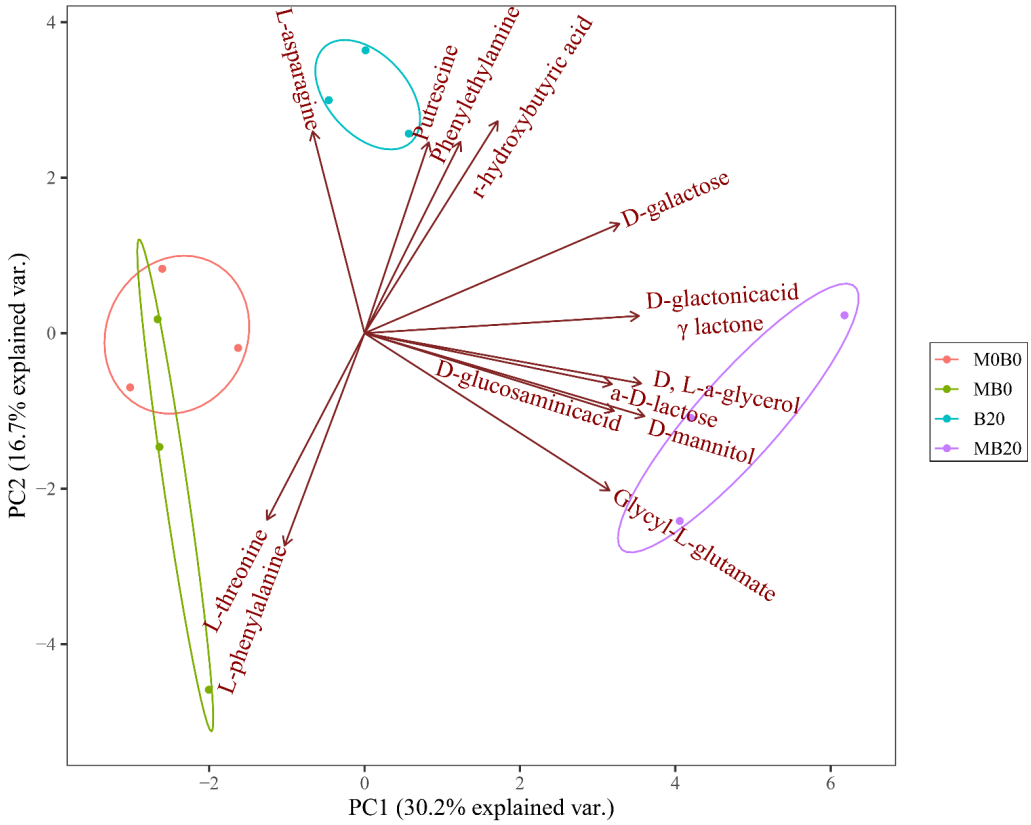

Figure 2. Principal Component Analysis (PCA) of carbon-use capacity in biochar and PGPR treated soils. PC1 accounted for 30.2% of the variance, and PC2 accounted for 16.7%. The carbon sources listed as red arrows in the figure represented the most contribution to PC1 & PC2.

The preferred carbon source in the soil was found to be carbohydrate, consistent with reports that carbohydrate acts as the preferred carbon source for soil microbes in the forestry ecosystem [47]. Our results may be influenced both by the altered physical structure [48], and the increased C: N ratio because of the total carbon supplied by biochars [49]. For example, carbohydrate is the preferred carbon source for microbes in farmland soil because of the sufficient carbohydrate supplied by the humidification of soil organic matter (SOM) [50]. In our case, the abundant SOM provided by the litterfall of eucalyptus plantations likely resulted in the soil microbes preferring carbohydrate as a carbon source.

Table 2. Carbon sources with contribution rates for principal component 1 (PC1) and principal component 2 (PC2) in soils treated by biochar-only, PGPR only, co-application of biochar and PGPR, and the control.

Carbon Type	Order	Carbon Source	PC1	PC2
Carbohydrate	C6	D-cellose	−0.11	0.033
	C7	a-D-lactose	−0.26	−0.072
	C8	ß-methyl D-glycoside	−0.25	0.056
	C9	D-xylose	−0.24	−0.15
	C10	L-erythritol	−0.059	−0.079
	C11	D-mannitol	−0.30	−0.12
	C12	N-acetyl-D-gluosamine	−0.18	0.061
	C14	Glucose−1-phosphate	−0.13	−0.24
	C15	D, L-a-glycerol	−0.29	−0.071
	C16	D-glactonicacid γ lactone	−0.29	0.025
Amino acid	C24	L-arginine	−0.14	0.25
	C25	L-asparagine	0.055	0.29
	C26	L-phenylalanine	0.084	−0.30
	C27	L-serine	−0.22	0.25
	C28	L-threonine	0.10	−0.27
	C29	Glycyl-L-glutamate	−0.26	−0.22
Carbonxylic acid	C1	Methyl pyruvate	0.0025	0.16
	C20	r-hydroxybutyric acid	−0.14	0.30
	C21	Itaconic acid	−0.022	0.15
	C22	a-ketobutyric acid	0.022	0.17
	C23	D-malic acid	0.034	0.15
	C13	D-glucosaminicacid	−0.26	−0.11
	C17	D-galactose	−0.27	0.16
Multipolymer	C2	Tween 40	−0.16	−0.029
	C3	Tween 80	−0.15	−0.087
	C4	a-cyclodextrin	−0.12	−0.048
	C5	Glycogenin	−0.18	−0.10
Phenoliacids	C18	2-hydroxy-benzoic acid	0.19	−0.12
	C19	4-hydroxy-benzoic acid	0.16	0.22
Amines	C30	Phenylethylamine	−0.10	0.27
	C31	Putrescine	−0.068	0.27

3.5. Plant Growth and Nutrient Status

There was no clear effect of PGPR and biochar amendments on plant-stem diameter (Table 3). For plant growth, MB20 had a significant ($p < 0.05$) effect on Eucalyptus height, whereas no significant difference occurred between MB0 and B20. Soil TN was significantly ($p < 0.001$) decreased by 20.03%, 25.48%, and 28.61% in MB0, B20, and MB20, respectively, relative to the control. For plant TP, the effect of MB20 was significant ($p < 0.01$), but there was no difference between either MB0 or B20 treatment and the control. For plant TK, pairwise tests showed significant ($p < 0.001$) increases in MB0, B20, and MB20 relative to the control.

Significant contrasting responses of plant height occurred between the PGPR plus biochar treatment and the control. Increased Eucalyptus height may result from increased CO_2 availability produced by soil respiration, which was accelerated by biochar and the stimulation of meristematic tissue division of plant-stem apex mediated by PGPR. All treatments appeared to have a negative effect on the TN concentration of plant foliar. This may be a result of the rapid adsorption of the available N by the plants, leading to a decreased level of soil inorganic N for all the treatments. The only statistically significant effect on plant foliar TP was its decrease in the biochar plus PGPR treatment relative to the control. The main reason could be the massive accumulation of nutrients in the stems of the eucalyptus for morphosis in the early growing stage, leading to a decrease of total foliar P. The trends toward increased plant foliar TK seen in all treatments suggested that increased soil TK may be contributing to increasing concentrations of plant TK, consistent

with the positive correlation between the availability of soil K and plant TK in our previous study [33].

Table 3. Means (±standard errors, N = 3) for plant growth and foliar nutrient concentrations. Differences in lowercase letters within rows indicate statistically significant differences among biochar and PGPR treatments at $\alpha = 0.05$ level.

Plant Variable	Unit	M0B0	MB0	B20	MB20
Height	cm	91 ± 6.24 b	96.67 ± 6.03 ab	90.33 ± 3.79 b	102 ± 2.65 a
Diameter	mm	10.14 ± 0.58	9.87 ± 1.14	9.17 ± 0.17	11.09 ± 0.98
TN	mg g^{-1}	14.68 ± 0.73 a	11.74 ± 0.59 b	10.94 ± 0.55 bc	10.48 ± 0.52 c
TP	mg g^{-1}	6.91 ± 0.8 a	9.52 ± 0.87 a	10.07 ± 0.1 a	6.28 ± 1.2 b
TK	mg g^{-1}	2.13± 0.023 c	2.27 ± 0.023 b	3.63 ± 0.031 a	2.26 ± 0.028 b

3.6. Relationship between Plant and Soil Parameters

The relationships among all soil and plant nutrient-response variables were investigated, and Figure 3 shows only the statistically significant results ($p < 0.05$; N = 12). Soil TP concentration was negatively correlated with foliar TP, and the predictive strength of the relationship was improved by including biochar and PGPR treatments in the model ($p = 0.0015$, adj. $R^2 = 0.62$) (Figure 3B). Besides, the soil NO_3^- concentration was positively correlated with foliage TN, and the correlation was relatively strong (Figure 3D) ($p = 0.0061$, adj. $R^2 = 0.5$).

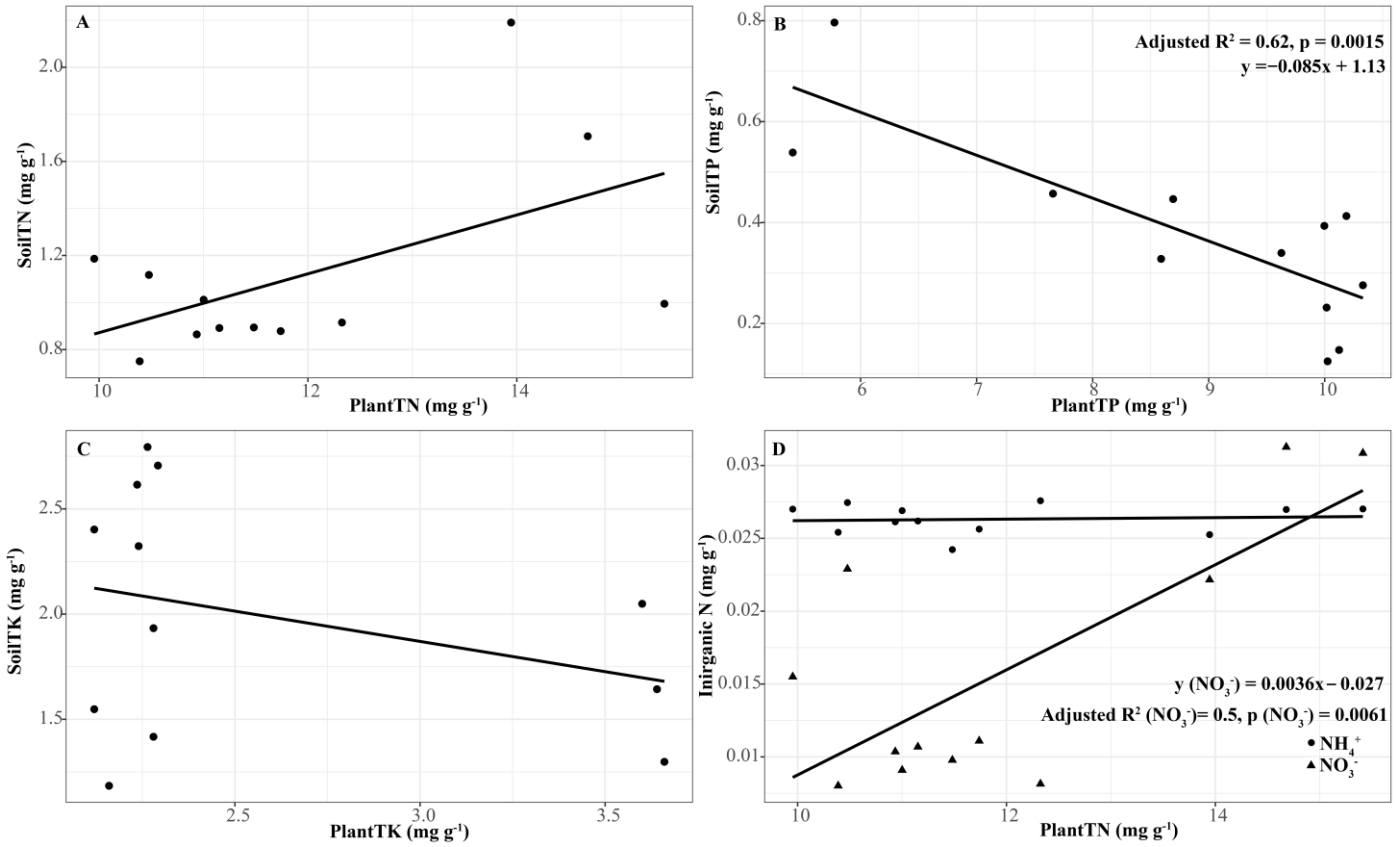

Figure 3. Statistically relationships between plant foliage nutrients and soil nutrient concentrations (N = 12) were determined. Significant effects of biochar and PGPR treatments are shown when adjusted R^2, *p*-value, and fitting equation are present in the figures. (**A**): the relationship between soil TN and plant TN; (**B**): the relationship between soil TP and plant TP; (**C**): the relationship between soil TK and plant TK; (**D**): the relationship between soil inorganic N and plant TN. TK: total potassium, TP: total phosphorus, TP: total nitrogen, Inorganic N: NO_3^- and NH_4^+.

The potential relationship between the carbon-usage capacity of the soil and the soil physiochemical property was analyzed using the canonical Redundancy Analysis (RDA) (Figure 4a). The ordination biplot revealed that the first ordination axis (RDA1) was mainly positively correlated with soil TK and TP and explained 53.92% of the total variability. The second ordination axis (RDA2) was predominantly negatively correlated with NO_3^- and explained 17% of the total variability. Furthermore, the Monte-Carlo permutation test indicated that soil TK (p = 0.004), TP (p = 0.006), and pH (p = 0.034) were significantly related to the utilization efficiency of soil microbial to carbon sources. Remarkably, the effect of soil TP and TK was significant and positive for the majority of microbial carbon source use (especially Carbohydrate), whereas soil pH showed a contrasting trend.

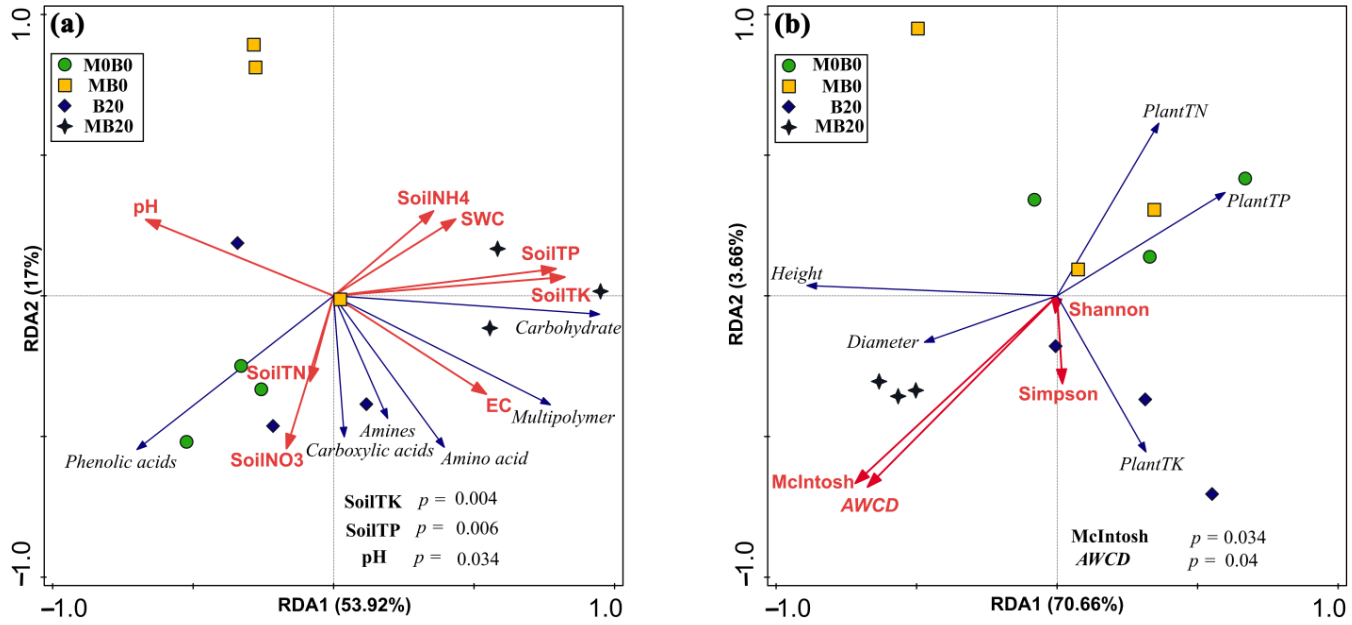

Figure 4. Redundancy analyses (RDA) of the soil carbon use factors and soil physicochemical property factors, as well as microbial-diversity indices and plant growth in biochar and PGPR treated soils. The explanatory variables are indicated by different arrows, soil carbon use factors are indicated by blue lines, and soil physicochemical property factors are indicated by red lines (**a**), while plant growth factors are indicated by blue lines, and soil microbial index factors are indicated by red lines (**b**). SoilTK: Soil total potassium; SoilTP: Soil total phosphorus; SoilTN: Soil total nitrogen; $SoilNO_3$: Soil nitrate-nitrogen; $SoilNH_4$: Soil ammonium nitrogen; EC: Electrical conductivity; SWC: Soil water content; *AWCD*: Average well color development.

RDA of plant parameters and soil microbial diversity (Figure 4b) revealed the first ordination axis to be mainly correlated with *AWCD* and McIntosh indices and explained 70.66% of the total variability. The second ordination axis was strongly associated with the Simpson index and explained 3.66% of the total variability. The Monte–Carlo permutation test indicated that McIntosh (p = 0.034) and *AWCD* (p = 0.034) indices were positively related with plant growth, but negatively with the plant nutrient concentrations.

The positive correlation between soil NO_3^- and plant TN was consistent with other studies that have demonstrated the effect of increased soil NO_3^- on the N concentration of seedling foliar following the application of biochar and PGPR [51,52]. Our data also indicated that soil NO_3^- was the preferred inorganic form of N for the growing eucalyptus, as NO_3^- could drive the accumulation of N in the plant. The negative correlation between soil TP and plant TP was also consistent with the report on the growth of young eucalyptuses, where the seedlings were found to be mainly restricted by the availability of soil P because of the competition in P acquisition between microorganisms and plants.

Biochar and PGPR amendments have been shown to influence the nutrient content, microbial diversity, and microbial community composition of the soil, indicating the possi-

ble existence of a close relationship between soil physicochemical property and the pattern of the microbial metabolism. The significant relationship found between soil TK/TP, and microbial utilization of the carbon (across all treatments) was consistent with other studies whereby the effects of increasing soil TK & TP contents have been shown to accelerate cell wall construction and cell division through low-molecular-weight organic acid (LMWOAs) synthesis, which can further affect soil microbial activity and carbon-usage efficiency [53]. However, the negative effect of soil pH on the carbon usage by soil microbes may provide some guidance for the maintenance of the soil-microbe interaction in the ecological environment of the soil.

The significantly positive relationships between soil microbial *AWCD* or McIntosh indices and plant growth agreed with other studies where increased soil microbial diversity was found to result in increased mung bean dry and wet biomass following the application of rhizobacteria Pb25 [54]. We also noted a negative correlation between soil microbial *AWCD* and plant TN & TP as well as between McIntosh indices and plant TN & TP. The competition between soil and plant P and N may exist because of the low N & P concentrations, whereas a relatively higher level of K available to the soil supplied by the biochar may reduce the competition [20].

4. Conclusions

Our study, which sought to pursue a comprehensive assessment of biochar/PGPR's effects on soil physicochemical property, microbial diversity, and plant performance, illustrates the variable outcomes that could result within six months after amendment. In general, the effect of PGPR plus biochar on the functional diversity response variables of the soil was maximum, as well as positively affecting the majority of soil nutrient accumulation. Carbohydrate was the preferred carbon source for microbial growth and reproduction. The positive correlations between the physicochemical property of the soil and the carbon usage of the microbes in the soil suggested that specific soil nutrients may serve as sensitive soil ecological stability indicators. The relationships between soil microbial diversity and plant growth and nutrient status suggested that soil microbial activity may directly affect plant performance and provide meaningful information on the soil fertility and plant productivity. These findings also verified the ability of biochar and PGPR to affect plant performance and soil microbial ecological stability by altering the physicochemical properties of the soil, pointing to the encouragement of the co-application of biochar and PGPR as a bio-fertilizer to improve the nutrient content as well as the microbial activity and diversity of the soil. Our present results represent the first growing season after biochar and PGPR applications and provide a valuable benchmark to evaluate longer-term response to bio-fertilizer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su141710922/s1. Figure S1: Experimental design in field site. The research site was plowed with a cultivator in July 2018 and then divided into three experimental units of 46 m × 10 m each. Each experimental unit was further divided into four blocks of 10 m × 10 m, separated by 2-m buffer strips, and one block was kept as the control while the other three were treated with biochar and PGPR. Each block consisted of 25 plots, each measuring 2 m × 2 m. To ensure no contamination in the blocks, a minimum distance of 2 m was kept between any two blocks. Figure S2: Carbon source type in the Biolog-Eco plate. There are 96 wells in the Biolog-Eco plate, and further divided into 3, 32 well plots. The plot is considered the unit of replication, and the first well of each plot is set as the blank control without carbon source, while other 31 wells contain tetrazolium blue and specified carbon sources.

Author Contributions: Conceptualization, H.R.; methodology, H.R.; software, H.R. and Z.L.; formal analysis, H.R.; investigation, H.R.; resources, C.L.; writing—original draft preparation, H.R.; writing—review and editing, H.R., Z.L. and H.C.; visualization, H.R.; project administration, J.Z.; funding acquisition, C.L. and H.R. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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