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Biochar-Based Compost Affects Bacterial Community Structure and Induces a Priming Effect on Soil Organic Carbon Mineralization

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Abstract: Urban forests are key to mitigating the Urban Heat Island Effect, which contributes to temperature increases in urban areas. However, the trees in these forests are usually under stress because urban soil is typically degraded. Biochar/compost amendments help with soil management by improving the physiochemical properties and bacterial communities of soil. Here, we compared the physiochemical properties and bacterial communities before and after (1) biochar-only and (2) biochar-based compost amendments. Our results suggested that biochar-only application did not improve soil properties after 1 year of treatment, whereas in the biochar-based compost treatment, the soil properties and bacterial communities changed after just four months. The increase in potassium and decrease in organic material, calcium, and available phosphorus in the soil of the former treatment indicated that the nutrient uptake of its trees had improved. Although there was no significant variation in the soil's total nitrogen, the higher abundance of potential nitrogen-fixing bacteria in the biochar-based treatment suggested that the soil contained a supplement to nitrogen. Our results show that biochar-based compost amendment improves soil quality and associated bacterial communities in urban forest management.

Keywords: biochar; compost; urban tree; soil bacterial community; soil physicochemical property

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1. Introduction

Urban forests have environmental and socio-cultural functions. These greenspaces adjust the microclimate and cool rising temperatures [1–3]. Urban areas are major contributors to air pollution and climate change. The high proportion of impervious surfaces in urban settings results in higher air temperatures than surrounding green fields, known as the urban heat island (UHI) effect [4]. Well-designed urban greenspaces can mitigate this undesirable condition [5]; specifically, plantations of various tree species in an urban forest can enhance the mitigation [6]. However, urban trees are usually planted in soil that is degraded and compacted due to abandoned architectural materials, thus hindering tree growth and reducing the ecological services from these forests.

Soil organic matter and nutrient levels are critical to maintaining urban trees [7–9]. Soil microorganisms play an essential role for mediating soil organic matter decomposition and nutrient cycling [10,11]. Numerous studies have shown that soil microbes are sensitive to plant species composition, biomass, diversity, and soil structure [12–14]. Recent studies also found that tree species diversity in an urban forest affect the soil bacterial and fungal communities, indicating that the design of urban greenspaces influences microbial communities of urban soil [15,16].

Biochar and organic compost amendment have been promoted to improve soil physical, chemical, and biological properties in agriculture for years [17–20]. Biochar as a carbon-rich material produced by pyrolysis can increase soil water-holding capacity [21] and nutrient availability in different types of soil [22,23], and pH in acidic soils [24]. For soil microbes, biochar provides metabolically available carbon sources, which alters the soil microbial activity and community structure [11,25,26]. In addition, the porous structure of biochar provides additional surfaces for microbial growth [17,27]. On the other side, applying organic composted amendment was shown to increase soil organic matter content, stimulating microbial activities and nutrient supply to plants [28,29]. Combined, biochar-based compost amendment improved soil more efficiently than the biochar alone in agriculture [30].

In urban forestry, biochar and organic compost experiments were conducted to evaluate their influences on soil properties and tree growth performance. One result was that a combination of the two had the strongest effects on urban tree soils and growth [31]. Although previous studies have researched the physical and chemical properties of soil after biochar/compost treatments on urban trees [19,31], no study has evaluated the impact of these treatments on the soil microbial community of urban forests.

This study investigated the effects of biochar and biochar-based compost amendments on soil physicochemical properties and bacterial communities associated with nine urban trees collected from two city centers. We added (1) biochar and (2) biochar-based compost to four *Diospyros blancoi* rhizosphere soils in the Kaohsiung Botanical Garden and on the other four urban tree species, including two *Ficus microcarpa*, one *Melia azedarach*, one *Swietenia macrophylla*, and one *Podocarpus nakaii* in Tainan's city center.

2. Materials and Methods

2.1. Preparation of Biochar and Organic Compost

Two biochars, Wood biochar 1 and Bamboo biochar 2, were produced at the pyrolyzing temperature of 500–600 °C and 650 °C, respectively. The two biochars were analyzed and found to have C:N ratios of 138 and 188 (Table 1), respectively. Organic compost was freshly produced from ingredients including mushroom sawdust waste, chicken manure, molasses, and fish powder (Fortune Life Enterprise Co., Ltd., Kaohsiung City, Taiwan); it had 36% organic carbon (C_{org}), 2% total nitrogen (N_{tot}), and 0.02% available phosphorus (Table 1). The biochars were mixed with self-produced organic compost for a final C/N of ca. 25; the ratio of biochar 1 and biochar 2 to organic compost was 1:14 and 1:18, respectively (Table 1). Biochar and organic compost were combined at least two weeks before use.

Table 1. The chemical properties of the biochar and compost in this study.

	N_{tot} (%)	C_{org} (%)	C/N	pH	S_{BET} (m^2 g^{-1})	Ash (%)	H (%)	P (%)
Biochar 1	0.55 ± 0.01	75.86 ± 1.14	138.17	6.55 ± 0.04	0.7	9.6	3.9	-
Biochar 2	0.53 ± 0.00	99.13 ± 0.88	188.27	8.20 ± 0.01	41.8	8.2	2.7	-
Compost	2	36	18	7.9				0.02

2.2. Study Site, Amendment Process, and Sampling Procedures

Nine urban trees distributed in four sites in Tainan or Kaohsiung city of Taiwan were selected for the study: four *Diospyros blancoi* in the Zuoying Indigenous Botanical Garden (22°40′51.3″ N 120°18′10.6″ E), two *Ficus microcarpa* in Tainan Municipal Jhongyi Elementary School (22°59′23.0″ N 120°12′11.1″ E), one *Melia azedarach* and one *Swietenia macrophylla* Tainan Municipal Jhongshan Junior High School (22°59′06.1″ N 120°12′11.8″ E), and one *Podocarpus nakaii* in Tainan Park (23°00′02.4″ N 120°12′38.3″ E). Bamboo biochar (biochar 2) was only added to the soil of two *Ficus macrocarpa* trees and wood biochar was applied in other urban trees.

We aimed to separately investigate the effects of biochar only and biochar with organic compost in combination. Urban forest soil was amended in two parts: first, four *Diospyros*

blancoi were amended with only biochar; then, biochar with compost was added after 1 year. Soil samples were collected for the first time 1 year after biochar amendment and for the second time 4 months after biochar/compost was added. Soil from the other five urban trees was collected directly before biochar/compost was added, and then 4 months later. Five soil cores (0–10 cm depth) were collected as five subsamples around each individual urban tree and then bulked into one composite sample of the spot. Homogenized samples were immediately hand-sieved in the field ($\leq$2 mm) to remove stones, roots, macrofauna, and litter material, and then a partial soil for the microbial analysis was collected in 50 mL Falcon tubes and immediately stored in liquid nitrogen until arriving at the lab. Samples were stored at −20 °C until molecular analysis. The remaining soil samples were air-dried for physicochemical analyses.

2.3. Soil and Biochar Physicochemical Parameters

Total carbon (C) and nitrogen (N) concentrations in biochar were measured by dry combustion at 1000 °C with a CHNS-Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The pH and electric conductivity of rhizosphere soil samples were determined using a WTW Multi 3510 IDS portable meter (Weilheim, Germany). In addition, soil was analyzed for total nitrogen using the Semimacro Kjeldahl method [32]; organic matter using the Walkley–Black procedure [33]; and soil texture [34], inorganic phosphorus, CEC [35], and exchangeable cations (K^+, Mg^{2+}, Ca^{2+}, and Na^+) [36] by atomic absorption spectrophotometry using a Z 5300 instrument following recommendations of the manufacturer (Hitachi—Science & Technology, Tokyo, Japan). The porosity of soil samples was determined by the method described in [37].

2.4. DNA Extraction, Bacterial Amplicon Libraries, and DNA Sequencing

Soil microbial genomic DNA was extracted from approximately 1 g of soil subsample using a MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carsbad, CA, USA) following the manufacturer's instructions. The presence and quantity of genomic DNA was checked using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany); then, the extracts were stored at −20 °C.

The bacterial communities were detected based on the 16S rRNA V6-V8 region. The amplicon libraries were generated using the universal primer pair, 968F/1391R. The PCR reaction was carried out in a total volume of 50 µL containing 1× Taq Master Mix (Genechain Industrial Co., Ltd., Kaohsiung City, Taiwan), 1 mg/mL of BSA, 0.2 µM of each primer, and 2–5 µg of diluted template DNA (final concentration, 100 ng). PCR was conducted using the following program: initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 20 s, 52 °C for 20 s, and 72 °C for 20 s; and a final step of 72 °C for 5 min, then cooling at 4 °C. The PCR products were checked with 1% agarose gel electrophoresis with 1× TAE buffer and SYBR® Green I. The band of expected size (about 424 bp) was cut and purified using a GeneKlean Gel Recovery Kit (Cyrusbioscience, Inc., Taipei City, Taiwan). The purified DNAs were quantified using a NanoDrop spectrophotometer (Thermo Scientific, Vantaa, Finland). In the second round of PCR, individual tags were added to the 5′ends of primers 968F/1391R. The PCR mixture contained 1× Taq Master Mix (Genechain Industrial Co., Ltd., Kaohsiung City, Taiwan), 0.4 µM of each tagged primer, and 100 ng of V6-V8 amplicon for a final volume of 50 µL. The PCR program for tag addition involved an initial denaturation at 94 for 3 min; 20 cycles of 94 °C for 20 s, 52 °C for 10 s, and 72 °C for 30 s; and a final step of 72 °C for 2 min, then cooling at 4 °C. The PCR products were purified and a 200 ng mixture of tagged products was subject to the Illumina HiSeq 2000 sequencing system (San Diego, CA, USA) at Genomics BioSci. & Tech. Co., Ltd. (Taipei, Taiwan).

2.5. Bioinformatics and Statistical Analyses

Paired-end reads were first merged using USEARCH (v8.0.1623) with default settings and then subjected to MOTHUR (v1.35.1) for quality control to retain sequences (1) 400–450 base pairs (bp) long, (2) with one or more homopolymers no longer than eight

bp, (3) without any ambiguous bases, and (4) of an average quality score greater than 20. Chimeric 16S rDNA sequences were detected and removed using UCHIME (implemented in USEARCH) in reference mode and with 3% minimum divergence. Quality-filtered and nonchimeric reads were further analyzed using the UPARSE pipeline [38] to generate Operational Taxonomic Units (OTUs) at the 97% identity level per sample. The OTU representative sequences were searched against the Greengenes 13_5 database using USEARCH global alignment to identify the best hit's corresponding taxonomy. OTUs with no hits or weak hits (i.e., average of sequence identity and alignment coverage less than 93%) were removed from downstream analysis.

We analyzed the bacterial diversity and community structure with R software [39]. Count data were transformed into relative abundance by dividing by sample size. Soil parameters were log(x + 1) transformed for all analyses. The clustering heat map was generated using a gplots package [40]. Nonmetric Multidimensional Scaling (NMDS) based on Bray–Curtis distances was performed using the Vegan package to visualize the distribution of the bacterial community composition, followed by post hoc regression of individual explanatory variables on the ordination scores. Goodness-of-fit values and their significance were calculated using 999 random permutations. Statistical analysis was carried out by one-way analysis of variance (ANOVA). Differences among treatments were examined using Tukey's HSD test.

3. Results

3.1. Effects of Biochar and Compost on Soil Physicochemical Properties

The pre-treatment soil from *D. blancoi* in the botanical garden was a slight alkaline sandy loam and with low total nitrogen (N_{tot}) (ca. 0.09 ± 0.01%), organic matter (OM) (ca. 1.22 ± 0.19%), and cation exchange capacity (CEC) (ca. 5.12 ± 0.14 cmol(+) kg^{-1}). After the amendment with biochar for 1 year, the soil physicochemical parameters collected from *D. blancoi* did not change significantly (Table 2a), but soil porosity seemed to have increased. In terms of the addition of biochar and compost in combination, most of the parameters including OM and phosphorus significantly decreased after 4 months, except K^+, which remarkably increased from *D. blancoi* 1 (Table 2b). In the other site, the soil was mainly slightly alkaline loamy sand with low N_{tot} (0.04–0.1%), OM (0.54–1.84%), and CEC (3.78–5.16 cmol(+) kg^{-1}) in the park (Table 3). After the combination of biochar and compost was added, the soil conductivity of *M. azedarach* and *F. microcarpa* 2 significantly increased, while those of other urban trees including *S. macrophylla* significantly decreased after 4 months. OM generally declined across all trees as phosphorus was only raised in soil of *M. azedarach*. In terms of cations, only K^+ and Mg^{2+} were significantly increased in soil of *M. azedarach*, *P. nakaii*, and *F. microcarpa* 2.

3.2. Response of Soil Microbial Community Composition to Biochar and Compost Addition

A total of 4,860,048 high-quality sequences were obtained across all urban trees; they were classified into 60 phyla, 204 classes, 425 orders, 688 families, 1361 genera, and 20,383 species. The bacterial communities were dominated by *Thermoleophilia*, followed by *Actinobacteria* and *Alphaproteobacteria* at the class level (Supplementary Figure S1). The OTU richness ranged between 4171 and 7845 and the Shannon (H′) diversity ranged between 6.29 and 6.94 (Supplementary Table S1). The OTU richness and Chao1 and Shannon H′ diversity indices of *D. blancoi*-associated bacteria did not significantly change after the addition of biochar or the combination of biochar/compost (Figure 1a). However, a trend of increasing OTU and Chao1 over the treatment period was observed. For the other four urban tree species, *F. microcarpa*, *M. azedarach*, and *S. macrophylla* showed a trend of increasing H′ diversity, whereas *P. nakaii* showed decreasing H′ diversity in soil (Figure 1b).

Table 2. Physicochemical properties of *Diospyros blancoi* soil responding to the amendment with (a) biochar and (b) biochar/compost in combination.

(a)	Conductivity (µS/cm)	pH	N_{tot} (%)	OM (%)	Phosphorus (mg/kg)	K^+ (cmol (+)kg^{-1})	Na^+ (cmol (+)kg^{-1})	Ca^{2+} (cmol (+)kg^{-1})	Mg^{2+} (cmol (+)kg^{-1})	CEC (cmol (+)kg^{-1})	Sand (%)	Clay (%)	Silt (%)	Porosity (%)
D. blancoi (0)	244.2 ± 36.16^{a}	7.89 ± 0.15^{a}	0.09 ± 0.01^{a}	1.22 ± 0.19^{a}	53.53 ± 3.75^{a}	0.39 ± 0.08^{a}	0.67 ± 0.19^{a}	9.24 ± 2.15^{a}	1.22 ± 0.01^{a}	5.12 ± 0.14^{a}	74 ± 3.0^{a}	14 ± 0.3^{a}	12 ± 2.6^{a}	47.59 ± 3.42^{a}
D. blancoi (+)	240.9 ± 10.24^{a}	8.03 ± 0.02^{a}	0.08 ± 0.00^{a}	1.11 ± 0.08^{a}	53.80 ± 1.99^{a}	0.29 ± 0.01^{a}	1.07 ± 0.30^{a}	13.53 ± 1.96^{a}	1.30 ± 0.03^{a}	5.53 ± 0.48^{a}	68 ± 3.0^{a}	14 ± 2.2^{a}	18 ± 1.3^{a}	50.06 ± 2.28^{a}
(b)														
D. blancoi 1I	235.7 ± 0.67^{a}	8.07 ± 0.01^{a}	0.08 ± 0.00^{a}	1.34 ± 0.05^{a}	58.27 ± 1.74^{a}	0.33 ± 0.24^{b}	0.45 ± 0.10^{a}	9.89 ± 4.54^{a}	1.29 ± 0.03^{a}	5.30 ± 0.35^{a}	74	10	16	
D. blancoi 1II	71.3 ± 3.3^{b}	7.80 ± 0.03^{b}	0.07 ± 0.01^{a}	0.75 ± 0.12^{b}	34.87 ± 6.33^{b}	1.59 ± 0.01^{a}	0.17 ± 0.01^{a}	0.87 ± 0.66^{b}	0.19 ± 0.13^{a}	2.54 ± 0.81^{a}	87	4	8	
D. blancoi 2I	217.3 ± 0.33^{a}	8.04 ± 0.02^{a}	0.07 ± 0.00^{a}	0.98 ± 0.01^{a}	49.82 ± 4.59^{a}	0.25 ± 0.08^{a}	1.79 ± 0.42^{a}	12.7 ± 0.23^{a}	1.24 ± 0.02^{a}	6.67 ± 1.32^{a}	71	14	15	
D. blancoi 2II	195.2 ± 7.9^{b}	7.86 ± 0.01^{b}	0.05 ± 0.01^{a}	0.79 ± 0.03^{b}	44.63 ± 4.67^{a}	1.19 ± 0.29^{a}	0.12 ± 0.05^{a}	0.26 ± 0.05^{b}	0.47 ± 0.35^{a}	3.06 ± 0.55^{a}	88	2	10	
D. blancoi 3I	266.7 ± 0.33^{a}	7.99 ± 0.03^{a}	0.07 ± 0.00^{a}	0.97 ± 0.21^{a}	55.94 ± 1.00^{a}	0.29 ± 0.12^{a}	0.75 ± 0.10^{a}	12.44 ± 0.64^{a}	1.31 ± 0.01^{a}	4.37 ± 0.23^{a}	68	12	20	
D. blancoi 3II	117.5 ± 5.4^{b}	7.95 ± 0.05^{a}	0.06 ± 0.01^{a}	0.78 ± 0.01^{a}	22.26 ± 1.22^{b}	0.08 ± 0.00^{a}	0.19 ± 0.03^{a}	0.27 ± 0.13^{b}	0.37 ± 0.21^{a}	5.37 ± 0.90^{a}	85	2	12	
D. blancoi 4I	243.7 ± 1.20^{a}	8.01 ± 0.04^{a}	0.08 ± 0.00^{a}	1.16 ± 0.06^{a}	51.16 ± 4.60^{a}	0.27 ± 0.04^{a}	1.29 ± 0.64^{a}	19.09 ± 0.94^{a}	1.37 ± 0.00^{a}	5.76 ± 1.16^{a}	60	20	20	
D. blancoi 4II	233.7 ± 1.7^{b}	8.12 ± 0.01^{a}	0.07 ± 0.01^{a}	1.04 ± 0.02^{a}	25.02 ± 5.15^{b}	2.24 ± 0.00^{a}	0.21 ± 0.01^{b}	0.34 ± 0.07^{b}	0.82 ± 0.18^{b}	3.48 ± 0.12^{a}	89	4	6	

(0) = before the addition of biochar; (+) = after the addition of biochar (n = 4); I = before the addition of biochar/compost in combination; II = after the addition of biochar/compost in combination; 1-4 = 4 individual *D. blancoi* urban trees. Different lower-case letters denote significant difference.

Table 3. Physicochemical properties of the soil of the urban tree species including *Swietenia macrophylla*, *Melia azedarach*, *Podocarpus nakaii*, and *Ficus microcarpa* responding to the amendment with biochar/compost in combination.

	Conductivity (µS/cm)	pH	N_{tot} (%)	OM (%)	Phosphorus (mg/kg)	K^+ (cmol (+)kg^{-1})	Na^+ (cmol (+)kg^{-1})	Ca^{2+} (cmol (+)kg^{-1})	Mg^{2+} (cmol (+)kg^{-1})	CEC (cmol (+)kg^{-1})	Sand (%)	Clay (%)	Silt (%)
S. macrophylla I	184.5 ± 0.69^{a}	7.81 ± 0.05^{a}	0.04 ± 0.00^{a}	0.83 ± 0.04^{a}	106.82 ± 3.19^{a}	0.25 ± 0.08^{a}	1.92 ± 0.36^{a}	18.12 ± 0.54^{a}	1.18 ± 0.13^{a}	5.06 ± 0.92^{a}	86	9	5
S. macrophylla II	134.4 ± 1.9^{b}	7.74 ± 0.09^{a}	0.06 ± 0.01^{a}	0.41 ± 0.21^{a}	79.48 ± 5.04^{b}	0.35 ± 0.03^{a}	0.26 ± 0.01^{a}	0.29 ± 0.03^{b}	0.87 ± 0.09^{b}	3.79 ± 0.50^{b}	88	6	6
M. azedarach I	139.9 ± 0.25^{b}	7.00 ± 0.02^{b}	0.10 ± 0.00^{a}	1.84 ± 0.03^{a}	78.45 ± 3.81^{b}	0.20 ± 0.05^{b}	0.81 ± 0.16^{a}	2.78 ± 1.47^{a}	0.71 ± 0.18^{a}	3.78 ± 0.98^{a}	85	7	8
M. azedarach II	163.5 ± 0.3^{a}	7.18 ± 0.04^{a}	0.08 ± 0.00^{b}	1.40 ± 0.08^{b}	126.42 ± 10.30^{a}	0.78 ± 0.00^{a}	0.28 ± 0.01^{a}	0.92 ± 0.11^{b}	1.23 ± 0.17^{a}	3.77 ± 0.21^{a}	88	8	4
P. nakaii I	134.1 ± 0.23^{a}	6.40 ± 0.04^{b}	0.07 ± 0.00^{a}	1.43 ± 0.04^{a}	145.06 ± 3.69^{a}	0.25 ± 0.05^{b}	0.82 ± 0.07^{a}	2.68 ± 0.56^{a}	1.07 ± 0.03^{b}	4.26 ± 0.01^{a}	81	9	9
P. nakaii II	112.3 ± 0.2^{b}	6.95 ± 0.08^{a}	0.08 ± 0.01^{a}	0.65 ± 0.22^{b}	101.46 ± 24.90^{b}	1.43 ± 0.43^{a}	0.16 ± 0.03^{a}	0.84 ± 0.22^{b}	1.36 ± 0.40^{a}	3.85 ± 0.88^{a}	89	6	5
F. microcarpa 1I	276.7 ± 0.33^{a}	7.94 ± 0.03^{a}	0.04 ± 0.00^{a}	0.54 ± 0.02^{a}	71.44 ± 2.07^{a}	0.45 ± 0.08^{a}	1.35 ± 0.76^{a}	10.00 ± 2.020^{a}	0.66 ± 0.02^{a}	4.23 ± 3.77^{a}	84	10	6
F. microcarpa 1II	230.7 ± 0.3^{b}	7.85 ± 0.04^{a}	0.06 ± 0.01^{a}	0.15 ± 0.04^{b}	72.70 ± 5.30^{a}	0.47 ± 0.15^{a}	0.4 ± 0.97^{b}	1.23 ± 0.49^{b}	0.97 ± 0.21^{a}	4.37 ± 0.63^{a}	84	12	4
F. microcarpa 2I	232.3 ± 0.33^{b}	7.83 ± 0.09^{a}	0.04 ± 0.00^{a}	0.78 ± 0.02^{a}	118.77 ± 3.94^{a}	0.50 ± 0.06^{a}	1.72 ± 0.28^{a}	8.80 ± 2.78^{a}	1.23 ± 0.01^{b}	5.16 ± 1.46^{a}	83	7	10
F. microcarpa 2II	282.3 ± 0.9^{a}	7.77 ± 0.01^{a}	0.05 ± 0.00^{a}	0.57 ± 0.06^{b}	106.55 ± 10.09^{a}	0.55 ± 0.02^{a}	0.55 ± 0.01^{b}	1.58 ± 0.38^{b}	1.28 ± 0.03^{a}	3.89 ± 0.53^{a}	74	18	8

I = before the addition of biochar/compost in combination; II = after the addition of biochar/compost in combination; 1-2 = 2 individual *F. microcarpa* urban trees. Different lower-case letters denote significant difference.

The NMDS ordination plot indicated that the urban tree-associated soil bacterial community composition changed when biochar only or a combination of biochar/compost was added (Figure 2) (Stress = 0.09). Specifically, the amendment with biochar/compost yielded a remarkable shift in the *D. blancoi* (Diospyros) soil. In addition, different biochar substances, i.e., bamboo and wood biochars, also influenced soil bacterial assemblages as *F. microcarpa* (Ficus) treated with bamboo biochar were discrete from other urban trees treated with wood biochar on the ordination plot. Furthermore, the heat map was applied to demonstrate the relative abundance of the 60 dominant bacteria species associated with individual urban trees across treatments (Figure 3). Specifically, the relative abundance of the following increased after the addition of biochar and compost in combination: *Bradyrhizobium elkanii* associated with *F. microcarpa* 1 (Ficus_1) while *Nitrospira* associated with *P. nakaii* (Podocarpus) and *Bacillus* with *M. azedarach* (Melia).

3.3. Soil Physicochemical Parameters Shaped Urban Tree-Associated Bacterial Community Composition

NMDS ordination based on bacterial community composition associated with the post hoc correlation among soil physicochemical parameters indicated that soil parameters—including pH, sand percentage, available phosphorus (P), total nitrogen (N_{tot}), organic matter (OM), and electrical conductivity (EC)—significantly triggered the bacterial community composition across treatments (Figure 4). Specifically, soil pH was the most significant variable explaining the shifts in bacterial assemblages across treatments, followed by P and silt percentage. In addition, biochar type also seemed to explain the variance in bacterial assemblages, as *F. microcarpa* (Ficus) was distributed separately from others on the ordination plot.

Furthermore, we found that the change in bacterial composition might have included the emergence of several potential nitrogen-fixing bacterial groups (Figure 5). In urban tree *D. blancoi* soil samples, *Paenibacillus*, *Rhizobiales*, and *Frankiaceae* had clearly increased after the treatment, whereas *Frankia*, *Hyphomicrobiaceae*, and *Azospirillum* were increased in the soil of the urban tree *F. microcarpa*. Similarly, the bacteria *Azospirillum* increased in the soil samples of *M. azedarach* and *P. nakaii*, but not in *S. macrophylla*, while the relative abundances of *Frankia* and *Hyphomicrobiaceae* increased dramatically in *S. macrophylla* after treatment.

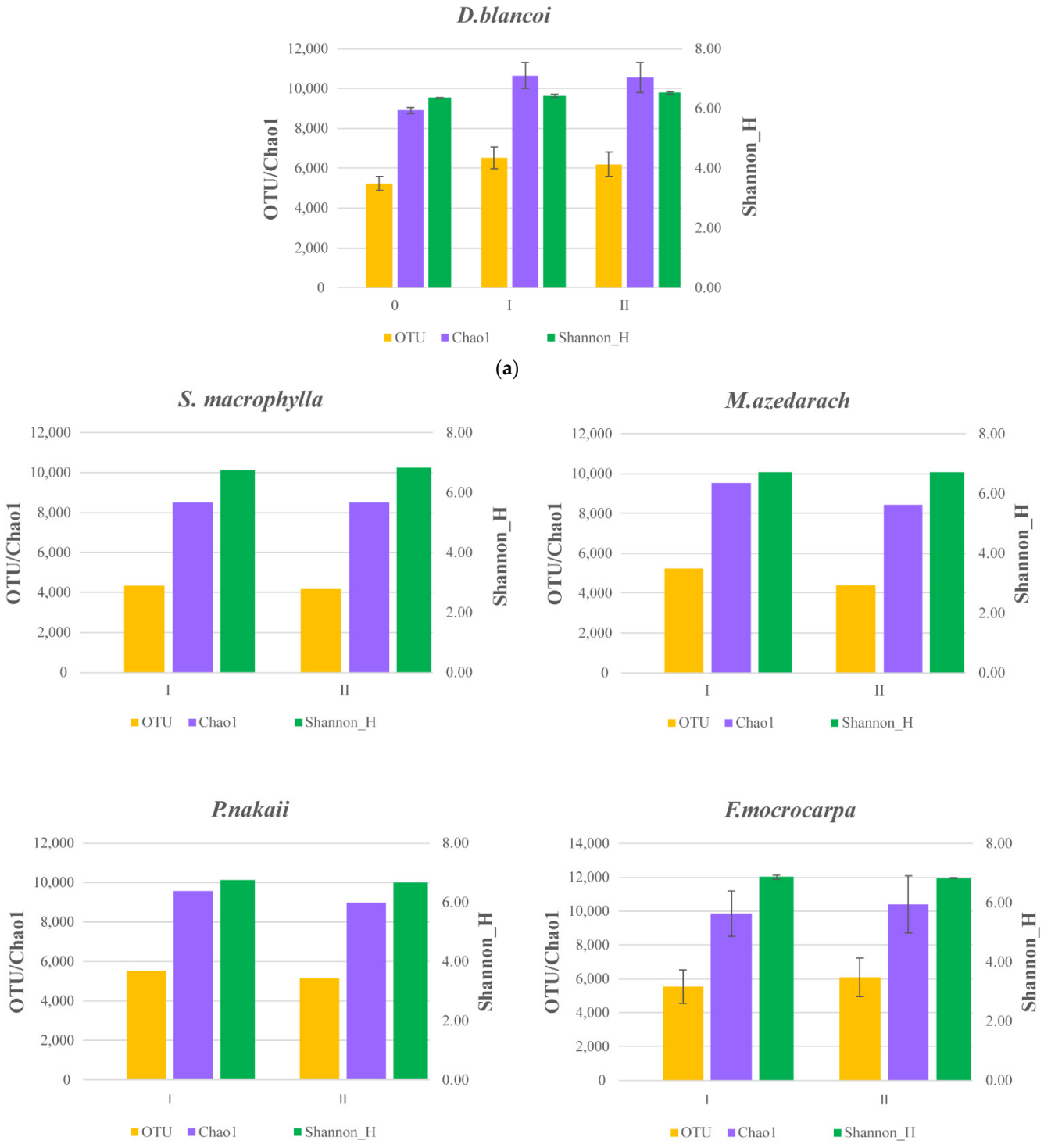

Figure 1. The change in the OTU richness, Chao1, and Shannon H′ of the bacterial community composition associated with (**a**) *D. blancoi* (*n* = 4) and (**b**) *S. macrophylla* (*n* = 1), *M. azedarach* (*n* = 1), *P. nakaii* (*n* = 1), and *F. microcarpa* (*n* = 2) across treatments at both sites. 0 denotes the original soil; I and II denote soil after the addition of biochar and biochar/compost in combination, respectively. Different lower-case letters denote significant difference.

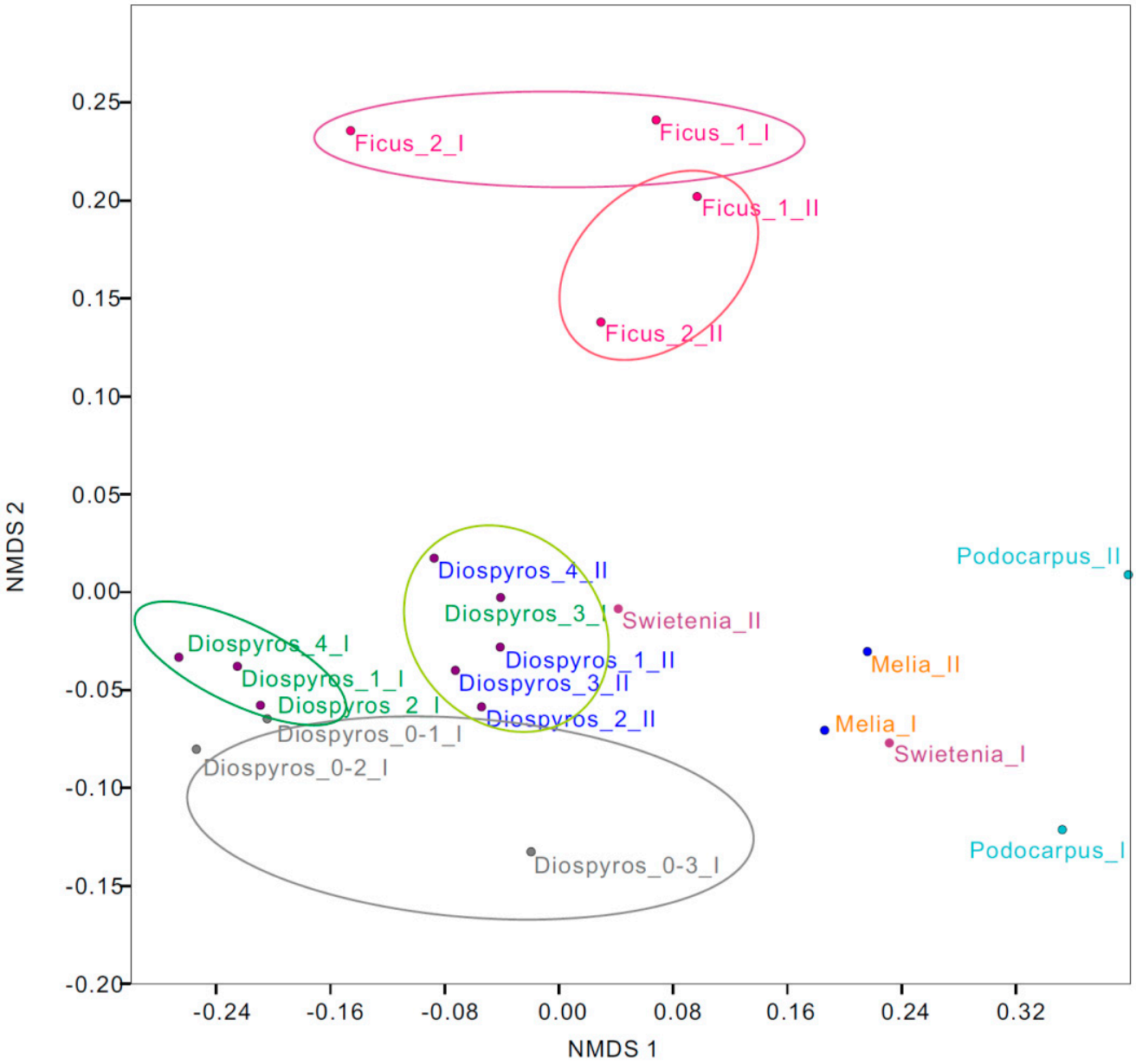

Figure 2. Nonmetric multidimensional scaling (NMDS) ordination of the nine urban trees at two study sites across the treatments based on bacterial communities. Diospyros = *D. blancoi*, Swietenia = *S. macrophylla*, Melia = *M. azedarach*, Podocarpus = *P. nakaii*, and Ficus = *F. microcarpa* across treatments at both sites. 0–1~0–3 denote the original soil; I and II denote soil after addition of biochar and of biochar/compost in combination, respectively. Stress = 0.09.

Figure 3. The heat map of the 60 dominant bacteria species associated with individual urban trees across treatments. Diospyros = *D. blancoi*, Swietenia = *S. macrophylla*, Melia = *M. azedarach*, Podocarpus = *P. nakaii*, and Ficus = *F. microcarpa*. Pre denotes the original soil; I and II denote soil after the addition of biochar and of biochar/compost in combination, respectively.

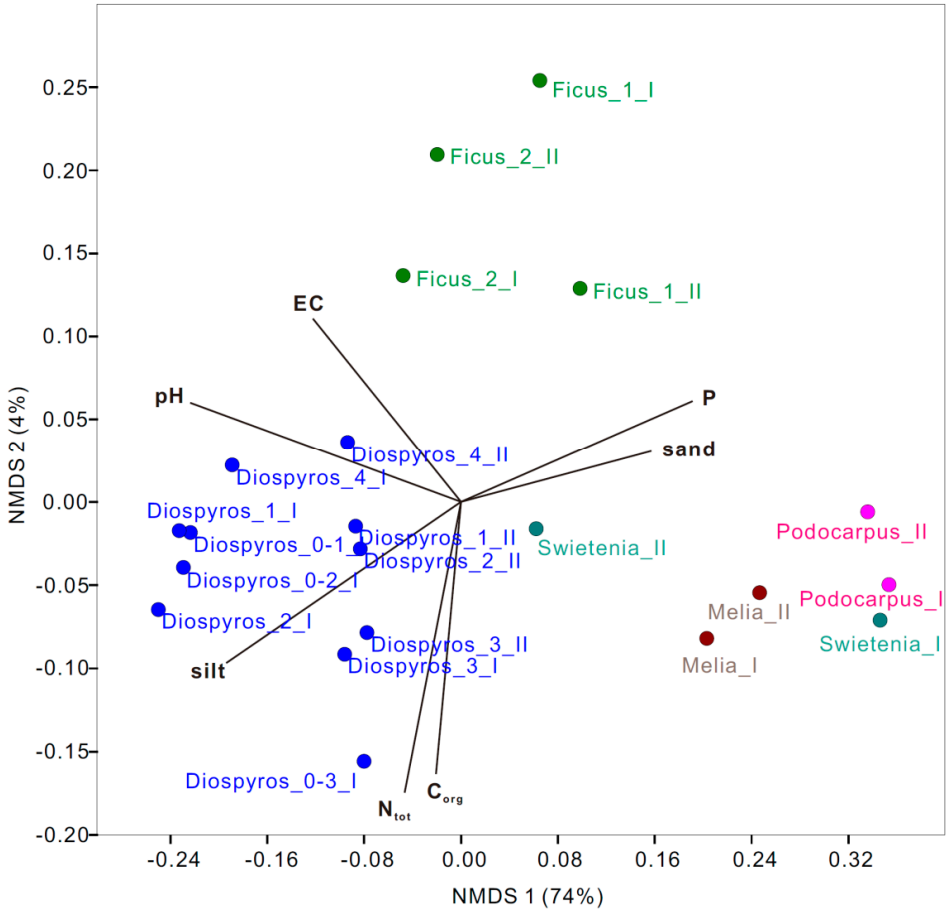

Figure 4. NMDS ordination of the nine urban trees at two study sites across the treatments based on bacterial community compositions at the species level (>1% relative abundance). Soil physicochemical parameters with a significant goodness of fit based on post hoc correlations ($p \leq 0.05$) are represented as vectors.

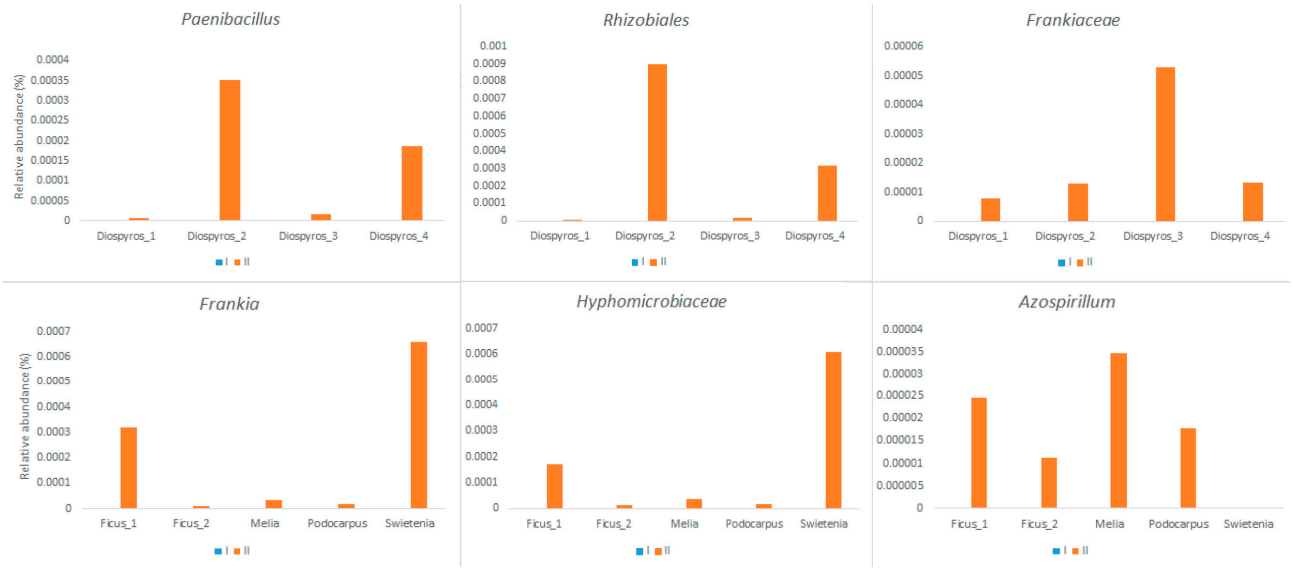

Figure 5. Potential nitrogen-fixing bacterial groups were detected in the rhizosphere soil after the amendment of biochar-based compost. Diospyros = *D. blancoi*, Swietenia = *S. macrophylla*, Melia = *M. azedarach*, Podocarpus = *P. nakaii*, and Ficus = *F. microcarpa*.

4. Discussion

4.1. Newly Developed Biochar-Based Compost Improved the Physicochemical Properties of Soil

Our result showed that biochar-based compost changed the soil properties more efficiently than biochar-only treatment. Although there was no significant increase in soil properties after 1 year of the biochar-only treatment, we suggest that the soil porosity would be significantly higher after repeating or extending the biochar amendments due to the porous internal structure of biochar [20]. This indicates that the water holding capacity would increase.

In general, the biochar-based compost amendment changed the following soil chemical properties: conductivity, the amount of organic matter, available phosphorus, calcium, and potassium (Tables 2b and 3). The treatment led to a decrease in organic matter, indicating a positive priming effect on SOC mineralization in the short term. This finding agrees with a hypothesis from a previous study: the presence of organic matter with biochar increases C mineralization in soil in the short term [41]. The mechanism may involve the biochar-promoting soil aggregation [17], which protects SOC from leaching [20] and highly stimulates bacteria to degrade organic carbon from the compost [28,29].

Moreover, the increased soil potassium after amendments may not only increase anti-stress and antipathogen responses, but also improve the osmotic uptake of other nutrients in water by roots, such as calcium and phosphorus. Consequently, we saw the calcium, available phosphorus, and conductivity decrease in the soil (Tables 2b and 3).

4.2. Microbial Community Structure Is Driven by Biochar/Compost Amendment

The bacterial alpha diversities in the soil of urban trees were high, based on the OTU richness and Shannon H' index (Figure 1). Previous studies [42,43] also found that the alpha diversities increase after biochar/compost treatment. Although there was no significant variation in the alpha diversities of soil bacterial communities, the bacterial community compositions changed after treatment (Figures 2–4).

Comparing the bacterial communities among all *D. blancoi* soil samples, the samples after biochar-based compost treatment were isolated from biochar-treated samples and control samples (samples marked in blue in Figure 2). This suggests that combined compost and biochar had stronger effects than the effects of biochar-only on the bacterial communities. This result is consistent with that of a previous study [31], which examined the tree growth and soil properties in a tropical urban environment. The other soil samples from different tree species had similar patterns as *D. blancoi*, and the samples after biochar-based compost treatment had different bacterial communities from the samples before treatment (Figures 2 and 3). Different biochar substances and tree species influenced soil bacterial assemblages as well. The *F. microcarpa* (Ficus) soil samples were isolated on the y-axis in the nMDS analysis (Figures 2 and 4), which was treated with different kinds of biochar from the other samples. In addition, *Nocardioidaceae*, *Gaiellaceae*, and *Chloroflexi* Ellin 6529 were more dominant in the *F. microcarpa* soil samples than other samples (Figure 3).

Furthermore, in the nMDS and heat map analyses (Figures 2 and 3), the samples were grouped by three of the four tree species (*S. macrophylla* was excluded). These findings agree with previous studies that concluded that the bacterial community structure in soil is related to urban tree species [16]. Interestingly, applying biochar-based compost increased the relative abundance of potential nitrogen-fixing bacterial groups (Figure 5), and the tree species affected the species of the nitrogen-fixing bacteria in the soil as well.

4.3. The Interaction between Bacterial Community Structure and Physicochemical Properties

The physicochemical properties—pH, phosphorus, and silt percentage in soil—were highly associated with the bacterial communities. They were potential key parameters shaping the bacterial communities. As mentioned in the previous paragraph, the aggregation of soil after biochar/compost amendment further enhances the activity of some bacteria in the soil. This consequently changes the pH value in soil, though there was no significant difference after 4 months of amendments. The activated bacteria may have come

from the compost, as they help decompose organic carbon and mineralize other nutrients. Lastly, the mineralized nutrients, such as nitrogen and phosphate, were absorbed by the roots of urban trees, resulting in a decrease in those concentrations in soil.

5. Conclusions

This study measured changes in soil physical and chemical properties and the variation in bacterial communities before and after applying biochar-based compost. We developed a new organic compost and applied biochar to it, which successfully improved the soil properties and fertility and increased the relative abundance of nitrogen-fixing bacteria in the soil. Finally, our results demonstrated that the biochar-based compost we used more efficiently remediated soil fertilities than biochar-only treatment in the urban forests.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10040682/s1, Table S1: The OTU richness, diversity indices, evenness, and estimated OTU richness of urban trees across treatments at both sites; Figure S1: The dominant bacterial community composition (>0.01) at class level across treatments of urban trees at both sites.

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