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Comparison of Bacterial Diversity in the Rhizosphere of *Chromolaena odorata* (L.) R.M. King and H.Rob. in Different Habitats

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Abstract: The invasion of *Chromolaena odorata* (L.) R.M. King and H.Rob. affects microbial community structure, but seldom do studies focus on bacterial diversity in the rhizosphere of *C. odorata* from different habitats. To reveal the impacts of *C. odorata* on bacterial communities in different habitats, diversity and functional groups of bacteria were compared in the rhizosphere soil of *C. odorata* collected from three habitats: abandoned land, a mature eucalyptus forest (eight-years), and a young eucalyptus forest (one-year). In this study, 3701 OTUs (operational taxonomic units) were obtained by next-generation sequencing. Simpson diversity indexes of bacteria in eucalyptus forests were significantly higher than that in abandoned land. Based on PCoA analysis, significant differences in the composition of bacterial communities were observed among the three habitats. Significant differences in bacterial communities among the three habitats were also demonstrated by biomarkers based on linear discriminant analysis effect size (LEfSe) analysis. Functional prediction analysis showed that the abundance of nitrogen-fixing functional genes in eucalyptus forests was greater than that in abandoned land. Canonical correspondence analysis (CCA) found that the biomass of *C. odorata*, soil pH, and total phosphorus were the main factors that impacted the microbial community structure. The results highlight the microbial community varied with habitats invaded by *C. odorata*, providing new evidence for understanding the invasion of *C. odorata* in different habitats.

Keywords: rhizosphere of *Chromolaena odorata*; soil bacterial; community structure; functional genes; different habitats

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Citation: Ni, X.; Zhao, C.; Li, J.; Li, B.; Zhu, J. Comparison of Bacterial Diversity in the Rhizosphere of *Chromolaena odorata* (L.) R.M. King and H.Rob. in Different Habitats. *Sustainability* **2023**, *15*, 2315. https://doi.org/10.3390/su15032315

Academic Editor: Julia Touza-Montero

Received: 9 December 2022
Revised: 17 January 2023
Accepted: 19 January 2023
Published: 27 January 2023

1. Introduction

Biological invasion is recognized globally as the second largest cause of biodiversity loss [1]. It directly threatens biodiversity and natural resource management in the invaded areas, especially in nature reserves [2]. Successful invasion is determined by various factors such as habitat type, human activities, and climatic conditions, as well as their interaction [3]. Soil microbial communities play an essential role in the successful invasion process of alien plants [4–6]. The enhanced mutualism hypothesis postulates that invasive plants promote self-growth by enriching beneficial microbes to establish positive soil feedback [7,8]. Microbes can facilitate nutrient acquisition in plants [9,10], defend against pests and diseases [11], and help plants withstand abiotic stresses [12]. Plant–soil feedback (PSF) is a plant-induced change in the abiotic soil environment and the soil community structure and function, which in turn affects the subsequent establishment and growth of plants [13]. Callaway et al. [14] found that the invasive plant *Centaurea maculosa* auct. non Lam. developed a unique rhizosphere microbial community that promoted its invasion. Plant-growth-promoting rhizobacteria (PGPR) is a class of naturally occurring beneficial soil bacteria that colonize the rhizosphere of plants. Many PGPR, such as *Bacillus*, *Klebsiella*,

and *Pseudomonas*, have been found to promote plant growth through nitrogen fixation, production of phytohormones (e.g., gibberellins), and increased abiotic stress tolerance [15–20]. However, the relationship between invasive plants and rhizosphere symbiotic microorganisms can cause functional differences due to habitat differences, geographical isolation, and competitive relationships [21]. A study of the aquatic invasive plant *Zizania latifolia* (Griseb.) Turcz.ex Stapf found that rhizosphere microorganisms varied widely among the four habitats and may have contributed to its invasion [22]. Research on the rhizosphere microorganisms of the same plant in different habitats deepens the understanding of the mechanism for the successful invasion of alien plants.

Chromolaena odorata, a perennial herb of the family Asteraceae, originated in southern Mexico, Central and South America, and the West Indies. It is considered a harmful invasive weed to agriculture and forestry in many countries in Asia, Oceania, and Africa [23]. Previous studies on the invasion mechanism of *C. odorata* have focused on phenotypic plasticity aspects, genetic differentiation, chemosensory effects, and microorganisms [24,25]. Among them, the evolution of increased competitive ability (EICA) hypothesis and the novel weapons hypothesis (NWH) are the main mechanisms for the invasion of *C. odorata*. *C. odorata* may evolve enhanced competitive ability by increasing the production of new allelochemicals [26]. The evolution of increased competitive ability can also be associated with soil microorganisms. Previous studies have compared the rhizosphere microbes of *C. odorata* with those of other invasive and native plants and found that the invasion of *C. odorata* can increase the number of bacteria associated with soil nutrient cycling, thus enhancing the ability of *C. odorata* to utilize soil nutrients and promote its growth and competition [27]. However, habitat heterogeneity of rhizosphere microorganisms and functional bacteria of *C. odorata* was still not reported, especially in nature reserves.

In this research, we hypothesized that the diversity and composition of bacterial communities in the rhizosphere of *C. odorata* varied with habitats. Concomitantly, we also hypothesized the invasion of *C. odorata* benefits from different bacterial functional groups in different habitats. Moreover, we supposed that the invasion of *C. odorata* and habitat characteristics all impacted the bacterial communities within three habitats. All these hypotheses were examined by collecting the rhizosphere soil of *C. odorata*, which had invaded three types of habitats in Guangxi Encheng National Nature Reserve: mature eucalyptus forests, young eucalyptus forests, and abandoned lands.

2. Materials and Methods

2.1. Study Area and Sampling

Our sampling site is located in Guangxi Encheng National Nature Reserve (106°58′16″ ~107°15′36″ E, 22°36′29″~22°50′5″ N) in southwest China (Figure 1). The reserve area is 258.176 km^2. The nature reserve has a northern tropical monsoon climate. Due to the complex and changeable terrain of the nature reserve, the climate differs in the low mountains, peaks, valleys, and depressions. The annual average sunshine hours are 1597 h, the annual average temperature is 21.3 °C, the annual average precipitation is 1362 mm, and there are obvious dry and rainy seasons. It is one of the areas with a serious invasion of *C. odorata* [28]. *C. odorata* invasion often form dominant communities not only in abandoned lands but also in plantation forests.

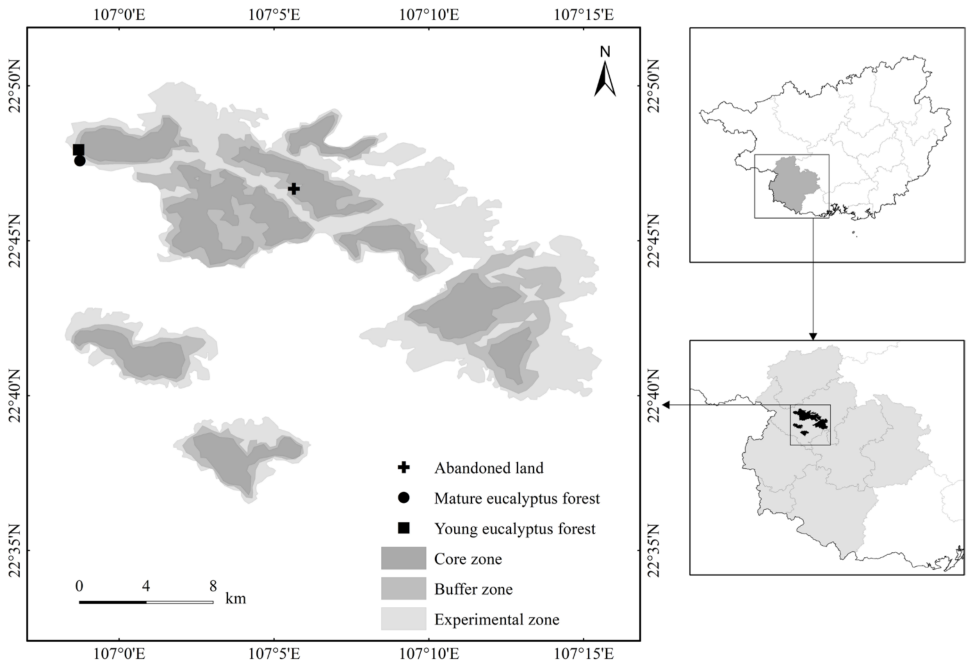


Figure 1. Sample sites in this study.

In July 2021, we selected three habitats in Encheng Nature Reserve: a young eucalyptus forest, a mature eucalyptus forest, and abandoned land. These three habitats are located in the experimental area and at lower altitudes (Table 1) with similar climate environments. In each habitat, three 4 × 4 m plots were set up in the invaded community of *C. odorata* and three 1 × 1 m subplots were sampled randomly. A total of 3 *C. odorata* individual samples were randomly selected in each subplot, and rhizospheric soil was collected after shaking the loose soil from the roots, resulting in a total of 27 soil samples. Then, the rhizospheric soil samples of three *C. odorata* individuals in each subplot were mixed into one soil sample. Thus, a total of 9 rhizospheric soil samples were finally collected. All the whole plants of *C. odorata* in the quadrate were pulled up, and the attached soil was shaken off and taken back to the laboratory. Then, three medium-sized *C. odorata* were selected and brought back to the laboratory. The whole plant was dried to a constant weight in an oven at 65 °C for 120 h until reaching a constant weight. Then the dry weight was used to calculate the total biomass of *C. odorata* in the quadrat. The number of individual *C. odorata* in each subplot was also counted as the density of *C. odorata*. The rhizosphere soil of *C. odorata* in the abandoned land was named A, that of *C. odorata* in the young eucalyptus forest was named B, and that of *C. odorata* in the mature eucalyptus forest was named C. The rhizosphere soils were stored at −20 °C for microbial analyses. The fine roots and impurities in each sample were removed by sieving (2 mm) and prepared for use. The physical and chemical parameters of the soil samples, including pH, soil organic carbon (SOC), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), and total potassium (TK), were measured. Soil pH was determined by the electrode method (distilled water extract, soil–water ratio of 1:5); SOC was determined by the potassium dichromate volumetric method; TN was determined using the Kjeldahl method; NH_4^+ and NO_3^- were determined by KCl solution extraction spectrophotometry; TP was determined using NaOH alkali fusion–molybdenum antimony spectrophotometry; and TK was determined by hydrofluoric acid–perchloric acid solution decoction and flame photometry [29,30].

Table 1. Summary of sample sites.

Sample	Longitude	Latitude	Altitude	Functional Zone	Other
A	107.094	22.778	229	Experimental	The abandonment time is unknown, without trees, and shrubs, with grazing activities.
B	106.978	22.798	378.53	Experimental	The trees are 8 years old, with a density of 0.342 trees/m^2, understory shrubs with *Mussaenda pubescens* Dryand.
C	106.979	22.793	368	Experimental	The trees are 1 year old, with a density of 0.188 trees/m^2, understory shrubs with *Vernicia fordii* (Hemsl.) Airy Shaw

A, abandoned land; B, young eucalyptus plantation; and C, mature eucalyptus plantation.

2.2. Soil Microbe Determination and Analysis

Total DNA was extracted from 200~–500 mg of each homogenized soil sample using the E.Z.N.A™ Mag-Bind Soil DNA Kit. DNA integrity was tested on agarose gels, and DNA sample concentration was quantified by Qubit. The V3–V4 region of the bacterial 16S rRNA gene was amplified using region universal primers (341F: 5′-CCTACGGGNGGCWGCAG-3′, 805R: 5′-GACTACHVGGGTATCTAATCC-3′). The original image data files obtained from Illumina Miseq™ 400 platformwere converted into sequenced reads by base calling analysis, and the sequenced sequences contained barcode sequences, as well as primer and splice sequences added during sequencing. Using Usearch, non-redundant sequences were extracted from the optimized sequences of each sample to facilitate the reduction of redundant computations in the middle of the analysis [31]. After the redundant sequences of all samples were combined, the non-redundant sequences (excluding single sequences) were clustered in an operational taxonomic unit (OTU) according to 97% similarity, and the chimeras were removed in the clustering process to obtain the representative sequences of the OTU. All the optimized sequences were compared to the OTU representative sequences, and sequences with 97% or more similarity to the representative sequences were selected to generate OTU tables. The RDP database was compared using the RDP classifier, which is based on Bergey's taxonomy, and the naïve Bayesian assignment algorithm was used to calculate the probability value of each sequence at different levels of the hierarchy for its assignment to a particular level [32]. All the above sequencing work was completed by Sangon Biotech (Shanghai, China) Co., Ltd.

2.3. Statistical Analysis

Stacked bar graphs with clustering trees were drawn at the phylum level. Select OTUs with relative abundance greater than 1% were analyzed at the genus level, and the rest were defined as others, then the relative abundance heat map was plotted [29]. Phylum and genus level analyses were performed on Tutools platform (https://www.cloudtutu.com (accessed on 5 January 2023)), an online data analysis website. The species richness, Shannon-Winner, Simpson index, and Pielou index were all used in the analysis of α-diversity, and only the distinguished and different index was presented in the text. Statistically significant differences in soil physicochemical properties and microbial community diversity indices among sample groups were assessed by ANOVA followed by Tukey's honest significant difference (HSD) post hoc test for multiple comparisons in SPSS v26.0. A principal coordinate analysis (PCoA) of microbial community composition was conducted to examine the differences between the microbial communities. Non-metric multidimensional scaling (NMDS) was used to evaluate the shifts of soil microbial community functions in different habitats. Weighted-normalized UniFrac distances were used in the PCoA and NMDS. We performed canonical correspondence analysis (CCA) with a forwarding selection of soil variables by variance inflation factors (VIFs) < 20. The above analyses were performed using the vegan package in R (version 4.2.1). The potential functions of the rhizosphere bacterial communities in different habitats were predicted using FAPROTAX analysis software(version 4.2.1); subsequently, the top ten functional annotated information of relative abundance were

selected to plot the relative abundance heat map. Linear discriminant analysis (LDA) used LEfSe software for biomarkers with statistical differences (LDA score > 3).

3. Results

3.1. Invasion Characteristics and Soil Physicochemical Properties of Chromolaena odorata in Three Habitats

The results of the soil physicochemical analysis showed that soil TK, pH, NH_4^+, and NO_3^- differed among the three habitats. TK and pH were significantly higher in the abandoned land habitat than in the two eucalyptus forest habitats and lower in the mature eucalyptus forest than in the young eucalyptus forest. NH_4^+ was significantly higher in the young eucalyptus forest than in the other two habitats, and NO_3^- was significantly higher in the mature eucalyptus forest than in the other two habitats. No difference was found for the SOC among the three habitats (Table 2). The biomass of *C. odorata* was highest in the abandoned land, reaching 0.64 ± 0.09 kg/m^2, followed by the mature eucalyptus forest. The density of *C. odorata* was highest in the mature eucalyptus forest, reaching 97.33 ± 32.04, and lowest in the abandoned land, 10.56 ± 2.99.

Table 2. Comparison of invasion characteristics and soil chemical properties of *C. odorata* in different habitats. The letters A, B, and C represent samples from abandoned land, young eucalyptus forest, and mature eucalyptus forest. Values (mean ± SE) followed by different letters in the same column indicate a significant difference at the $p < 0.05$ level.

Sample	pH	SOC (g·kg^{-1})	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	TK (g·kg^{-1})	Biomass (kg)	Density
A	8.00 ± 0.04 a	40.97 ± 7.75 a	8.61 ± 1.43 b	2.82 ± 1.57 b	12.61 ± 0.49 a	0.64 ± 0.09 a	10.56 ± 2.99 b
B	5.37 ± 0.14 b	32.58 ± 6.26 a	19.72 ± 4.52 a	4.25 ± 0.95 b	6.29 ± 0.41 b	0.04 ± 0.02 b	31.00 ± 7.81 b
C	4.99 ± 0.09 c	40.64 ± 0.64 a	17.97 ± 1 ab	13.65 ± 1.92 a	6.26 ± 0.09 b	0.19 ± 0.02 b	97.33 ± 32.04 a

3.2. Community Composition of Rhizosphere Bacteria of Chromolaena odorata

The microbial sequencing data of the soil samples were processed to obtain 715,672 total bacterial sequences with an average sequence length of 415.12 bp. A total of 3701 OTUs were obtained from the sequence partitioning, with 1335 OTUs in the three habitats, including 549 OTUs specific to abandoned land, 177 OTUs specific to young eucalyptus forest, and 204 OTUs specific to mature eucalyptus forest, 310 OTUs shared between abandoned land and young eucalyptus forest, 229 OTUs shared between abandoned land and mature eucalyptus forest, and 897 OTUs shared between the two eucalyptus forests (Figure 2).

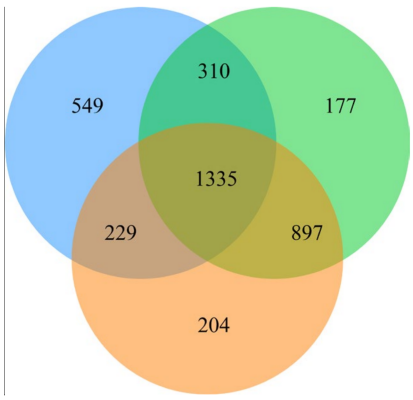
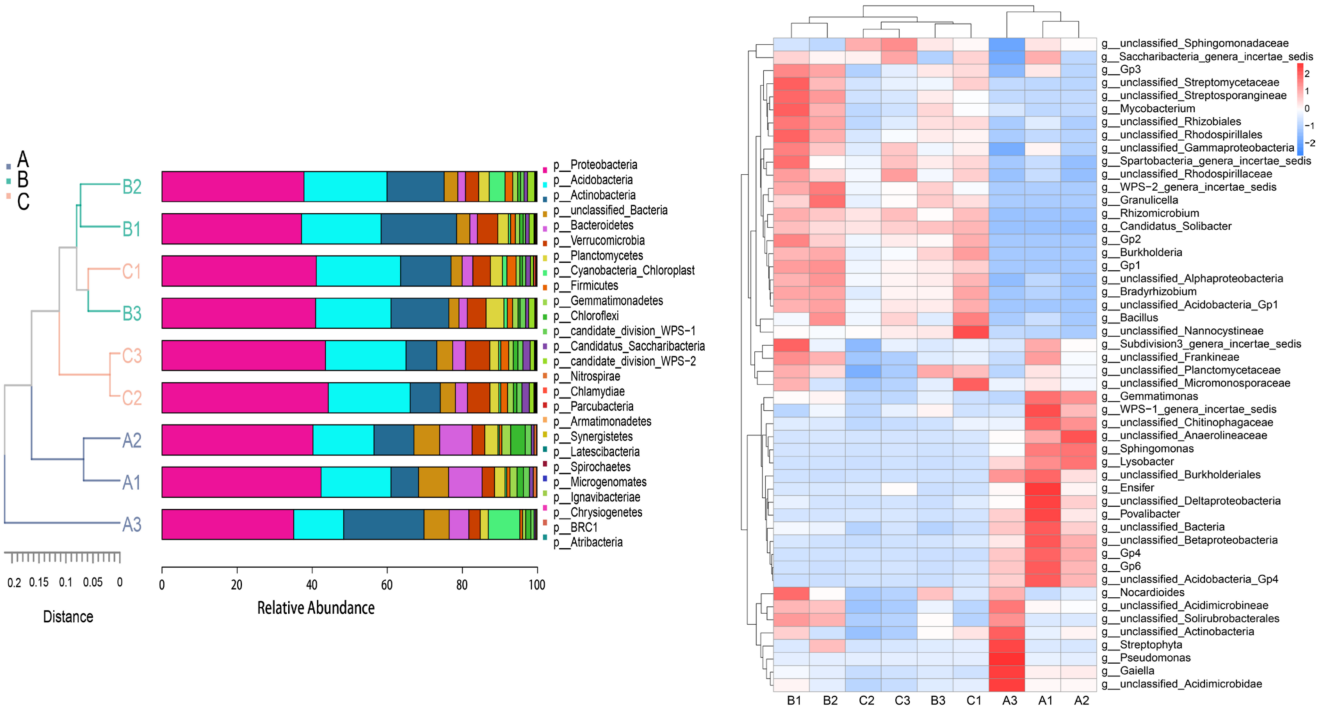


Figure 2. Venn diagram of microbial OTUs in the rhizosphere (blue: abandoned land; green: young eucalyptus forest; orange: mature eucalyptus forest).

The results showed that the number of phyla measured in the three habitats was similar with Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia and Planctomycetes as the dominant phyla (Figure 3A). However, the relative abundances of Acidobacteria and

Planctomycetes in the rhizosphere soil of *C. odorata* in the abandoned land were lower than those in both the young and mature eucalyptus forests.



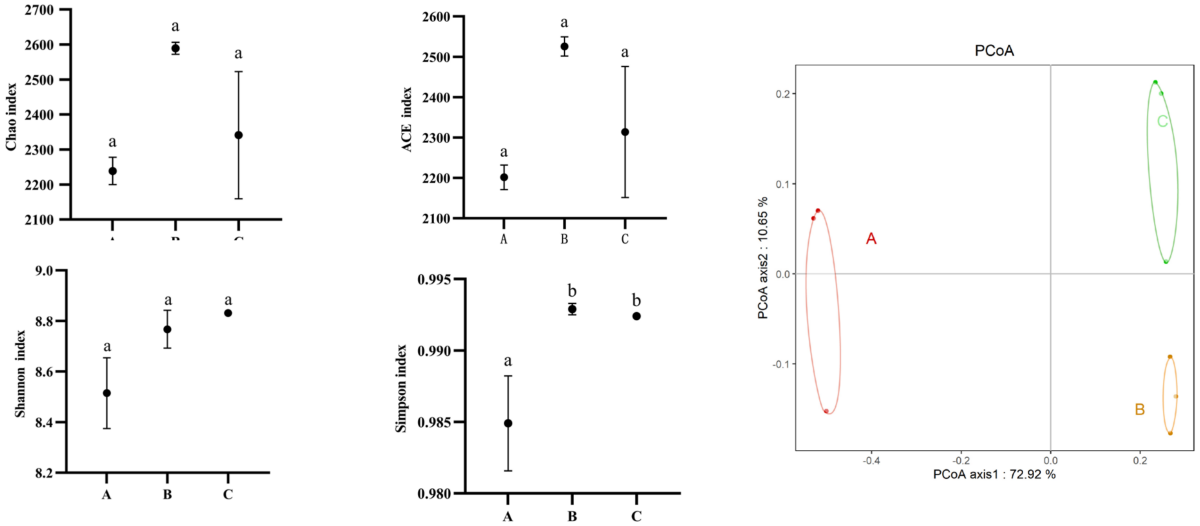
(**A**) (**B**)

Figure 3. Bacterial community composition at different taxonomic levels: (**A**) phylum; (**B**) genus. A1–A3 represent samples from the abandoned land, B1–B3 represent samples from the young eucalyptus forest, and C1–C3 represent samples from the mature eucalyptus forest.

The results of the rhizosphere bacteria genus level analysis of *C. odorata* in the three habitats showed that the bacterial abundance of the two eucalyptus plantations was similar, with *GP1*, *unclassified Rhizobiales*, *Burkholderia*, *Bradyrhizobium*, and *GP2* as the dominant genera. The abundance of bacteria in the abandoned land was significantly different from that in the eucalyptus forests. The relative abundance of *Sphingomonas*, *unclassified_Bacteria*, *unclassified Betaproteobacteria*, *GP6*, and *GP4* in the abandoned land was relatively high (Figure 3B).

3.3. Diversity of Rhizosphere Bacterial Community of Chromolaena odorata

One-way ANOVA showed that the Simpson index was significantly lower in the abandoned land (0.9849 ± 0.0033) than in the young eucalyptus forest (0.9929 ± 0.0004, $p = 0.027$) and the mature eucalyptus forest (0.9924 ± 0.0002, $p = 0.034$), while there was no significant difference between the two eucalyptus forests ($p = 0.861$). The other indices were not significantly different (Figure 4A). The results of the principal coordinate analysis (Figure 4B) showed that the sum of the two principal axes explained 83.57% of the variation in microbial diversity. The rhizospheric bacterial communities of *C. odorata* in the different habitats did not overlap with each other, indicating differences in the species composition of *C. odorata* and rhizospheric bacteria in the different habitats. The young and mature eucalyptus forest samples are clustered to each other in the figure, which indicates a more similar species composition between the two types of soil.

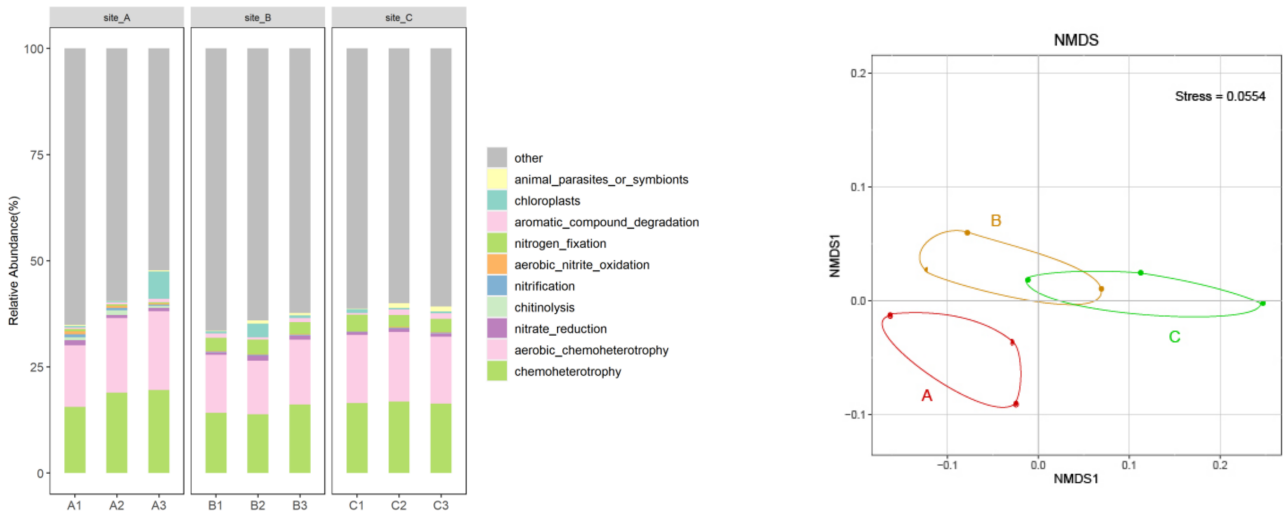


(A) **(B)**

Figure 4. Alpha diversity in different habitats (**A**). Principal coordinate analysis of different soil microbial communities based on OTU data (**B**). The letters A, B, and C represent samples from the abandoned land, young eucalyptus forest, and mature eucalyptus forest. Different lowercase letters showed significant differences in diversity index ($p < 0.05$).

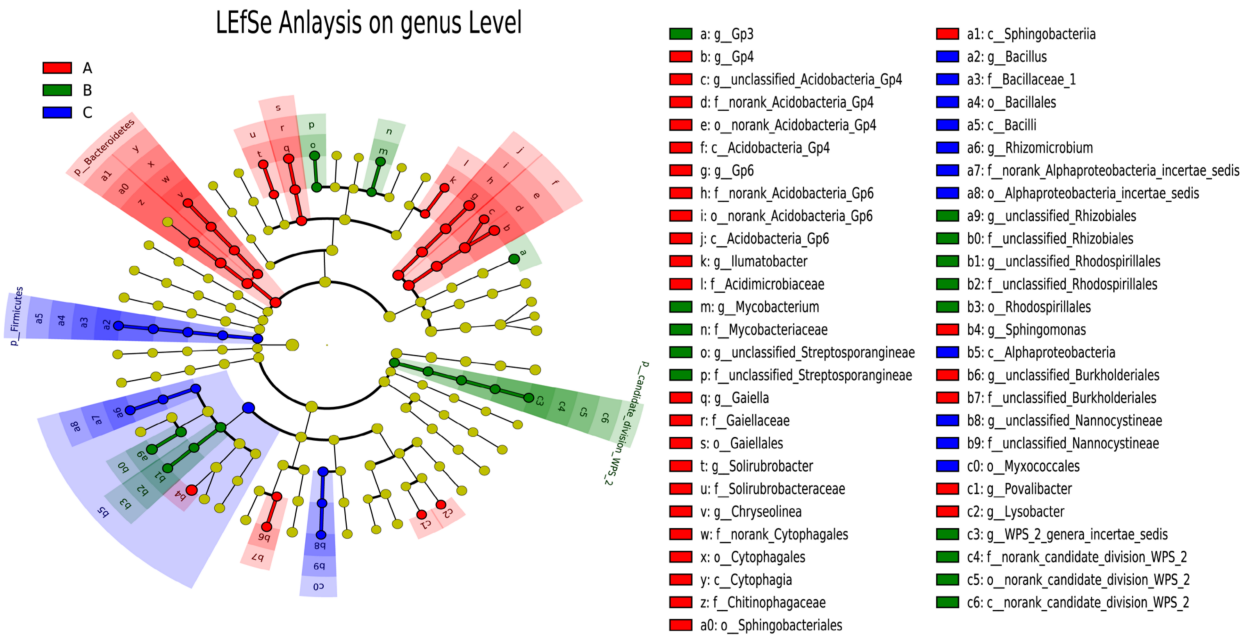
3.4. Functional Prediction of Rhizosphere Bacteria of Chromolaena odorata

The functional compositions of all samples from the three habitats were essentially unchanged, but there were differences in the relative abundance of different functional genes (Figure 5A). Chemoheterotrophy, aerobic chemoheterotrophy, nitrate reduction, chitinolysis, nitrification, etc., were mainly present in the three habitats. The sequencing of predicted metabolic functions indicated that the functional genes varied significantly between the eucalyptus forests and the abandoned land (Figure 5B).



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

Figure 5. *Cont.*



(**C**)

Figure 5. Functional composition of the soil bacterial communities (**A**); NMDS analysis of different soil bacterial communities based on functional prediction data (**B**); LEfSe analysis of differential bacterial species (**C**). The letters A, B, and C represent samples from the abandoned land, young eucalyptus forest, and mature eucalyptus forest.

In the LEfSe analysis plot (Figure 5C), the biomarkers of significant differences in the abandoned land were concentrated in *GP6*, *GP4*, *Ilumatobacter*, *Gaiella*, *Solirubrobacter*, *Chryseolinea*, *Sphingomonas*, *Povalibacter*, and *Lysobacter*. The biomarkers with significant differences in the young eucalyptus forest were concentrated in *WPS-2 genera incertae sedis*, *unclassified Rhodospirillales*, *unclassified Rhizobiales*, *GP3*, *Mycobacterium*, *unclassified streptosporangneae*, while the biomarkers in the mature eucalyptus forest were concentrated in Alphaproteobacteria, *unclassified Nannocystineae*, *Bacillus*, and *Rhizomicrobium*.

3.5. Analysis of Influence Factors of Rhizosphere Bacterial Community

In the CCA analysis of the *C. odorata* rhizospheric soil microbiome, Axis 1 explained 41.28% of the total variance, while Axis 2 explained 13.28% (Figure 6). The results showed that soil pH, the biomass of *C. odorata*, and TP showed stronger correlations with the first canonical axis (CCA1). The biomass of *C. odorata*, soil pH, and TP had strong positive correlation effects on the bacterial community structure of the rhizosphere soil samples from the abandoned land. In contrast, these environmental factors negatively correlated with the bacterial community structures of the two eucalyptus forests.

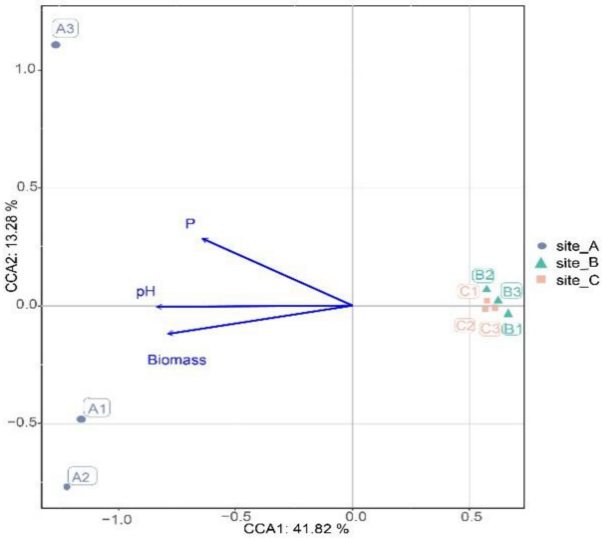


Figure 6. Canonical correspondence analysis of the influencing factors of the rhizosphere microbial communities.

4. Discussion

Soil microorganisms often change their communities to adapt to the physical and chemical environment of their habitats for better growth and development [33], and the rhizosphere microorganisms of invasive species are not only influenced by their habitats but also closely related to the invasive species [22]. In this study, the Simpson diversity index was found to be significantly lower in the abandoned land than in the eucalyptus forests, indicating higher diversity in the eucalyptus forests. High plant rhizosphere bacterial diversity can improve plant nutrient use efficiency and increase plant biomass through synergistic interactions between different microorganisms [34]. The experimental results of Zhang et al. similarly showed that invasive plants were able to influence the soil biotope through the root system, thereby enhancing the effect of plant invasion on the abundance or biomass of certain components of the community and enhancing nutrient cycling, thus facilitating the invasion of invasive alien plants [35].

Linear discriminant analysis effect size (LEfSe) can help reveal differences between groups across different samples or environments, and find biomarkers with statistical differences among groups [36]. Based on LEfSe analysis, it was evident that the rhizosphere of *C. odorata* in different habitats contains specific microbiomes. In addition, a variety of PGPR may be associated with the successful invasion of *C. odorata* [37]. Plant-associated bacteria often have some plant-growth-promoting traits (PGPTs), such as nitrogen metabolism, production of IAA, and phosphorus metabolism [38]. In the analysis of key species in the mature forest of eucalyptus, mainly *Bacillus* and others were the key species. Among them, the *Bacillus* species are an important agricultural microbial resource that are commonly found in soil, animal guts, air, water, and plants, solubilizing insoluble phosphorus, potassium, and other compounds, and are defined as important beneficial bacteria [39,40]. It has been found that the invasion of *Ageratina adenophora* promoted an increase in *Bacillus cereus*, a common plant rhizosphere nitrogen-fixing bacterium, which in turn exerted a positive feedback effect on the growth of *A. adenophora* [41,42]. Thus, the accumulation of *Bacillus* in the mature eucalyptus forest may increase the efficiency of nitrogen fixation in the rhizosphere soil of *C. odorata*, thereby promoting growth. In contrast, the significantly different species in the abandoned land were concentrated in *GP6*, *GP4*, *Gaiella*, *Sphingomonas*, and *Lysobacter*. Most of these key species may help *C. odorata* adapt to the poor soil environment. Firstly, it has been demonstrated that *Gaiella* species, as beneficial bacteria in the plant rhizosphere, contribute to the ability of *Boehmeria nivea* to tolerate poor soils [43]. Therefore, we deduced that the enrichment of *Gaiella* may contribute to the successful invasion of *C. odorata* in abandoned land. Secondly, Acidobacteria, as one of the most abundant phyla

in the plant rhizosphere, plays an important role in soil carbon and nitrogen cycling [44]. Acidobacteria is clearly defined as an oligotrophic bacterial phylum, and oligotrophic taxa dominate in soils with low organic carbon availability [45]. *Gp4,* isolated from African savanna soils, has shown cytoplasmic extension morphologically, thus suggesting adaptations in nutrient uptake by *Gp4* in nutrient-poor environments, similar to our findings [46]. Therefore, rhizosphere aggregation of specific functional bacteria in different habitats of *C. odorata* could help *C. odorata* adapt to different environmental conditions, such as the accumulation of microorganisms more adapted to survive in poor soil environments in abandoned land, and the accumulation of microorganisms related to the nitrogen cycle in eucalyptus forests, thus promoting its invasive expansion. This is consistent with our hypothesis that the diversity and composition of bacterial communities in the rhizosphere of *C. odorata* varied with habitats.

FAPROTAX analysis mapped the classification annotation results of 16S rRNA amplification sequencing to the database, so as to annotate and predict the microbial community metabolism or ecologically relevant functions. Many papers have well annotated the function of soil microorganisms through FAPROTAX analysis [47]. In this study, differences were found in the function of the rhizosphere bacterial communities of *C. odorata* in three habitats. The abundance of nitrogen fixation function in the eucalyptus forests was greater than that in the abandoned land, which was consistent with the results of NH_4^+ content in the soil. In general, in the process of alien plant invasion, the functional groups of soil microorganisms play an important role because they can alter soil nutrient cycling and affect soil nitrogen mineralization [48,49]. Previous research has determined that alien plants can alter the diversity and abundance of specific functional groups of soil microorganisms [50,51]. Changes in soil microbial communities that regulate soil nutrient effectiveness may affect nutrient acquisition capacity and thus produce positive feedback on the growth of invasive plants. Invasive alien plants affect the abundance and diversity of nitrogen-fixing soil bacteria, which may cause changes in the available N content in soil [52]. For example, *Acacia dealbata* increases the available soil carbon source and enhances biological nitrogen fixation efficiency through a large number of dead leaves, thus changing soil nutrients and affecting the structure and function of soil microbial communities [53]. In our study, the difference in the functional abundance of nitrogen fixation may be due to the aggregation of PGPR, such as *Rhizobium* and *Bacillus*, that promote plant growth by performing nitrogen fixation in *C. odorata* in eucalyptus forests [54,55]. Moseman also demonstrated that the increase in the abundance and diversity of nitrogen-fixing bacteria (NFB) could facilitate plant invasion by increasing nitrogen input [56,57]. Therefore, consistent with our hypothesis, the difference of nitrogen fixation function between eucalyptus forests and abandoned land may be closely related to the difference of soil nutrient content and the growth of *C. odorata*.

In this study, we found that nitrate and ammonia nitrogen were significantly higher in the eucalyptus habitats than in the abandoned land habitat, and light intensity was lower in the eucalyptus forests than in the abandoned land, while *C. odorata* was able to adjust and respond positively to changes in light intensity in some physiological characteristics (e.g., regulation of photosynthetic capacity, biomass allocation, and phenotypic plasticity) [25,58]. Because the shaded environmental conditions of the eucalyptus forests were not conducive to the growth of *C. odorata*, we can deduce that more complex bacterial communities are needed to promote the soil N use efficiency of *C. odorata* in eucalyptus forests. In the present study, we also found that the nitrogen-fixing functional group increased significantly in the eucalyptus forests, so it is possible that the nitrogen-fixing bacterial community enhanced the soil fast-acting N content and allocated more N to photosynthesis [29], thus facilitating the adaptation of *C. odorata* to unfavorable environmental conditions [59]. In previous studies, nitrogen-fixing bacteria played a key role in the process of plant invasion; for example, *Mikania micrantha*-enriched microorganisms participated in the N cycle and led to increased nitrogen effectiveness, which, then, increased the N content of the plants' photosynthetic tissues, thus promoting the growth of *M. micrantha* [60]. The key roles of

soil characteristics and plant species in shaping rhizosphere microbial communities have been mentioned in many studies. Therefore, the differences in the rhizosphere bacteria in this study may be explained by a combination of habitat and *C. odorata* invasion, and the differences in the functional bacterial communities, especially the enrichment of nitrogen-fixing bacteria in eucalyptus forests, may promote the adaptation of *C. odorata* to different habitats.

Of course, some limitations still existed in this study. First, although we found bacterial communities varied in different habitats related to the biomass of *C. odorata* and soil physicochemical properties, such as total phosphorus and pH, we cannot disentangle the effects of the habitats and invasion of *C. odorata*. Controlled experiments were needed to explore the relationship between rhizosphere microorganisms and invasion of *C. odorata*. Second, it remained unclear whether changes of soil bacterial communities induced feedback to the growth of the invader. Therefore, future large-scale studies are required to test whether the rhizospheric soil of *C. odorata* promotes plant growth compared to non-rhizospheric soil.

5. Conclusions

Our study found that the diversity and composition of the rhizosphere soil bacterial communities of *C. odorata* in the three habitats differed significantly and that the composition of the functional groups might help *C. odorata* adapt to different habitats, which is consistent with our hypothesis. The results suggested that *C. odorata* invasion can accumulate different functional bacteria to adapt to different habitat conditions, alter soil nutrient cycling, especially N use, and thus facilitate invasion. We also found that the biomass of *C. odorata* can explain the variation of soil bacterial communities in different habitats. This result indicates the various impacts of the invasion of *C. odorata*. However, knowledge of the plant-soil feedback between bacterial communities and *C. odorata* must be improved. We suggest that considering a habitat's heterogeneity of plant-soil feedbacks can help to understand the successful invasion of *C. odorata*. Moreover, the response of native plants and ecosystems to the modifications of the soil environment initiated by invasions of *C. odorata* should be studied in the future.

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

Funding: This research was funded by the National Key Research and Development Program of China (2020YFC1806300, 2016YFC1201100, 2017YFC0506200) and the Special Fund of Chinese Central Government for Basic Scientific Research Operation in Commonweal Research Institutes (2022YSKY-08).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (e-mail: zhaocy@craes.org.cn).

Conflicts of Interest: The authors declare no conflict of interest.

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