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Differences in rhizosphere soil microbial function and community structure in invasive weed *Bidens pilosa* and native weeds

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ABSTRACT: As an invasive weed, *Bidens pilosa* seriously threatens native ecosystem functions in beach regions in South China. This study aimed to explore the differences in soil nutrients and microorganisms between the invasive weed *B. pilosa* and native weeds (*Cynodon dactylon*, *Portulaca oleracea*, and *Eleusine indica*), exploring variations in soil functions after *B. pilosa* invasion. The results showed that soil nutrients varied by the weed species. The total and unique bacterial and fungal amplicon sequence variant (ASV) numbers in *B. pilosa* were higher than those in the other three weeds. The bacterial composition related to soil carbon metabolism differed between *B. pilosa* and the other three native weeds. Analysis of bacterial metabolic pathways showed that their soil carbon metabolism ability was higher than that of the other three native weeds. Some pathogenic fungi and *Lophotrichus* (dark septate endophytes) are enriched in the rhizosphere of *B. pilosa*. Increasing the relative abundance of beneficial soil bacteria and decreasing the relative abundance of pathogenic soil fungi may be beneficial for resisting the invasion of *B. pilosa*. In addition, the soil total phosphorus, available phosphorus, and soil organic matter contents were identified as the most important edaphic factors shaping microbial community structure and function in the context of *B. pilosa* invasion. This study revealed differences in soil microorganisms between *B. pilosa* and native weeds, and these differences potentially affected the native ecosystem function.

KEYWORDS: *B. pilosa* invasion, native dominant weeds, soil microbial community structure, metabolic pathway

INTRODUCTION

B. pilosa is an annual plant that belongs to the Asteraceae family and is native to Central America. It is a harmful, strongly invasive weed that has extensively colonized China, resulting in serious losses in agriculture, forestry, and animal husbandry [1]. Effective control of the expansion of *B. pilosa* is important for maintaining local biodiversity and ecosystem functioning. The success of an invasion is often dictated by the ability of the invasive plant to influence underground processes [2]. Different root exudates and litter inputs from invasive plants influence soil nutrient cycling and soil microbial communities [3, 4]. These changes influence the formation of aboveground plant communities [5].

Soil microorganisms play a crucial role in driving soil processes and plant growth, owing to their important role in the cycling of soil nutrients and decomposition of organic matter [6]. Invasive plants can benefit from soil microbial communities, for example, they can strengthen the interaction among soil bacterial communities [7]; shift the soil microbial community composition and its functions related to C, N, S, and other compound cycling [8, 9]; increase arbuscular mycorrhizal fungi root colonization during competition with native plants [10]; and inhibit the growth of pathogenic fungi [11]. One of the main reasons for successful invasion is that invasive plants and soil microbial communities form multiple feedback mechanisms [12]. Therefore, assessing the effects of plant invasion on soil microbial communities is essential to improve the elucidation of the mechanisms of invasive plants.

River beaches are characterized by diverse species and serve as important habitats for biodiversity conservation. The invasion of *B. pilosa* reduces the native plant richness and evenness of the local river beach, which is unfavorable for local ecological diversity. An investigation showed that rapid adventitious root generation and high root dehydrogenase activity improve the waterlogging resistance of *B. pilosa*, and short-term waterlogging can significantly improve the competitiveness of invasive *B. pilosa* [13]. *B. pilosa* has greater photosynthetic characteristic parameters, leaf characteristic indices, and energy utilization efficiency than co-occurring native Asteraceae plants [14]. However, the effect of *B. pilosa* invasion on the composition and function of soil microbial communities, in contrast with native plants, remains uncertain. The structure and function of soil microorganisms are highly sensitive to changes in plant species, and the analysis of soil microbes has gained importance for estimating soil quality and functional changes caused by plant invasion [15].

In this study, we aimed to improve the understanding of the mechanisms of invasion by *B. pilosa*. Thus, we proposed the following hypothesis: The rhizosphere soil microbial function and community structure were different between invasive weed *B. pilosa* and native weeds. To test this hypothesis, we collected the soils of the main invasive weed, *B. pilosa*, and three dominant native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, from the beach of the Liuxi River in South China and studied the differences in soil nutrients and rhizosphere soil microorganisms between invasive and native weeds. Illumina MiSeq sequencing of bacterial 16S rRNA and fungal ITS genes was performed to analyze variations in soil microbial diversity, community composition, and metabolic pathways.

MATERIAL AND METHODS

Field sites

The experiment was conducted at Liuxi River Beach, Guangzhou City, Guangdong Province (23°31′ N, 113°28′ E), which has a subtropical monsoon climate. In this area, in 2020, the sunshine duration in this area was 1641.7 h, the precipitation was 1890.3 mm, and the average temperature was 22.7 °C. The main vegetation composition was a natural mixed community of the invasive weed *B. pilosa* (annual herb Bidens) and native weeds, with plants distributed in patches. The dominant native weeds were *C. dactylon* (annual herb, Bermuda grass), *P. oleracea* (annual herb, purslane), and *P. oleracea* (annual herb, Trifolium). The coverages of *B. pilosa*, *C. dactylon*, *P. oleracea*, and *E. indica* were approximately 40%, 10%, 20%, and 20%, respectively. The sample site was more than 50 m from the main road, and no control measures had been implemented in the area during the last five years. A diagram of the sampling area is shown in Fig. S1.

Soil sample collection

Soil samples were collected in May 2020. The sampling area was approximately 500 m long and 50 m wide. Five sampling plots were selected for each weed species. Five plants were randomly excavated from each plot, and the loosely adhering soil was shaken. The shaken soil was collected as the non-rhizosphere soil for subsequent analysis. In total, 20 sampling plots were selected (five replicates from four weed species) for soil sample collection, and each sampling plot contained only one weed species, which was separated from the other sampling plot by approximately 1 m to minimize the influence of the rhizosphere. Non-rhizosphere soil was used to analyze physical and chemical properties. Rhizosphere soil was collected using the root-washing method according to the following steps: (1) The roots of weeds were cut and placed in a 250 ml flask with 50 ml PBS-S buffer solution (containing 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 130 mM NaCl, and 0.5% Tween 80; pH adjusted to 7.0). (2) The samples were shaken at 180 rpm for approximately 30 min, poured into centrifuge tubes, and centrifuged at low temperature for 20 min. (3) The supernatant was removed, and step 2 was repeated thrice. (4) The collected soil samples were placed in frozen storage tubes and then stored at −30 °C until further molecular analysis. In total, 20 mixed non-rhizosphere soil samples (five replicates from four weed species) and 20 mixed rhizosphere soil samples (five replicates from four weed species) were collected.

Soil physical and chemical properties analysis

The analyzed parameters were measured according to Bao's [16] method. Soil pH and electrical conductivity (EC) were measured using an electrode pH meter and conductivity meter, respectively, at a soil: water ratio of 1:5 weight/volume. Soil organic matter (SOM) was detected using a volumetric method after oxidation with potassium dichromate at high temperatures. Total nitrogen (TN) was detected using the semi-micro Kjeldahl method with a nitrogen determinator after heat digestion using a catalyzer (potassium sulfate:copper sulfate:selenium = 100:10:1). Total phosphorus (TP) was detected using the molybdenum-antimony colorimetry method after heat digestion with 8 ml concentrated sulfuric acid and 0.5 ml perchloric acid. Total potassium (TK) was detected using flame photometry after heat digestion with 3 ml perchloric acid and 5 ml hydrofluoric acid. Available N (AN) was detected using an alkaline hydrolysis diffusion method after hydrolysis with 1.0 mol/l NaOH. Available P (AP) was detected by molybdenum-antimony colorimetry after extraction with solvents (0.03 mol/l ammonium fluoride and 0.025 mol/l hydrochloric acid). Available K (AK) was detected using flame photometry after extraction with 1 mol/l ammonium acetate solution.

Soil DNA extraction and Illumina MiSeq Sequencing

Total soil DNA was extracted from 0.25 g (fresh weight) of rhizospheric soil samples by using the OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. A NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis were used to measure the quantity and quality of extracted DNA. The hypervariable regions (V3–V4) of the bacterial 16S rRNA were amplified with specific primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [17], and the ITS1 region of the fungus was amplified with specific primers ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′) [18]. Polymerase chain reactions (PCRs) were performed in triplicate using 25 µl mixtures containing 5 µl 5× Buffer, 2 µl NTP (2.5 mM), 1 µl forward primer

(10 µm), 1 µl reverse primer (10 µm), 1 µl template DNA, 14.75 µl ddH_2O, and 0.25 µl fast pfu DNA polymerase. To amplify the bacteria 16S V3–V4, PCRs were conducted using the following programme: 5 min of initial denaturation at 98 °C followed by 24 cycles of 30 s at 98 °C, 30 s at 52 °C, and 45 s at 72 °C, and a final extension at 72 °C for 5 min. To amplify the fungi ITS1, PCRs were conducted using the following programme: 5 min of initial denaturation at 98 °C followed by 28 cycles of 30 s at 98 °C, 45 s at 52 °C, and 45 s at 72 °C, and a final extension at 72 °C for 5 min. PCR amplicons were purified and determined using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, the amplicons were pooled in equal amounts, and paired-end sequencing was performed using the Illumina MiSeq platform (2*250) with the MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd., Shanghai, China). Raw amplicon sequence data from this study were submitted to the National Center for Biotechnology Information Sequence Read Archive database under Bioprojects PRJNA755140 (bacteria) and PRJNA756140 (fungi).

Sequence data analysis

Microbiome bioinformatics was performed using QIIME2 2019.4 with slight modifications according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). In brief, raw sequence data were demultiplexed using the Demux plugin, followed by primer cutting using the Cutadapt plugin. Sequences were then quality filtered, denoised, and merged, and chimeras were removed using the DADA2 plugin. Non-singleton amplicon sequence variants (ASVs) were aligned with MAFFT and used to construct a phylogeny using FastTree2. Alpha diversity was estimated using the diversity plugin. Taxonomy was assigned to ASVs by using the classify-sklearn naïve Bayes taxonomy classifier in the feature classifier plugin against the SILVA Release 132 (bacteria, Release132, http://www.arb-silva.de) /UNITE Release 8.0 (fungi, Release 8.0, https://unite.ut.ee/) database. We used the QIIME feature table rarefy function (QIIME2) to flatten the generated ASV table and set the flattening depth to 95% of the minimum sample sequence. In total, 20 rhizospheric soil DNA samples from five replicates of the four weeds were used to sequence bacterial 16S rRNA (V3–V4) and fungal ITS1 communities. The 1786192 bacterial and 1856434 fungal raw sequences contained 705747 bacterial and 1534642 fungal effective sequences. The effective sequences generated 52519 bacterial and 4416 fungal ASVs (amplicon sequence variants) based on dereplication. The rarefaction curves of the 16S rRNA (V3–4) and ITS (ITS1) amplicon libraries showed that all sequenced bacterial (Fig. S2A) and fungal (Fig. S2B) samples tended toward a plateau, indicating that the amount and depth of the sequencing data were reasonable and reliable. Microbial functions were predicted by PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states) in the MetaCyc (https://metacyc.org/) database.

Statistical analysis

Analysis of variance (ANOVA) was used to detect the differences in the soil physical and chemical properties and metabolic pathways of the bacteria and fungi in the rhizosphere soil (first functional layer) of the four weeds. A *T*-test was used to detect differences in alpha diversity between two weeds. ANOVA and *t*-tests were performed using the statistical software SPSS 19.0. Data for assumptions were checked using SPSS 19.0, a descriptive statistics function. The relevant data were normally distributed (Shapiro-Wilk), and the variance was homogeneous. Alpha diversity was determined using the Chao1 richness estimator and Shannon diversity index, and alpha diversity was calculated using the ASV table in QIIME2 and visualized as box plots. A Venn diagram was generated to visualize the number of ASVs in the treatment groups. Linear discriminant analysis effect size (LEfSe) was used to detect differentially abundant taxa across groups by using default parameters. Principal coordinate analysis (PCoA) and cluster analysis were conducted on the relative abundance data to visualize the shifted patterns of the microbial community and metabolic pathways among treatment groups based on the Bray-Curtis distance. A volcano map was generated to visualize the differential metabolic pathways between treatment groups. Alpha diversity, Venn diagrams, LEfSe, PCoA, cluster analysis, and volcano mapping were performed using the GeneScloud platform (https://www.genescloud.cn/login). The Mantel test and redundancy analysis (RDA) were performed to examine the correlation between the microbial community and soil chemical properties. The Mantel test and RDA were performed in R v.2.5-5 by using the 'vegan' package.

RESULTS AND DISCUSSION

Effect of the four weeds on soil physical and chemical properties

Significant differences were observed in the soil physical and chemical properties among the four weeds. The soil pH, EC, and AK content of *B. pilosa* were higher than those of the other three native weeds, and the SOM, TN, TP, AN, and AP contents of *B. pilosa* and *C. dactylon* were lower than those of *E. indica* and *P. oleracea* (Table 1). This finding is consistent with that in the literature that invasive plants can invade and expand in soils with low nutrient content [3]. Moreover, the decreased SOM, TN, TP, AN, and AP contents associated with *B. pilosa* and *C. dactylon* may

Table 1 Non-rhizosphere soil physical and chemical properties in four different wees at Liuxi River Beach. Four weeds –three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Groups	pH	EC	SOM (g/kg)	TN (g/kg)	TP (g/kg)
C. dactylon	6.40 ± 0.06^{d}	0.04 ± 0.01^{b}	11.81 ± 0.11^{c}	1.20 ± 0.06^{bc}	0.51 ± 0.03^{c}
P. oleracea	7.35 ± 0.11^{b}	0.10 ± 0.00^{a}	15.33 ± 0.28^{a}	1.43 ± 0.03^{a}	0.68 ± 0.02^{b}
E. indica	6.96 ± 0.07^{c}	0.06 ± 0.01^{b}	14.40 ± 0.19^{b}	1.35 ± 0.06^{ab}	0.82 ± 0.03^{a}
B. pilosa	7.90 ± 0.07^{a}	0.11 ± 0.01^{a}	12.57 ± 0.46^{c}	1.16 ± 0.06^{c}	0.54 ± 0.02^{c}
Groups	TK (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	
C. dactylon	33.96 ± 0.76^{a}	91.09 ± 10.88^{c}	81.75 ± 9.86^{c}	112.80 ± 7.59^{c}	
P. oleracea	32.57 ± 0.38^{a}	140.99 ± 8.83^{ab}	196.18 ± 4.73^{b}	102.40 ± 13.84^{c}	
E. indica	32.61 ± 0.83^{a}	157.10 ± 4.09^{a}	337.30 ± 20.99^{a}	183.40 ± 6.43^{a}	
B. pilosa	34.61 ± 0.62^{a}	124.13 ± 6.51^{b}	95.71 ± 12.81^{c}	144.60 ± 21.66^{ab}	

Data are shown as the means ± standard errors of five replicates ($n = 5$). Values followed by a different letter in the same column indicate significant differences at $p < 0.05$ using Duncan's test. The soil physical and chemical properties included soil pH, electrical conductivity (EC), soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available N (AN), available P (AP), and available K (AK).

be because these two weeds have higher soil nutrient cycling rates than the other native weeds, promoting soil nutrient uptake by the weeds. Studies have also suggested that invasive plants have higher soil N [19] and C [20] cycling rates than native plants do. However, the similarity in soil physical and chemical properties between *B. pilosa* and *C. dactylon* indicates that competition between native and invasive weeds is complex. The functional similarity between the two weeds may imply that *C. dactylon* was more resistant to invasion by *B. pilosa*. This view was supported by Zheng et al [21], who found that the more similar the functional characteristics of the species in the native community were to those of plane grass, the stronger the ability to resist the invasion of *Chromolaena odorata*. Therefore, the use of *C. dactylon* for the ecological defence of *B. pilosa* may be a new measure for reducing weed invasion in the study area.

Sequencing results and α-diversity of bacterial and fungal communities

The Venn diagram shows that there were 15043, 15877, 12563, and 17011 bacterial ASVs and 1258, 1355, 1378, and 1858 fungal ASVs in *C. dactylon*, *P. oleracea*, *E. indica*, and *B. pilosa*, respectively. Each weed had unique and common bacterial and fungal ASVs. The total and unique bacterial and fungal ASV numbers in *B. pilosa* were higher than those in the other three native weeds (Fig. 1). This finding indicates that the rhizosphere bacterial and fungal species of *B. pilosa* were richer than those of the other three weeds. This may be beneficial to the invasion of *B. pilosa* because the increase in total and unique bacterial and fungal ASVs increased number of niches where nutrients could be available [22], and rare microbial populations represent the 'seed bank' increasing the stability of microbial community [23]. Xiong et al [24] confirmed that rare taxa play important roles in fungal co-occurrence networks and ecosystem functions. Furthermore, rare fungal taxa are influenced more by host selection than by environmental interference. Consequently, our results suggest that the invasion of *B. pilosa* increases rare microbial groups, which improves the stability of the soil microbial community and expands the scope of resource utilization.

The richness estimator Chao1 index and the abundance-based Shannon index were used to show the alpha diversity of the soil microbial communities. The bacterial Chao1 and Shannon indices of *B. pilosa* were not significantly different from those of *P. oleracea* and *C. dactylon* but were significantly higher than those of *E. indica*. There were no significant differences in the fungal Chao1 and Shannon indices among the weeds (Fig. 2). The results indicated that soil microbial alpha diversity was relatively stable in the study area. This finding was consistent with that of Custer and Diepen [25]: soil microbial α-diversity remains constant after invasion, contrary to the aboveground counterparts. The relative stability of α-diversity may occur because increasing or reducing the microbial group may be replaced by the reducing or increasing of groups.

Response of bacterial and fungal community composition to the four weeds

NMDS and cluster analysis showed that the bacterial and fungal community structures were clearly divided into four categories among the four weeds, which indicated that the four weeds had a significant influence on the microbial community structure (Fig. S3). The dominant bacterial phyla (relative abundance > 1%) were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Firmicutes, and Rokubacteria, accounting for 96% of the total sequences. There were no significant differences among the four weeds for Proteobacteria, but the abundance of other dominant bacteria varied by the weed species ($p < 0.05$) (Table S1).

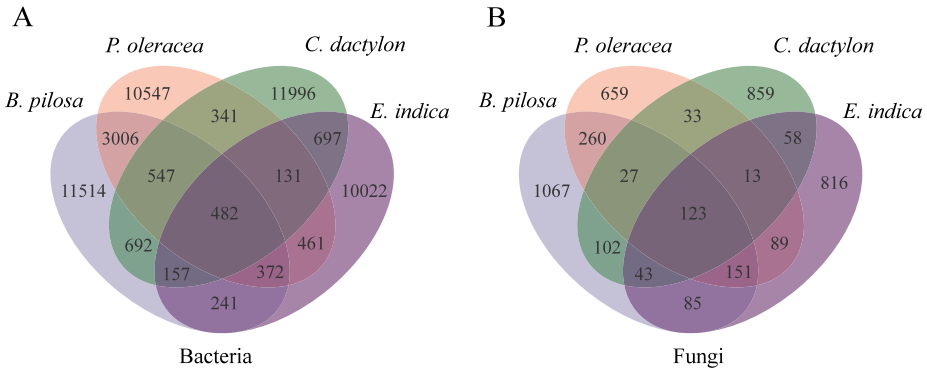

Fig. 1 Bacterial (A) and fungal (B) Venn diagram of ASVs in four different rhizosphere soil of four weed species at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used. Data are shown in means of five replicates ($n = 5$).

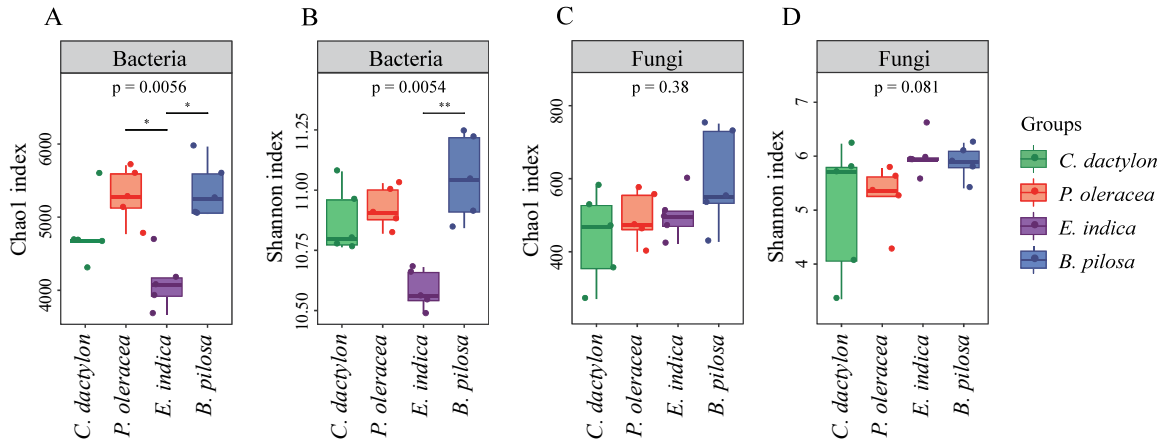

Fig. 2 Bacterial and fungal α-diversity (Chao1 index (A, C) and Shannon index (B, D)) from rhizosphere soil of four different weed species at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used. The asterisks on the bars indicate significant differences in different weeds using the *T*-test. * Significant differences at $p < 0.05$, ** Significant differences at $p < 0.01$.

The dominant fungal phylum, Ascomycota, accounted for 57.07–90.12% of the total soil fungi, followed by Mortierellomycota and Basidiomycota with 0.97–3.14% and 2.13–3.62%, respectively. The abundances of Ascomycota, Mortierellomycota, Chytridiomycota, Rozellomycota, and Olpidiomycota varied by the weed species (Table S2). This finding is consistent with host species being the most important factors in determining microbiome assembly [26].

LEfSe was used to explore the indicator taxa of bacterial (Fig. 3) and fungal (Fig. 4) community structures among the different weeds. The presence of bacterial and fungal indicator taxa in the rhizosphere of *B. pilosa* suggests that this invasive weed formed a specific microbiome that may be involved in invasion success and changes in soil function. For bacterial community composition at the genus level, the abundance of *Nocardioides* and *Gaiella* was higher in *B. pilosa* than in *P. oleracea* and *E. indica*. *Nocardioides* contain many bacteria that degrade xenobiotic compounds, such as deoxynivalenol [27] and atrazine [28]. *Gaiella* was reported to have survival advantages in the presence of high metal (loid) contents [23]. The metabolic versatility of these bacteria might improve the efficiency of carbon resource utilization, which is conducive to the growth of *B. pilosa* because it has a larger biomass and requires more resources than native weeds. Additionally, these bacteria might influence the soil carbon cycling function and promote the expansion of *B. pilosa* as the genera *Nocardioides* and *Gaiella* might adapt to uncommon carbon sources, improving their ability to withstand abiotic perturbations.

Furthermore, *Nocardioides* and *Gaiella* have the ability to directly and indirectly inhibit *Fusarium oxysporum* f. sp. lycopersici abundance [29]. *Enterobacter*, a plant growth-promoting bacterium [30], and *Pseu-*

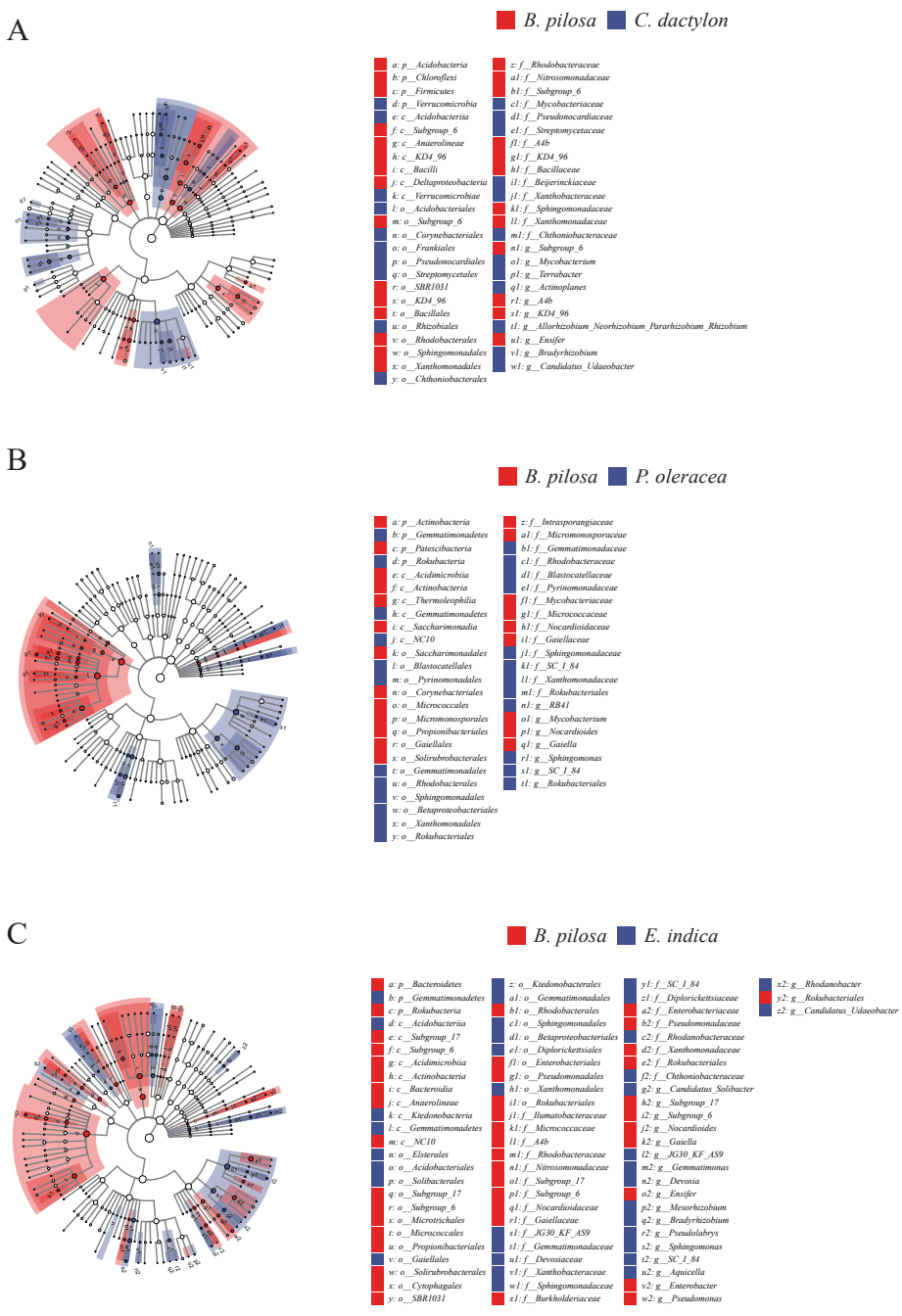

Fig. 3 Linear discriminant analysis effect size analysis (LEfSe) of bacterial taxa in rhizosphere soil of (A) *B. pilosa* vs. *C. dactylon*, (B) *B. pilosa* vs. *P. oleracea*, and (C) *B. pilosa* vs. *E. indica* at Liuxi River Beach. ASVs with LDA scores > 3.5 were used in the analysis. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

domonas, a plant disease-suppressing bacterium [31], are more abundant in *B. pilosa* than in *E. indica*. *Ensifer*, a plant growth-promoting bacterium [32] was more abundant in *B. pilosa* than in *C. dactylon* or *E. indica*. However, the abundance of *Rhizobium* and *Bradyrhizobium* was lower in *B. pilosa* than in *C. dactylon*; the abundance of *Sphingomonas* was lower in *B. pilosa* than in *P. oleracea*; and the abundances of *Devosia*, *Mesorhizobium*, *Bradyrhizobium*, and *Sphingomonas* were lower in *B. pilosa* than in *E. indica*.

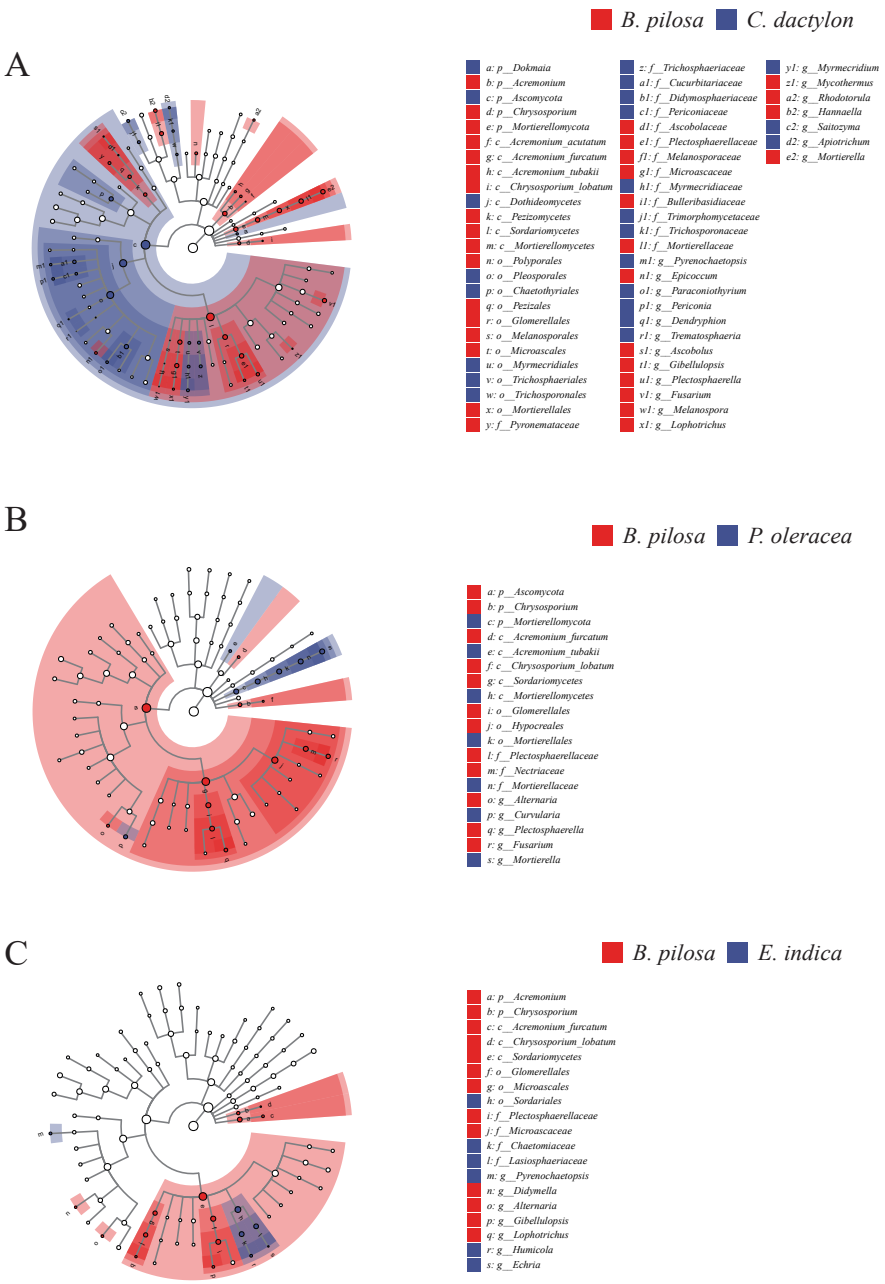

Fig. 4 Linear discriminant analysis effect size analysis (LEfSe) of fungal taxa in rhizosphere soil of (A) *B. pilosa* vs. *C. dactylon*, (B) *B. pilosa* vs. *P. oleracea*, and (C) *B. pilosa* vs. *E. indica* at Liuxi River Beach. ASVs with LDA scores > 3.5 were used in the analysis. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

These genera are plant growth-promoting bacterium, which could increase nutrients (e.g. micronutrients and phosphorous); promote potassium solubilization and uptake in plants; release chemical substances, such as ethylene, IAA, GA, and cytokinins; and improve plant growth under stress conditions [33–35]. The enrichment of these beneficial bacteria in native weeds may play an influential role in resisting the invasion of *B. pilosa*. Investigating the effects of these genera on the germination and growth of *B. pilosa* may provide a new tool for the ecological control of *B. pilosa* invasions. However, this multi-site study was conducted in

South China for one year; thus, multi-annual studies should be conducted to confirm the differences in soil microbial communities between native and invasive weeds.

Regarding fungal community composition at the genus level, the abundance of some pathogenic fungi in the rhizosphere soil of *B. pilosa* was higher than that of the other three native weeds. *Plectosphaerella* and *Fusarium* was higher in *B. pilosa* than in *C. dactylon* and *P. oleracea*. *Didymella* was higher in *B. pilosa* than in *E. indica*. The accumulation of soil pathogens may restrain the growth of native plant species without affecting invaders [36] because exotic invaders can escape the control of local soil pathogens [37]. This finding may be one reason for the successful invasion and expansion of *B. pilosa*. Therefore, we speculate that improving soil microbial diversity and reducing the relative abundance of pathogenic fungi are beneficial for the growth of local plants and inhibit the invasion of *B. pilosa*.

Notably, the abundance of *Lophotrichus*, a dark septate endophyte that can colonize plant roots, improving plant stress tolerance and enhancing the ecological adaptability of the host [38], was higher in the rhizosphere soil of *B. pilosa* than in that of *C. dactylon* and *P. oleracea*. The presence of *Lophotrichus* provided favorable conditions for the invasion of *B. pilosa*. In general, changes in microbial community composition might lead to feedback that influences the aboveground plant community, influencing various ecosystem services and dynamics.

Analysis of microbial metabolic pathways in different weeds

For bacteria, the prediction results based on the MetaCyc database showed seven metabolic pathways in the first functional layer and 60 metabolic pathways in the second functional layer. The first functional layer was Biosynthesis (62.51–66.75%), Degradation/Utilization/Assimilation (14.08–18.13%), Detoxification (0.03–0.29%), Generation of Precursor Metabolite and Energy (14.59–15.89%), Glycan Pathways (0.68–0.86%), Macromolecule Modification (0.57–0.80%), and Metabolic Clusters (2.29–2.55%) (Table 2). For fungi, there were five metabolic pathways in the first functional layer and 29 metabolic pathways in the second. The first functional layer was Biosynthesis (62.51–66.75%), Degradation/Utilization/Assimilation (14.08–18.13%), Detoxification (0.03–0.29%), Generation of Precursor Metabolite and Energy (14.59–15.89%), Glycan Pathways (0.68–0.86%), Macromolecule Modification (0.57–0.80%), and Metabolic Clusters (2.29–2.55%) (Table S3). The microbial metabolic pathways varied by the weed species because invasive plants might influence soil nutrient cycling and soil microbial communities through litter accumulation and rhizosphere interactions, influencing the metabolic functional diversity of the soil microecosystem [39]. PCoA analysis of functional units showed that the bacterial metabolic pathway was clearly divided into four categories under four weeds, whereas there was no obvious clustering of fungal metabolic pathways (Fig. S4).

Volcano plots (Fig. 5) were used to show the differences in metabolic function of the second functional layer. No significant differences between *C. dactylon* and *B. pilosa* in the second metabolic pathway were observed (Fig. 5A). Pyrimidine deoxyribonucleotide biosynthesis from CTP and *de novo* pyrimidine deoxyribonucleotide biosynthesis IV belonging to Metabolic Clusters were lower in *B. pilosa* than that of in *E. indica* and *P. oleracea* (Fig. 5B,C). Because the main function of deoxyribonucleotides is to guide protein synthesis, the decrease in these two pathways suggests that *B. pilosa* potentially reduces protein metabolism.

The antibiotic resistance of *B. pilosa* was greater than that of *E. indica* and *P. oleracea*; the increase of antibiotic resistance might improve the competitiveness of *B. pilosa*. The alcohol and aldehyde degradation of *E. indica*, methanol oxidation to carbon dioxide of *P. oleracea*, Entner-Doudoroff pathways, and methyl ketone biosynthesis of *E. indica* and *P. oleracea* were lower than those of *B. pilosa*. These pathways were related to the carbon cycle, which indicated that the rhizosphere soil microorganisms of *B. pilosa* had a strong carbon metabolism ability. This phenomenon was beneficial for improving the carbon metabolism capacity of *B. pilosa*, promoting its invasion and expansion. Rodríguez-Caballero also found that invasive *N. glauca* shifted the soil microbial community composition and its function related to C and S cycling [8].

In this study, the soil carbon metabolism ability in *B. pilosa* rhizosphere soil was higher than in the other three native weeds may be related to the abundance of carbon-metabolizing bacteria in *B. pilosa* rhizosphere soil was higher than in the other three native weeds. A high soil carbon metabolic rate was conducive to promoting the growth *B. pilosa*; however, for an undisturbed natural environment, a fast soil metabolic rate may be detrimental to the storage of soil carbon. McLeod et al [40] also found that the invasion of *knapweed* reduced soil carbon storage.

Redundancy analysis of soil physical and chemical properties, microbial communities, and bacterial metabolic pathways

RDA (Fig. 6) was used to explain the relationship between the soil physical and chemical properties and bacterial and fungal community structures. The results showed that the soil physical and chemical properties might explain 79.13%, 69.73%, and 68.20% of the variation in the bacterial community structure,

Table 2 Abundance of rhizosphere soil bacterial metabolic pathways in the first functional layer in four different wees at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Groups	Biosynthesis	Degradation/Utilization/ Assimilation	Detoxification	Generation of precursor metabolite and energy
C. dactylon	154496.71 ± 995.42^{c}	42982.92 ± 699.86^{a}	563.94 ± 42.02^{a}	38133.42 ± 349.71^{a}
P. oleracea	163709.99 ± 722.49^{a}	39666.54 ± 1380.24^{b}	200.65 ± 30.87^{b}	37294.57 ± 124.36^{a}
E. indica	162431.15 ± 740.80^{a}	36846.62 ± 640.26^{c}	223.40 ± 38.45^{b}	37425.50 ± 178.76^{a}
B. pilosa	158759.42 ± 651.07^{b}	42167.14 ± 653.51^{ab}	464.18 ± 53.81^{a}	37967.21 ± 403.41^{a}
Groups	Glycan pathways	Macromolecule modification	Metabolic clusters	
C. dactylon	1942.23 ± 28.87^{a}	1387.85 ± 39.38^{b}	5672.10 ± 37.5^{c}	
P. oleracea	1827.25 ± 29.59^{b}	1795.45 ± 36.82^{a}	5990.04 ± 57.41^{b}	
E. indica	1939.43 ± 49.45^{a}	1742.28 ± 64.23^{a}	6184.42 ± 32.08^{a}	
B. pilosa	1744.64 ± 28.23^{b}	1477.20 ± 42.86^{b}	$5870.30 \pm 30.22b$	

Data are shown as the means ± standard errors of five replicates ($n = 5$). Values followed by a different letter in the same column indicate significant differences at $p < 0.05$ using Duncan's test.

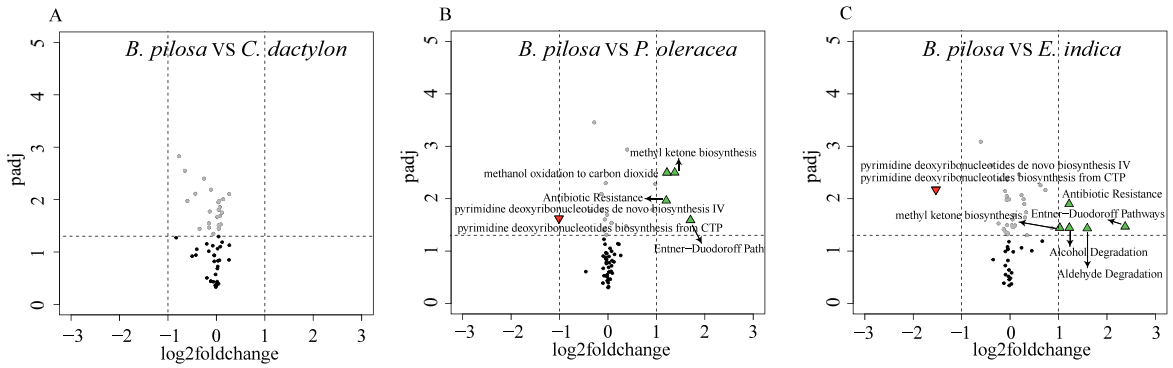

Fig. 5 Volcano map of rhizosphere soil bacterial metabolic pathways among (A) *B. pilosa* vs. *C. dactylon*, *B. pilosa* vs. *P. oleracea*, and (C) *B. pilosa* vs. *E. indica*. The red solid triangle symbols represent that these metabolic pathways of *B. pilosa* were lower than those of native weeds. The green solid triangle symbols represent that these metabolic pathways of *B. pilosa* were higher than those of native weeds. The gray solid dot symbols represent that these metabolic pathways between *B. pilosa* and native weeds were not significantly different.

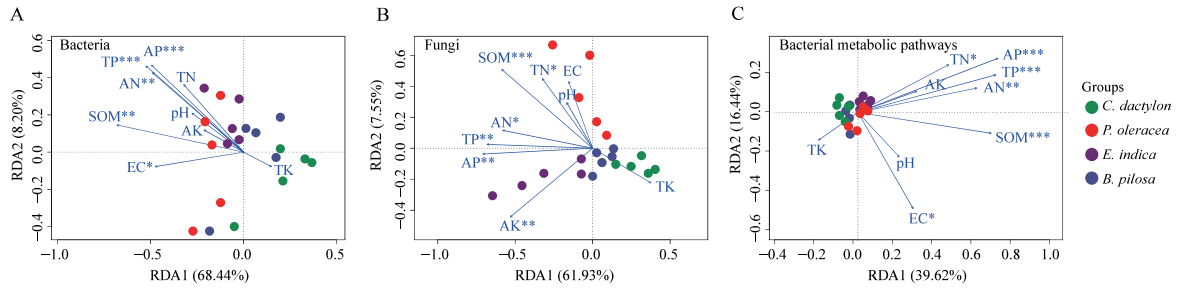

Fig. 6 Redundancy analysis (RDA) of soil physical and chemical properties and soil bacterial (A) and fungal (B) community structure and bacterial metabolic pathways (C) in four different weed rhizospheres. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used. The selected variables included pH, EC, SOM, TN, TP, TK, AN, AP, AK. The asterisks indicate that this index had a significant influence on community structure and bacterial metabolic pathways. * Significant differences at $p < 0.05$, ** Significant differences at $p < 0.01$, *** Significant differences at $p < 0.001$.

fungal community structure, and bacterial metabolic pathways, respectively. These results indicate that the soil microbial community structure and metabolic pathways are closely related to soil physical and chemical properties. TP, AP, and SOM contents were the primary edaphic factors that influenced the structure of the microbial community in our study. This finding may have been observed because the TP, AP, and SOM contents of *B. pilosa* and *C. dactylon* were significantly lower than those of *E. indica* and *P. oleracea*. Low TP, AP, and SOM content may inhibit the growth of other plants. This finding was consistent with that of Shrader-Frechette [3], who reported that invasive plants might inhibit the growth of local plants by reducing the nutrient levels of the soil and changing the pH value of the soil. Notably, the physical and chemical soil properties of *C. dactylon* were similar to those of *B. pilosa*; however, the microbial community structures and functions of the two weeds were significantly different. This finding further confirmed that the host species was the primary variable responsible for the structure and function of the microbial community. Thus, the invasion of *B. pilosa* would affect the soil environment and function.

CONCLUSION

In summary, although the three native weeds originated from different families, the differences in the rhizosphere soil microorganisms between *B. pilosa* and the other three native weeds had characteristics in common. A higher total and unique bacterial and fungal ASV numbers in *B. pilosa* rhizosphere resulted in a higher number of niches where nutrients were available and increased the stability of the microbial community. The enrichment of beneficial bacteria in native weeds may provide a new tool for the ecological control of the invasion of *B. pilosa*. The enrichment of pathogenic fungi in *B. pilosa* rhizosphere soil might inhibit the growth of other plants, and the existence of *Lophotrichus* may enhance the ecological adaptability of *B. pilosa*. Because the composition and function of the soil microbial community might be predictors of soil function, these results imply that the invasion of *B. pilosa* may have an important influence on native ecosystem function. However, the differences in metabolic pathways in this study were predicted by PICRUSt2 software, which may not fully reflect the metabolic information of soil microorganisms between native weeds and *B. pilosa*. Thus, further research utilizing metagenomics and metabolomics should be conducted to study the relationship between microbial function and root exudates of weeds.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.099.

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Appendix A. Supplementary data

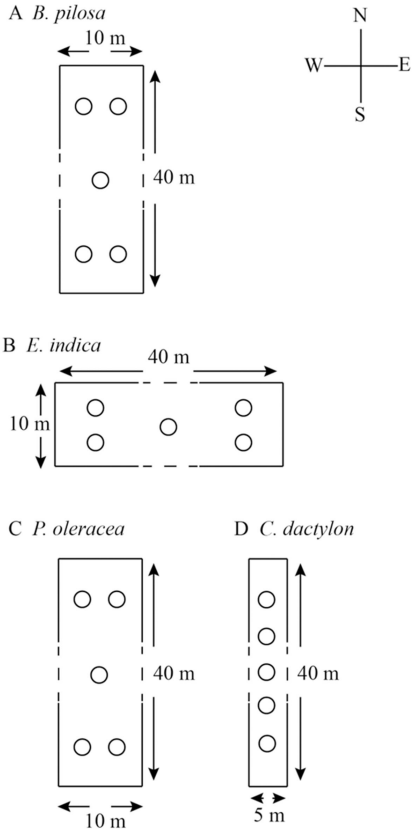

Fig. S1 Diagram of the sampling area of four weeds: three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa*. The circles in different boxes represent different weed sampling points.

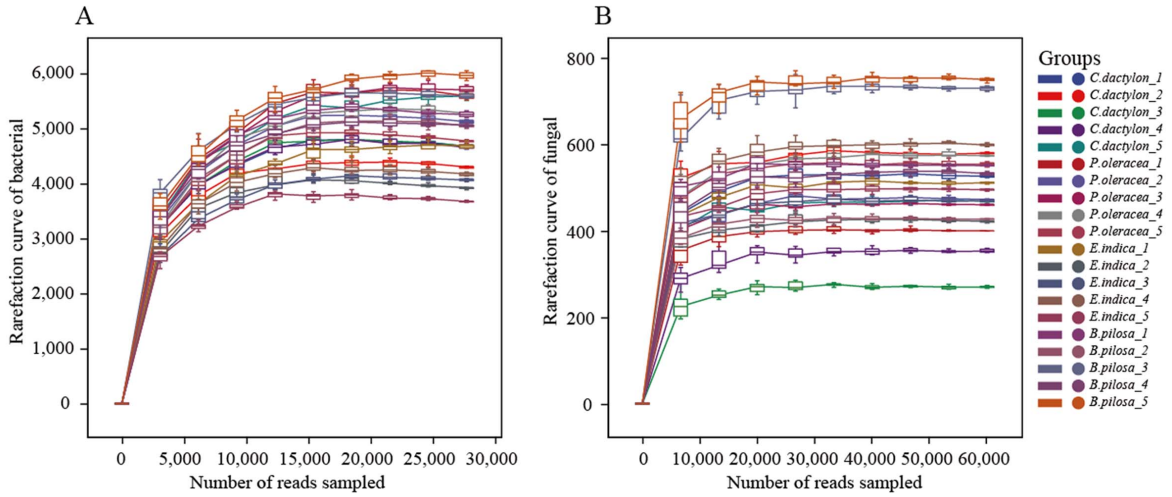

Fig. S2 Rarefaction curve of bacterial (A) and fungal (B) sequence numbers from four different weed rhizosphere soils at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

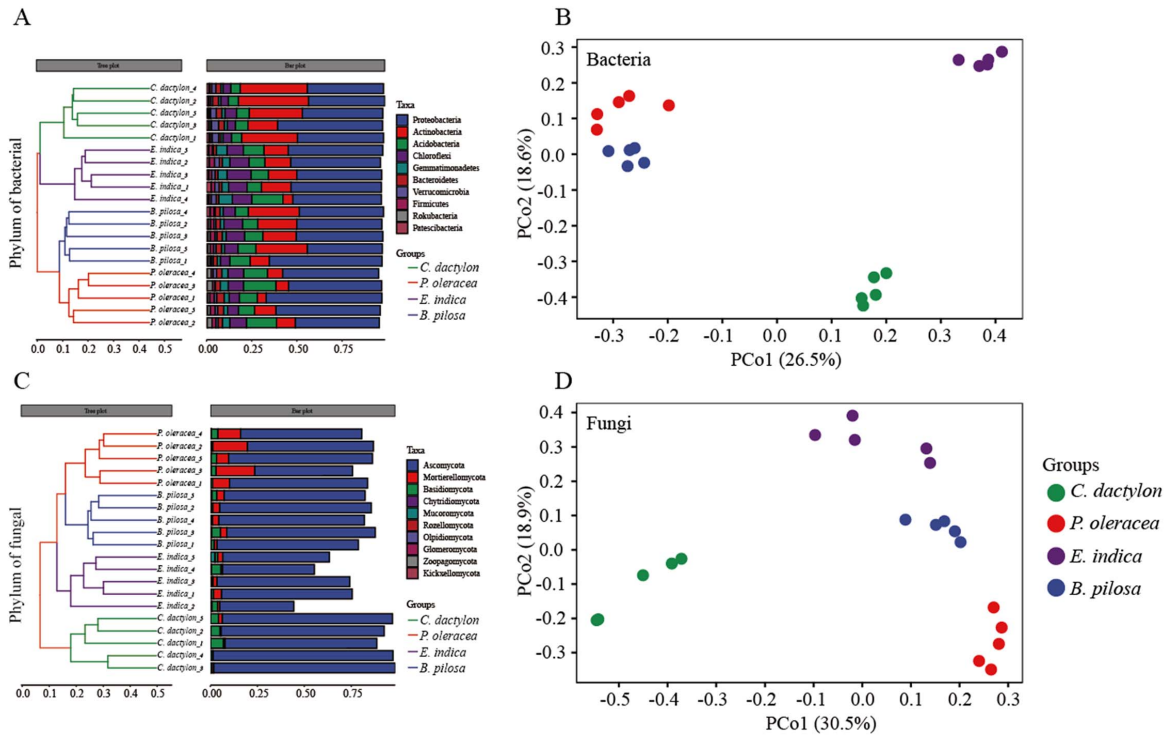

Fig. S3 Cluster analysis at the phylum level (A, C) and principal coordinate analysis (PCoA) (B, D) at the ASV level of soil bacterial and fungal community structure in four different weed rhizospheres in the Liuxi River beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

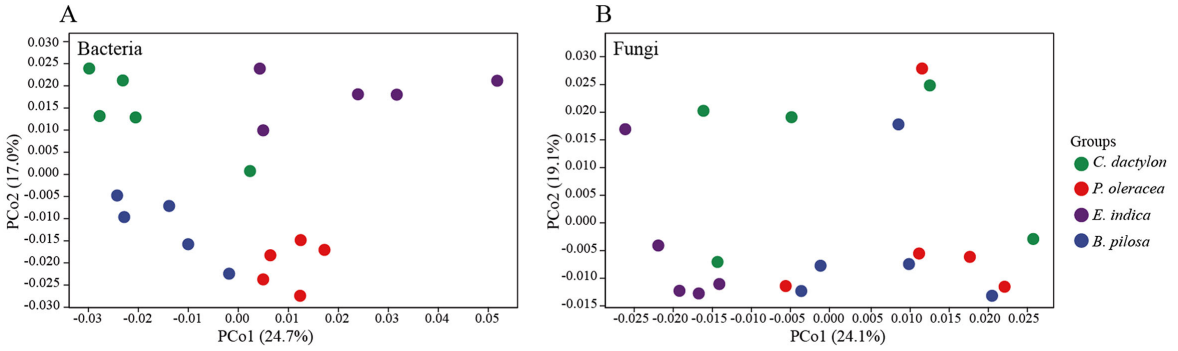

Fig. S4 Principal coordinate analysis (PCoA) of soil bacterial (A) and fungal (B) metabolic pathways in four different weed rhizospheres at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Table S1 Relative abundance of bacteria at the phylum classification rank of rhizosphere soil in four different weed species at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Groups	Proteobacteria	Actinobacteria	Acidobacteria	Chloroflexi	Gemmatimonadetes
C. dactylon	0.4687 ± 0.02942^{a}	0.3049 ± 0.03921^{a}	0.0605 ± 0.00376^{b}	0.0482 ± 0.00414^{c}	0.0143 ± 0.00141^{c}
P. oleracea	0.545 ± 0.02971^{a}	0.085 ± 0.01253^{c}	0.133 ± 0.01788^{a}	0.0761 ± 0.00739^{b}	0.0337 ± 0.00412^{b}
E. indica	0.4954 ± 0.00857^{a}	0.1305 ± 0.01956^{bc}	0.1101 ± 0.01648^{b}	0.1074 ± 0.00717^{a}	0.0531 ± 0.00653^{a}
B. pilosa	0.4909 ± 0.03505^{a}	0.214 ± 0.03332^{b}	0.094 ± 0.00716^{ab}	0.0797 ± 0.01001^{b}	0.0186 ± 0.001^{c}
Groups	Bacteroidetes	Verrucomicrobia	Firmicutes	Rokubacteria	Others
C. dactylon	0.0268 ± 0.00367^{a}	0.0285 ± 0.00519^{a}	0.0086 ± 0.00114^{b}	0.0066 ± 0.00128^{bc}	0.0295 ± 0.00218^{b}
P. oleracea	0.0309 ± 0.00268^{a}	0.0126 ± 0.00247^{b}	0.0195 ± 0.00311^{a}	0.0209 ± 0.00342^{a}	0.0361 ± 0.00312^{ab}
E. indica	0.0153 ± 0.00053^{b}	0.0207 ± 0.00405^{ab}	0.0186 ± 0.00289^{a}	0.0034 ± 0.00086^{c}	0.0398 ± 0.00445^{a}
B. pilosa	0.0266 ± 0.00148^{a}	0.0105 ± 0.00147^{b}	0.0185 ± 0.00277^{a}	0.0105 ± 0.00198^{b}	0.0313 ± 0.00172^{ab}

Data are shown as the means ± standard errors of five replicates ($n = 5$). Values followed by a different letter in the same column indicate significant differences at $p < 0.05$ using Duncan's test.

Table S2 The relative abundance of fungi at the phylum classification rank of rhizosphere soil in four different weed species at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Groups	Ascomycota	Mortierellomycota	Basidiomycota	Chytridiomycota
C. dactylon	90.12 ± 2.924^{a}	0.97 ± 0.294^{b}	3.62 ± 1.14^{a}	0.05 ± 0.04^{b}
P. oleracea	66.79 ± 4.27^{bc}	13.32 ± 2.706^{a}	2.13 ± 0.58^{a}	0.17 ± 0.06^{b}
E. indica	57.07 ± 6.00^{c}	2.23 ± 0.60^{b}	2.20 ± 0.81^{a}	0.12 ± 0.06^{b}
B. pilosa	77.49 ± 1.09^{b}	3.14 ± 0.42^{b}	2.13 ± 0.70^{a}	0.40 ± 0.12^{a}
Groups	Mucoromycota	Rozellomycota	Olpidiomycota	Others
C. dactylon	0.00 ± 0.00001^{a}	0.0001 ± 0.00008^{b}	0.00 ± 0.00001^{b}	5.22 ± 1.82^{c}
P. oleracea	0.0004 ± 0.00006^{a}	0.0003 ± 0.0002^{b}	0.0014 ± 0.0003^{a}	17.39 ± 2.02^{b}
E. indica	0.0062 ± 0.004^{a}	0.0035 ± 0.002^{a}	0.00 ± 0.00002^{b}	37.41 ± 5.85^{a}
B. pilosa	0.0004 ± 0.0002^{a}	0.0002 ± 0.0001^{b}	0.0004 ± 0.000223^{b}	16.74 ± 1.55^{b}

Data are shown as the means ± standard errors of five replicates ($n = 5$). Values followed by a different letter in the same column indicate significant differences at $p < 0.05$ using Duncan's test.

Table S3 Abundance of rhizosphere soil fungal metabolic pathways in the first functional layer in four different weed species at Liuxi River Beach. Four weeds – three native weeds, *C. dactylon*, *P. oleracea*, and *E. indica*, and one invasive weed, *B. pilosa* – were used.

Groups	Biosynthesis	Degradation/Utilization/ Assimilation	Generation of precursor metabolite and energy	Glycan pathways	Metabolic clusters
C. dactylon	23368.24 ± 157.36^{c}	6540.27 ± 160.67^{b}	18466.68 ± 176.69^{bc}	786.01 ± 5.44^{b}	2873.25 ± 12.13^{b}
P. oleracea	26108.80 ± 840.27^{a}	7347.45 ± 327.10^{a}	19143.84 ± 184.88^{a}	808.79 ± 6.37^{a}	3330.52 ± 143.52^{a}
E. indica	25198.20 ± 414.28^{ab}	7396.04 ± 91.17^{a}	19015.32 ± 320.52^{ab}	785.44 ± 6.83^{b}	3325.24 ± 65.66^{a}
B. pilosa	24216.60 ± 234.10^{bd}	7075.05 ± 156.04^{ab}	18257.30 ± 93.10^{c}	786.33 ± 2.96^{b}	3090.22 ± 58.27^{ab}

Data are shown as the means ± standard errors of five replicates ($n = 5$). Values followed by a different letter in the same column indicate significant differences at $p < 0.05$ using Duncan's test.