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REGULAR ARTICLE

Soil fungi rather than bacteria were modified by invasive plants, and that benefited invasive plant growth

Hai Feng Xiao · Yu Long Feng · Douglas A. Schaefer · Xiao Dong Yang

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Abstract
Background and aims Successful invasion by exotic plant species can modify the abundance and composition of soil microbial communities. *Eupatorium adenophora* and *Chromolaena odorata* are exotics that have become highly invasive plants in China. Several studies have investigated mechanisms of their successful invasions including phenotypic plasticity, genetic differentiation, and allelopathy, but little is known about their effects on soil microorganisms. Moreover, whether soil microbial community changes could cause feedback effects on these plant species is also not known. We seek a belowground microbiological mechanism supporting successful invasions by these exotic plants.
Methods In this study, two invasive (*E. adenophora* and *C. odorata*) and two native plant species (*Eupatorium japonicum* and *Eupatorium heterophyllum*) were used to compare the soil feedback (on plant growth) before and after soil sterilization and from plant-root exudates. Bacterial and fungal biomass and community composition were also examined.
Results We found that soil sterilization significantly increased biomass of native species and did not affect the invasive species' biomass. After root exudates from these plants had acted on the soil biota for 10 months, soil sterilization significantly decreased the growth of *E. adenophora* and *C. odorata* and continued to significantly increase the biomass of two native species. Denaturing gradient gel electrophoresis revealed that these four plant species modified fungal rather than bacterial communities in soil.
Conclusions Higher abundance of *Paraglomus sp*. in soil with *C. odorata* is likely to provide *C. odorata* roots with more soil nutrients. Considered together, these results strongly suggest that invasive *E. adenophora* and *C. odorata* created a belowground feedback that may be a mechanism contributing to their success as invasive species.

Keywords *Eupatorium adenophora* · *Chromolaena odorata* · *Eupatorium japonicum* · *Eupatorium heterophyllum* · Plant invasion · Microbial biomass · Microbial community composition · AMF

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Introduction

Alteration of belowground microbial communities has been reported to contribute to the invasion of many exotic plant species (Kourtev et al. 2002; Li et al. 2006; Kao-Kniffin and Balser 2007; Sanon et al.

2009). However, most studies focus on microbial biomass, abundance or diversity (Belnap and Phillips 2001; Li et al. 2007), relatively few studies examine microbial community composition changes with molecular methods to explore mechanisms related to successful invasion by exotic plants (Lorenzo et al. 2010).

There is evidence that exotic invasive plants modify soil biological communities, and may establish a positive feedback favoring exotic plants over natives (Callaway et al. 2004). For example, Hahn (2003) found that the invasive plant *Zostera japonica* altered microbial community composition and accelerated rates of decomposition, thereby facilitating higher carbon and nutrient turnover. Moreover, symbiosis of exotic invasive plants with fungi, which increase nutrient supply, is another typical positive feedback strategy (Reynolds et al. 2003; Callaway et al. 2004). Positive feedback strategies involving soil microbes may be tightly linked with successful invasions by exotic plant species.

Eupatorium adenophora Spengel and *Chromolaena odorata* (L.) R. M. King and H. Robinson are native to Mexico but are considered as noxious invasive perennial herbs or sub-shrubs in numerous countries in Asia, Oceania, and Africa. These plants were first found in Yunnan Province of southwest China more than 70 years ago and have recently become two of the most noxious invasive plants in China (Feng et al. 2009; Li et al. 2012). These plants can reduce aboveground vegetation diversity and cause diseases after consumption by domestic animals (Feng et al. 2009). Thus, mechanisms contributing to the invasive success of *E. adenophora* and *C. odorata* require investigation.

Previous studies reported explained successful invasions by *E. adenophora* and *C. odorata* in terms of aboveground phenotypic plasticity, genetic differentiation, and allelopathy (Feng et al. 2007; Li and Feng 2009; Sangakkara et al. 2008). However, effects of these plants on soil are rarely investigated (Niu et al. 2007; Mangla et al. 2008). Plants provide C for soil microorganisms through root exudates and litter (Kao-Kniffin and Balser 2007; Drigo et al. 2010). Therefore, we hypothesize that *E. adenophora* and *C. odorata* can modify soil microbial communities to their own benefit.

Microbial community responses to *E. adenophora* and *C. odorata* and microbially induced soil feedbacks have not been investigated. DNA-based techniques can measure the diversity and composition of soil microbial communities, including non-culturable species. Although denaturing gradient gel electrophoresis (DGGE) method provides only a limited description of diversity, this DNA-based technique can show dominant microbial community composition differences among multiple treatments (Kirk et al. 2004). In this study, DGGE was employed to analyze dominant bacterial and fungal communities by using eubacteria-specific primers for 16S rRNA genes (Muyzer et al. 1993) and primers for 18S rRNA fungal genes (May et al. 2001).

Our objectives were (1) to test the hypothesis whether invasive and native plants have different effects on bacterial and fungal community, (2) to detect feedbacks of soil organisms on invasive and native plant species, and (3) to further explore belowground microbiological mechanisms behind successful invasions by *E. adenophora* and *C. odorata*.

Materials and methods

Experiment design and sample collection

The study was conducted in the Xishuangbanna Tropical Botanical Garden, a long-term ecological experimental station of the Chinese Academy of Sciences in Yunnan Province, China (21.41° N, 101.25° E). The soil used in this experiment was sandy loam, composed of 50.5 % sand, 27.6 % silt, and 21.9 % clay. The soil contained 31.04 g kg^{-1} organic C, 2.61 g kg^{-1} total N, 19.45 mg kg^{-1} NH_4^+, 3.23 mg kg^{-1} NO_3^-, 196 mg kg^{-1} available P, and 134 mg kg^{-1} available K. The pH (H_2O) was 6.5. These soils were obtained from a nearby vegetable field and not previously invaded by *E. adenophora* or *C. odorata*.

Experiment 1 The soil described above was divided into two parts, one part was sterilized (121 °C, 30 min, three times, 24 h between sterilizations, following Callaway et al. 2004) and the other part was not sterilized. Soil C, N, and available P and K were measured in both sterilized and unsterilized soil (four replicates) (Table S1). Soil total C and N was measured by Vario MAX CN (Germany) using 1 g air-dried soil passed through a 0.15 mm diameter mesh. NH_4^+-N and NO_3^--N were extracted with 2 M KCl (soil:KCl 1:5, 30 min shaking), and measured by continuous flow auto-analysis (AA3, Germany) (Mao et al. 2006). Available P was extracted with 0.025 mol L^{-1} HCl and 0.03 mol L^{-1} NH_4F (soil: solution 1:10, 30 min shaking), and also measured by continuous flow auto-analysis. Available K was

extracted with 1 mol L^{-1} CH_3COONH_4 (soil: solution 1:10, 30 min shaking), and measured by iCAP6300 (US) (Luo et al. 2011). Then, both sterilized and non-sterilized soils were transferred into 30-cm diameter and 40-cm high pots and stored until planting the seedlings. Four plant species, including two invasives, *E. adenophora* and *C. odorata*, and two natives, *E. japonicum* Thunb. and *E. heterophyllum* DC, were used in this study. Native *E. japonicum* and *E. heterophyllum* were selected because of their close resemblance to the two invasives. These two natives are also perennial herbs.

All plant seeds were disinfected with NaOCl (60 mg L^{-1}) for 20 min, and then washed five times with sterile water (Sauer and Burroughs 1986). After this, disinfected plant seeds were spread evenly in sterilized humus soil mixed with vermiculite. Then, the seeds were covered with a thin soil layer and received sufficient water. All the containers were placed under 50 % relative light intensity in an incubator. After the seedlings reached a height of approximately 8 cm, those of similar size in healthy condition were selected and transplanted into pots (one seedling per pot). The plants were irrigated with sterile water as frequently as necessary to keep the soil moist. A total of 64 pots consisting of two treatments (sterilized and non-sterilized), four plant species (two invasives and two natives) with eight replicates for each plant species were used. The seedlings were transplanted in February 2011 and harvested in December 2011 after a growth period of 10 months in a greenhouse. No additional illumination or fertilization was provided during the 10 months growing period. Finally, all the pots were harvested and plant biomass was measured (after drying at 80 °C to constant weight), and the soil in each pot was homogenized immediately for microbial biomass and community analyses. However, we did not obtain PCR production from sterilized soils, although we extracted sterilized and non-sterilized soil DNA under the same condition simultaneously, and we amplified PCR production also at the same condition. This may be due to the inadequate microbial template in the sterilized soil samples or that sterilization affects DNA extraction efficiency from soil. Thus, sterilized soils were not sampled for microbial biomass and DGGE analyses. Finally, four non-sterilized soil samples of each plant species were randomly selected for soil microbial biomass analysis (totally 16), and three of these four non-sterilized soil samples in each plant species were randomly selected for soil microbial community analyses. At the end of experiment 1, we stored some soil samples from each pot at −80 °C prior to soil-nutrient measurements.

Experiment 2 We collected all the unsterilized soil from the pots having previously contained these plants. These soils were again divided into two parts, one part was sterilized and the other part was not. Then, both sterilized and non-sterilized soils were transferred into new pots and plant seedlings were transplanted into them. A total of 48 pots that contained the two soil treatments (sterilized and non-sterilized), four plant species with six replicates each were used. The growth conditions were as described above, and after 10 months of growth, we harvested plants and measured their biomass as previously. Soil samples from each pot were also stored for soil-nutrient measurements as described above.

Bacterial and fungal biomass

Glucosamine and muramic acid were used to assess fungal and bacterial biomass, respectively. Glucosamine and muramic acid concentrations were measured according to the procedure described by Appuhn et al. (2004). Briefly, a sieved 500 mg moist soil sample was weighed and placed into a 20 mL test tube. The sample was mixed with 10 mL 6 M HCl and heated for 6 h at 105 °C. After the HCl was removed and the soil sample was centrifuged, the supernatant was transferred into vials and stored at −18 °C until high-performance liquid chromatography measurements were performed. Approximately 5 μl sample and 20 μl *ortho*-phthalaldehyde reagent were extracted with a sample injector. Fluorometric emission of amino sugars was measured at a wavelength of 445 nm with an excitation wavelength of 340 nm (Agilent 1200). Fungal C (mg g^{-1} dry weight) was calculated by subtracting bacterial glucosamine from total glucosamine as an index for fungal residues, assuming that muramic acid and glucosamine occur at a 1 to 1 molar ratio in bacterial cells (Appuhn and Joergensen 2006). Fungal C (μg g^{-1} dry soil) = (mol glucosamine – mol muramic acid) × 179.2 (molecular weight of glucosamine) × 9 (an average conversion value from fungal glucosamine to fungal C). Bacterial C (μg g^{-1} dry soil) was calculated as an index for bacterial residues by multiplying the content of muramic acid in μg g^{-1} dry weight by 45 (an average conversion value from muramic acid to bacterial C) (Appuhn and Joergensen 2006).

Extraction and purification of DNA from soil samples

Microbial community structure was determined by DNA-based analysis using polymerase chain reaction (PCR)-DGGE. DNA was extracted by mixing a 5 g soil sample with 13.5 mL DNA extraction buffer containing 100 mmol Tris·HCl, 100 mol EDTA·Na_2, 100 mmol Na_3PO_4, 1.5 mol NaCl, and 10 g L^{-1} CTAB at pH 8.0. The samples were incubated for 30 min at 37 °C in a horizontal shaking bath at 225 rev min^{-1}. After three alternating cycles of freezing and thawing in liquid nitrogen and a water bath at 65 °C, 1.5 mL 200 g L^{-1} sodium dodecyl sulfate was added, and the samples were further incubated for 2 h at 65 °C with manual agitation every 15 min. The samples were then centrifuged (6,000×*g*) at room temperature for 10 min to collect the supernatant. The supernatant was then transferred to another 50 mL centrifuge tube for phenol extraction, followed by chloroform-isoamyl alcohol (v: v=24:1) purification. The aqueous phase was transferred to a 50 mL centrifuge tube, and 0.6 volumes of isopropanol were added to the tube, which was incubated at room temperature for 1 h. The samples were centrifuged (6,000×*g*) at room temperature for 20 min, and then nucleic acids were collected and washed with cold 70 % ethanol dissolved in 300 μl sterile ultra-pure water. The crude DNA product was purified by using an OMEGA (Omega Bio-Tek, USA) purification kit, and the operating procedures were following the manual.

PCR and DGGE

The primers for 16s rRNA gene amplification of bacteria were 338GC: 5′ CC TAC GGG AGG CAG CAG 3′ 518R: 5′ ATT ACC GCG GCT GCT GG 3′ (Muyzer et al. 1993), and those for the 18s rRNA gene amplification of fungi were Fung GC: 5′ ATT CCC CGT TAC CCG TTG 3′ NS1: 5′GTA GTC ATA TGC TTG TCT C 3′ (May et al. 2001). A 40 bp GC clamp was added at the 5′ end of the forward primers. For bacterial amplification, the PCR protocol included a 5-min initial denaturation at 94 °C; 30 cycles at 94 °C for 30 s, at 61 °C for 30 s, and at 72 °C for 30 s; followed by a final extension at 72 °C for 5 min. For fungal amplification, the PCR protocol included a 5-min initial denaturation at 94 °C; 30 cycles at 94 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 45 s; followed by a final extension at 72 °C for 5 min. The 50 μL reaction mixtures contained 1×PCR reaction buffer (TaKaRa, Japan), 100 ng DNA template (DNA quantity was determined by using Qubit® 2.0 (Life Technologies, Invitrogen, USA)), 10 pmol L^{-1} of forward and reverse primers, 200 μmol L^{-1} dNTP mix, and 2.5 U of Ex Taq DNA Polymerase (TaKaRa, Japan).

DGGE of PCR products was conducted by using a DCode mutation detection system (Bio-Rad, USA). Polyacrylamide gels (8 % of a 37.5:1 acrylamide–bisacrylamide mixture in 1×TAE buffer) with gradient ranging from 40 to 60 % denaturant for bacteria and from 20 to 40 % for fungi were used, with 100 % denaturant being defined as 7 mol L^{-1} urea and 40 % (V/V) formamide (Muyzer et al. 1993). Approximately 200 ng of each PCR product was loaded, and then the gels were electrophoresed for 5 h at 200 V and at 60 °C. The gel was then silver stained and prepared for image analysis by using Quantity One gel analysis software, version 4.62 (Bio-Rad, USA).

Cloning and sequencing

The selected bands from DGGE gel were cut out and re-amplified using a primer without GC clamp, and the PCR products were purified by using the OMEGA quick PCR purification kit prior to cloning. Approximately 4 μl of purified products were ligated into the PMD-19T vector (TaKaRa Cloning® Kit) and then further transformed into *Escherichia coli* competent cells DH5α (TaKaRa). White colonies were randomly selected from each cloned sample, grown overnight, and then sequentially reacted on an ABI 377 apparatus (BGI Company). The nucleotide sequences were deposited in the GenBank database and assigned with accession numbers JX126739 to JX126762.

Statistical analysis

To examine impacts of sterilization and plant species on plant biomass and soil nutrients, we log- or square-root transformed biomass and soil nutrients data as needed to achieve normality and eliminate heteroscedasticity. Two-way ANOVA (on sterilization and plant species) was used to analyze differences in plant biomass and soil nutrients, and multiple comparisons were performed using the Duncan's test to determine significant differences. One-way ANOVA was used to analyze the difference between bacterial and fungal biomass. Duncan's test was employed to determine significant differences among treatments. The significance level was set at $P<0.05$. Statistical analysis was performed by using

SPSS 13.0. Quantity one software was used to digitize the fungal DGGE profile according to the relative band intensity. Finally, principal-component analysis (PCA) was employed to analyze the digitized data.

Results

Plant biomass and soil nutrient

In the first experiment, soil sterilization differently affected plant biomass (Fig. 1a). No significant differences were observed between invasive *E. adenophora* and *C. odorata* biomass in sterilized and non-sterilized soil. However, sterilization significantly increased the biomass of native *E. japonicum* and *E. heterophyllum*. At the end of experiment 1, analysis of soil nutrients (Table 1) showed that ammonium and nitrate in sterilized were significantly lower than in non-sterilized soils. In addition, nitrate concentrations in non-sterilized soils in two invasive plants were significantly higher than in two native species (F=330.623, P<0.01). In the second experiment (Fig. 1b), sterilization significantly decreases the plant biomass of *E. adenophora* and *C. odorata* but still significantly increased the biomass of two native species. Soil levels of ammonium, available P and K concentration in sterilized soils were significantly lower than in non-sterilized soils (Table 2). Compared to native species, invasive species had higher nitrate concentrations in non-sterilized soils (F=311.161, P<0.01).

Bacterial and fungal biomass

In the non-sterilized soil, bacterial biomass did not significantly differ among species (Fig. 2). Fungal biomass in the soil with *C. odorata* was significantly higher than with the other plant species.

DGGE analysis of bacterial and fungal communities

In the non-sterilized soil, DGGE was run on both bacterial 16s rRNA and fungal 18s rRNA amplicons from the four plant treatments to reveal band-pattern differences. The band pattern for fungal 18s rRNA amplicons were generally more complex than those obtained for the bacterial 16s rRNA amplicons (Figs. 3 and 4). Few differences were observed among the four plant treatments in terms of bacterial community composition (Fig. 3). However, strong differences were observed in fungal community composition (Figs. 4 and 5). The 1, 4, 7, 8, 9, 13, 14, and 15 bands were dominant in all soil treatments. The 3, 6, 10, 12, and 20 bands were intense in the treatment plant with *C. odorata* but relatively

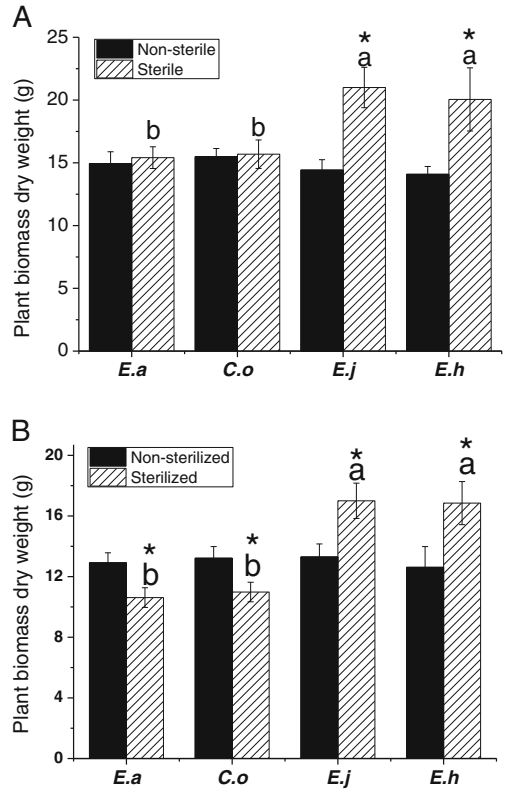

Fig. 1 **a** Total plant biomass of four plant species in non-sterilized and sterilized soil. *Lower case letters* indicates significant differences (P<0.05) among plant species. *Asterisks* indicate significant difference between non-sterilized and sterilized soil with the same plant species. *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.h Eupatorium heterophyllum*, and *E.j Eupatorium japonicum. Error bars* represent standard errors (n=8). In a two-way ANOVA (sterilization and plant species) $F_{(\text{sterilization})}$ =65.917, P<0.01; $F_{(\text{plant species})}$ =24.105, P<0.01. $F_{(\text{sterilization} \times \text{plant species})}$ =42.150, P<0.01. **b** Plant biomass of four plant species in non-sterilized and sterilized soil previously planted with these species 10 months before. *Lower case letters* indicate significant differences (P<0.05) among plant species. *Asterisks* indicate significant difference between non-sterilized and sterilized soil with the same plant species. *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.h Eupatorium heterophyllum*, and *E.j Eupatorium japonicum. Error bars* represent standard errors (n=6). In a two-way ANOVA (sterilization and plant species) $F_{(\text{sterilization})}$ =9.143, P<0.01; $F_{(\text{plant species})}$ =37.872, P<0.01. $F_{(\text{sterilization} \times \text{plant species})}$ =40.237, P<0.01

Table 1 Soil nutrient content in sterilized and non-sterilized soils after the plants had been incubated for 10 months in experiment 1

	Organic C (kg^{-1})		Total N (kg^{-1})		NH_4^+-N (mg kg^{-1})		NO_3^-N (mg kg^{-1})		Available P (mg kg^{-1})		Available K (mg kg^{-1})	
	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized
E. a	30.15±1.4	28.89±1.43	2.42±0.32	2.27±0.34	26.4±4.31	15.58±2.03*	3.25±0.08b	1.14±0.04*	74.30±4.95b	79.55±4.21	104±4.40	95.36±5.44
C. o	29.77±0.66	30.07±0.86	2.55±0.41	2.29±0.26	21.69±3.25	14.75±1.09*	7.02±0.58a	1.38±0.25*	103.55±6.53a	77.94±5.20*	97.77±5.33	91.1±4.31
E. j	30.48±0.74	29.23±0.65	2.29±0.39	2.37±0.31	22.06±1.54	14.28±1.28*	1.39±0.06c	1.09±0.02*	75.25±3.34b	74.21±2.77	101.54±4.47	94.67±5.71
E. h	29.56±1.12	29.85±0.88	2.32±0.23	2.30±0.19	24.33±3.08	13.77±1.41*	1.38±0.10c	1.17±0.05*	69.78±3.47b	73.47±3.05	103.69±4.05	90.04±4.39*

Lowercase letters indicates the significant difference among different plant species. * indicates the significant difference between sterilized and non-sterilized soil. ± represent standard deviation (n=8). In a two-way ANOVA NH_4^+-N $F_{(sterilization)}$=279.077, P<0.01. $F_{(plant\ species)}$=3.462, P=0.07. $F_{(sterilization \times plant\ species)}$ =1.450, P=0.173. NO_3^-N $F_{(sterilization)}$=479.310, P<0.01. $F_{(plant\ species)}$=377.736, P<0.01. $F_{(sterilization \times plant\ species)}$=283.299, P<0.01

E.a indicates *Eupatorium adenophora*, *C.o* indicates *Chromolaena odorata*, *E.j* indicates *Eupatorium japonicum*; *E.h* indicates *Eupatorium heterophyllum*

Table 2 Soil nutrient content in sterilized and non-sterilized soils after the plants had been incubated for 10 months in experiment 2

	Organic C (kg^{-1})		Total N (kg^{-1})		NH_4^+-N (mg kg^{-1})		NO_3^-N (mg kg^{-1})		Available P (mg kg^{-1})		Available K (mg kg^{-1})	
	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized
E. a	31.07±1.03	30.54±1.05	2.18±0.45	2.20±0.49	19.57±2.04	6.38±0.43*	3.06±0.07b	0.88±0.03b*	54.21±3.25ab	41.87±3.05*	72.58±3.69	63.58±3.07*
C. o	29.55±0.88	30.07±1.02	2.26±0.53	2.27±0.32	18.69±2.07	6.71±0.87*	4.30±0.38a	1.23±0.19a*	67.06±5.01a	43.56±2.19*	69.96±4.01	61.40±4.13*
E. j	28.78±0.67	29.14±0.58	2.14±0.27	2.09±0.28	18.88±1.64	6.04±0.52*	0.89±0.07c	0.78±0.07b	49.25±3.38b	40.47±2.93*	75.17±3.50	62.30±2.55*
E. h	30.54±1.17	29.67±0.97	2.29±0.36	2.17±0.19	18.77±1.37	6.17±0.43*	1.04±0.06c	0.94±0.06b	46.73±3.47bc	39.69±2.59*	76.63±3.71	64.76±3.79*

Lowercase letters indicates the significant difference among different plant species. * indicates the significant difference between sterilized and non-sterilized soil. ± represent standard deviation (n=6). In a two-way ANOVA NH_4^+-N $F_{(sterilization)}$=607.075, P<0.01. $F_{(plant\ species)}$=0.395, P=0.757. $F_{(sterilization \times plant\ species)}$=0.430, P=0.733. Available P $F_{(sterilization)}$= 171.628, P<0.01. $F_{(plant\ species)}$=29.236, P<0.01. $F_{(sterilization \times plant\ species)}$=15.365, P<0.01. Available K $F_{(sterilization)}$=104.099, P<0.01. $F_{(plant\ species)}$=3.986, P=0.014. $F_{(sterilization \times plant\ species)}$=1.047, P=0.382

E.a indicates *Eupatorium adenophora*, *C.o* indicates *Chromolaena odorata*, *E.j* indicates *Eupatorium japonicum*, *E.h* indicates *Eupatorium heterophyllum*

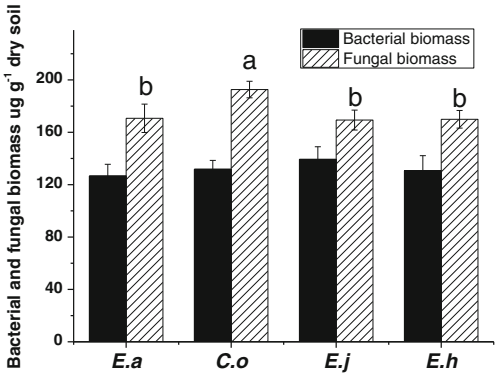

Fig. 2 Bacterial and fungal biomass in four treatments in non-sterilized soil. *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.j Eupatorium japonicum*, *E.h Eupatorium heterophyllum. Lower case letters* indicate significant differences among plant species; *Vertical bars* represent standard errors (n=4). In a one-way ANOVA for bacterial biomass F=1.283, P=0.325. Fungal biomass F=7.919, P<0.01

weak in the other treatments. Band 5 was only observed in soil with *E. japonicum*. Band 11 was intense in soil with two native plant treatments but weak in two invasive plant treatments. To examine possible functions of these fungal species, we cloned and sequenced all the labeled bands in Fig. 4. The sequences were aligned with previously published sequences, and the results are shown in Table 3. Subsequent sequencing results showed that *C. odorata* soils had higher abundance of *Paraglomus* sp. compared with soils in which other plant species were grown.

Discussion

In our investigation of alteration of soil microbial communities by invasive species (*E. adenophora* and *C. odorata*) and native species (*E. japonicum* and *E. heterophyllum*), we found that the soil fungal community was altered more than was the bacterial community. Invasive species C. *odorata* led to higher fungal biomass than did other plant species. According to our hypothesis, root exudations by different plant species into soil may create different microbial communities (Kao-Kniffin and Balser 2007; Drigo et al. 2010). In this study, different response patterns of bacterial and fungal communities were observed. No significant difference was observed in bacterial biomass and community composition among four plant species (Figs. 2 and 3). However, fungal biomass was higher in the soil with *C. odorata* and there were significant differences in soil fungal community composition among these four plant species (Figs. 4 and 5). These different responses may have occurred because fungi were more closely associated with plant roots and thus more readily affected than soil bacterial communities (Drigo et al. 2010).

Previous studies regarding invasive plants' effects on soil microbial biomass are inconsistent, with some studies finding increased microbial biomass (e.g., Liao and Boutton 2008; Marchante et al. 2008a), no significant change (e.g., Hahn 2003), and others reporting reduced microbial biomass (e.g., Broz et al. 2007). Additionally, in this experiment *E. adenophora* did not alter fungal

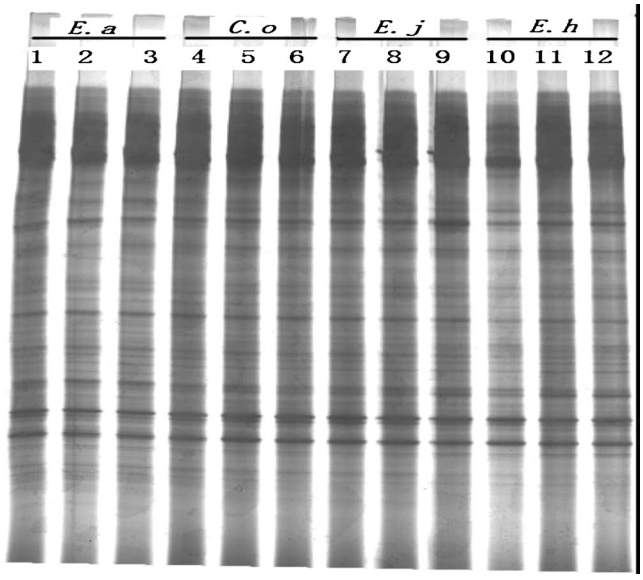

Fig. 3 DGGE profiles of 16s rRNA genes of soil bacterial communities in non-sterilized soil planted with: *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.j Eupatorium japonicum*, *E.h Eupatorium heterophyllum*

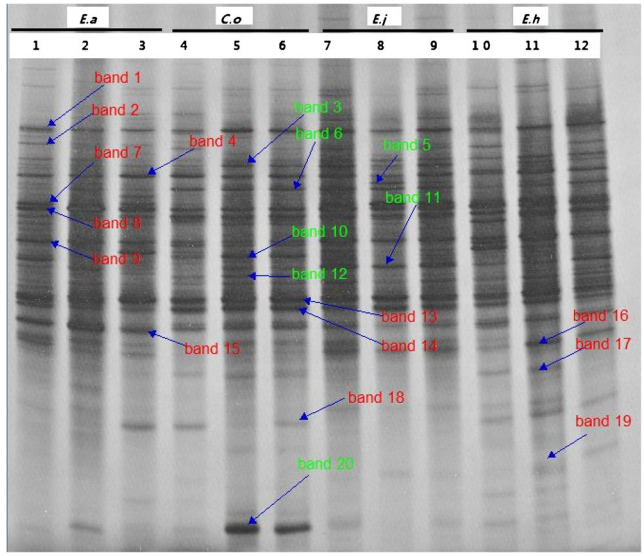

Fig. 4 DGGE profiles of 18s rRNA gene of soil fungal communities in non-sterilized soil planted with: *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.j Eupatorium japonicum*, *E.h Eupatorium heterophyllum*. The *green bands* were dominant species in certain plant species; most of the *red bands* were dominant in all the treatments

biomass, although it is also considered to be an invasive plant. This result suggests that the response of microbial biomass to plants is species-specific (Belnap and Phillips 2001).

For microbial community composition analysis, we assumed that DGGE band numbers and intensities reflected the diversity and relative abundance of the dominant microbial communities (Kirk et al. 2004). Our results showed very similar bacterial but significantly different fungal communities among the plant species (Figs. 3 and 4). Soil fungal community changes induced by plants would probably result in changes in soil functions and feedback, such as soil nutrient dynamics (Hahn 2003), plant diseases caused by pathogenic fungi (Mangla et al. 2008), and nutrient uptake by arbuscular mycorrhizal fungi (AMF) (Reinhart and Callaway 2006).

To understand further functional differences among treatments induced by the soil fungal community change, bands differing among four plant species were sequenced and aligned with previously published sequences. The dominant fungal species of band 6 with *C. odorata* was closest to *Auricularia* sp. (Table 3). The fungal species of band 10 with *C. odorata* was closest to *Paraglomus* sp. (Table 3). *Paraglomus* sp. could induce symbiosis with plant roots to form AMF (Blaszkowski

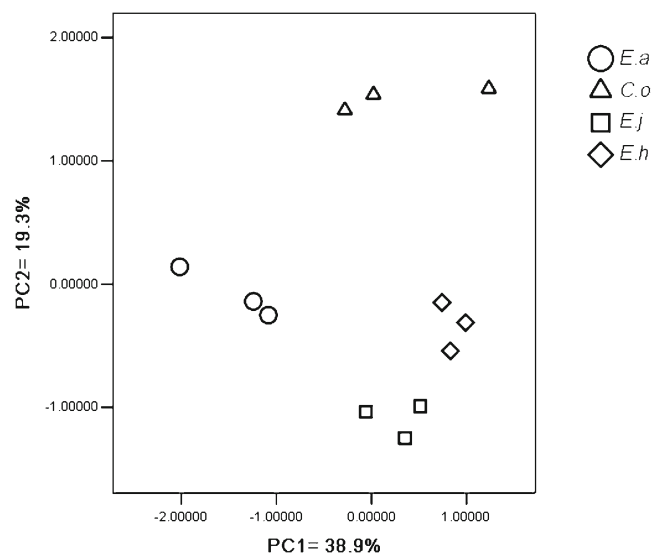

Fig. 5 Principal-component (PC) analysis of DGGE bands patterns representing fungal community structure. *E.a Eupatorium adenophora*, *C.o Chromolaena odorata*, *E.h Eupatorium heterophyllum*, and *E.j Eupatorium japonicum*

Table 3 Identification of selected DGGE bands in non-sterilized soil

DGGE bands	GenBank accession number	Closest match from GenBank	Sequence similarity by BLAST (%)
Band 1	JX126739	*Phialophora* sp	99 %
	JX126740	*Phialophora* sp	99 %
Band 2	JX126747	Uncultured *Orbiliaceae* clone	97 %
Band 3	JX126748	*Candida*	100 %
Band 4	JX126741	Uncultured *Ascomycota*	100 %
Band 5	JX126760	Uncultured *Chytridiomycota* clone	88 %
Band 6	JX126751	*Auricularia* sp	99 %
Band 7	JX126749	*Acaulospora* sp	98 %
Band 8	JX126742	*Teratosphaeriaceae* sp	99 %
	JX126743	*Penicillium* sp	99 %
Band 9	JX126744	*Coniosporium* sp	98 %
	JX126745	*Pesotum* sp	98 %
Band 10	JX126757	*Paraglomus* sp	99 %
Band 11	JX126758	Uncultured *Scolecobasidium* clone	96 %
Band 12	JX126761	*Pterocystis* sp	94 %
Band 13	JX126759	*Alicorhagia* sp	99 %
Band 14	JX126755	Uncultured *Tremellaceae* clone	99 %
Band 15	JX126752	Uncultured *Auriculariaceae* clone	98 %
Band 16	JX126762	Uncultured eukaryote clone	98 %
Band 17	JX126754	Uncultured soil *basidiomycete*	99 %
Band 18	JX126750	*Archaeospora*	97 %
Band 19	JX126746	*Nanoscypha tetraspora* 18S rRNA gene	99 %
Band 20	JX126753	*Botryosphaeria*	98 %

et al. 2012). Several studies have also reported that invasive plant symbiosis with native fungi formed AMF (Richardson et al. 2000; Reynolds et al. 2003; Callaway et al. 2004). Nijjer et al. (2008) found that high levels of AMF colonization on invasive *Sapium sebiferum* increased the plant's growth rates and facilitated its invasion into mesic temperate forests. Besides *Paraglomus* sp., *Glomus* sp., *Acaulospora* sp., *Entrophospora* sp., and *Archaeospora* sp. are also common AMF in plant rhizospheres (Gai and Liu 2000). *Acaulospora* sp. (band 7) and *Archaeospora* sp. (band 18) were also observed in this study. Although band 7 was observed in all the plant species and band 18 was observed with *E. adenophora*, *C. odorata*, and *E. heterophyllum*, band 10 (*Paraglomus* sp.) was only abundant with *C. odorata*, which indicates that there is higher abundance of *Paraglomus* sp. in soil with *C. odorata* compared with other treatments. Higher abundance of particular AMF species such as *Paraglomus* sp. is likely to provide more nutrients and promote growth of *C. odorata* (Reynolds et al. 2003). As a result, *C. odorata* may develop a competitive advantage over native plant species.

The fungal species with band 20 in *C. odorata* closely resembles *Botryosphaeria* (Table 3). *Botryosphaeria* sp. is considered to be a plant pathogen that causes leaf blotch and bark disease (Maharachchikumbura and Adikaram 2009). The accumulation of pathogens is generally harmful to plants. However, studies have indicated that *C. odorata* could accumulate soil pathogens (*Fusarium semitectum*) that can inhibit native plants without being affected (Mangla et al. 2008). This phenomenon suggests an additional invasive pathway for *C. odorata*. We did not determine whether *Botryosphaeria* sp. may negatively affects native plant species. However, *C. odorata* exhibited no sign of disease and may thus be unaffected by *Botryosphaeria* sp. Other fungal species were also detected in this study,

including band 5 (uncultured *Chytridiomycota* clone), band 11 (uncultured *Scolecobasidium* clone, dominant in native but weak in invasive), band 12 (*Pterocystis* sp.). Functions of these fungal species remain undetermined, but substantial shifts in soil fungal composition were clearly demonstrated.

Theoretically, DGGE can distinguish a single base difference between two different sequences. However, a co-migration phenomenon was observed in this study (Sekiguchi et al. 2001), with bands 1, 8, and 9 including two distinct sequences despite being unresolved by DGGE. Similar migration behaviour of these bands under denaturing conditions is probably linked to similar G/C ratios (Gelsomino et al. 1999).

Autoclaving sterilization affects soil properties and subsequently affects plant growth and acquisition of nutrients (Troelstra et al. 2001; De Deyn et al. 2004). In this study, no significant soil nutrient difference was initially observed between sterilized and non-sterilized soils (Table S1). However, most soil nutrients were lower in sterilized than in non-sterilized soil after 10 months of subsequent plant growth. This result suggests that sterilization-induced death of soil organisms decreased soil nutrient availability, or turnover or sterilization may increase plant uptake of some soil nutrients (Hayat et al. 2010). Additionally, in non-sterilized soil, nitrate concentrations of two invasive plants were significantly higher than those of two native species. However, significant nitrate-concentration differences were not observed between natives and invasives in sterilized soil. This indicated that invasive *E. adenophora* and *C. odorata* may promote microbial nitrification (Hawkes et al. 2005). Alteration of soil microbial community composition or functional diversity accompanied with soil nutrient increases by invasive plants has been demonstrated before (Marchante et al. 2004, 2008b).

In the first experiment (Fig. 1a), sterilization significantly increased biomass of two native plant species but had no significant effect on the two invasives. Meanwhile, no significant soil nutrient differences were observed in sterilized soil between native and invasive species (Table 1). These results suggested that increased biomass of native species may not be due to soil nutrients but instead due to soil biotal feedback. Native organisms such as pathogenic microbes or soil animals in the soil may restrain the growth of native plant species while not affecting invaders (Callaway et al. 2004; van de Voorde et al. 2012; Xiao et al. 2013). These results support the hypothesis that some exotic invaders can escape control by local soil pathogens (Klironomos 2002).

The second experiment examined the response of plant species to soil biota communities altered by a previous growing phase. It showed that soil sterilization significantly decreased plant biomass of two invasive species, yet still significantly increased the biomass of two native species. Thus, invasive *E. adenophora* and *C. odorata* changed fungal communities which may have favored plant growth. We suggest that invasive *C. odorata* increased abundance of microorganisms such as AMF and made them more beneficial to plant growth. However, sterilization killed all the microorganisms including AMF, which is likely to decrease biomass of the invasives. It should be noted that, like *C. odorata*, biomass of *E. adenophora* also was significantly decreased. However, different from *C. odorata*, increased abundance of known AMF species was not observed in soil planted with *E. adenophora*. It is possible that some other microorganisms beneficial to *E. adenophora* were not detected due to limitations of the DGGE method in diversity analysis.

In summary, three important points were demonstrated. First, native soil biota caused plant-soil feedbacks unfavorable for native *E. japonicum* and *E. heterophyllum*, but not for invasive *E. adenophora* or *C. odorata*. Second, these four plant species mainly influenced soil fungal rather than bacterial communities. Third, soil communities modified by invasive *E. adenophora* or *C. odorata* had positive effect on growth of these invaders. Higher abundance of particular AMF species such as *Paraglomus* sp. in soil modified by *C. odorata* may be one of the mechanisms contributing to its success as an invasive plant species.

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Conflict of interest All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

Author contributions Y.L Feng contributed to experimental design. Hai F. Xiao performed experiments and wrote the manuscript. D.S. Schaefer and X.D. Yang provided technical support.

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