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Soil microbes alleviate allelopathy of invasive plants

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Abstract Soil microbes are one of the most important determinants of allelopathic effects in the field. However, most studies testing the role of allelopathy in biological invasions did not consider the roles of soil microbes. Here we tested the hypothesis that soil microbes which can degrade allelochemicals may accumulate in soils over time by adaptation and therefore increase the degradation of allelochemicals and alleviate the allelopathic effects in biological invasions. As expected, soil microbes significantly decreased the allelopathic effects of leaf leachates of eight in the nine invasive plant species studied. In addition, *Ageratina adenophora* showed lower allelopathic effects in soil with long or intermediately invasion history than those in soil with short invasion history. The two main allelochemicals of the invader were degraded more rapidly with increasing invasion history in the soil. Correspondingly, biomass and activity of the soil microbes were higher in the soils with long invasion history than in that with short invasion history. Our results indicate that soil microbes may gradually adapt to the allelochemicals of *Ageratina* and alleviate its allelopathic effects and thus support the above hypothesis. It is necessary to consider the effects of soil microbes when testing the roles of allelopathy or the novel weapons hypothesis in biological invasions.

Keywords Adaptation · Allelopathy · Degradation of allelochemicals · Soil microbes · Invasive plants

1 Introduction

Allelopathy has been widely accepted as a mechanism underlying invasion success of introduced plants [1–5]. Introduced plants may succeed due to possessing unique allelochemicals, to which naïve natives have not adapted (the novel weapons hypothesis) [1]. However, the hypothesis was only tested using a few invasive exotic plants, and some of the studies were carried out in laboratories, not considering potential influences of other biotic and abiotic factors [6–8]. To show allelopathic effects, allelochemicals must be released into soils by leaves leaching, root exudation, or degradation of plant residues, accumulate, and persist at phytotoxic levels in the field and come in contact with target plants [9]. Many factors influence accumulation of allelochemicals in soils, such as soil texture, chemical character, and microbial community [7, 10–12]. Without considering the effects of these factors, we could not accurately evaluate the importance of allelopathy in invasion success of introduced plants, and evidence for the role of allelopathy in biological invasions is controversial [13, 14]. Thus, understanding how biotic and abiotic factors affect

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allelopathic effect between invasive and native plants may yield important insight into the role of allelopathy in biological invasions.

Of these factors, soil microbes are an important determinant factor of allelopathic effect [15–17]. It has been reported that allelopathic activity of carvacrol is stronger in sterilized soils than that in unsterilized soils [18]. Blum [19] found that soil microbes are able to degrade phenolic acid and then decrease its phytotoxicity and duration. Chen et al. [20] isolated a fungus, *Trichoderma harzianum* SQR-T037, which rapidly degrades allelochemicals released into rhizospheres by cucumber.

Short generation times and high genomic plasticity allow microbes to evolve new genes at a relatively high rate [21]. Soil microbial communities are able to adapt to the novel chemicals applied into environments by human via mutation, horizontal gene transfer, and DNA rearrangement [22, 23]. Aelion et al. [24] found that soil microbes acquire the ability to degrade p-nitrophenol after exposing to this compound for a time, and the rate of mineralization of p-nitrophenol and the amount of specific degrader increase with time. It has been widely reported that soil microbes evolve the ability of rapid degradation of many xenobiotic compounds including pesticides, fungicides, and herbicides, which were synthesized by human and did not exist previously in soils. For example, biodegradations of herbicide 2,4-dichlorophenoxy and nematicide fenamiphos are faster in soils previously experienced these chemicals than in naïve soils [21, 25, 26]. In this study, we hypothesized that naïve soil microbes may evolve the ability to degrade allelochemicals of invasive plants (natural products) more rapidly compared with the human-synthesized xenobiotic compounds and that the ability may increase with increasing invasion time. Little effort has been made to study the effects of soil microbes on accumulation of allelochemicals of invasive plants in soils, and the results in allelopathy of invasive plants are inconsistent [15, 16]. For example, Perry et al. [27] found that catechin was potential allelochemicals of *Centaurea maculosa* and maintain high concentration in field soil near *C. maculosa.* Perry et al. [28] collected 402 soil samples from 11 sites with invasive *C. maculosa* in two growing seasons and detected low level of catechin (0.65 ± 0.45 mg g^{-1}) in only 20 samples collected from one site at one sampling time [28]. Similarly, Inderjit et al. [29] found that catechin is dynamic in natural soils, but inhibit *Koeleria macrantha* at low concentration in some soils. These results suggest that allelopathy is context dependent [29].

Here we studied the generality of allelopathic effect using nine invasive plant species, the effects of invasion history using one invader, and the ability of soil microbes to degrade allelochemicals of the invader. First, we studied the effects of soil microbes (with or without) on allelopathy of nine invasive species. We also measured the effects of the amount of soil microbes on allelopathy using five of the nine invaders. Then we studied how invasion history influences the effects of soils on allelopathy and degradation of allelochemicals using a subtropical invader. We also estimated biomass and activity of the microbes that are able to degrade the allelochemicals by measuring soil substrate-induced respiration rate [30]. We predicted that soil microbes may decrease allelopathic effects for all invasive plants studied here (a general phenomenon), and the effects of soil microbes may increase with increasing invasion history by accumulating allelochemical-degrading soil microbes.

2 Materials and methods

2.1 Plant materials

To test the effects of soil microbes on allelopathic effects of leaf leachates, nine noxious invasive plant species were used in this experiment. They are *Ageratina adenophora* (Sprengel) R. M. King and H. Robinson (a perennial herb or subshrub native to Mexico and Central America; Asteraceae), *Ageratum conyzoides* L. (an annual herb native to Tropical America, especially Brazil; Asteraceae), *Bidens pilosa* L. (an annual herb native to Central and Tropical America; Asteraceae), *Chromolaena odorata* (L.) R. M. King and H. Robinson (a perennial herb or subshrub native to Americas; Asteraceae), *Gynura crepidioides* Benth. (an annual herb native to Tropical Africa; Asteraceae), *Lantana camara* L. (a perennial subshrub native to Mexico and Central America; Verbenaceae), *Mikania micrantha* (L.) Kunth. (a perennial creeper native to the subtropical zones of North, Central, and South America; Asteraceae), *Tithonia diversifolia* (Hemsl.) Gray (a perennial herb native to eastern Mexico and Central America; Asteraceae), and *Wedelia trilobata* (L.) Hitchc (a spreading, mat-forming perennial herb native to Mexico, Central America; Asteraceae). These invaders have allelopathic potential (Table S1).

2.2 Leaf leachate preparations

In July 2012, fully expanded leaves were collected from more than ten individuals of a natural population for each of the nine invasive species in Xishuangbanna (21°41′N, 101°25′E, 570 m asl), Yunnan Province, southwest China. Fresh leaves of each species were immersed in distilled water (2.5 % based on leaf dry mass) for 36 h. The 2.5 % aqueous leaf leachates were concentrated to 5 % for *A. conyzoides*, *B. pilosa*, *C. odorata*, *G. crepidioides*, *M. micrantha*, and *T. diversifolia* and to 10 % using rotary

evaporators for *L. camara* and *W. trilobata*. We did not compare the differences in allelopathic effects among the invaders, and thus, the interspecific differences in the concentrations of the leaf leachates were not a matter. The leaf leachates were filtered through four layers of filter paper, then through 0.45-μm Micro PES membranes (Jinteng Experiment Equipment Co., Ltd, Tianjin, China) to get rid of microbes, and were kept at 4 °C until used.

2.3 Soil samples

Soils under *C. odorata*, *T. diversifolia*, *W. trilobata*, L. *camara*, *B. pilosa*, *A. conyzoides*, and *G. crepidioides* were also collected in Xishuangbanna in August 2012. Soils under *A. adenophora* were collected in Ailao Mountain (24°31′N, 101°00′E, 2,430 m asl), Jingdong County, Yunnan Province, southwest China. Soils under *M. micrantha* were collected in Danzhou (19°30′N, 109°29′E, 299 m asl), Hainan Province, south China. For each species, we selected a site with a high population density (coverage > 70 %) and randomly established three 0.5 m × 0.5 m plots. In each plot, 0–10 cm topsoil was collected after removing aboveground vegetation and litter. Roots, residues, and gravels were removed from the soil using 10-mesh (2 mm) sieves. Soils from three plots were mixed and kept in 4 °C until used.

We collected soils using the method described as above in three sites with different invaded history by *A. adenophora* in Ailao Mountain. At site I, *A. adenophora* had invaded for more than 30 years and formed a dense monoculture with 100 % cover and 1.6–2.0 m height. At site II, *A. adenophora* had invaded for about 15 years and formed a mono-dominant community with 70 % cover and 1.0–1.5 m height. Some native herbs such as *Coelachne simpliciuscula* and *Hemiphragma heterophyllum* grew with the invader. At site III, *A. adenophora* had invaded for 5 years and sparsely co-occurred with native herbs, such as *C. simpliciuscula*, *Digitaria cruciata*, and *H. heterophyllum*. The cover and height of the invader were 20 % and 0.5–1.0 m, respectively. Information on the invasion history by *A. adenophora* in each site was provided by Dawen Li, a staff in Ailaoshan Station for Subtropical Forest Ecosystem Studies, Chinese Academy of Sciences. Soil microbial biomass carbon was extracted using chloroform fumigation-extraction method [31] and measured using Vario TOC analyzer (Elementar Analysensysteme GmbH, Hanau, Germany; Table S2).

2.4 Soil sterilization experiments

To test the effect of soil microbes on allelopathic effect of nine invasive plants, the soil from each invasive plant was divided into two samples: One was sterilized by autoclaving (121 °C, 0.105 MPa, 1 h) for three times at 24-h interval, and another was used as unsterilized control. The soil from each species and sterilization treatment was divided into 16 shares, 25 g per share, and put into 16 plastic cups (220 mL). Twenty seeds of *Oryza sativa* L. were sown in each cup after being sterilized using 0.1 % HgCl for 5 min and washed three times using distilled water. Sixteen cups were randomly divided into two groups: one was treated with 12 mL distilled water per pot, and another was treated with 12 mL leaf leachate of the same species at three intervals. In total, there were 288 cups (soils from nine species × two sterilization treatments × two leaf leachate treatments × eight replicates). Seeds were germinated in LRH-250 incubator (Yiheng Scientific Instruments Co., Ltd, Shanghai, China), in which temperature was 30/20 °C (day/night) and light intensity was 250 μmol m^{-2} s^{-1} with 12-h photoperiod.

To test the effect of amount of soil microbe on allelopathic effect of invasive plants, five perennial invasive species (*A. adenophora*, *C. odorata*, L. *camara*, *T. diversifolia*, and *W. trilobata*) were used in this experiment. Soil sample from each species was mixed evenly with the soil deep below ground (>2 m; collected in Xishuangbanna and sterilized before used) to obtain the soil mixtures with 0, 10 %, 30 %, 50 %, 70 %, and 100 % of the soil sample from each invader, respectively. The methods of seed germination were the same as above experiment. In total, there were 240 cups (soils from five species × six soil treatments × two leaf leachate treatments × four replicates).

To test the effect of invasion history on allelopathy, we tested the effect of soil microbes with different invasion history of *A. adenophora* on allelopathic inhibition of the invader on shoot and root length of *O. sativa*. The methods of soil sterilization and seed germination were the same as above experiment. In total, there were 96 cups (soils from three sites × two sterilization treatments × two leaf leachate treatments × eight replicates).

Oryza sativa (upland rice in our study) was used as a test plant species because it had been used in related studies for at least five of the nine invasive species (Table S1). Upland rice is widely cultivated in mountain area in Yunnan, China, and *A. adenophora* often invades upland rice fields. In addition, oxo-10,11-dehydroageraphorone (DTD) and 9β-hydroxyageraphorone (HHO), two main allelochemicals of *A. adenophera*, were purified and identified using *O. sativa* as a test plant [32].

Ten days later, seed germination rate and shoot and root length of seedlings of *O. sativa* were measured. The experiment lasted for only 10 days because seeds of *O. sativa* germinated fast. Sterilization treatment may increase soil nutrient availability and thus increase *O. sativa* seedling growth. In order to exclude (1) the confounding effects

of the potential change in soil nutrients in soil sterilization treatment and (2) the effects of the differences in soil nutrients among soil sources on comparison of absolute growth of *O. sativa* seedlings, we calculated the response index (RI) of *O. sativa* to leaf leachate of each invader in each treatment and each soil source. It was calculated according to Williamson and Richardson [33]: ($variable_{leachate} - variable_{water}$)/$variable_{water}$ [33]. In this study, $variable_{water}$ was the average of all replicates per species per treatment and $variable_{leachate}$ was the value of each replicate. $RI > 0$ indicates improvement of growth, i.e., positive allelopathic effect; $RI < 0$ indicates inhibition of growth, i.e., inhibitory allelopathic effect; $RI = 0$ indicates no allelopathic effect.

For nine invasive plants, the effect of soil sterilization treatment on RI for each invader was tested using one-way ANOVAs. To evaluate the effects of the amount of soil microbes (soil from each invader), we analyzed the regression between RI and soil sample concentration using SigmaPlot 10.0 (Systat Software Inc. Erkrath, Germany). For *A. adneophora*, two-way ANOVAs were used to test the effect of soil sterilization and soil source on RI, and one-way ANOVAs (Duncan's test) were used to test the difference in RI among soil sterilization treatment and soil sources. All analyses in this and the following experiments were performed with SPSS 17.0 (SPSS Inc. Chicago, IL, USA).

2.5 Chemical analyses

To evaluate the effect of invasion history on allelopathic effect of *A. adneophora*, we measured degradation of DTD and HHO, the main allelochemicals of *A. adenophora* in soil with different invasion history. Soil from each site was divided into two groups. One was sterilized by autoclaving (121 °C, 0.105 MPa, 1 h) for three times at 24-h intervals, and another was used as unsterilized control. The soil from each site and sterilization treatment was put into 18 centrifuge tubes (50 mL), 10 g per tube. Four milliliters of 2.5 % leaf leachate of *A. adenophora* was added into each tube. Three tubes per soil sample from each site each treatment were randomly chosen after 0, 24, 48, 72, 96, and 120 h of incubation in chambers (conditions were the same as for seed germination). Ten milliliters of absolute methanol was added into each tube and mixed in a vortex for 5 min. Then the tubes were put in a chamber (25 °C) for 24 h and mixed in a vortex twice during this time and centrifuged at 2,500 *g* for 10 min. The supernatant was filtered using 0.45 μm Micro PES membrane (Jinteng Experiment Equipment Co., Ltd, Tianjin, China) for analyzing DTD and HHO. There were 108 soil samples in total for allelochemicals analyses (three soil samples × two soil sterilization treatments × six incubation time × three replicates).

ACQUITY Ultra Performance Liquid Chromatography (Waters Corporation, Miller, Massachusetts, USA) was used for analyzing the allelochemicals. The mobile phases included (A) 0.1 % formic acid and (B) acetonitrile containing 0.1 % formic acid. The elution program was as follows: 0–3.0 min, 30 %–45 % of eluent B and 70 %–55 % of eluent A; 3.0–3.5 min, 45 %–30 % of eluent B and 55 %–70 % of eluent A. The flow rate of the eluent was 0.5 mL min^{-1}; the injection volume of the allelochemical extract was 2 μL; and the column oven was set at 30 °C. The eluent was monitored with SYNAPT G2 (Q-TOF) mass spectrometer. Contents of the two allelochemicals in each sample were determined using external standard allelochemicals (BioBioPha Co. Ltd, Kunming, China).

Three-way ANOVAs were used to test the effects of soil sterilization treatment, soil sources, and incubation time on degradation of the allelochemicals.

2.6 Soil microbial activity

To test the effect of invasion history on soil microbes that were able to degrade or use DTD and HHO, we measured soil respiration rates induced mainly by the allelochemicals. Substrate-induced respiration rate is often used to estimate biomass and activity of target soil microbes [30]. Soil collected from each site was immediately put into three 12.5-cm Petri dishes, 50 g per dish, sprayed with 3 mL *n*-hexane extract of concentrated leaf leachate of *A. adenophora* (containing 2.83 mg mL^{-1} DTD and 0.25 mg mL^{-1} HHO), and mixed evenly. Two allelochemicals were the main compounds in the *n*-hexane extract according to our measurements using ACQUITY Ultra Performance Liquid Chromatography and Yang et al. [32]. Sterilized soil was used as a control. The dishes were placed in a fume hood for 30 min to volatilize *n*-hexane. A 3-cm beaker containing 10 mL 0.1 mol L^{-1} NaOH was put into each Petri dish. The Petri dishes were then sealed to avoid CO_2 loss and were incubated at 27 °C. Twenty-four hours later, 1 mL of 0.1 mol L^{-1} $BaCl_2$ was added into each beaker containing NaOH to terminate respiration measurement. We used 0.1 mol L^{-1} HCl to titrate the NaOH solution and calculated the amount of CO_2 released from each soil sample and the substrate-induced respiration rate [34].

One-way ANOVAs (Duncan's test) were used to test the differences in soil substrate-induced respiration rates among soil sources.

3 Results

3.1 Effects of soil microbes on allelopathy

Soil microbes significantly decreased allelopathic effects for eight of the nine invasive plant species evaluated in this study. Soil sterilization significantly decreased RI of shoot and root length of *O. sativa* to leaf leachates of *A. adenophora*, *B. pilosa*, *C. odorata*, *G. crepidioides*, L. *camara* (marginally), *M. micrantha*, *T. diversifolia*, and *W. trilobata* (Table 1). According to the RI of shoot and root length of *O. sativa*, the effect of soil sterilization on allelopathy was not significant only for *A. conyzoides*. In addition, the negative effects of soil microbes on allelopathy increased significantly with increasing amount of the microbes (soil sample concentration) for all five perennial invaders evaluated in our study (Fig. S1). Compared with seedling growth, seed germination of *O. sativa* was much less vulnerable to leaf leachates of the nine invasive species. According to the RI of seed germination of *O. sativa*, the effect of soil sterilization on allelopathy was not significant for six of the nine species (significant for *A. adenophora*, *B. polisa,* and *C. odorata* only).

3.2 Effects of invasion history on allelopathy

Soil sources and soil sterilization significantly affected RI of shoot and root lengths (Table S3). In soils with microbes (unsterilized), allelopathic effects of leaf leachate of *A. adenophora* on both shoot and root lengths of *O. sativa* were lower (RI was higher) in soils from the site invaded long and intermediately than in soil from the site invaded newly (Fig. 1). However, the pattern disappeared in soils

Table 1 Response index of *Oryza sativa* to leaf leachates of nine invasive plant species in sterilized and unsterilized soils and the effect of sterilization treatment according to one-way ANOVAs

Species	Concentration of leaf leachate (%)	Based on	Soil treatment		*F* value	*P* value
			Sterilized	Nonsterilized		
Ageratina adenophora	2.5	Germination	−0.05 ± 0.02	0.03 ± 0.00	13.862	0.010
		Shoot length	−0.56 ± 0.01	−0.17 ± 0.01	453.302	<0.001
		Root length	−0.53 ± 0.03	−0.10 ± 0.04	60.714	<0.001
Ageratum conyzoides	5	Germination	−0.02 ± 0.03	−0.05 ± 0.04	0.215	0.659
		Shoot length	−0.16 ± 0.08	0.04 ± 0.10	2.496	0.165
		Root length	−0.47 ± 0.09	−0.50 ± 0.05	0.100	0.762
Bidens pilosa	5	Germination	−0.03 ± 0.03	0.08 ± 0.03	7.150	0.037
		Shoot length	−0.08 ± 0.04	0.26 ± 0.04	29.308	0.002
		Root length	−0.30 ± 0.04	−0.02 ± 0.07	10.920	0.016
Chromolaena odorata	5	Germination	−0.03 ± 0.03	0.06 ± 0.003	9.111	0.023
		Shoot length	−0.47 ± 0.05	−0.10 ± 0.05	24.226	0.003
		Root length	−0.73 ± 0.03	−0.41 ± 0.05	26.799	0.002
Gynura crepidioides	5	Germination	0.03 ± 0.05	0.03 ± 0.03	0.809	0.403
		Shoot length	−0.30 ± 0.02	0.02 ± 0.02	29.819	0.002
		Root length	−0.41 ± 0.05	−0.01 ± 0.02	49.830	<0.001
Lantana camara	10	Germination	0.05 ± 0.03	−0.03 ± 0.05	1.914	0.216
		Shoot length	−0.15 ± 0.11	0.12 ± 0.06	4.843	0.070
		Root length	−0.27 ± 0.09	0.05 ± 0.13	3.809	0.099
Mikania micrantha	5	Germination	−0.03 ± 0.03	−0.05 ± 0.05	0.183	0.684
		Shoot length	−0.14 ± 0.08	0.23 ± 0.07	13.650	0.010
		Root length	−0.30 ± 0.05	0.08 ± 0.08	17.022	0.006
Tihonia diversifolia	5	Germination	0.01 ± 0.05	−0.06 ± 0.07	0.638	0.445
		Shoot length	−0.17 ± 0.01	0.17 ± 0.05	42.145	0.001
		Root length	−0.13 ± 0.04	0.02 ± 0.04	6.267	0.046
Wedelia trilobata	10	Germination	0.08 ± 0.03	−0.05 ± 0.10	1.610	0.251
		Shoot length	−0.02 ± 0.14	0.29 ± 0.05	4.651	0.074
		Root length	−0.22 ± 0.05	0.02 ± 0.04	13.042	0.011

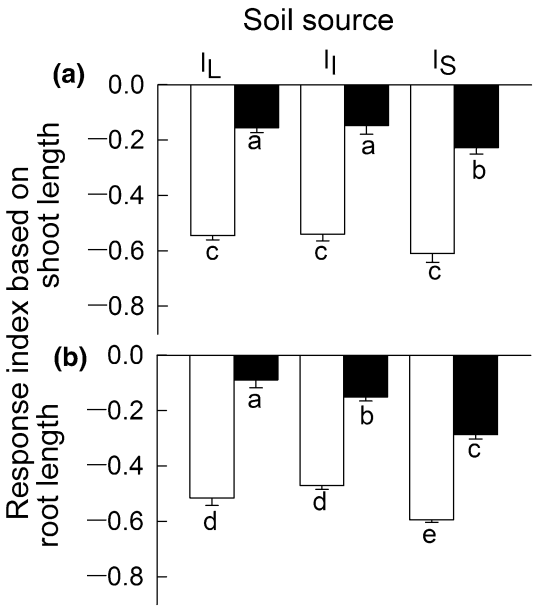

Fig. 1 Response index of *Oryza sativa* to leaf leachate of *Ageratina adenophora* in sterilized (white bars) and unsterilized (black bars) soils collected from different sites. I_L, soil from site invaded long by *A. adenophora*; I_I, soils from site invaded intermediately by *A. adenophora*; I_S, soils from site invaded shortly by *A. adenophora*. Different letters indicate significant differences ($P < 0.05$) among soil sources and treatments according to one-way ANOVA (Duncan's test)

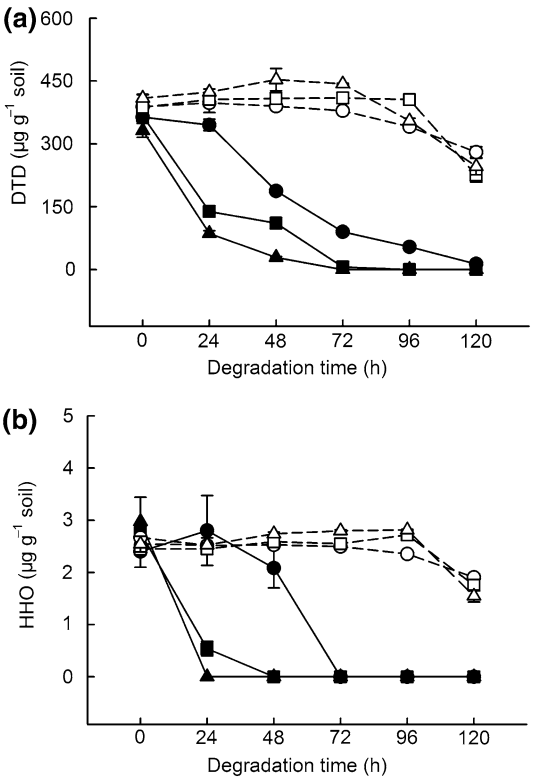

Fig. 2 Degradation of 9-oxo-10, 11-dehydroageraphorone (DTD; **a**) and 9β-hydroxyageraphorone (HHO; **b**) in soils from different sites. Full circles, squares, and triangles indicate soil from site invaded shortly, intermediately, and long by *Ageratina adenophora*, respectively; empty circles, squares, and triangles indicate soil from site invaded shortly, intermediately, and long by *A. adenophora* in soil sterilized, respectively. Solid lines indicate unsterilized soil, and dash lines indicate sterilized soil

without microbes (sterilized) for RI of shoot length. Soil sterilization treatment significantly increased allelopathic effect of leaf leachate of *A. adenophora* in soils from all sites, as judged by the decreased RI of *O. sativa* (Fig. 1; Table S3). The effect of sterilization was stronger for soils from the site invaded long and intermediately than for soil from the site invaded newly by *A. adenophora* (Fig. 1), consistent with the significant interaction between soil source and sterilization treatment (Table S3). For example, soil sterilization-driven decreases in RI of root length were 82 %, 68 %, and 51 % in soils from sites invaded long, intermediately, and newly by the invader, respectively. The results indicate that invasion history may influence allelopathic effect between *A. adenophora* and co-occurring natives by influencing soil microbes.

Soil sterilization treatment, soil source, and degradation time significantly influenced the contents of the two allelochemicals (DTD and HHO; Table S4). Soil sterilization treatment reduced degradation of two allelochemicals (Fig. 2). Two allelochemicals still maintained high content in sterilized soil in 120 h, while they did not be detected in unsterilized soil in 72 h (Fig. 2). In unsterilized soil, degradation of the allelochemicals (especially HHO) was faster in soils from the site invaded long and intermediately than in soil from the site invaded newly by *A. adenophora* (Fig. 2); in sterile soil, the differences was not been observed (Fig. 2). Similarly, soil respiration rates induced by the main allelochemicals (DTD and HHO) of *A. adenophora* were also higher in soils from the site invaded long and intermediately than in soil from the site invaded newly by *A. adenophora* (Fig. 3).

4 Discussion

Our results provide evidence that soil microbes have the ability to degrade the novel allelochemicals of invasive plants, decreasing their allelopathic effects in the field. Soil sterilization increased allelopathic effects of leaf leachates of eight of the nine plant species evaluated in this study (Table 1). In addition, the effect of sterilization (microbes) on allelopathic effect and degradation of two allelochemicals of *A. adenophora* was found in soils with different invasion history (Figs. 1, 2). Similar results were also found for other invasive plants [35–37]. For example, allelopathic inhibition of invasive *Alliaria petiolata* on a

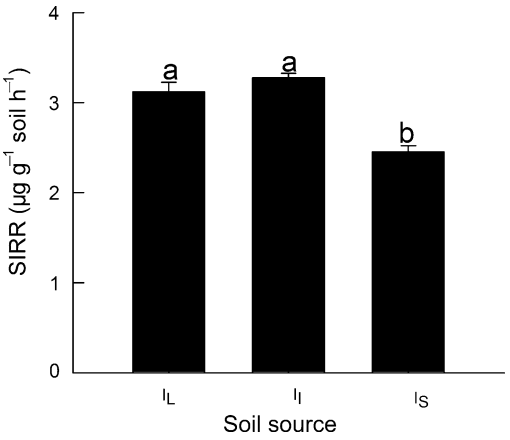

Fig. 3 Substrate-induced respiration rate (SIRR) in soils from different sites. I_L, soil from site invaded long by *Ageratina adenophora*; I_I, soils from site invaded intermediately by *A. adenophora*; I_S, soils from site invaded shortly by *A. adenophora*. Different letters indicate significant differences ($P < 0.05$) among soil sources and treatments according to one-way ANOVA (Duncan's test)

native plant was observed in sterilized soil but not in unsterilized soil [35]. These results indicate that the influences of soil microbes on allelopathic effect of invasive on native plants may be a general phenomenon in the field.

Increasing the number of degraders is an important reflection of microbial adaptation to degrade a specific chemical in soil [21]. In the present study, we further found that biomass and activity of allelochemical-degrading soil microbes were higher, degradations of DTD and HHO (main allelochemicals of *A. adenophora*) were faster, and allelopathic effect of leaf leachates of the invader was lower in soil collected from site invaded long or intermediately than in soil collected from site invaded shortly (Figs. 1, 2, 3). The results indicate that soil microbes may gradually adapt to the allelochemicals of *A. adenophora*, increasing degradation of allelochemicals of the invader and therefore decreasing its allelopathic effect in the field. Allelopathic effects of leaf leachates of five perennial invasive plants in soils collected beneath corresponding invader declined significantly with increasing amount of soil microbes (Fig. S1), also confirming the role of soil microbes in mediating allelopathic effects of invasive on native plants. We did not know how invasion history affects soil microbes, allelochemical degradation, and allelopathic effects in other invasive plants. Our study was the first to estimate allelochemical-degrading soil microbes and evaluate the effects of invasion history. However, it has been documented that xenobiotic compound-degrading soil microbes and degradation of the human-synthesized compounds increase gradually with time after application of the xenobiotic compounds [24, 38]. In addition, native plants can also adapt to allelochemicals of invasive plants over time [39].

Inconsistent with our expectation, soil sterilization did not influence allelopathic effect of *A. conyzoides*; leaf leachate of the invader still inhibited root growth of upland rice by 50 % in unsterilized soils (Table 1). Kong et al. [40] found that ageratochromene, the main allelochemical of *A. conyzoides*, was degraded very slowly in fertile soils, which may be associated with its existence form (dimers). Ageratochromene could be degraded slowly into benzoic acid and its derivatives (2 methyl propanoic acid and acetic acid) when the dimers were depolymerized [40]. And aenzoic acid and its derivatives also had allelopathic potential [40]. The result was meaningful, especially considering the fact that *A. conyzoides* had been introduced into China (i.e., coevolved with native soil microbes) for more than 100 years. Leaf leachate of *C. odorata* also strongly inhibited root growth of *O. sativa* (by 41 %) in unsterilized soils (Table 1). Zheng et al. [5] also found that allelochemicals of the invader could persist at phytotoxic levels in soils. The results indicate that allelopathic effects are complex in the field and highly context dependent.

In this study, we only studied the effects of soil microbes on allelopathic effects of leaf leachates of invasive plants. Soil characters also affect allelopathic effects of plants by influencing allelochemicals, seed germination and growth of target species, such as soil nutrient and pH [41]. In this study, to exclude the effect of the differences in soil chemical traits among soil sources on allelopathic effect of *A. adenophora*, we used RI of *O. sativa* to evaluate allelopathic effect. Other factors also mediate allelopathic effects of invasive plants by influencing allelochemicals. For example, sagebrush produces volatiles to inhibit germination and establishment of neighboring plants after clipped [42]. The roots of *Centaurea diffusa* increase release of 8-hydroxyquinoline under iron limitation [10]. Eight days of competition induce the seaweed *Galaxaura filamentosa* to increase the release of allelochemicals [43]. Thus, future studies with more realistic allelochemical inputs and more ecologically relevant interactions between plants are required to enhance our understanding of the complex allelopathy between invasive and native plants.

In summary, our results indicate that microbial degradation of allelochemicals of invasive plants may be common in the field and the effects of soil microbes may

increase with increasing invasion time and challenge the importance of allelopathic effects in invasion success of introduced plants. However, novel allelochemicals of some invasive plants may not be degraded by soil microbes in some environments or specific place such as rhizosphere [28, 29, 40]. In addition, some invasive plants release volatile allelochemicals, inhibiting co-occurring natives directly through air [7]. Anyway, it is necessary to consider the effects of soil microbes when testing the roles of allelopathy in biological invasions or the novel weapons hypothesis. Allelopathic effects may not be detected in some invasive plants in the field, even if the effects are strong in Petri dishes.

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Conflict of interest The authors declare that they have no conflict of interest.

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