Journal of Applied Ecology

Journal of Applied Ecology 2017, **54,** 1281–1290 doi: 10.1111/1365-2664.12878

Changes in soil microbial communities due to biological invasions can reduce allelopathic effects

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Summary

1. Soil microbes are important in mediating allelopathic interactions between invasive and native plants in the field. However, it was not known how these interactions vary in the process of biological invasions and the effects of soil microbes; this knowledge may facilitate understanding the dynamics and mechanisms of biological invasions and managing invaded ecosystems.

2. We conducted competition and seed germination experiments to determine the allelopathic effects of *Ageratina adenophora* in soils from 42 sites with varying abundances of the invasive plant. Then we isolated the microbes that could degrade the allelochemicals of the invasive plant and tested their functions.

3. In both experiments, the allelopathic effects of the invasive plant were much stronger in soils from non-invaded sites than in soils from invaded sites. Activities of the allelochemical-degrading microbes were higher and degradation of the allelochemicals of the invasive plant was faster in soils from invaded sites than in soils from non-invaded sites. In living soils from 30 sites with increasing abundance of *A. adenophora*, the allelopathic effects of the invasive plant decreased and degradation of the allelochemicals and activity of the allelochemical-degrading microbes gradually increased.

4. Two bacterial strains were isolated from the soils. Inoculation of *Arthrobacter* sp. ZS, which was isolated from soil invaded by *A. adenophora*, greatly increased the degradation of the allelochemicals, thereby decreasing its allelopathic effects.

5. *Synthesis and applications*. Our results indicate that soils may accumulate microbes that can degrade allelochemicals in the process of biological invasions, gradually reducing the allelopathic effects of invasive species. The effects of soil microbes should be considered when studying dynamics and mechanisms of biological invasions. Application of allelochemical-degrading microbes may facilitate ecological restoration of invaded or newly disturbed ecosystems by alleviating allelopathic inhibition of invasive plants on native plants.

Key-words: *Ageratina adenophora*, allelopathic interaction, degradation of allelochemicals, inoculation, invasive plant, invasive species abundance, soil microbes

Introduction

Allelopathy has been widely studied as one of the mechanisms underlying the invasion success of exotic plant species (Callaway & Ridenour 2004; Callaway *et al.* 2008, 2012; Qin *et al.* 2013; Zheng *et al.* 2015). Allelopathic interactions between organisms are complex because many abiotic and biotic factors affect the synthesis, release, accumulation, fate and functioning of allelochemicals (Lankau 2010; Inderjit *et al.* 2011; Asaduzzaman *et al.* 2014; Kaur, Callaway & Inderjit 2014). A growing number of studies have found that allelopathy might be context-dependent, rather than an invariable trait of a species (Lankau 2010; Cipollini, Rigsby & Barto 2012; Li *et al.* 2015a; Vestergård, Rønn & Ekelund 2015). For example, Perry *et al.* (2007) collected 402 soil samples over two growing seasons from 11 sites invaded by the invasive *Centaurea maculosa*, and detected only a low level of

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catechin, the main allelochemical in 20 samples collected from one site in one season. The results are interesting, because *C. maculosa* has long been thought to benefit from allelopathy. The spatial and temporal variations in allelopathy may be associated with differences in soil conditions and allelochemical production among invasive populations. However, the exact mechanisms underlying the context-dependency of allelopathy are still not clear.

Allelochemicals are generally released into soils by leaf leaching, root exudation or degradation of plant residues, before any allelopathic effects become evident (Inderjit & Nilsen 2003). Allelopathic interactions may not occur if the allelochemicals are rapidly degraded after being released into the soil. Soil microbes play a very important role in mediating allelopathic interactions between organisms by transforming allelochemicals into less or more toxic chemicals (Inderjit 2005; Lau 2008; Lankau 2010; Cipollini, Rigsby & Barto 2012; Achatz & Rillig 2014; Li *et al*. 2015a). For example, *Pseudomonas putida* can degrade juglone, the main allelochemical of *Juglans nigra*, thereby decreasing allelopathic effects (Schmidt 1988). Microbial degradation of allelochemicals was also documented for *Festuca rubra* ssp. *commutata* (Kaur *et al*. 2009), *Alliaria petiolata* (Lankau 2010), *Ageratina adenophora* (Zhu, Zhang & Ma 2011; Li *et al*. 2015a). Soil microbes may acquire the ability to degrade the allelochemicals of invasive plants through adaptive changes or cross acclimation to similar chemicals released by phylogenetically related native plants. It is known that soil microbes are able to rapidly adapt to novel chemicals (Top & Springael 2003; Inderjit & Cahill 2015). Soils that are exposed to one chemical may acquire the ability to degrade similar chemicals (Arbeli & Fuentes 2007). Further studies are needed to explore the changes in the allelopathic interactions between invasive and native plants in the process of biological invasions and the effects of soil microbes. Such studies may facilitate understanding dynamics and mechanisms of biological invasions and managing invaded ecosystems.

Ageratina adenophora (Spreng.) R. M. King and H. Rob. is native to Central America but a noxious invasive perennial weed in Asia, Africa, Oceania and Hawaii. It was first found in China in the 1940s, and is now established in six provinces in Southwest China. It has shown strong allelopathic potential in petri-dish laboratory experiments (Zheng & Feng 2005) and pot experiments (Wan, Liu & Wan 2011; Hu *et al*. 2016). Furthermore, it has been documented that *A. adenophora* accumulates allelochemicals at phytotoxic level in field soils in Lancang County, Yunnan Province, Southwest China (Tian, Feng & Liu 2007). In addition, two main allelochemicals, 9-Oxo-10, 11-dehydroageraphorone (DTD) and 9β-hydroxyageraphorone (HHO), have been identified in the leaf leachate and root exudate of *A. adenophora* (Yang *et al*. 2008; Jin 2010). Yang *et al*. (2016) found that the amounts of DTD and HHO in soils collected from some sites were sufficient to inhibit seedling growth of native species, but not in soils from other sites. Allelopathic effects were also not found for *A. adenophora* in a common garden experiment in Xishuangbanna, Yunnan, Southwest China (Wang & Feng 2006). These results suggest that the allelopathic effects of *A. adenophora* are context-dependent. Two recent studies showed that DTD and HHO degraded faster in live soil than they did in sterile soil, suggesting a limiting impact of soil microbes on allelopathic effects (Zhu, Zhang & Ma 2011; Li *et al*. 2015a).

To evaluate the effects of soil microbes on the allelopathic effects of the invasive species, we first conducted a competition experiment using soils collected from six invaded and six non-invaded sites. Activated carbon was used to diminish the allelopathic effects of the invasive species (Lankau 2010; Zheng *et al*. 2015). Our next experiment investigated the effects of the abundance of *A. adenophora* on the allelopathic effects of its leaf leachate, degradation of DTD and HHO, and the allelochemical-degrading soil microbes by using soils collected from 30 sites with varying abundances of *A. adenophora*. Finally, we isolated the allelochemical-degrading microbes from the invaded and non-invaded soils and determined their effects on the degradation of DTD and HHO, and on the allelopathic effects of the invasive species. We hypothesized that (i) the allelopathic effects of *A. adenophora* would be lower, degradation of DTD and HHO would be faster, and activity of allelochemical-degrading soil microbes would be higher, in soils from invaded sites than in soils from non-invaded sites; (ii) with increasing abundance of *A. adenophora*, activity of allelochemical-degrading soil microbes would increase, promoting degradation of the allelochemicals and decreasing the allelopathic effects of the invasive species; and (iii) application of allelochemical-degrading soil microbes isolated from invaded soils would increase degradation of DTD and HHO, and therefore decrease the allelopathic effects of invasive species.

Materials and methods

EXPERIMENT I: COMPETITION AND ALLELOPATHIC EFFECTS

Soil collection

We collected soils from 12 sites in Lancang County (22°33′ N, 99°55′ E, 1000–1700 m a.s.l.), Yunnan Province, Southwest China. Six sites, three in secondary forests and three along roadsides, had been invaded by *A. adenophora*; the remaining six sites had not invaded. The forests were secondary forests recovering from logging and subsequent planting in 1980s. The road was built of stone in the 1960s and improved to asphalt in 2000. The cover of *A. adenophora* was c. 80% across the six invaded sites (visually estimated). The main forest species were *Eucalyptus robusta*, *Pinus kesiya*, *Hedychium villosum*, *Sambucus chinensis*, *Cornus wilsoniana*, *Cyclobalanopsis glauca*, *Quercus chenii* and *Q. acutissima*. Along the roadsides, *Digitaria sanguinalis*, *Wedelia trilobata*, *Crassocephalum crepidioides* and other Gramineae species were prominent.

The sites were spaced at least 1 km from one another. At each site, we randomly established three 1 m × 1 m plots with similar cover of *A. adenophora*, from which the top 10 cm of soil was collected after removing above-ground plants and litter. Roots, residues and small stones were removed from the soil using a 2-mm sieve. The soil from the three plots at each site was mixed evenly and used within 3 days after collection.

Competition experiment

The soil from each site was divided into two samples: one sample was unaltered, and the other sample was evenly mixed with 2·0% activated carbon to diminish any allelopathic interactions (Lankau 2010; Zheng *et al.* 2015). The soil sample from each site and activated carbon treatment was put into 5-L pots, 4 kg of soil per pot. In February 2013, 15-cm-tall seedlings of *A. adenophora* and 10-cm-tall seedlings of *Oryza sativa* L. upland rice were transplanted into the pots singly or mixed (one plant of each species, placed 5 cm apart). The 360 pots (three competition treatments × two activated carbon treatments × 12 sites × five replicates) were placed in a greenhouse in Xishuangbanna Tropical Botanical Garden (21°21′ N, 101°51′ E, 750 m a.s.l.), Chinese Academy Science, located in Yunnan, Southwest China. The pots were allotted into five plots, 72 pots (a replicate of all treatments) per plot, and were randomly arranged (50 cm apart from one another) in each plot. When necessary, the seedlings were watered with sprinklers.

Oryza sativa was used in this study because it is the plant in which DTD and HHO were first purified and identified (Yang *et al.* 2008). *Oryza sativa* is widely cultivated in mountain areas in Yunnan, China, and *A. adenophora* often invades *O. sativa* fields. Wild rice, which can be outcompeted by *A. adenophora*, is naturally distributed in Yunnan (Dai *et al.* 2004).

After 6 months, all plants (including the roots) were harvested, dried at 60 °C for 48 h and weighed. The competition response of *O. sativa* and *A. adenophora* was measured as per cent change in biomass when grown with competition $(P_{comp} - P_{single})/P_{single}$, where P_{comp} and P_{single} were the average biomass for each soil sample when grown with and without competition, respectively.

Statistical analyses

Two-way ANOVA was used to test the effects of habitats (invaded vs. non-invaded), activated carbon treatments and their interactions on biomass and per cent change in biomass of *O. sativa* and *A. adenophora*. A Duncan's multiple range test was used to compare the differences among habitats and activated carbon treatments. All analyses in this and the following experiments were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

EXPERIMENT II: INVADER ABUNDANCE AND ALLELOPATHIC EFFECTS

Leaf and soil collections

Fully expanded *A. adenophora* leaves were collected from 12 individuals in a natural population in Xishuangbanna, Yunnan Province, Southwest China. Fresh leaves were immersed in distilled water (1 : 10 ratio; 2·5% based on dry mass) for 36 h and filtered through four layers of filter paper, then through 0·45 μm Micro PES membrane (Jinteng Experiment Equipment Co., Ltd, Tianjin, China) to remove microbes. The leaf leachate was kept at 4 °C until used.

We collected soils from 30 sites with varying abundances of *A. adenophora* in Lancang County, Yunnan Province, Southwest China, using the same method as in the competition experiment. Twenty-four sites were invaded by *A. adenophora*, nine of which were in forests, six in wastelands and nine along roadsides; six sites were not invaded (see Table S1, Supporting Information). The sites were spaced at least 1 km from one another. To evaluate invasive species abundance, we visually estimated the cover of *A. adenophora* and measured above-ground biomass of the invasive plant, using the harvest method, from three plots in each site. The number of the non-invaded sites was much less than that of the invaded sites because collecting soils along a roughly, well-distributed abundance gradient of *A. adenophora* would enable us to evaluate the effects of invasive species abundance on the accumulation and activity of allelochemical-degrading soil microbes.

Seed germination

The soil from each site was divided into two samples, one of which was sterilized by autoclaving (121 °C, 0·105 MPa, 1 h) three times at 24-h intervals in order to eliminate microbial degradation of allelochemicals, and therefore increase allelopathic interactions. The other sample was used as a non-sterilized control. The soil from each sterilization treatment was divided into eight equal portions of 25 g each and put into eight plastic cups (220 mL). Twenty *O. sativa* seeds were sown in each cup after being sterilized (using 0·1% HgCl for 5 min) and washed three times using distilled water. The eight cups were randomly divided into two groups, one of which was treated with 12 mL distilled water per cup and the other treated with 12 mL 2·5% of leaf leachate from *A. adenophora*. Water or leaf leachate was added into each cup at three different times. Seeds were germinated in an LRH-250 growth chamber (Yiheng Scientific Instruments Co., Ltd, Shanghai, China), with a temperature of 30/20 °C (day per night) and light intensity of 250 μmol m^{-2} s^{-1} for a 12 h photoperiod. In total, there were 480 cups (soils from 30 sites × two sterilization treatments × two leaf leachate treatments × four replicates).

After 10 days, the shoot and root lengths of *O. sativa* seedlings were measured, and mean values were calculated for each cup. We recognized that soil nutrient availability may differ among sites and sterilization treatment may also influence soil nutrient availability. In order to exclude the confounding effects of potential differences in soil nutrients between sterilization treatments and sites on shoot and root lengths, we calculated a response index (RI) to evaluate the allelopathic effects of leaf leachate in each soil and sterilization treatment (Li *et al.* 2015a). Response index was calculated as follows: $(variable_{leachate} - variable_{water})/variable_{water}$ (*sensu* Williamson & Richardson 1988), where $variable_{water}$ and $variable_{leachate}$ were the average root or shoot length of four cups (replicates), with water and leaf leachate addition, respectively. An RI > 0 indicated growth improvement, i.e., positive allelopathic interaction; an RI < 0 indicated growth inhibition, i.e., inhibitory allelopathic interaction; an RI = 0 indicated no allelopathic interaction.

Degradation of allelochemicals and activity of degrading microbes

DTD and HHO were added into non-sterilized soil from each of the 30 sites (three replicates per site) and incubated for 48 h in a

chamber maintained at 25 °C. DTD and HHO contents in the soils were then measured using ACQUITY Ultra Performance Liquid Chromatography (APLC; Waters Corporation, Miller, MA, USA), according to the method in Li *et al.* (2015a). In order to estimate biomass and activity of the microbes that could degrade the allelochemicals, we measured the allelochemical-induced soil respiration rate for soil from each of the 30 sites (three replicates) according to the method in Li *et al.* (2015a). Substrate-induced respiration rate is often used to estimate target microbes, especially in soils (Kaur *et al.* 2009; Li *et al.* 2015a). Because the allelochemicals degraded very fast and the initial contents were very low (Li *et al.* 2015a), they did not influence measurements of substrate-induced respiration rates.

Statistical analyses

Two-way ANOVA was used to test the effects of habitats (invaded vs. non-invaded), soil sterilization treatments and their interactions on RI for *O. sativa* with leaf leachate of *A. adenophora*. A Duncan's multiple range test was used to compare differences among habitats and soil sterilization treatments. One-way ANOVA was used to test the effects of habitat types on DTD and HHO contents and substrate-induced respiration rates. Response indices of shoot and root length were log transformed, and the substrate-induced respiration rates were square-root transformed to meet the assumptions of ANOVA. To evaluate the effects of *A. adenophora* abundance on the allelopathic effects of leaf leachate, we analysed regressions between the RI of *O. sativa*, DTD content, allelochemical-induced respiration rate and *A. adenophora* above-ground biomass or cover. We also analysed regressions between the RI of *O. sativa*, DTD content and allelochemical-induced soil respiration rate.

EXPERIMENT III: ALLELOCHEMICAL-DEGRADING MICROBES

Isolation and purification

Soil samples (1 g) from invaded and non-invaded habitats were added to 100 mL of autoclave-sterilized inorganic culture solution containing 1 mg L^{-1} DTD and HHO as a sole carbon source and incubated for 5 days at 30 °C in shaking incubator (150 r.p.m.; Guowang Experiment Equipment Co., Ltd, Changzhou, China). The culture solution contained 0·5 g NH_4NO_3 + 0·5 g K_2HPO_4 + 0·5 g KH_2PO_4 + 0·008 g $MgSO_4{\cdot}7H_2O$ + 0·002 g $CuSO_4$ + 0·002 g $MnSO_4$ + 0·002 g $FeSO_4$ + 0·002 g $CaCl_2$ per litre, at pH 7·5. Subsequently, 100 μL of the incubated solution was streaked onto a solidified inorganic medium containing 1 mg L^{-1} DTD and HHO. After 5 days of growth at 30 °C, individual microbial colonies were successively streaked, until pure cultures were obtained, on a solidified Luria-Bertani (LB) medium (10 g trypsin, 5 g yeast extract, 10 g NaCl per litre; pH 7·2). The pure isolates were then plated on the solidified inorganic culture medium containing DTD and HHO. The isolates that grew well were selected and added into an inorganic culture solution containing DTD and HHO and incubated at 30 °C with shaking (150 r.p.m.). After 5 days, the incubated solution of each isolate was streaked onto a solidified inorganic culture medium containing DTD and HHO. The purpose of the last three steps was to confirm the stability of the DTD- and/or HHO-degrading microbes. Two isolates that grew well were transferred onto a solidified LB medium, incubated for 2 days and stored at 4 °C for further experiments.

Identification

Total DNA of each isolated microbe was extracted using TaKaRa Lysis Buffer for Microorganism to Direct (Code No. 9164). Amplification of the 16 S rDNA was performed using TaKaRa 16 S rDNA Bacterial Identification PCR Kit (Code No. RR176). The PCR products were purified using agarose gel electrophoresis, cloned using TaKaRa pMD®18-T Vector (Code No. D101A) and sequenced using M13 ± primer (Sangon Biotech Co., Ltd, Shanghai, China). The sequences were compared with sequences registered in GenBank using the Blast programs in NCBI's website.

Effects of the isolated strains on degradation of DTD and HHO

The roadside soil was sterilized using the same method applied in Experiment II, put into nine, 50-mL tubes (10 g per tube) and incubated for 1 week at 25 °C. The tubes were divided into three groups, each treated with 5 mL suspensions of the two isolated strains (OD_{600} = 1) with water as the control and then incubated at 30 °C for 1 week. Four mL of sterilized leaf leachate of *A. adenophora* (containing 85·6 μg mL^{-1} DTD, 8·38 μg mL^{-1} HHO) were added into each tube. After 48 h of degradation of the allelochemicals, DTD and HHO contents in each tube were measured using ACQUITY Ultra Performance Liquid Chromatography (Li *et al.* 2015a).

Effects of the isolated strains on allelopathy

The soil was treated using the same method applied in Experiment II and placed into 15 pots (220 mL), 25 g per pot. The pots were divided into three groups, each treated with 15 mL suspensions of the two isolated strains (OD_{600} = 1) with water as a control, and incubated at 30 °C for 1 week. Thirty *Brassica pekinensis* seeds were sown into each pot, and 12 mL sterilized leaf leachate of *A. adenophora* (containing 85·6 μg mL^{-1} DTD, 8·38 μg mL^{-1} HHO) was added into each pot at three different times. The seeds were germinated under the conditions described above. Germination of *B. pekinensis* seeds is sensitive to *A. adenophora* leaf leachate (Zhu, Zhang & Ma 2011). After 10 days, germination rates of *B. pekinensis* seeds were measured.

Statistical analyses

One-way ANOVA was used to test the effects of isolated strains on DTD and HHO content and on the germination rate of *B. pekinensis*.

Results

EXPERIMENT I: COMPETITION AND ALLELOPATHIC EFFECTS

When grown without competition, activated carbon addition did not influence the biomass of *O. sativa* ($F_{1,20}$ = 0·002, P = 0·961) or *A. adenophora* ($F_{1,20}$ = 0·166, P = 0·688) in soils from both invaded and non-invaded

habitats (Fig. 1; see Table S2). Biomass of *A. adenophora* was significantly higher in soils from invaded habitats than it was in soils from non-invaded habitats ($F_{1,20}$ = 29·936, P < 0·001; Fig. 1; see Table S2). When grown with competition, the effects of activated carbon treatment were significant for *O. sativa* ($F_{1,20}$ = 5·814, P = 0·026) but not for *A. adenophora* ($F_{1,20}$ = 3·027, P = 0·097; see Table S2). In addition, activated carbon increased the biomass of *O. sativa* much more in soils from non-invaded habitats than it did in soils from invaded habitats (Fig. 1). In fact, the effects of activated carbon addition were not significant for *O. sativa* in soils from invaded habitats according to Duncan's test.

EXPERIMENT II: INVADER ABUNDANCE AND ALLELOPATHIC EFFECTS

Soil sterilization significantly increased the allelopathic inhibition of *A. adenophora* leaf leachate on both shoot ($F_{1,56}$ = 39·403, P < 0·001) and root ($F_{1,56}$ = 18·820, P < 0·001) growth of *O. sativa* (decreased RI; Fig. 2; see Table S3). The effects of sterilization were greater for soils from invaded habitats than it was for soils from non-invaded habitats (Fig. 2; see Table S3). For example, sterilization decreased the RI of shoot and root lengths by 55·0% vs. 34·1% and 50·1% vs. 22·8% in invaded vs. non-invaded habitats, respectively. In fact, the effects of sterilization were not significant for the RI of root in non-invaded habitats according to Duncan's test. In addition, the effects of sterilization were significant for soils from all 24 invaded sites, while it was significant for soils from only three of the six non-invaded sites (data not shown).

Degradation of DTD was more rapid in soils from invaded habitats than that in soils from non-invaded habitats ($F_{1,28}$ = 13·869, P = 0·001; Fig. 3). HHO degraded too quickly to be detected in some soil samples, most of which were collected from invaded habitats (data not shown). Similarly, soil respiration rate induced by DTD and HHO was significantly higher in soils from invaded habitats than it was in soils from non-invaded habitats ($F_{1,28}$ = 4·807, P = 0·037; Fig. 3).

The allelopathic effects of *A. adenophora* leaf leachate decreased (RI increased) linearly with increasing abundance (both above-ground biomass and cover) of *A. adenophora* in non-sterilized soils (with microbes) from the 30 sites (Fig. 4). The correlations were not significant when the soil samples were sterilized (without microbes; data not shown).

Degradation of DTD increased significantly in soils from sites with higher invasive species abundances, as evidenced by the significantly negative correlation between DTD content and *A. adenophora* biomass or cover (Fig. 4). Substrate-induced soil respiration rate (a measure of allelochemical-degrading microbes) also increased significantly with increasing *A. adenophora* abundance (Fig. 4). With higher substrate-induced soil respiration rates, soil DTD content and the allelopathic effects of *A. adenophora* were significantly lower (Fig. 5), further indicating that soil microbes degrade DTD and therefore reduce the allelopathic effects of *A. adenophora*.

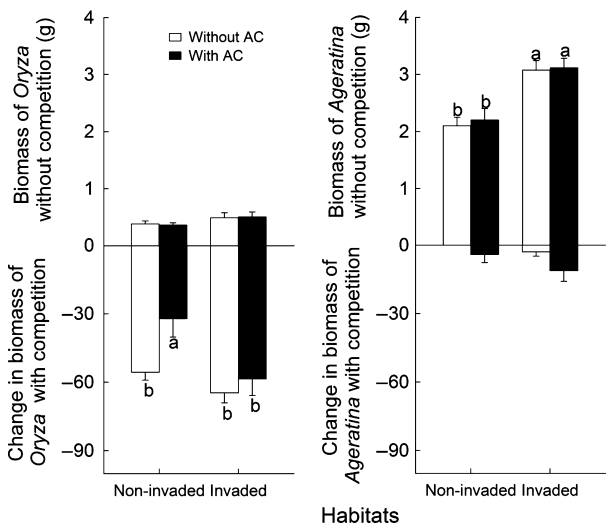

Fig. 1. Biomass and per cent change in biomass for *Oryza sativa* and *Ageratina adenophora* grown in soils from 12 non-invaded and invaded sites without and with activated carbon (AC), means + 1 SE (n = 6). Different letters indicate significant differences between habitats and activated carbon treatments according to Duncan's test.

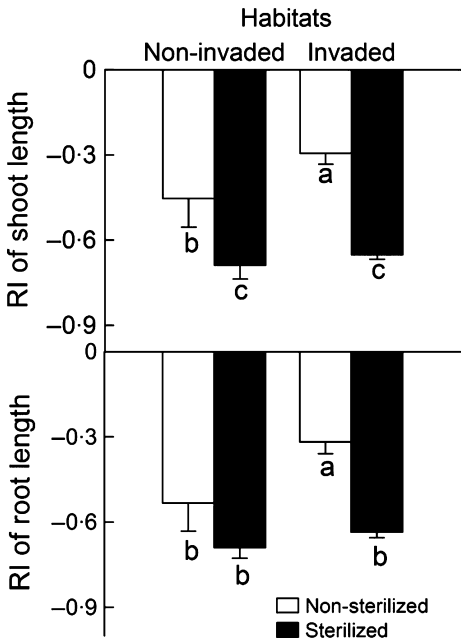

Fig. 2. Response indices (RI) of shoot and root lengths of *Oryza sativa* to leaf leachate of *Ageratina adenophora* in non-sterilized and sterilized soils collected from habitats not invaded and invaded by *A. adenophora*, means + 1 SE (n = 24 for invaded habitat; n = 6 for non-invaded habitat). Different letters indicate significant differences between habitats and sterilized treatments according to Duncan's test.

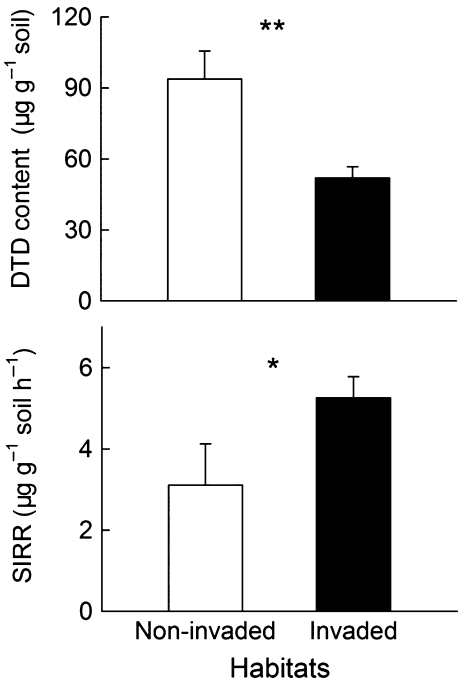

Fig. 3. Content of 9-Oxo-10, 11-dehydroageraphorone (DTD) and substrate-induced soil respiration rate (SIRR) in soils from habitats not invaded ($n = 6$) and invaded ($n = 24$) by *Ageratina adenophora*, means + 1 SE. Asterisks indicate level of significant differences between the non-invaded and invaded habitats at $P < 0{\cdot}05$ (*) and $P < 0{\cdot}01$ level (**), according to one-way ANOVA.

EXPERIMENT III: ALLELOCHEMICAL-DEGRADING MICROBES

With DTD and HHO as the sole carbon sources, we purified two bacterial strains, one from soil collected in the habitats invaded by *A. adenophora* and one from soil collected in the non-invaded habitats (Fig. S1). Both strains grew rapidly on solidified inorganic medium, forming visible colonies in 72 h. According to the sequences of full-length 16 S rDNA (≈1500 bp; >96% similarity), the two strains belonged to *Arthrobacter* sp. ZS (from the invaded habitats) and *Rhodococcus* sp. BS (from the non-invaded habitats). The allelochemical DTD ($F_{2,6} = 41{\cdot}946$, $P < 0{\cdot}001$) degraded faster in sterilized soil inoculated with the bacteria than it did in soil without inoculation, and degradation of HHO ($F_{2,6} = 2{\cdot}643$, $P = 0{\cdot}150$) was not significantly different between inoculation treatments (Fig. 6). The effects of the microbe strain from the invaded habitat were much stronger than that of the microbe strain from the non-invaded habitat. Inoculation of *Arthrobacter* sp. ZS increased DTD degradation by 31% and increased *B. pekinensis* germination rate by 86%.

Discussion

Our results showed that soil microbes may decrease the allelopathic effects of invasive plants by degrading their allelochemicals, and that the effects of soil microbes may increase with increasing invasive species abundance, gradually decreasing the allelopathic inhibition on co-occurring native plants. In our competition experiment, the allelopathic effects of *A. adenophora* were detected in soils collected from the non-invaded habitats but not in soils from the invaded habitats, suggesting that allelochemicals degraded faster in soils from the invaded habitats. In our seed germination experiment, soil microbes reduced the allelopathic effects of *A. adenophora* leaf leachate in soils from all 24 sites invaded by *A. adenophora* and in soils from only three of the six non-invaded sites. The overall effects of soil microbes were greater for soils from the invaded habitats than it was for soils from the non-invaded habitats. Consistently, degradation of *A. adenophora* allelochemicals was faster, and activity of the allelochemical-degrading microbes was higher, in soils from the invaded habitats than they were in soils from the non-invaded habitats. Other researchers have also found that soil microbes mediate allelopathic interactions between invasive and native plants (Lankau 2010; Zhu, Zhang & Ma 2011; Li *et al.* 2015a). In the present study, we further found that allelochemical-degrading microbes increased gradually with increasing invasive species abundance, promoting degradation of the allelochemicals and therefore decreasing the allelopathic effects of invasive plants.

We could not determine how the naïve soil microbes acquired the ability to degrade allelochemicals of *A. adenophora*. *Rhodococcus* sp. BS, which was isolated in the soil collected from the non-invaded habitat, may have obtained this ability by experiencing chemicals similar to the allelochemicals of the invasive species. *Ageratina adenophora* (also known as *Eupatorium adenophorum*) has several phylogenetically related native species, such as *E. japonicum* and *E. lindleyanum*, within the region invaded by *A. adenophora* in China. These native species may release chemicals similar to the ones released by *A. adenophora*. *Arthrobacter* sp. ZS, which was isolated from the soil invaded by *A. adenophora*, may acquire a much stronger ability to degrade DTD than does *Rhodococcus* sp. BS, and may therefore decrease the allelopathic effects of the invader by adaptive evolution.

Soil microbes are able to rapidly adapt to novel chemicals (Top & Springael 2003). For example, hydrocarbon-degrading microbes increased gradually in soil contaminated by crude oil (Oudot *et al.* 1989). Naïve soil microbes gradually acquired the ability to degrade xenobiotic compounds by adaptation after repeated application (Aelion, Swindoll & Pfaender 1987; Hole, McClure & Powles 2001). Lankau (2011) found that soil microbe communities showed increasing resistance to allelochemicals of *A. petiolata* over time in terms of taxa richness and community composition of bacteria, fungi and arbuscular mycorrhizal fungi. Soil pathogens also increased with increasing abundance or residence time of invasive

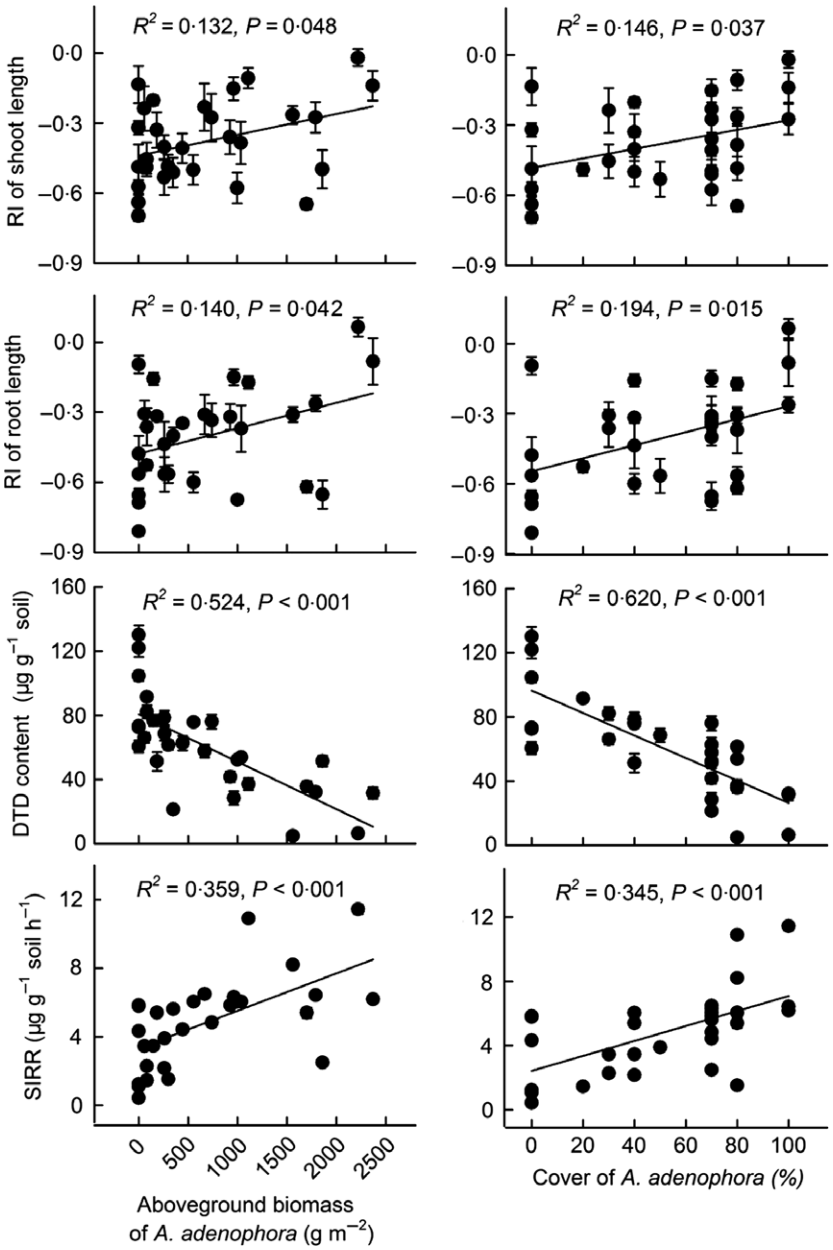

Fig. 4. Response indices (RI) of shoot and root lengths of *Oryza sativa* to leaf leachate of *Ageratina adenophora*, content of 9-Oxo-10, 11-dehydroageraphorone (DTD), and substrate-induced respiration rate (SIRR) as functions of above-ground biomass and cover of *A. adenophora*.

plants (Diez *et al.* 2010; Michell *et al.* 2010; Dostál *et al.* 2013; Flory & Clay 2013).

The method that we used in the seed germination experiment, of adding leaf leachate into the soil at three different times, differed from field conditions where allelochemicals are released into the soil, or even onto neighbouring plants, continuously through root exudate and leaf leachate. The continuous input of allelochemicals may inhibit seed germination even in soils with active microbes. In addition, seeds of *O. sativa* were large and so less vulnerable to the allelochemicals of invasive plants than were the smaller-seeded, native herbaceous plants (Zheng & Feng 2005; Qin *et al.* 2013). Although soil microbes may degrade part of the allelochemicals of invasive plants, the decreased levels of allelochemicals may still have allelopathic effects on sensitive native plants (Yang *et al.* 2016). Furthermore, rhizosphere soil may have quite high concentrations of allelochemicals as roots produce and exude them continuously. This may help exclude the roots of competing species from a set volume of soil, thereby allowing invasive species exclusive access to nutrients in the surrounding soil. For example, strong allelopathic effects on eight native species were found for the rhizosphere soil from *C. odorata* (Zheng *et al.* 2015). However, in our competition experiment, which had more realistic allelochemical inputs and ecologically relevant

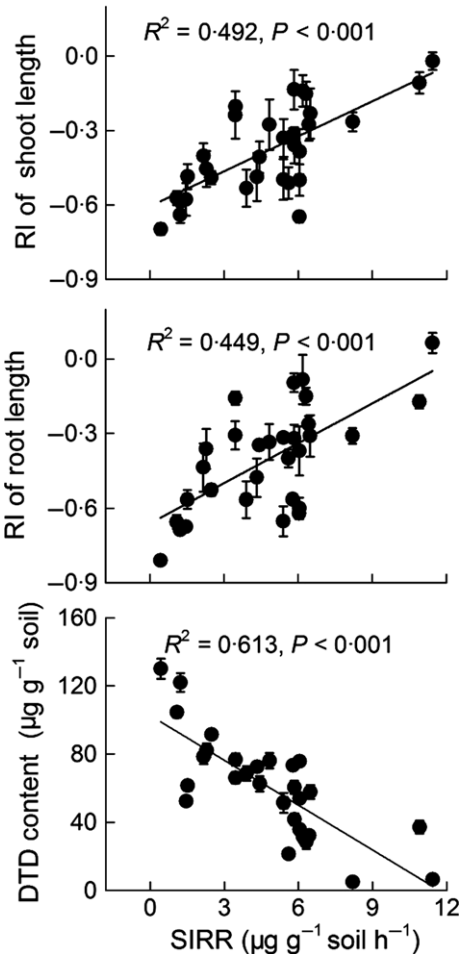

Fig. 5. Response indices (RI) of shoot and root lengths of *Oryza sativa* to leaf leachate of *Ageratina adenophora* and content of 9-Oxo-10, 11-dehydroageraphorone (DTD) as functions of substrate-induced respiration rates (SIRR).

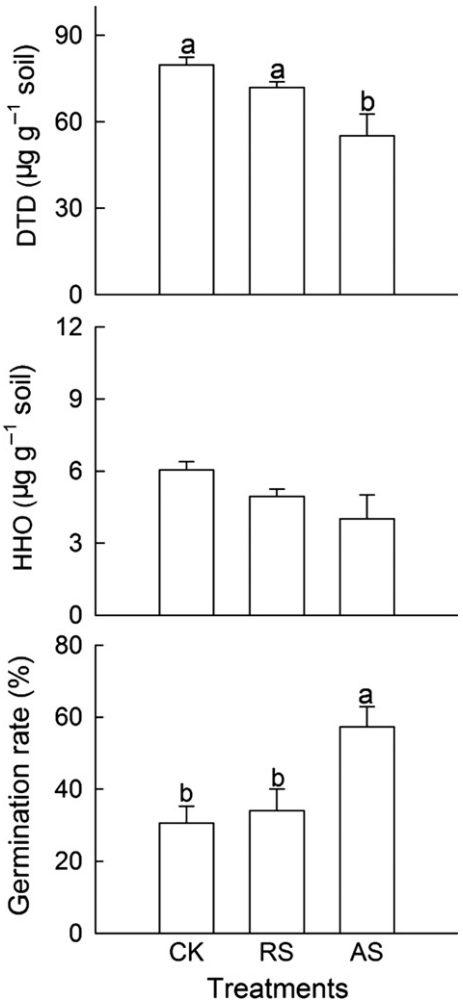

Fig. 6. Effects of the two isolated microbe strains on the contents of 9-Oxo-10, 11-dehydroageraphorone (DTD) and 9β-hydroxyageraphorone (HHO), and *Brassica pekinensis* seed germination rate. CK, control; RS, *Rhodococcus* sp. BS; AS, *Arthrobacter* sp. ZS. Different letters indicate significant differences between treatments according to Duncan's test.

interaction between plants, we did not find allelopathic effects for *A. adenophora* in soils from the invaded habitats. These results indicate that allelopathic interactions in the field are complex and context-dependent, providing an alternative explanation to the phenomenon that disturbed sites (with strong allelopathic interactions) are easily invaded by *A. adenophora* (Sun, Lu & Sang 2004).

Our results have significant implications for the ecological restoration of invaded or newly disturbed ecosystems. Replacement of invasive plants with native species is a sustainable method for managing invasive plants (Li *et al.* 2015b). The addition of allelochemical-degrading microbes into soils may improve establishment and growth of native plants by alleviating allelopathic inhibition of invasive plants on native plants. With the acceleration of infrastructure improvements, such as construction of high-speed railways in recent years, newly disturbed habitats have increased greatly in China. Native plants are often grown in these altered habitats for various purposes. Applying allelochemical-degrading microbes may facilitate the establishment of the native plants in such areas.

In summary, our results indicate that soil microbes decrease the allelopathic effects of invasive plants by degrading allelochemicals, and that the effects of soil microbes may increase with increasing invader abundance, thereby gradually decreasing the invasive plant allelopathic inhibition on co-occurring native plants. Our study also indicates that application of the allelochemical-degrading microbes may facilitate ecological restoration of invaded or newly disturbed ecosystems. However, soil microbes may not rapidly degrade novel allelochemicals of invasive plants in early stages of invasions and in some environments, or in specific places such as in the rhizosphere. In addition, some invasive plants may release volatile chemicals that have strong allelopathic effects on native plants. It is therefore necessary to consider the effects of soil microbes when testing the novel-weapons hypothesis or the role of allelopathy in biological invasions and when replacing invasive species with native plants.

Authors' contributions

Y.P.L. and Y.L.F. conceived the ideas and designed study. Y.P.L., Z.L.K., Y.L.Z., J.L.Z. and Y.J.C. performed the experiments and collected data. Y.P.L. and Y.L.F. analysed the data. Y.P.L. and Y.L.F. interpreted the data and wrote the paper. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

The authors declare that they have no conflicts of interest. This work was supported by National Key Research and development Program of China (2016YFC1200601), the National Natural Science Foundation of China (31100410, 31470575, 31670545 and 31270582), West Light Foundation of the Chinese Academy of Sciences (CAS), and CAS 135 program (XTBG-T01, F01). We are grateful to the Public Technical Service Center of Xishuangbanna Tropical Botanical Garden for measuring the allelochemical contents of *A. adenophora*.

Data accessibility

Competition and germination data are available from Dryad Digital Repository https://doi.org/10.5061/dryad.nc69n (Li *et al.* 2017).

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Received 10 August 2016; accepted 24 January 2017
Handling Editor: Luke Flory

Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Information on the 30 sampling sites in Lancang County, Yunnan, Southwest China.

Table S2. Effects of habitats, activated carbon treatments and their interactions on biomass and per cent change in biomass of *Oryza sativa* and *Ageratina adenophora*.

Table S3. Effects of habitats, soil sterilization treatments and their interactions on response indices (RI) of shoot and root lengths of *Oryza sativa*.

Fig. S1. Colonies and unicellular thallis of the two strains isolated.