An Invasive Plant Promotes Its Arbuscular Mycorrhizal Symbioses and Competitiveness through Its Secondary Metabolites: Indirect Evidence from Activated Carbon

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Abstract
Secondary metabolites released by invasive plants can increase their competitive ability by affecting native plants, herbivores, and pathogens at the invaded land. Whether these secondary metabolites affect the invasive plant itself, directly or indirectly through microorganisms, however, has not been well documented. Here we tested whether activated carbon (AC), a well-known absorbent for secondary metabolites, affect arbuscular mycorrhizal (AM) symbioses and competitive ability in an invasive plant. We conducted three experiments (experiments 1–3) with the invasive forb Solidago canadensis and the native Kummerowia striata. Experiment 1 determined whether AC altered soil properties, levels of the main secondary metabolites in the soil, plant growth, and AMF communities associated with S. canadensis and K. striata. Experiment 2 determined whether AC affected colonization of S. canadensis by five AMF, which were added to sterilized soil. Experiment 3 determined the competitive ability of S. canadensis in the presence and absence of AMF and AC. In experiment 1, AC greatly decreased the concentrations of the main secondary metabolites in soil, and the changes in concentrations were closely related with the changes of AMF in S. canadensis roots. In experiment 2, AC inhibited the AMF Glomus versiforme and G. geosporum but promoted G. mosseae and G. diaphanum in the soil and also in S. canadensis roots. In experiment 3, AC reduced S. canadensis competitive ability in the presence but not in the absence of AMF. Our results provided indirect evidence that the secondary metabolites (which can be absorbed by AC) of the invasive plant S. canadensis may promote S. canadensis competitiveness by enhancing its own AMF symbionts.

Introduction
Why invasive plants have increased competitive ability at their introduced range is the central question in understanding the mechanism of successful plant invasion [1–3]. Published studies have shown that invasive plants enhanced their competitive ability via the production of secondary metabolites [4,5]. Secondary metabolites can mediate invasive plants’ competition via strong effects on nutrient cycling [6], native plants [7], and soil microbes [8]. These influences could be mediated through directly and indirect ways.

The direct mediation of secondary metabolites from invasive plants on plant-plant competition can be best illustrated by observing native plants inhibiting their neighboring plants [9,10]. For example, roots exudes phytotoxin (→)-catechin from the invasive Centaurea maculosa directly inhibited the growth of native plants [7]. Secondary metabolites released from other invasive plants also directly inhibited seed germination of native plants [9,10]. The inhibition resulting from such direct effects can obviously increase the competitive ability of the invasive plant [11]. Secondary metabolites released by invasive plants also mediate competition indirectly. For example, secondary metabolites can indirectly promote the growth of invaders via influencing the nutrient cycling, like the pools and fluxes of inorganic and organic soil nutrient [6], or indirectly mediate competition via native soil microorganisms. Plant pathogens and symbionts [12–17], and the soil community [18–20] can be altered by secondary metabolites in general. Such effects on soil microorganisms may alter the competition between invasive and native plants [21].

Arbuscular mycorrhizal fungi (AMF) are important and ubiquitous soil microorganisms that form symbioses with many plant species in terrestrial ecosystems [22,23]. Interestingly, AMF also influence invasive plants’ competition [24,25]. For example, invasive Asian knapweed (Centaurea maculosa) in North America is able to tap into and benefit from the mycorrhizal network linking native plant roots [26,27]. Invasive Solidago canadensis in China can alter the AMF composition in the introduced range in ways that promote its competition against native plants [20]. In some cases, the secondary compounds released by invasive plants disrupt the symbioses between the AMF and native plants. For example, secondary chemicals released by the invasive plant Alliaria petiolata,
which cannot form symbioses with the AMF, disrupted the symbioses between native canopy tree seedlings and AMF [28]. A specific flavonoid fraction released by A. petiolata has a far stronger inhibitory effect on AMF in the invaded soil than in its original soils [29]. Additionally, the secondary compounds from A. petiolata not only inhibited AMF hyphal growth and spore germination [30] but also altered the AMF community associated with native sugar maple seedlings [31].

Although substantial evidence demonstrates that invasive plants can affect AMF symbioses of local hosts via secondary metabolites [30,32–36], whether those secondary metabolites can affect symbioses between AMF and the invasive plants themselves has not been documented. We address this question by using activated carbon (AC) to absorb secondary metabolites. Generally the effect of AC addition on plant growth and the formation of mutualistic relationships can reflect the putative role of secondary metabolites [37].

We used the perennial herb Solidago canadensis L. (goldenrod) as a model plant in our study. S. canadensis is native to North America but forms a near monoculture in areas of China where it has invaded [38]. S. canadensis has a strong allelopathic effect on native plants and is always associated with AMF, making it suitable for testing our hypothesis [39]. In a previous study, we found that the concentrations of three main secondary metabolites in S. canadensis plants were greater in the invaded range than in its native range and that these compounds substantially increased the competitive ability of S. canadensis in the invaded range [11]. Experiments have also shown that invasive S. canadensis is always associated with AMF and can alter the AMF community in ways that enhance its competitive ability [20,40]. In the current study, we separated the indirect effects (i.e., those mediated by AMF) and direct effects (i.e., those not mediated by AMF) of secondary metabolites from S. canadensis on the competition between S. canadensis and a native plant.

Materials and Methods

Activated Carbon

Activated Carbon (AC) is often used as an experimental tool to absorb secondary metabolites in soil [41–43]. Researchers have noted, however, that even in the absence of secondary metabolites, AC can affect plant growth by altering nutrient availability and other soil properties [44,45]. In a preliminary experiment, we tested whether AC affected soil properties with and without plants. There were five replicates for each treatment. The experiment lasted for six months. Soil samples were air-dried and sieved carefully, prior to testing. The concentration of soil organic matter (SOM) was measured by the K2Cr2O7–H2SO4 oxidation method of Walkey and Black [46]. Total nitrogen (TN) and total phosphorus (TP) in soil samples (1.0 g per sampling treatment) was determined with a San++ Continuous Flow Analyzer (Skalar, Netherlands). The pH of the soil from each sampling plot was measured using a soil–water slurry (1:5 soil:water). Soil available phosphorus (AP) was analyzed by the Olsen method [47]. AC at a rate of 20 ml l−1 did not affect soil properties (Table 1), and we therefore used AC at 20 ml l−1 in this study. The P and N concentration in the AC was 0 and 0.18%, respectively, and the pH was 6.0. The effect of AC on soil properties was also evaluated in experiment 1 of the current study, as described later.

Plant Species

In a previous study with six S. canadensis populations (three invasive populations from China and three native populations from America) and one K. striata population (from the area invaded by S. canadensis in China), we determined that the enhanced competitive ability of S. canadensis relative to K. striata in China resulted from increased root exudates [11]. Because neither biomass nor height of S. canadensis in our previous study was significantly affected by the source of the S. canadensis population (either from the original or invaded lands), the current study used one invasive S. canadensis population originating from the invaded area in China. One native K. striata population from China was also used. Seeds of S. canadensis and K. striata were collected in the same locations (30°16′N, 120°11′E) in Hangzhou, Zhejiang province (China), where the area was invaded by S. canadensis. Seeds of S. canadensis and K. striata were air-dried and stored at 4°C.

Experiment 1

A greenhouse microcosm experiment was performed to determine whether AC affects soil properties, the levels of three groups of secondary metabolites (total flavones, total phenolics, and total saponins) in soil, plant growth, and the AMF community associated with invasive S. canadensis and native K. striata.

The experiment was a two factorial design, with two AC treatments (with and without AC), two host plants (invasive S. canadensis and native K. striata), and five replicates (five blocks). Each microcosm measured 20×15×20 cm (length×width×height) and contained 6 kg of soil. The soil used in the experiment was collected from Guxi City, Zhejiang Province, China (30°18′N, 121°10′E), where S. canadensis had invaded the locality.

To ensure the initial consistency of plants in the experiment, we germinated and pre-cultured the plants in a plastic mesh plate with vermiculite and peat in the greenhouse with natural light and temperature [11]. When seedlings were 4 cm tall, two seedlings were transplanted into each microcosm so that the microcosm contained two S. canadensis seedlings or two K. striata seedlings. For the AC treatment, finely ground AC was mixed into the soil at 20 ml l−1 [11].

Microcosms were randomly arranged in each block in a greenhouse with natural light and with average daily temperatures ranging from 18–30°C during the experiment, which began in April and ended in October. Each microcosm was watered daily to maintain soil moisture at 70–90% of water-holding capacity. No additional nutrients were added during the experiment.

Experiment 1 was terminated 6 months after transplanting, which coincided with floral initiation. Root systems were separated from shoots. Half of each root sample was frozen at −80°C for molecular analysis. The remaining half of each root sample was used for quantification of AMF colonization. AMF colonization of roots was quantified using a microscope (<20 magnification) and utilizing the gridline intersection method [48], and 200 transects were examined per replicate.

Soil samples in each microscope were air-dried and sieved for the test of soil properties. Soil properties were measured with the methods described in the preliminary experiment. Soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP) and pH were tested. Three main secondary metabolites (total flavones, total phenolics, and total saponins) in the soil were also quantified using the methods described by Zhang et al. [49]. First, the crude soil extracts were obtained by placing the 10-g sample in 100 ml of 70% ethanol and later, filtering the sample through a Whatman no. 44 filter paper. Following which, total flavones in the crude extracts were determined by employing the NaNO2–Al(NO3)3–NaOH colorimetric assay with rutin as the reference substance. Total phenolic acids were measured by the Folin-Ciocalteu assay with gallic acid as the reference substance. The total saponins were detected using vanillin–HClO4 as the chromogenic reagent.

Secondary Metabolites, AMF and Invasive Plant
To examine the effects of AC on AMF in roots of *S. canadensis* and *K. striata*, we used a nested PCR-denaturing gradient gel electrophoresis (DGGE)-cloning-sequencing method. Each plant species was represented by three replicates. The details of the methods are described in Methods S1. The DGGE banding pattern obtained using this method was analyzed with Quantity-One software (Bio-Rad).

Each DNA sequence obtained from the AMF was subjected to similarity comparison using an online program (BLAST, http://www.ncbi.nlm.nih.gov/BLAST). Sequences of possible chimeric origin were detected using the online CHIMERA DETECTION program (http://rdp8.cme.msu.edu/html/analyses.html). Sequences were subsequently registered in the GenBank database under accession numbers KC507871–KC507891. For the phylogenetic analysis, sequences with gaps were treated as missing data, and then a neighbor-joining tree (1,000 replicates) including the obtained sequences and their closest relatives from GenBank was constructed using MEGA version 4.0 with the Kimura 2-Parameter model. Sequences that were 2% different from each other were subsequently assigned to separate phylotypes. It is noteworthy that the tree topology and bootstrap value were always included in the analysis.

To analyze the potential influence of secondary metabolites and soil properties on the AMF community composition, we

| Soil property | Without plants | With plants | significance |
|---------------|----------------|-------------|--------------|
|               | No AC | AC |       | No AC | AC |       |
| pH            | 8.18  | 8.19 | ns   | 7.96  | 8.03 | ns   |
| TN (g/kg)     | 0.55  | 0.56 | ns   | 0.58  | 0.61 | ns   |
| TP (g/kg)     | 0.62  | 0.63 | ns   | 0.49  | 0.50 | ns   |
| SOM (g/kg)    | 12.23 | 13.25 | ns   | 12.34 | 11.80 | ns   |
| AP (mg/kg)    | 21.87 | 21.08 | ns   | 18.06 | 18.69 | ns   |

TN refers to total nitrogen; TP refers to total phosphorous; SOM refers to soil organic matter; AP refers to available phosphorous. No effect was significant at *P*<0.05. ns, not significant.

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Figure 1. Effects of activated carbon (AC) on shoot biomass and AMF community of plants (experiment 1). Effects of AC on shoot biomass of *S. canadensis* and *K. striata* (A), AMF colonization rate (B) and on the phylotypes of AMF communities in the roots of *S. canadensis* and *K. striata* (C) growing in field soil in experiment 1. GA1–GA6 are phylotypes (Fig. S2). Values are means ± SE. *P*-value: *, *P*<0.05; **, *P*<0.01; ns, not significant. Means with different letters are significantly different.
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Figure 2. Factors affecting the changes of AMF communities of host plants (experiment 1). CCA-biplot depicting the relationship between AMF communities and soil properties. Circles represent *S. canadensis*, and triangles represent *K. striata*; open symbols indicate No AC treatment while filled symbols indicate AC treatment. TN refers to total nitrogen; TP refers to total phosphorus; SOM refers to soil organic matter; AP refers to available P; Tph refers to total phenolics; Tfl refers to total flavones; Tsa refers to total saponins. The length of the arrows indicates the relative influence of the factor on community structure, while the angle between the arrows indicates the degree to which factors are correlated.
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performed a canonical correspondence analysis (CCA) with Monte-Carlo permutation tests (n = 499) using the Canoco for Windows version 4.52. In the CCA plot, the length of the arrows illustrated the relative importance of factors affecting community structure, while the angle between the arrows indicated the degree to which factors are correlated.

The presence (or absence) and intensity of the bands in a DGGE profile were used to construct a two-dimensional matrix.

Two-way analysis of similarity (ANOSIM) was performed using the Past, version 1.91 program [50] to analyze AMF communities differences subjected to different AC treatments.

After the data for soil properties, shoot biomass, and AMF colonization were tested for normality and homogeneity of variances, we used a one-way ANOVA in the general linear model to determine the effects of AC on soil properties, shoot biomass, and AMF colonization of *S. canadensis* and *K. striata*.

**Experiment 2**

This experiment was conducted to study the effects of AC on the symbioses between *S. canadensis* and five AMF species respectively. In contrast to experiment 1, experiment 2 used sterilized soil that was subsequently inoculated with selected AMF. Based on the results from experiment 1 and the experiments of Zhang et al. [20], which indicated that *Glomus* was the dominant AMF genus in *S. canadensis* roots, we used the following five *Glomus* species in experiment 2: *G. mosseae* (BGC501, XJ-01), *G. versiforme* (BGC504, BJ08), *G. diaphanum* (BGC506, SC05), *G. geosporum* (BGC507, GZ01), and *G. etunicatum* (BGC505, TW01). The AMF species were provided by the Glomales Germplasm Bank in China. These five *Glomus* species existed naturally in abandoned fields where the seeds of *S. canadensis* and *K. striata* were collected [20,51]. Soil with spores was used as the inoculum for the experiment.

Experiment 2 employed the following experimental design: two factors (AC and AMF) with five replicates (five blocks). The soil was the same as in experiment 1. AC was added to half of the microcosms at 20 ml l⁻¹ to absorb secondary compounds. After each microcosm was filled with 3 kg soil that had been sterilized by γ-radiation, an equal number of spores of each AMF species was mixed into the soil (one species per microcosm).

Seeds of *S. canadensis* were germinated and pre-cultured as described in experiment 1. Two seedlings were transplanted into each microcosm. The microcosms were randomly arranged in each block in the greenhouse. All growth conditions were the same as in experiment 1. Six months after transplanting, the plants were...
Table 2. Effects of AC treatments and plants (invasive S. canadensis or native K. striata) on soil properties in experiment 1.

| Soil property | S. canadensis No AC | AC | significance | S. canadensis No AC | AC | significance |
|---------------|---------------------|----|-------------|---------------------|----|-------------|
| pH            | 7.77                | 7.78| ns          | 7.77                | 7.77| ns          |
| TN (g/kg)     | 1.03                | 1.01| ns          | 1.02                | 1.05| ns          |
| TP (g/kg)     | 0.50                | 0.48| ns          | 0.49                | 0.50| ns          |
| SOM (g/kg)    | 20.54               | 23.04| *           | 20.34               | 21.20| ns          |
| AP (mg/kg)    | 49.02               | 48.57| ns          | 48.06               | 48.69| ns          |
| Tph (ppm)     | 204.40              | 5.61| **          | 107.81              | 2.24| **          |
| Tfl (ppm)     | 47.67               | 0.48| **          | 23.50               | 0.17| **          |
| Tsa (ppm)     | 467.67              | 23.80| **          | 231.00              | 19.9| **          |

TN refers to total nitrogen; TP refers to total phosphorous; SOM refers to soil organic matter; AP refers to available phosphorous; Tph refers to total phenolics; Tfl refers to total flavones; Tsa refers to total saponins. For each plant species, a significant difference between No AC and AC is indicated as follows:

*P<0.05;

**P<0.01;

ns P>0.05 (not significant).

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Results

Effects of AC on Soil Properties, Plant Growth, and AM Symbiosis (Experiment 1)

AC did not affect the key attributes of soil properties (soil organic matter, total nitrogen, total phosphorus, available phosphorus and pH) (P>0.05) but significantly decreased the levels of the main groups of secondary compounds (total flavones, total phenolics, and total saponins) (P<0.01) in soil planted with S. canadensis or K. striata (Table 2).

The addition of AC to soil significantly reduced the shoot biomass of S. canadensis (F1,11 = 58.169, P=0.007) but increased the shoot biomass of K. striata (F1,7 = 9.561, P=0.018) (Fig. 1A). AC reduced AMF colonization of S. canadensis (F1,11 = 47.287, P<0.001) but did not affect AMF colonization of K. striata (F1,7 = 2.523, P=0.131) (Fig. 1B).

DGGE profiles showed that the AMF composition differed in invasive S. canadensis versus native K. striata. AC affected the pattern and signal intensity of bands of the 18S rDNA fragments of AMF in roots of S. canadensis and K. striata (Fig. S1). The phylogenetic analysis indicated that sequences retrieved from all root samples could be separated into six AMF groups (GA1–GA6) (Fig. S2).
treatments affected AMF phylotypes in *S. canadensis* roots but not in *K. striata* roots (Fig. 1C). Roots of *S. canadensis* were colonized by five AMF phylotypes when AC was not added (group GA4 was missing) and by four phylotypes when AC was added (GA2 and GA5 were missing) (Fig. 1C). AMF phylotypes GA1, GA3, and GA6 in roots of *S. canadensis* or *K. striata* were not affected by the addition of AC (Fig. 1C).

The ANOSIM output showed that the AMF communities was significantly different with different AC treatments (*P* = 0.011) and host plants (*P* = 0.010).

The CCA-biplot showed that AC treatment significantly altered the AMF community structure of *S. canadensis* but not of *K. striata* (Fig. 2). The CCA-biplot also demonstrated that the differences in AMF community structures of *S. canadensis* between the No AC and AC treatments were more closely related to the three groups of secondary compounds (total flavones, total phenolics, and total saponins) than to soil properties.

**Effects of AC on Growth of *S. canadensis* and AMF, when Five AMF Species were Added to Sterilized Soil (Experiment 2)**

AC treatment reduced the total biomass of *S. canadensis* when inoculated with *G. geosporum* and *G. versiforme* (*P* < 0.05), but did not affect the total biomass of *S. canadensis* when inoculated with *G. mosseae*, *G. diaphanum* and *G. etunicatum* (*P* > 0.05). In the absence of AC, *S. canadensis* attained higher total biomass with *G. geosporum* and *G. versiforme* than with other three AMF species (*G. mosseae*, *G. diaphanum* and *G. etunicatum*) (Fig. 3).

Spore density in soil and colonization of *S. canadensis* roots were high for *G. geosporum* and *G. versiforme* in the absence of AC but were reduced (*P* < 0.05) when AC was added (Fig. 4). Addition of AC increased (*P* < 0.05) the spore density for *G. mosseae* and *G. diaphanum* (Fig. 4A). By contrast, the addition of AC did not significantly affect (*P* > 0.05) spore density or colonization for *G. etunicatum* (Fig. 4).
Effects of AC on the Competition between *S. canadensis* and *K. striata* in the Presence and Absence of AMF in Soil that had been Previously Sterilized (Experiment 3)

In the absence of AMF, the addition of AC increased *S. canadensis* shoot biomass in monoculture (*F*$_{1,8} = 39.526, P<0.001$) (Fig. 5A) and in mixture (*F*$_{1,8} = 8.914, P = 0.013$) (Fig. 5A). In the presence of AMF, addition of AC decreased *S. canadensis* shoot biomass in monoculture (*F*$_{1,8} = 9.429, P = 0.017$) (Fig. 5A) but did not cause a reduction in biomass when planted in a mixture (*F*$_{1,8} = 0.022, P = 0.883$) (Fig. 5A).

No AMF spores or any other evidence of AMF colonization were detected in microcosms that were not inoculated with AMF. In soil inoculated with AMF, adding AC reduced AMF colonization of *S. canadensis* in monoculture (*F*$_{1,8} = 19.253, P<0.001$) (Fig. 5B) and in mixture (*F*$_{1,8} = 21.452, P<0.001$) (Fig. 5B) but did not affect AMF colonization of *K. striata* in monoculture (*F*$_{1,8} = 0.088, P = 0.775$) (Fig. 6) and in mixture (*F*$_{1,8} = 0.782, P = 0.080$) (Fig. 6).

When AMF were not added, AC did not affect the competitive ability (as indicated by AI values) of *S. canadensis* (*F*$_{1,8} = 0.023, P = 0.745$), i.e., the AI values were near zero with or without AC (Fig. 7). When AMF were added, *S. canadensis* AI values were lower (indicating higher competitive ability) without AC than with AC (*F*$_{1,8} = 13.776, P = 0.012$) (Fig. 7). Regardless of AC addition, AI values were much lower with AMF than without AMF (without AC, *F*$_{1,8} = 17.851, P<0.001$; with AC, *F*$_{1,8} = 10.415, P<0.001$) (Fig. 7).

**Discussion**

Because activated carbon (AC) absorbs secondary metabolites in soil, it is often used to test the effects of secondary metabolites in situ [3,41–43]. AC, however, may also affect nutrient availability [54] and soil physio-chemical properties [45,55,56]. Thus, when AC is used in research concerning plant secondary compounds, its putative effects should be checked carefully. In our experiment 1, addition of AC did not change the general soil properties (soil organic matter, total nitrogen, total phosphorus, available phosphorus and pH) but greatly reduced the concentrations of total flavones, phenolics, and saponins. CCA also indicated that variation in the AMF community in *S. canadensis* roots was more closely related to these secondary metabolites than to other soil properties and that these secondary metabolites enhanced the colonization of *S. canadensis* roots by certain AMF species (experiment 2). We therefore deduced that the effects of AC in our study resulted predominantly from the absorption of secondary metabolites by AC.

Our community similarity analysis demonstrated that through the absorption of secondary metabolites, AC affected the AMF community in the roots of *S. canadensis*. Although it was documented that secondary metabolites from invasive plants can affect AM symbiosis of neighboring plants [28,30], in our study, AC effects suggest that secondary metabolites from an invasive plant may promote its own AM symbiosis and thereby enhancing its own growth.

It remains unclear how plant secondary metabolites affect the process of AMF symbiosis [29,30,32,33]. One plausible avenue could be that some compounds (e.g., flavones, sesquiterpenes, and strigolactones) could act as signals that induce AMF spore germination [57], hyphal branching [32], and/or AM symbiosis formation [36]. Flavones, phenolics, and saponins are commonly produced by many plants [58–60]. These three types of compounds had been reported to accumulate in the soil where *S. canadensis* had invaded [49]. Phenolics have been shown to reduce AMF colonization [35], while flavones like chrysin and luteolin can promote AM symbiosis [36]. In this study, AC greatly reduced the levels of these three types of secondary metabolites, indicating that the effects of AC on AMF symbiosis in *S. canadensis* may be due to the decrease in the concentrations of secondary metabolites.

Interestingly, not all AMF species were affected in the same way by the secondary metabolites from *S. canadensis*. In experiment 2, for example, without the addition of AC, *S. canadensis* secondary compounds greatly enhanced the growth (as indicated by spore density and root colonization) of *G. geosporum* and *G. versiforme* but did not affect the growth of *G. etunicatum*. The DGGE data and the phylogenetic tree also indicated some AMF phylotypes were present in *S. canadensis* roots only when secondary metabolites had

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**Figure 6. Effects of AC treatments on AMF colonization of *K. striata* (experiment 3).** AMF colonization of *K. striata* under monoculture or mixture as affected by AC treatments in experiment 3. Values are means ± SE. For comparison of paired bars (No AC vs. AC), ns indicates not significant.

**Figure 7. Effects of AMF and AC treatments on the competition of *S. canadensis* (experiment 3).** Competitive ability (as indicated by the aggressivity index) of *S. canadensis* as affected by AMF (AMF or No AMF) and activated carbon (AC or No AC) (experiment 3). A lower value for the index indicates greater competitive ability. Values are means ± SE. Means for No AC with different uppercase letters and means for AC with different lowercase letters are significantly different at *P*<0.05. For comparison of paired bars (No AC vs. AC), asterisks indicate significant differences (*<0.05), and ns indicates not significant.

**Figure 5A** and in mixture (indicating higher competitive ability) without AC than with AC (*P* = 0.080) (Fig. 6).
been absorbed by AC. These differential effects on AMF growth confirm that different AMF species can respond differently to the same chemicals. For example, spore germination of Glomus intraradices and G. claroideum can respond differently to strigolactones [57]. These results suggested that the development of symbioses between specific AMF and S. canadensis was mediated by S. canadensis secondary metabolites; this selective effect resulted in changes in the AMF community in the roots and also in the soil. In experiment 2, the beneficial AMF species G. geosporum and G. versiforme were able to promote greater growth of S. canadensis than the AMF species that were considered to be less beneficial (Fig. 3). These observations implied that S. canadensis’ secondary metabolites (which can be absorbed by AC in the soil) may specifically favor certain compatible AMF partners.

Results from experiment 3 showed that the competitive ability of invasive S. canadensis was influenced by both AC and AMF, i.e., release of chemicals by S. canadensis enhanced AMF colonization of S. canadensis roots. When AMF were absent, secondary metabolites did not increase the competitive ability of S. canadensis and autotoxicity might have been generated instead (Fig. 5A). As a consequence, S. canadensis grew better with AC treatment than without AC treatment in the absence of AMF. Autotoxicity is also exhibited by other exotic plants, including Centaurea maculosa [61], and this phenomenon is a way to reduce intraspecific competition. When AMF inoculum was added to the soil, however, the autotoxic effect was not evident (Fig. 5A), and secondary metabolites enhanced the competitive ability of S. canadensis (Fig. 7). These results suggested that AMF can offset the negative effects of secondary metabolites on S. canadensis. In other words, secondary metabolites indirectly promoted the competitive ability of S. canadensis via AMF. Given that secondary metabolites affected the degree of AMF colonization and the composition of the AMF community in the roots of the invasive S. canadensis but had little effect on AMF in the roots of the native K. striata in experiments 1 and 3, we concluded that the enhanced competitive ability of S. canadensis might have been the consequence of its own secondary metabolites affecting its own AM symbioses. Secondary metabolites released by an invasive plant may enhance invasiveness by both direct and indirect effects. The direct effect involves the inhibition of native competitors through secondary metabolites without mediation by microorganisms. The indirect effect involves microorganisms that inhibit the native competitors or enhancing the invader [21,62]. Although both field and common garden experiments have demonstrated that plant secondary metabolites can promote an invader’s competitive ability, past research was unable to separate the direct effects from indirect, microbially mediated effects of invasive non-indigenous plants [14]. It follows that the reported impact of secondary metabolites on competition may in many or all cases have resulted from combined direct and indirect effects. In this study, we separated the direct and indirect effects of secondary metabolites on plant-plant competition using the intrinsic properties of activated carbon. Although our study was performed in microcosms and not in the field, it provided authentic evidence that plant secondary metabolites are able to enhance the competitive ability of an invader by increasing AMF colonization of the invader itself.

In conclusion, the effects of secondary metabolites produced by invasive plants on local plants have been well documented [29], but few studies had focused on how secondary chemicals released by an invasive plant affect that invasive plant itself. Our study demonstrated an interesting phenomenon of ‘self-promotion’ by an plant invader resulting from the positive effects of its own secondary metabolites on the AMF that colonize its roots. The results presented here further expanded our understanding of the interesting role of secondary metabolites during plant invasion.

Supporting Information

Figure S1 Effect of AC on DGGE pattern of AMF communities in experiment 1. DGGE pattern of 18S rDNA fragments of AMF in roots of S. canadensis and K. striata as affected by addition of activated carbon (AC and No AC) in experiment 1. (TIF)

Figure S2 Phylogenetic tree of AMF communities in experiment 1. Neighbor-joining phylogenetic tree based on partial SSU rRNA gene sequences of all identified AMF and referenced sequences in the root samples. Numbers above branches indicate bootstrap values from 1,000 replicates. The internal identification number represents sequences retrieved from specific DGGE profile bands. A total of 21 partial SSU rDNA sequences were thus obtained in the present study. They are shown in bold and labeled with the GeneBank database accession numbers (i.e., KC507871–KC507891). Sequence groups (GA1, GA2, etc.) identify distinct clusters of sequences with similarity ≥ 99%. (TIF)

Methods S1 Methods used for DNA isolation, PGR amplification, DGGE, cloning, and sequencing analysis of AMF. (DOC)

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Author Contributions

Conceived and designed the experiments: XC SH. Performed the experiments: YY JT DL. Analyzed the data: YY JW HY. Contributed reagents/materials/analysis tools: YY JT DL. Wrote the paper: YY XC SH.

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