Greater chemical signaling in root exudates enhances soil mutualistic associations in invasive plants compared to natives

Hongwei Yu[1*], Yingying He[1*], Wen Zhang[1], Li Chen[1], Junli Zhang[1], Xuebin Zhang[1], Wayne Dawson[2], Jianqing Ding[1]

[1] State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475004, China

[2] Department of Biosciences, Durham University, Durham DH1 3LE, UK

Author for correspondence

Jianqing Ding, Email: jding@henu.edu.cn

[*]These authors contributed equally to this work.

ORCID

Hongwei Yu 0000-0001-8075-9209

Junli Zhang 0000-0003-3159-8829

Xuebin Zhang 0000-0002-6089-4339

Wayne Dawson 0000-0003-3402-0774

Jianqing Ding 0000-0002-7399-7388

Summary

- Invasive plants can change soil properties resulting in improved growth. Although invaders are known to alter soil chemistry, it remains unclear if chemicals secreted by roots facilitate invasive plant-soil mutualisms.
- With up to 19 confamilial pairs of invasive and native plants, and most of which were congeners, we explored the root exudate-induced changes in plant-arbuscular mycorrhizal (AM) fungal mutualisms.
- We found that, relative to natives, invaders had greater AM colonization, greater biomass and their root exudates contained higher concentrations of two common chemical signals- quercetin and strigolactones- which are known to stimulate AM fungal growth and root colonization. An exudate exchange experiment showed that root exudates from invaders increased AM colonization more than exudates from natives. However, application of activated carbon led to greater reduction in AM colonization and plant biomass for invaders than natives, suggesting stronger effects of chemical signals in root exudates from invaders.
- We show that non-native plants promote interactions with soil mutualists via enhancing root exudate chemicals, which could have important implications for invasion success.

Key words: arbuscular mycorrhizal fungi, flavonoid, plant-AM fungal association, plant invasion, root exudates, strigolactones.

Introduction

Associations of plants with soil microbes can drive plant population and community dynamics and determine plant invasion success (van der Putten *et al*., 2007; Callaway & Lucero, 2020; Tedersoo *et al*., 2020). Invasive plants may escape from co-evolved soil microbes in their native ranges and form new associations with soil microbes when introduced into new areas (Callaway *et al*., 2004; Callaway & Rout, 2011; Lekberg *et al*., 2013; Pei *et al*., 2020; Waller *et al*., 2020). These altered plant-microbe associations could improve invasive plant performance, for example, by enhancing plant-soil mutualism associations (Reinhart & Callaway, 2006; Sun & He, 2010; Dickie *et al*., 2017). As root exudate chemicals are reported to play a critical role in mediating plant-soil mutualism associations (Haichar *et al*., 2014; Rasmann & Turlings, 2016; Tian *et al*., 2021), studies of root exudate chemicals produced by invasive plants are needed to understand whether they result in enhanced mutualisms, and if this in turn leads to greater plant growth over natives.

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with most terrestrial plants, and play an important role in plant growth and development by facilitating nutrient and water uptake from soil and promoting tolerance to abiotic/biotic stress (Koltai & Kapulnik, 2010; Wipf *et al*., 2019; Frew *et al*., 2020). Previous studies have found that, relative to native species, increased association with AM fungi can promote the performance of many introduced plants (Pringle *et al*., 2009; Yang *et al*., 2015; Zhang *et al*., 2018). Thus, higher AM colonization of invasive plant roots may lead to growth advantages over native plants (Zhang *et al*., 2017; Sielaff *et al*., 2019). In a meta-analysis of plant-AM fungi interactions, Bunn *et al.* (2015) found that the strength of invasive plant-AM fungal mutualisms may depend on species and functional group. Although many studies have examined the difference in plant-AM fungal mutualism between native and invasive species (Reinhart & Callaway, 2006; Awaydul *et al*., 2019), the role that chemicals released by plants play is unclear (Inderjit *et al.*, 2021).

A growing number of studies report that AM colonization could be largely determined by root exudate chemicals (Rasmann & Turlings, 2016; Tian *et al.*, 2021). For example, some secondary metabolites (mostly flavonoids), such as quercetin and quercitrin, have been shown to stimulate spore germination, hyphal growth and enhance root colonization (Scervino *et al*., 2005; Abdel-Lateif *et al*., 2012; Tian *et al*., 2021). Similarly, some plant hormones such as strigolactones, can induce hyphal branching and promote AM colonization (Akiyama *et al*., 2005; Lanfranco *et al*., 2018). Recently Tian *et al*. (2021) found that greater flavonoid concentrations in root exudates of the invasive plant *Triadica sebifera* increased AM colonization in introduced populations more than native ones, suggesting genetic differences in root exudate flavonoids play an important role in enhancing AM fungal associations and invasive plant performance. However, there is no study testing whether such a finding also applies more generally to invasive plant species and whether other root exudate chemicals also contribute to enhanced invasive plant-AM fungal mutualism.

In this study, we performed experiments to examine the effects of root exudates on AM colonization and plant performance using multiple invasive species in China paired with phylogenetically related native species. We also conducted laboratory analyses of root exudate chemicals (quercetin, quercitrin and strigolactones) and employed manipulation experiments to investigate their potential roles in improving AM colonization. We predicted that, relative to phylogenetically related native species, invasive plants produce higher concentrations of root exudate chemical signals that enhance associations with AM fungi. Specifically, we tested whether invaders show higher AM colonization than phylogenetically related natives, and if so, how root exudates affect AM colonization and whether the root exudate impact differs between native and invasive species. Our work provides clear evidence that root exudates of invasive species can enhance AM colonization, and that this effect is linked to enhanced plant performance.

Materials and Methods

Study species

We used multiple pairs of native and invasive species in our experiment, with species in each pair comprising confamilial or congeneric species. Nineteen pairs were used for Experiment 1 and seven pairs were used for Experiments 2, 3, 4 and 5 (Table 1). Species were selected from three families: Asteraceae, Convolvulaceae and Solanaceae. We aimed to include one invader and one native congener per pair. However, due to a lack of native congeners for some invasive species (only one invasive species in the genus), only 13 congeneric pairs were available. The remaining six pairs comprised invasive species and a confamilial native species in a different genus. We selected the species based on the following criteria: (1) invasive species are listed in the Database of Invasive Alien Species in China (Center for Management of Invasive Alien Species Ministry of Agriculture, 2010), (2) invasive species have invaded large areas and diverse habitats in China (Table 1), (3) native species occur commonly in the range invaded by its respective invasive species, (4) both the native and invasive species have similar growth habit and could survive in similar habitats, and (5) all the species can be propagated by seeds. For each species, we collected seeds from one to three populations in fields, and seeds from more than one population were pooled together and used for the following experiments.

Experiment 1: plant performance, AM colonization and root exudate chemicals

Nineteen plant species pairs were used to evaluate the differences in AM colonization and root exudate chemicals between invasive and native species (Table 1). The topsoil (10–15 cm depth) from an abandoned field at Henan University, Kaifeng, China (34°82′N, 114°29′E), where annual native and invasive plants co-occurred sporadically, was collected and thoroughly mixed with equal volume of sand as growth medium. Growth medium composition was as follows: 11.5 ± 0.13 $g \cdot kg^{-1}$ carbon, 0.2 ± 0.01 $g \cdot kg^{-1}$ total nitrogen, 36.3 ± 2.63 $mg \cdot kg^{-1}$ available nitrogen, 3.5 ± 0.21 $mg \cdot kg^{-1}$ available phosphorus, 256.8 ± 2.86 $mg \cdot kg^{-1}$ available potassium, and pH of 8.5 ± 0.01. We first planted seeds in a tray filled with the growth medium. After 1–3 wk, we transplanted native and invasive seedlings of similar size within each

129 species pair into individual 2 l pots filled with the above growth medium on the same
130 day. Each treatment comprised 15 replicates, resulting in 570 pots. All plants were
131 grown alone and placed in a glass shade house at Henan University in Kaifeng, Henan
132 Province, China. The location is in a temperate monsoon climate region characterized
133 by hot and rainy summers, cold and dry winters, receiving a mean annual
134 precipitation of 650 mm and mean annual temperature of 14 ℃
135 (http://ha.cma.gov.cn/kaifeng/). The glass shade house was open on four sides to
136 permit air flow and maintained light and temperature at ambient levels. Pots were
137 positioned randomly in three 3 m × 4 m × 2 m nylon cages to exclude herbivores and
138 were re-randomized on a weekly basis within the cages. All plants were watered daily
139 during the experiment.
140 We harvested the plants at 30, 60 and 90 d after transplanting seedlings. Before
141 harvest, we collected root exudates and fine root fragments from each pot and species.
142 Root exudates were sampled using the soil-hydroponic-hybrid approach (Oburger &
143 Jones, 2018). Briefly, plants were first washed with deionized water to remove
144 rhizosphere soil and then placed in a beaker containing 500 ml deionized water. The
145 beaker was wrapped in foil to prevent light entry, while the top of the beaker was
146 covered with a foam board, which had a hole in the center to let the plant root dip into
147 the deionized water. We used an air pump to add air to the solutions, ensuring that the
148 roots received adequate oxygen. After 3 d, the deionized water containing exudates
149 was collected and filtered through filter paper (50 μm), then stored at -20 ℃ for
150 further analyses. To determine AM colonization, fine root segments of 1 cm length
151 from each plant were collected and then stained with Trypan blue. Ten segments per
152 sample were mounted on a microscope slide and examined under a fluorescence
153 stereomicroscope at 200× magnification (Liang *et al.*, 2015). The AM colonization
154 rate was calculated using the gridline intersect method for 100 intersections per
155 sample, where arbuscules, vesicles and intraradical hyphae within roots were recorded
156 as colonized. The harvested plants were dried at 65 ℃ for 48 h and weighed to
157 determine total plant biomass. As some plants died and some roots were damaged
158 during the bleaching processes, biomass for 531 plants and AM colonization rate for

497 plants were obtained finally.

The exudates were dried at 45 ℃ under a vacuum with rotary evaporators, dissolved with 2 ml methanol solution and filtered through 0.22 μm hydrophobic membranes. The flavonoids (quercetin and quercitrin), which have been reported to stimulate AM fungal growth (Tsai & Phillips, 1991; Scervino *et al*., 2005; Abdel-Lateif *et al*., 2012), were quantified using high-performance liquid chromatography (HPLC) methods as described by Wang *et al.* (2012). Briefly, the filtered solutions (20 μl per sample) were injected into a 1260 Infinity II HPLC system (Agilent, CA, USA) and compounds were separated by a ZORBAX SB-C18 column (4.6 × 250 mm, 5 μm; Agilent Technologies). The mobile phase flow rate was set as 1.0 ml min^{-1} with a 0.4% phosphoric acid:100% methanol in water gradient as follows: 0-10 min, 52:48; 10-24 min, 48:52. Ultraviolet (UV) absorbance spectra were recorded at 210 nm. Flavonoid standards were obtained from Macklin Biochemical Company, Shanghai, China. Flavonoid compound concentrations were calculated and standardized using peak areas of standards with known concentrations.

We attempted to quantify root exudate concentrations of strigolactones using standard separation and ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods (Supporting Information: Methods S1), but this yielded no detectable strigolactones. Instead, we conducted further analysis for strigolactones from roots of invasive and native species using the synthetic strigolactone analogue GR24 as standard (Table S1). Briefly, 1 gram of ground fresh root tissues from each species was extracted in the dark with 6 ml of ethyl acetate at 4 ℃ for 24 h. After filtration, samples were evaporated with a Termovap sample concentrator, and dissolved with 1 ml methanol solution, and filtered through an 0.22 μm organic membrane. Instrument parameters and analyzing procedures were the same as for root exudates.

Experiment 2: effects of AM fungi inoculation on plant performance

To assess the dependence of the growth of native and invasive plants on AM fungi, we conducted an AM fungi inoculation experiment using seven of the 19 pairs of invasive

and native plants, based on the representativeness and plant material availability in the glasshouse (Methods S2; Fig. S1; Table 1). The experiment involved two factors: species origin (invasive or native) and AM fungi inoculation (without or with AM fungi inoculation). Each treatment was replicated five times, resulting in 140 pots.

In this experiment, we first sterilized the growth medium (the same as used in the Experiment 1) with 25 kGy gamma-irradiation. For the inoculation treatment, we extracted two microbial fractions (1) AM fungi communities and (2) pathogen/saprobe communities from soil using a wet-sieving method (Klironomos, 2002; Liang *et al*., 2015). The topsoil (10–15 cm depth) from a corn field was collected and divided into 100 g aliquots, which were then passed through a 250 μm sieve into 100 ml suspension before inoculation. Arbuscular mycorrhizal fungal spores were sieved out using 45 μm mesh and added to half of the pots as a 100 ml suspension (AM fungi inoculation), and the other half of the pots received an equal volume of deionized water as control. Pathogen/saprobe communities from the filtrates that passed through a 20 μm sieve were added to all the pots as a 100 ml suspension. Prior to planting, seeds were surfaced-sterilized with 2% NaClO for 2 min and germinated in sterilized growth medium as in Experiment 1. Similar-sized seedlings within each species pair were then transplanted into individual pots and watered daily. All other procedures followed Experiment 1. At the end of the experiment, 139 plants (excluding one dead *Bidens biternata* plant) were harvested, and total biomass and AM colonization were measured.

Experiment 3: effects of root exudates on AM colonization

To assess the effects of root exudates on plant-AM fungal colonization, we conducted a root exudate addition experiment in which exudates from each species were collected in deionized water and added to the soils of the same species or their counterpart within a species pair. For this experiment, we used the same seven pairs of invasive and native plants in Experiment 2 (Table 1). Each species received deionized water, root exudates from the same species, or root exudates from their invasive / native counterpart in the same pair, creating six types of combination: (1) native

217 species received deionized water, (2) invasive species received deionized water, (3)
218 native species received root exudates from the same native species, (4) native species
219 received root exudates from their invasive counterpart species, (5) invasive species
220 received root exudates from their native counterpart species, (6) invasive species
221 received root exudates from the same invasive species. Each treatment was replicated
222 five times, resulting in 210 pots.
223 All experimental plants were grown for 15 d, and then supplemented with root
224 exudate treatments. Ten exudate donor plants of each species were first cultured in a
225 beaker with 500 ml full-strength Hoagland nutrient solution. Four days later, the
226 Hoagland nutrient solution was changed to deionized water for collection of root
227 exudates. To provide oxygen to the roots, we used air pump to pump air into the
228 solutions. After 3 d, the water samples containing root exudates were collected and
229 transferred to receiver plants. Each receiver plant received exudates from an
230 individual donor plant. Control plants received the same volume of water. The
231 procedures were repeated by supplementing donor plants with Hoagland nutrient
232 solution for growth and changing to deionized water for collection of root exudates.
233 As some plants died, this procedure was repeated for a total of 45 d, resulting in 6
234 exudates transfers per plant. The growth medium and other growth procedures
235 followed Experiment 1. At the end of the experiment, 177 plants (excluding a few
236 dead plants) were harvested and AM colonization of individual plants was determined
237 as in Experiment 1.

238 **Experiment 4: effects of activated carbon on AM colonization**
239 To further confirm the role of root exudate chemicals in mediating interactions
240 between plants and AM fungi, we conducted an activated carbon (AC) addition
241 experiment using the same seven plant pairs as Experiment 2 (Table 1) to test the
242 effect of root exudates after chemicals are removed by AC. Activated carbon is known
243 to adsorb chemicals in soil released by plants and is used in ecological studies to test
244 for invasive plant allelopathy and competition (Inderjit & Callaway, 2003). This
245 experiment included two AC treatments: no AC addition (control) and AC addition.

All plants were grown individually in 2 l pots filled with the same growth medium as in Experiment 1. For the AC addition treatment, we added AC at 20 ml l^{-1} to the substrate in each pot. Yuan *et al*. (2014) had demonstrated that 20 ml l^{-1} AC addition does not alter soil nutrient properties, but significantly reduced secondary compound concentrations (i.e. flavones, phenolics and saponins) in soil. However, previous studies suggested that AC addition may yield potential unwanted side effects on nutrient availability and other soil properties (Lau *et al*., 2008; Weisshuhn & Prati, 2009). Thus we supplied 100 ml Hoagland's nutrient solution weekly to the substrate to minimize the potential side effects of AC on soil nutrient availability (Ning *et al.*, 2016). Each treatment was replicated five times, resulting in 140 pots. The experiment lasted for 60 d. At the end of the experiment, 124 plants (i.e. 16 plants died) were harvested, and AM colonization and total biomass were measured as described in Experiment 1.

Experiment 5: strigolactones in root exudates and plant-AM fungal associations

Because we failed to detect strigolactones in root exudates in Experiment 1, to obtain further evidence for the potential role of strigolactones from root exudates in plant-AM fungi interactions, we conducted an additional experiment using the same seven pairs of invasive and native plants in Experiment 2 (Table 1). While we focused on the concentration of strigolactones in root exudates, we also examined whether plant-AM fungal associations and strigolactones varied with growth period. The plants were cultivated in the same sized pots and placed in the same glass shade house as Experiment 1. The other growth procedures were the same as Experiment 1 and all plants were grown for 30, 60 and 90 d with five replicates for each treatment. Before harvest, root samples and root exudates were collected as in Experiment 1, and AM colonization was determined as in Experiment 1.

Root exudates were separated by a solid phase C18 column, eluted with 3 ml ethyl formate and 3 ml acetonitrile, concentrated using a Termovap sample concentrator, and filtered through 0.22 μm organic membranes. Strigolactones in root exudates were quantified by UPLC-MS/MS, using a natural strigolactone strigol as standard

and followed a modified procedure based on Experiment 1. Samples were analyzed using an Xevo TQ-XS system (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) ion source. The data were collected under the positive ion mode. Chromatographic separation was conducted by a Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm). The mobile phase, composed of solvent A (0.1% formic acid, water) and solvent B (methanol), flow rate was set as 0.3 ml min^{-1}. The linear gradient system was set as following: 0-2 min, 2% B; 2-10 min, to 80% B; 10-12 min, 80% B; 12-13 min, to 2% B; 13-15 min, 2% B. The autosampler temperature was set to 4 °C and sample injection volume was 10 μl.

Data analyses

As some plants died during the course of experiments and some roots were damaged during the bleaching process, we ran a non-parametric Pearson chi-square test to assess whether the death of plants and loss of AM colonization were distributed equally among the treatments. The results showed the death and loss were distributed evenly across treatments, suggesting they did not skew the statistical analyses (Methods S3; Tables S2, S3).

For data from Experiment 1, we first fitted linear mixed effects models to examine whether growth and AM colonization differed between native and invasive plants. In our models, species origin (native or invasive), growth time (30, 60 or 90 d) and their interactions were set as fixed factors, with taxonomic pairs and species identity treated as random factors. We only included 18 species pairs in the analysis of the third growth period as all *Senecio vulgaris* plants died. Total biomass was natural log transformed and AM colonization was square root transformed to improve normality. The relationship between total biomass and AM colonization was also assessed using linear mixed effects model, with taxonomic pairs and species identity treated as random factors. Total biomass was square root transformed at this time to improve normality. We then ran *t*-tests to determine the differences in flavonoid compounds between native and invasive plants at each growth period. Average values for quercetin and quercitrin in native and invasive plants are presented in Fig. S2. For

GR24-referenced strigolactones in roots, we ran *t*-tests to examine the difference in average (at species level) values using the available 13 pairs of data from native and invasive plants. Spearman correlation tests were also performed to explore the relationships of AM colonization and root exudate chemicals (i.e. quercetin and GR24-referenced strigolactones) using means per species.

For the data of Experiment 2, linear mixed effects models were used to test the effects of species origin, AM fungi inoculation, and their interactions on total biomass and AM colonization, with taxonomic pairs and species identity treated as random terms. Arbuscular mycorrhizal colonization was square root transformed to improve normality.

To assess the effects of root exudate addition on AM colonization between native and invasive plants in Experiment 3, we performed linear mixed effects modeling to test the effects of species origin, root exudates (deionized water, root exudates from native or invasive plants), and their interactions on AM colonization, with taxonomic pairs and species identity treated as random terms. Arbuscular mycorrhizal colonization was square root transformed to improve normality.

For the data of Experiment 4, linear mixed effects models were used to test the effects of species origin, AC treatment, and their interactions on total biomass and AM colonization, with taxonomic pairs and species identity treated as random terms. Total biomass and AM colonization were square root transformed to improve normality. To quantify the effects of AC on plant-AM fungi interaction, we first calculated the change in total biomass and AM colonization and then used a linear mixed effects model to determine the relationship between the change in total biomass and the change in AM colonization, with taxonomic pairs and species identity treated as random factors.

For Experiment 5, linear mixed effects models were used to determine whether AM colonization and strigolactones (i.e. strigol) content in root exudates differed between seven pairs of native and invasive plants. Fixed and random terms were the same as Experiment 1. Arbuscular mycorrhizal colonization was square root transformed and strigolactones content in root exudates was natural log transformed to improve

normality. The relationship between AM colonization and strigolactones (i.e. strigol) content in root exudates was also assessed using linear mixed effects model, with taxonomic pairs and species identity treated as random factors.

All statistical analyses were conducted using R v.3.5.2 (R Core Team, 2018). Linear mixed effects models were implemented using the lmer function in the lme4 package (Bates *et al.*, 2015) and the significance of fixed components was assessed by Wald chi-square tests using the Anova function in the car package (Fox & Weisberg, 2011). The goodness-of-fit of models was assessed by the marginal coefficient of determination (R-squared) using the r.squaredGLMM function in the MuMIn package (Bartoń, 2018). Pair-wise *post-hoc* tests with Benjamini-Hochberg correction for *p* values were made using the predictmeans function in the predictmeans package (Luo *et al.*, 2018). Correlation tests were performed using the corr.test function in the psych package (Revelle, 2018).

Results

Experiment 1: total biomass, AM colonization and root exudate chemicals

In this experiment we aimed to evaluate differences in plant-AM fungal mutualisms and root exudate chemicals between native and invasive plants. On average, invasive species had greater total biomass and AM colonization than native species, and these differences strongly depended on growth period (Fig. 1; Table S4). Specifically, invasive plants grew larger than native plants at 30 d (Fig. 1a, $P = 0.076$); this difference increased at 60 and 90 d (Fig. 1a, all $P < 0.010$). Invasive plants also had greater AM colonization at 30 and 60 d than native plants (Fig. 1b, all $P < 0.010$), but this difference decreased at 90 d (Fig. 1b, $P = 0.065$). Moreover, the linear mixed effects model indicated that species that had higher AM colonization tended to achieve greater biomass ($\chi^2 = 34.48$, $P < 0.001$).

Root exudate chemical composition varied substantially among species and over time (Table S1). Due to the difficulty in detecting each flavonoid in root exudates and GR24-referenced strigolactones in fresh roots for each plant, we used average values

362 at the species level and only included paired data in the final analysis. Quercitrin was
363 only found in a few samples, thus we did not include it in the analysis. The
364 concentrations of quercetin in root exudates of invasive plants were significantly
365 greater at 30 d ($P = 0.005$) and marginally greater at 60 d ($P = 0.055$) than those in
366 their native counterparts (Fig. 1c), but there was no difference at 90 d (Fig. 1c, $P =$
367 0.444). The GR24-referenced strigolactones concentration in roots was marginally
368 greater in invasive plants than in native plants after 60 d of growth (Fig. 1d, $P =$
369 0.058). The correlation test indicated that species that secreted a greater concentration
370 of quercetin tended to have higher AM colonization ($r = 0.328$, $P = 0.009$), while no
371 significant relationship was observed between the concentration of GR24-referenced
372 strigolactones in fresh roots and AM colonization ($r = 0.176$, $P = 0.390$).

373 **Experiment 2: effects of AM fungi inoculation on AM colonization and plant**
374 **performance**

375 In this experiment we inoculated AM fungi into soils to examine the growth
376 dependence of native and invasive plants on AM fungi. Species origin and AM fungi
377 inoculation significantly affected AM colonization and total biomass (Table S5).
378 Regardless of species origin, AM colonization in roots inoculated with AM fungi was
379 greater than in the control treatment (Fig. 2a; Table S5), indicating effective AM fungi
380 inoculation in our experiment. Arbuscular mycorrhizal fungi inoculation significantly
381 increased the total biomass of both native and invasive species (Table S5, AM fungi
382 inoculation effects: $\chi^2 = 60.78$, $P < 0.001$), and also amplified the biomass difference
383 between native and invasive species from 0.28 g to 0.51 g, implying that invaders
384 benefited more than natives (Fig. 2b).

385 **Experiment 3: effects of adding root exudates on AM colonization**

386 The exudate complementation experiment aimed to examine whether the observed
387 differences in AM colonization between native and invasive plants in Experiment 1
388 can be explained by differences in root exudate composition. Overall, invasive species
389 had higher AM colonization than native species (Fig. 3; Table S6). Plants receiving
390 invasive root exudates had the highest AM colonization (mean ± standard error (SE):

391 19.31 ± 1.01%), which was greater than for plants receiving native root exudates
392 (mean ± SE: 15.25 ± 0.75%) or deionized water (mean ± SE: 16.31 ± 0.74%) (Fig. 3;
393 Table S6).

394 **Experiment 4: effects of adding AC on total biomass and AM colonization**
395 To further assess root exudates as mediators of the plant-AM fungal mutualism, we
396 added AC to adsorb and potentially ameliorate exudates. Invasive plants had more
397 biomass and higher AM colonization than native plants (Fig. 4a,b; Table S7).
398 Regardless of species origin, AC addition significantly decreased the total biomass
399 and AM colonization of both native and invasive plants (Fig. 4a,b; Table S7). Also,
400 the decreases of total biomass and AM colonization were greater for invasive plants
401 than for native plants (Fig. 4a,b; Table S7). Moreover, the change in total biomass was
402 positively and marginally correlated with change in AM colonization, regardless of
403 species origin (Fig. 4c: $\chi^2 = 3.715$, $P = 0.054$).

404 **Experiment 5: root-AM fungal associations and strigolactones in root exudate**
405 Using a natural strigolactone strigol as standard, this supplementary experiment aimed
406 to test potential role of strigolactones from root exudates in plant-AM fungi
407 interactions. The differences in AM colonization between seven pairs of native and
408 invasive plants grown for 30, 60 and 90 d were similar to Experiment 1 (Fig. 5a;
409 Table S8). At 30 d, the concentration of strigol in root exudates of invasive plants was
410 greater on average than for their native counterparts ($P = 0.040$), but there were no
411 difference at 60 and 90 d (Fig. 5b). Overall, the result from the linear mixed effects
412 model indicated that the AM colonization marginally increased with the increasing
413 concentration of strigol in root exudates ($\chi^2 = 2.904$, $P = 0.088$).

414 ## Discussion

415 We showed that invasive plants had higher AM colonization than phylogenetically
416 related native species and variation in root exudate chemicals might account for some
417 of these differences. We found invasive plants had higher concentrations of quercetin

418 and strigolactones in root exudates than their native counterparts. Furthermore, our
419 exudate exchange and exudate removal experiments indicate that quantitative and
420 qualitative changes in exudate chemicals might alter these plant-AM fungal
421 mutualisms. Together, our findings suggest that differences in root exudate chemicals
422 may result in different AM fungal associations for native and invasive plants, and that
423 root exudates are a mechanism behind enhanced plant-AM fungal mutualisms in
424 invasive plants (Fig. 6).

425 **Root exudate-mediated interactions of AM fungi and invasive and native plants**
426 Root exudates have been shown to mediate plant-soil microbe interactions (Haichar *et*
427 *al*., 2014; Rasmann & Turlings, 2016; Zhalnina *et al*., 2018), and changes in
428 quantities and composition of root exudates may alter AM fungal associations (Badri
429 & Vivanco, 2009; Hu *et al*., 2018), and therefore plant fitness. By inoculating plants
430 with AM fungi, we found that AM fungi benefit both native and invasive species in a
431 similar way, but this positive effect was stronger in invasive plants than native plants
432 (i.e. invaders overall have larger biomass with AM fungi). In our root exudate
433 addition experiment, plants that received root exudates from invasive plants, rather
434 than those from natives, showed a significant increase in AM colonization, suggesting
435 that root exudates from invasive plants had stronger effects on AM fungi. These
436 results supported the hypothesis that differences in root exudates secreted by native
437 and invasive species accounted for the differing plant-AM fungal mutualisms for
438 invasive compared to native plants.
439 Our activated carbon addition experiment further suggested the role of root
440 exudates in mediating interactions of AM fungi and native and invasive plants. We
441 found both total biomass and AM colonization in native and invasive plants were
442 reduced when adding AC to soils and these effects were greater for invasive plants
443 than for native plants. This suggests that invasive plant-AM fungal mutualisms are
444 more sensitive to root exudate chemical changes than native ones. Activated carbon is
445 known to adsorb chemicals, which is why it has been used in many previous studies
446 focusing on the allelopathic effects of invasive plants (Callaway & Aschehoug, 2000;

Abhilasha *et al*., 2008). Some studies have suggested the effects of activated carbon on plant growth can be explained by adsorption of nutrients or chemical signaling compounds that interfere the communication between plants and microbes (Lau *et al*., 2008; Wurst *et al*., 2010). The amounts of root exudate chemicals adsorbed by AC were not measured in this study, however, we added fertilizer to alleviate changes in nutrient availability, thus the negative effect of activated carbon on plant-AM fungal mutualisms in this study is likely due to chemical signaling in root exudates being adsorbed and thereby reducing AM colonization. With the AC addition experiment, our study further suggests that differences in root exudate chemicals between invasive and native plants may lead to different AM colonization rates.

Root exudate chemicals and their stimulating effects on AM fungi of invasive and native plants

Of the factors affecting AM colonization, nutrients and chemical signals both play major roles in enhancing or stimulating AM fungal spore germination, hyphal growth and branching (Steinkellner *et al*., 2007; Koltai & Kapulnik, 2010; Nagahashi & Douds, 2011). Our chemical analysis results show that invaders' root exudates promote AM colonization and that this could be due to the higher concentrations of quercetin and strigolactones, as evidenced by the significant correlations between AM colonization and concentrations of these exudates.

Flavonoids, such as quercetin, are well known to be able to stimulate AM fungal spore germination, hyphal growth and branching (Tsai & Phillips, 1991; Scervino *et al*., 2007; Steinkellner *et al*., 2007). In this study, we found higher concentrations of quercetin in invasive plant root exudates than in their native counterparts at the early and mid-stages of plant growth (30 and 60 d), which is in accordance with the higher AM colonization in invasive plants than native plants, suggesting increasing quercetin could increase AM fungal growth during the growth period of invasive plants. Once the plant-AM fungal association is established, the greater quercetin exudation by invasive plants might stimulate AM fungal formation at early stages of establishment and growth, giving them a head-start over natives. We also found that the quercetin

concentration decreased by the end of the growing season while there was no difference between invasive and native plants, suggesting that the chemical-mediated plant-AM fungi interactions were time-dependent. As common secondary metabolite chemicals, the concentration of quercetin and other flavonoids may vary with plant species and environmental conditions (Mierziak *et al.*, 2014; Mouradov & Spangenberg, 2014). In the present study, the amount of root exudate flavonoids and number of species in which flavonoids were detected at each growth period varied with species origin (Fig. S2), which may lead to different AM fungal relationships for native and invasive plants. Future work needs to identify factors triggering high quercetin concentration in invasive plant root exudates.

With a similar function to quercetin, strigolactones act as chemical signals that induce hyphal branching, mitochondrial metabolism, transcriptional reprogramming and production of chitin oligosaccharides, which in turn facilitate plant-AM fungal mutualism (Steinkellner *et al*., 2007; Lanfranco *et al*., 2018). These plant hormones are carotenoid-derived molecules that enable AM fungi to detect host plants. In this study, we found strigolactone concentration in both roots and root exudates at early growth stages were higher in invasive plants than in their native counterparts, suggesting strigolactones may stimulate AM fungal association in establishing invasive plants more than native ones. Since strigolactones are relatively newly discovered plant chemical signals (Akiyama *et al*., 2005; Koltai & Kapulnik, 2010), we still need further work to unravel their roles in mediating invasive plant-soil microbe interactions.

Chemicals in root exudates and their functions may vary, depending on plant species, plant growth stage and environment (Bais *et al*., 2006; De-la-Pena *et al*., 2010; Chaparro *et al*., 2013; Canarini *et al*., 2019). We found the differences between invasive and native plants were consistent for quercetin and strigolactones, two key signals that mediate plant-AM fungal interactions. However, we could not rule out that other chemicals may also be able to enhance AM fungal growth and association with host plants. Moreover, many environmental factors such as temperature, nutrient availability, soil water and other microbes, and even plant neighbors, may also affect

root exudation and chemicals as well as AM fungal growth (Badri & Vivanco, 2009; Yoneyama *et al*., 2012; Kong *et al*., 2018; de Vries *et al*., 2019; Inderjit *et al.*, 2021). Furthermore, for plant invasion, selection may favor species that have high root exudation in non-native ranges (Callaway & Ridenour, 2004), and escape from natural enemies (herbivores and pathogens) may alter plant chemical signals that are associated with root chemical exudates (Tian *et al.*, 2021). Therefore, field surveys and laboratory experiments on AM fungi and root chemical exudates with different environments may further reveal the driving factors that affect root exudation and invasive plant-AM fungal mutualisms.

Unlike biogeographical studies comparing shifts in AM colonization within species (Yang *et al.*, 2015; Waller *et al.*, 2016; Filep *et al.*, 2021; Tian *et al.*, 2021), we focused on the plant-AM fungal mutualisms in native and invasive species in the introduced range. Our results on plant-AM fungal association were different from those of Bunn *et al.* (2015) who found that invasive forbs were more colonized than native grasses but not native forbs. This might be largely due to the fact that multiple functional groups (i.e. forb, grass, shrub and tree) were included in the meta-analysis by Bunn *et al.* (2015), but they did not control phylogenetic relatedness in their analysis. In our study, while we did not consider groups other than forbs, multiple pairs of closely related native and invasive plants were used, which minimized trait differences associated with comparing unrelated and functionally different species (Funk *et al.*, 2015). Thus, future work that includes other functional groups such as grasses and trees would allow more general inference regarding the differences in exudates and plant-microbe interactions between native and invasive plants.

While enhanced mycorrhizal colonization resulting from root exudate chemicals might be partially responsible for the superior growth of invasive plants, it is important to consider that other mechanisms, such as difference in plant traits (van Kleunen *et al.*, 2010), fast growth rate (Dawson *et al.*, 2011), high seed production (Mason *et al.*, 2008), and escape from herbivores and pathogens (Keane & Crawley, 2002; Mitchell & Power, 2003), could explain invasive species performance. Additionally, it should be noted that the effect of AM symbiosis on plants can range

from mutualism to parasitism, and is greatly influenced by environmental conditions, plant genotype, and their interactions (Johnson *et al.*, 1997; Chen *et al.*, 2020; Berger & Gutjahr, 2021). Notwithstanding these points, the differences in root exudate chemicals detected between invasive and native plants correspond to differences in AM colonization and plant performance at an early growth stage, suggesting that invaders may have the advantage over natives when colonizing and establishing in a new location.

Using multiple native and invasive species, we provided evidence that root exudates of invasive plants enhance AM colonization, likely through increased levels of signaling compounds, and this may have consequences for plant invasion success. Many previous studies on invasive plant root chemicals focused on allelopathic effects (Abhilasha *et al.*, 2008; Thorpe *et al*., 2009; Jandová *et al*., 2015), however, our study demonstrates that greater concentrations of root exudate chemicals in invasive plants could enhance plant-AM fungal mutualisms, pointing towards a wider role of root exudates in plant invasions. These findings have profound implications for understanding plant-AM fungal communication, and future work should explore which and how exudate compounds specifically lead to enhanced AM fungal association and subsequent growth advantages in invasive plants.

Acknowledgements

We are grateful to Xuefang Yang, Baoliang Tian, Yingchun Pei, Lifeng Zhou, Yali Xiao, Pinpin Ma and Jiahua Lu for their help in the course of our experiments. We thank Matthias Erb for the valuable comments and suggestions on this article. Sincere thanks to Editor Ian Dickie and the three anonymous reviewers for their valuable and constructive comments that improve the manuscript. The study was funded by the National Natural Science Foundation of China (32001233, U21A20190), the Major Public Welfare Projects in Henan Province (201300311300), the China Postdoctoral Science Foundation (2019M662526) and the Henan Province Postdoctoral Science

Foundation.

Author contributions

JD designed the study. YH, WZ, LC, HY and JZ conducted experiments. HY and YH performed data analyses. HY, YH, WZ, XZ, WD and JD drafted the manuscript. All authors contributed substantially to revisions and approved the final version of the manuscript. HY and YH contributed equally to this work.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abdel-Lateif K, Bogusz D, Hocher V. 2012. The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and *Frankia* bacteria. *Plant Signaling & Behavior* **7**: 636–641.

Abhilasha D, Quintana N, Vivanco J, Joshi J. 2008. Do allelopathic compounds in invasive *Solidago canadensis* s.l. restrain the native European flora? *Journal of Ecology* **96**: 993–1001.

Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824–827.

Awaydul A, Zhu W, Yuan Y, Xiao J, Hu H, Chen X, Koide RT, Cheng L. 2019. Common mycorrhizal networks influence the distribution of mineral nutrients between an invasive plant, *Solidago canadensis*, and a native plant, *Kummerowa striata*. *Mycorrhiza* **29**: 29–38.

Badri DV, Vivanco JM. 2009. Regulation and function of root exudates. *Plant, Cell and Environment* **32**: 666–681.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere interations with plants and other organisms. *Annual Review of Plant Biology* **57**: 233–266.

Bartoń K. 2018. *MuMIn: Multi-Model Inference*. R package version 1.42.1. URL https://CRAN.R-project.org/package=MuMIn.

Bates D, Mäechler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Berger F, Gutjahr C. 2021. Factors affecting plant responsiveness to arbuscular mycorrhiza. *Current Opinion in Plant Biology* **59**: 101994.

Bunn RA, Ramsey PW, Lekberg Y. 2015. Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. *Journal of Ecology* **103**: 1547–1556.

Callaway RM, Aschehoug ET. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**: 521–523.

Callaway RM, Lucero JE. 2020. Soil biota and non-native plant invasions. In: Traveset A, Richardson DM, eds. *Plant invasions: the role of biotic interactions*. Wallingford, Oxford, UK: CABI, 45–66.

Callaway RM, Thelen GC, Rodriguez A, Holben WE. 2004. Soil biota and exotic plant invasion. *Nature* **427**: 731–733.

Callaway RM, Ridenour WM. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* **2**: 436–443.

Callaway RM, Rout ME. 2011. Soil biota and plant invasion: biogeographical effects on plant-microbe interactions. In: Richardson DM, eds. *Fifty years of invasion ecology–The legacy of Charles Elton*. Oxford, UK: Wiley-Blackwell, 131–142.

Canarini A, Kaiser C, Merchant A, Richter A, Wanek W. 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science* **10**: 157.

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM. 2013. Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are

developmentally programmed and correlate with soil microbial functions. *PLoS One* **8**: e55731.

Center for Management of Invasive Alien Species, Ministry of Agriculture, China (CMIAS). 2010. *Database of Invasive Alien Species in China*. URL http://www.chinaias.cn/wjPart/index.aspx.

Chen E, Liao H, Chen B, Peng S. 2020. Arbuscular mycorrhizal fungi are a double-edged sword in plant invasion controlled by phosphorus concentration. *New Phytologist* **226**: 295–300.

Dawson W, Fischer M, van Kleunen M. 2011. The maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. *Global Ecology and Biogeography* **20**: 299–306.

De-la-Pena C, Badri DV, Lei Z, Watson BS, Brandao MM, Silva-Filho MC, Summer LW, Vivanco JM. 2010. Root secretion of defense-related proteins is development-dependent and correlated with flowering time. *Journal of Biological Chemistry* **285**: 30654–30665.

Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau ML, Grelet G, Hulme PE, Klironomos J, Makiola A, Nuñez MA, Pringle A *et al.* 2017. The emerging science of linked plant-fungal invasions. *New Phytologist* **215**: 1314–1332.

Editorial Board of Flora of China (EBFC). 1985. *Flora of China*. Beijing, China: Science Press.

Fox J, Weisberg S. 2011. *An R companion to applied regression*, *2nd edn*. California: SAGE Publications Inc..

Filep R, Lengyel A, Cook BJ, Farkas Á, Nagy K, Nagy DU, Imri Á, Czakó-Vér K, Pal RW. 2021. *Helianthus tuberosus* at home and away: stronger ecological impacts in invaded than in native range are not explained by arbuscular mycorrhizal colonization. *Preslia* **93**: 363–376.

Frew A, Powell JR, Johnson SN. 2020. Aboveground resource allocation in response to root herbivory as affected by the arbuscular mycorrhizal symbiosis. *Plant and Soil* **447**: 463–473.

Funk JL, Rakovski CS, Macpherson JM. 2015. On the analysis of phylogenetically

651 paired designs. *Ecology and Evolution* **5**: 940–947.
652 **Haichar FZ, Santaella C, Heulin T, Achoua W. 2014.** Root exudates mediated
653 interactions belowground. *Soil Biology and Biochemistry* **77**: 69–80.
654 **Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger**
655 **T, van der Heijden MGA *et al.* 2018.** Root exudate metabolites drive plant-soil
656 feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature*
657 *Communications* **9**: 2738.
658 **Inderjit, Callaway RM. 2003.** Experimental designs for the study of allelopathy. *Plant*
659 *and Soil* **256**: 1–11.
660 **Inderjit, Simberloff D, Kaur H, Kalisz S, Bezemer TM. 2021.** Novel chemicals
661 engender myriad invasion mechanisms. *New Phytologist* **232**: 1184–1200.
662 **Jandová K, Dostál P, Cajthaml T, Kameník Z. 2015.** Intraspecific variability in
663 allelopathy of *Heracleum mantegazzianum* is linked to the metabolic profile of root
664 exudates. *Annals of Botany* **115**: 821–831.
665 **Johnson NC, Graham JH, Smith FA. 1997.** Functioning of mycorrhizal associations
666 along the mutualism-parasitism continuum. *New Phytologist* **135**: 575–586.
667 **Keane RM, Crawley MJ. 2002.** Exotic plant invasions and the enemy release
668 hypothesis. *Trends in Ecology & Evolution* **17**: 164–170.
669 **van Kleunen M, Weber E, Fischer M. 2010.** A meta-analysis of trait differences
670 between invasive and non-invasive plant species. *Ecology Letters* **13**: 235–245.
671 **Klironomos JN. 2002.** Feedback with soil biota contributes to plant rarity and
672 invasiveness in communities. *Nature* **417**: 67–70.
673 **Koltai H, Kapulnik Y. 2010.** *Arbuscular mycorrhizas: physiology and function*, *2nd*
674 *edn*. London: Springer.
675 **Kong CH, Zhang SZ, Li YH, Xia ZC, Yang XF, Meiners SJ, Wang P. 2018.** Plant
676 neighbor detection and allelochemical response are driven by root-secreted signaling
677 chemicals. *Nature Communications* **9**: 3867.
678 **Lanfranco L, Fiorilli V, Venice F, Bonfante P. 2018.** Strigolactones cross the
679 kingdoms: plants, fungi, and bacteria in the arbuscular mycorrhizal symbiosis.
680 *Journal of Experimental Botany* **69**: 2175–2188.

Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist* **178**: 412–423.

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW. 2013. Severe plant invasion can increase mycorrhizal fungal abundance and diversity. *ISME Journal* **7**: 1424–1433.

Liang MX, Liu XB, Etienne RS, Huang FM, Wang YF, Yu SX. **2015.** Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology* **96**: 562–574.

Luo D, Ganesh S, Koolaard J. 2018. *predictmeans: calculate predicted means for linear models*. R package version 1.0.4. URL https://cran.r-project.org/package=predictmeans.

Mierziak J, Kostyn K, Kulma A. 2014. Flavonoids as important molecules of plant interactions with the environment. *Molecules* **19**: 16240–16265.

Mitchell CE, Power AG. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* **421**: 625–627.

Mason RAB, Cooke J, Moles AT, Leishman MR. 2008. Reproductive output of invasive versus native plants. *Global Ecology and Biogeography* **17**: 663–640.

Mouradov A, Spangenberg G. 2014. Flavonoids: a metabolic network mediating plants adaptation to their real estate. *Frontiers in Plant Science* **5**: 620.

Nagahashi G, Douds DD. 2011. The effects of hydroxy fatty acids on the hyphal branching of germinated spores of AM fungi. *Fungal Biology* **115**: 351–358.

Ning L, Yu F, van Kleunen M. 2016. Allelopathy of a native grassland community as a potential mechanism of resistance against invasion by introduced plants. *Biological Invasions* **18**: 3481–3493.

Oburger E, Jones DL. 2018. Sampling root exudates - mission impossible? *Rhizosphere* **6**: 116–133.

Pei Y, Siemann E, Tian B, Ding J. 2020. Root flavonoids are related to enhanced AMF colonization of an invasive tree. *AoB Plants* **12**: plaa002.

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN. 2009. Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology, Evolution,*

711 *and Systematics* **40**: 699–715.
712 **van der Putten WH, Klironomos JN, Wardle DA. 2007.** Microbial ecology of
713 biological invasions. *ISME Journal* **1**: 28–37.
714 **R Core Team. 2018.** *R: a language and environment for statistical computing, v.3.5.2.*
715 Vienna, Austria: R Foundation for Statistical Computing. URL http://www.r-
716 project.org.
717 **Rasmann S, Turlings TCJ. 2016.** Root signals that mediate mutualistic interactions in
718 the rhizosphere. *Current Opinion in Plant Biology* **32**: 62–68.
719 **Reinhart KO, Callaway RM. 2006.** Soil biota and invasive plants. *New Phytologist*
720 **170**: 445–457.
721 **Revelle W. 2018.** *psych: procedures for personality and psychological research*, USA:
722 Northwestern University. R package version 1.8.12. URL https://CRAN.R-
723 project.org/package=psych.
724 **Scervino JM, Ponce MA, Erra-Bassells R, Vierheilig H, Ocampo JA, Godeas A.**
725 **2005.** Flavonoids exhibit fungal species and genus specific effects on the
726 presymbiotic growth of *Gigaspora* and *Glomus*. *Mycological Research* **109**: 789–
727 794.
728 **Scervino JM, Ponce MA, Erra-Bassells R, Bornpadre J, Vierheilig H, Ocampo JA.**
729 **Godeas A. 2007.** The effect of flavones and flavonols on colonization of tomato
730 plants by arbuscular mycorrhizal fungi of the genera *Gigaspora* and *Glomus*.
731 *Canadian Journal of Microbiology* **53**: 702–709.
732 **Sielaff AC, Polley HW, Fuentes-Ramirez A, Hofmockel K, Wilsey BJ. 2019.**
733 Mycorrhizal colonization and its relationship with plant performance differs between
734 exotic and native grassland plant species. *Biological Invasions* **21**: 1981–1991.
735 **Steinkellner S, Lendzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP,**
736 **Vierheilig H. 2007.** Flavonoids and strigolactones in root exudates as signals in
737 symbiotic and pathogenic plant-fungus interactions. *Molecules* **12**: 1290–1306.
738 **Sun ZK, He WM. 2010.** Evidence for enhanced mutualism hypothesis: *Solidago*
739 *canadensis* plants from regular soils perform better. *PLoS One* **5**: e15418.
740 **Tedersoo L, Bahram M, Zobel M. 2020.** How mycorrhizal associations drive plant

741 population and community biology. *Science* **367**: eaba1223.
742 **Thorpe AS, Thelen GC, Diaconu A, Callaway RM. 2009.** Root exudate is
743 allelopathic in invaded community but not in native community: field evidence for
744 the novel weapons hypothesis. *Journal of Ecology* **97**: 641–645.
745 **Tian B, Pei Y, Huang W, Ding J, Siemann E. 2021.** Increasing flavonoid
746 concentrations in root exudates enhance associations between arbuscular
747 mycorrhizal fungi and an invasive plant. *ISME Journal* **15**: 1919–1930.
748 **Tsai SM, Phillips DA. 1991.** Flavonoids released naturally from alfalfa promote
749 development of symbiotic *Glomus* spores in vitro. *Applied Environmental*
750 *Microbiology* **57**: 1485–1488.
751 **de Vries FT, Williams A, Stringer F, Willcocks R, McEwing R, Langridge H,**
752 **Straathofv AL. 2019.** Changes in root-exudate-induced respiration reveal a novel
753 mechanism through which drought affects ecosystem carbon cycling. *New*
754 *Phytologist* **224**: 132–145.
755 **Waller LP, Allen WJ, Barratt BIP, Condron LM, Franca FM, Hunt JE, Koele N,**
756 **Orwin KH, Steel GS, Tylianakis JM *et al*. 2020.** Biotic interactions drive
757 ecosystem responses to exotic plant invaders. *Science* **368**: 967–972.
758 **Waller LP, Callaway RM, Klironomos JN, Ortega YK, Maron JL. 2016.** Reduced
759 mycorrhizal responsiveness leads to increased competitive tolerance in an invasive
760 exotic plant. *Journal of Ecology* **104**: 1599–1607.
761 **Wan FH, Liu QR, Xie M. 2012.** *Biological invasions: color illustrations of invasive*
762 *alien plants in China*. Beijing, China: Science Press.
763 **Wang Y, Siemann E, Wheeler GS, Zhu L, Gu X, Ding J. 2012.** Genetic variation in
764 anti-herbivore chemical defences in an invasive plant. *Journal of Ecology* **100**: 894–
765 904.
766 **Weisshuhn K, Prati D. 2009.** Activated carbon may have undesired side effects for
767 testing allelopathy in invasive plants. *Basic and Applied Ecology* **10**: 500–507.
768 **Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty P. 2019.** Trading on the
769 arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks.
770 *New Phytologist* **223**: 1127–1142.

Wurst S, Vender V, Rillig M. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology* **211**: 19–26.

Xu HG, Qiang S. 2018. *China's invasive alien species*. Beijing: Science Press.

Yang Q, Wei S, Shang L, Carrillo J, Gabler CA, Nijjer S, Li B, Siemann E. 2015. Mycorrhizal associations of an invasive tree are enhanced by both genetic and environmental mechanisms. *Ecography* **38**: 1112–1118.

Yoneyama K, Xie X, Kim HI, Kisugi T, Nomura T, Sekimoto H, Yokota T, Yoneyama K. 2012. How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation? *Planta* **235**: 1197–1207.

Yuan Y, Tang J, Leng D, Hu S, Yong JWH, Chen X. 2014. An invasive plant promotes its arbuscular mycorrhizal symbioses and competitiveness through its secondary metabolites: indirect evidence from activated carbon. *PLoS One* **9**: e97163.

Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho HJ, Karaoz U, Loqué D, Bowen BP *et al.* 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology* **3**: 470–480.

Zhang FJ, Li Q, Chen FX, Xu HY, Inderjit, Wan FH. 2017. Arbuscular mycorrhizal fungi facilitate growth and competitive ability of an exotic species *Flaveria bidentis*. *Soil Biology and Biochem*istry **115**: 275–284.

Zhang F, Li Q, Yerger EH, Chen X, Shi Q, Wan F. 2018. AM fungi facilitate the competitive growth of two invasive plant species, *Ambrosia artemisiifolia* and *Bidens pilosa*. *Mycorrhiza* **28**: 703–715.

Figure legends

Fig. 1 (a) Total biomass, (b) arbuscular mycorrhizal (AM) colonization, the concentrations of (c) quercetin in root exudates from paired native and invasive plants grown for 30, 60 and 90 d, and (d) GR24-referenced strigolactones in fresh root (FW) from paired native and invasive plants grown for 60 d in Experiment 1. The line in the box represents the median value, box boundaries indicate the value in the 25^{th}–75^{th} percentile range, whiskers indicate the 95% confidence interval. The colored points represent the mean values of different species in the respective treatment, with species in each pair sharing the same color. The value *n* represents the number of paired species used in the analysis. The asterisk denotes pairwise difference between native and invasive species in the respective growth time with Benjamini-Hochberg correction for *P* values. *: $P < 0.10$; **: $P < 0.05$; ***: $P < 0.01$.

Fig. 2 (a) Arbuscular mycorrhizal (AM) colonization and (b) total biomass of native and invasive plants grown without (control) or with AM fungi inoculation in Experiment 2. Data from seven pairs of native and invasive plants were used in the analysis. The line in the box represents the median value, box boundaries indicate the value in the 25^{th}–75^{th} percentile range, whiskers indicate the 95% confidence interval. The colored points represent the mean values of different species in the respective treatment, with species in each pair sharing the same color. Different lowercase letters indicate significant difference between the two AM fungi inoculation treatments at $P = 0.05$ level with Benjamini-Hochberg corrections. The asterisk denotes pairwise difference between native and invasive species in the respective AM fungi inoculation treatment with Benjamini-Hochberg correction for *P* values. *: $P < 0.10$; **: $P < 0.05$; ***: $P < 0.01$.

Fig. 3 Arbuscular mycorrhizal (AM) colonization of native and invasive plants receiving deionized water, native or invasive root exudates in Experiment 3. Data from seven pairs of native and invasive plants were used in these analyses. The line in the box represents the median value, box boundaries indicate the value in the 25^{th}–75^{th}

821 percentile range, whiskers indicate the 95% confidence interval. The colored points
822 represent the mean values of different species in the respective treatment, with species
823 in each pair sharing the same color. Different lowercase letters indicate significant
824 difference among three exudates addition treatments at $P = 0.05$ level with Benjamini-
825 Hochberg corrections. The asterisk denotes pairwise difference between native and
826 invasive species in the respective exudate addition treatment with Benjamini-Hochberg
827 correction for P values. **: $P < 0.05$; ***: $P < 0.01$.
828 **Fig. 4** (a) Total biomass and (b) arbuscular mycorrhizal (AM) colonization of native
829 and invasive plants grown without (control) or with activated carbon in Experiment 4.
830 The line in the box represents the median value, box boundaries indicate the value in
831 the 25^{th}–75^{th} percentile range, whiskers indicate the 95% confidence interval. The
832 colored points represent the mean values of different species in the respective treatment,
833 with species in each pair sharing the same color. Different lowercase letters indicate
834 significant difference between two activated carbon (AC) treatments at $P = 0.05$ level
835 with Benjamini-Hochberg corrections. The asterisk denotes pairwise difference
836 between native and invasive species in the respective AC treatment with Benjamini-
837 Hochberg correction for P values. ***: $P < 0.01$. (c) Relationship between the change
838 in total biomass and change in AM colonization for native and invasive plants. Data
839 from seven pairs of native and invasive plants were used in these analyses. Blue dot:
840 native value. Red dot: invasive value. The grey ribbons represent the 95% confidence
841 intervals.
842 **Fig. 5** (a) Arbuscular mycorrhizal (AM) colonization and (b) strigolactones (i.e. strigol)
843 concentration in root exudates of native and invasive plants grown for 30, 60 and 90 d
844 in Experiment 5. Data from seven pairs of native and invasive plants were used in these
845 analyses. The line in the box represents the median value, box boundaries indicate the
846 value in the 25^{th}–75^{th} percentile range, whiskers indicate the 95% confidence interval.
847 The colored points represent the mean values of different species in the respective
848 treatment, with species in each pair sharing the same color. Different lowercase letters
849 indicate significant difference among three growth time treatments at $P = 0.05$ level
850 with Benjamini-Hochberg corrections. The asterisk denotes pairwise difference

between native and invasive species in the respective growth time with Benjamini-Hochberg correction for *P* values. **: $P < 0.05$; ***: $P < 0.01$.

Fig. 6 A diagram summarizing the potential ways in which root exudate chemicals regulate plant-arbuscular mycorrhizal (AM) fungal mutualisms. In the introduced area, invasive species are expected to release more chemicals and establish strong mutualistic relationships with AM fungi. However, whether the enhanced mutualism between invasive species and AM fungi is induced by the chemicals released by the invasive species is unclear. With five complementary experiments, we found that AM fungi benefit both native and invasive species (Experiment 2), however, invaders had greater AM colonization, greater biomass and their root exudates contained higher concentrations of quercetin and strigolactones (Experiments 1 and 5) than native plants. The root exudates exchange experiment (Experiment 3) and activated carbon addition experiment (Experiment 4) further suggested greater effects of root exudate chemicals from invaders on AM fungal association than that from natives. Overall, this study provides evidence that root exudates of invasive plants can enhance AM colonization, and that this effect is linked to enhanced plant performance. The value of *n* represents the number of paired species used in the experiment.

869 **Table 1** Detailed information on invasive and native species used in five experiments
870 in the study.

Pair	Species	Family	Origin†, ‡, §	Habitat type	Experiment				
					1	2	3	4	5
1	*Ambrosia artemisiifolia**	Asteraceae	North America	i, ii, iii, vi	+				
	Siegesbeckia pubescens								
2	*Aster subulatus**	Asteraceae	North America	i, ii, iii, vi, vii	+	+	+	+	+
	Aster ageratoides								
3	*Bidens frondosa**	Asteraceae	North America	i, ii, iii, vi	+				
	Bidens tripartita								
4	*Bidens pilosa**	Asteraceae	America	i, ii, iii, v, vii, viii	+	+	+	+	+
	Bidens biternata								
5	*Crassocephalum crepidioides**	Asteraceae	Africa	i, ii, iii, iv, vi, vii	+				
	Emilia sonchifolia								
6	*Mikania micrantha**	Asteraceae	America	ii, iii, vi, vii	+				
	Eupatorium heterophyllum								
7	*Eupatorium odoratum**	Asteraceae	South America	i, ii, iii, vi, vii	+	+	+	+	+
	Eupatorium chinense								
8	*Eupatorium adenophora**	Asteraceae	America	i, ii, iii, v, vi, vii, viii	+				
	Eupatorium japonicum								
9	*Eupatorium catarium**	Asteraceae	South America	i, ii, iii, vi, vii, viii	+	+	+	+	+
	Eupatorium fortunei								
10	*Flaveria bidentis**	Asteraceae	South America	i, ii, iii, vii, viii	+	+	+	+	+
	Eclipta prostrata								
11	*Galinsoga quadriradiata**	Asteraceae	America	i, ii, iii, v, vi, vii	+				
	Kalimeris lautureana								
12	*Nicandra physalodes**	Solanaceae	South America	i, ii, iii, v	+				
	Solanum spirale								
13	*Ipomoea cairica**	Convolvulaceae	Asia/Africa	iii, vi, vii, ix	+	+	+	+	+
	Ipomoea aquatica								
14	*Senecio vulgaris**	Asteraceae	Europe	i, ii, iv, vi, viii	+				
	Senecio scandens								
15	*Silybum marianum**	Asteraceae	Europe	i, ii, iii, vi	+				
	Cirsium japonicum								
16	*Solanum aculeatissimum**	Solanaceae	South America	i, ii, iii, vii	+				
	Solanum americanum								
17	*Solidago canadensis**	Asteraceae	North America	i, ii, iii, v, vi, vii	+				
	Solidago decurrens								

871

872 **Table 1 – continued**.

Pair	Species	Family	Origin†, ‡, §	Habitat type	Experiment				
					1	2	3	4	5
18	*Sonchus asper** *Sonchus arvensis*	Asteraceae	Europe	i, ii, iii, iv	+				
19	*Xanthium italicum** *Xanthium sibiricum*	Asteraceae	America/Europe	i, ii, iii, vi, viii	+	+	+	+	+

873 Experiment: '+' indicates the pair was used in the corresponding experiment.

874 Habitat type: i, farmland; ii, roadside; iii, abandoned field; iv, vegetable patch; v, residential areas; vi,
875 wetland; vii, forested land; viii, grassland; ix, mountainous areas.

876 *Indicates the invasive species.

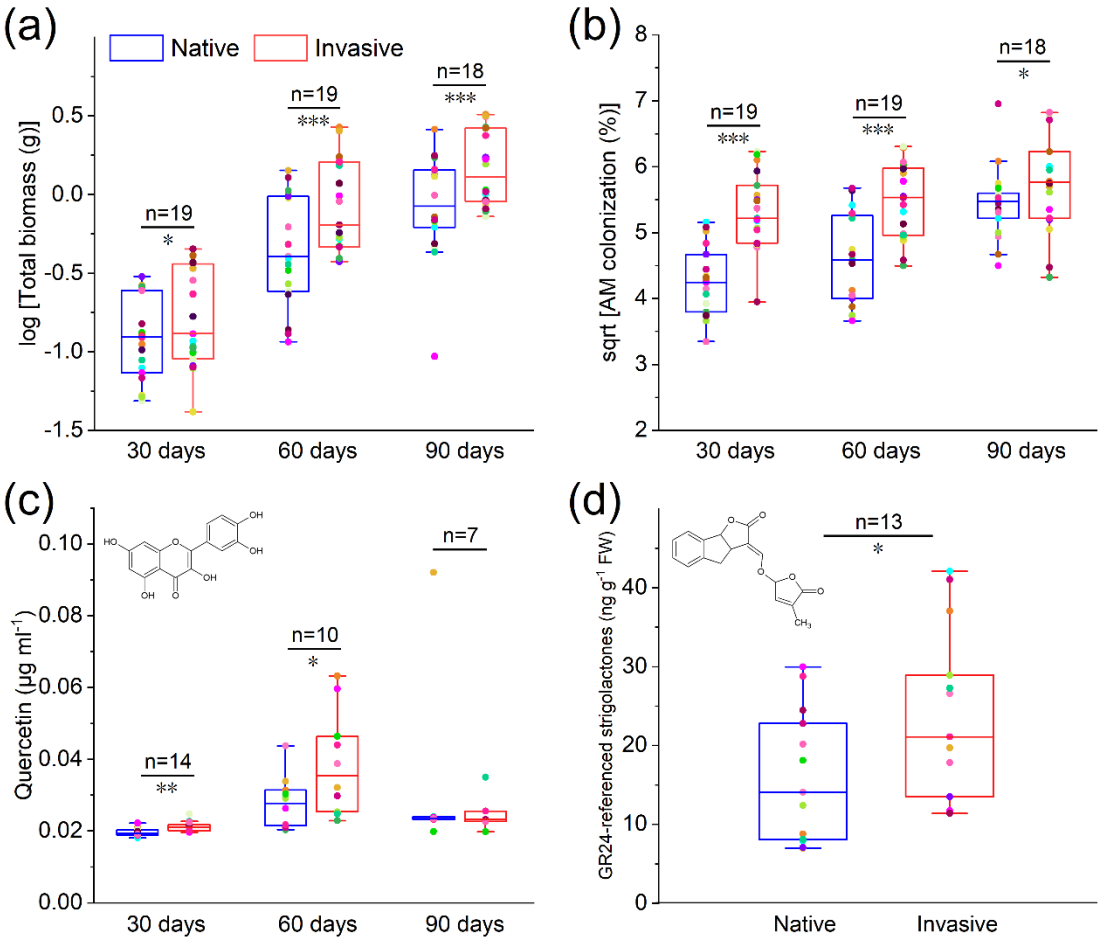
877 †EBFC (1985).

878 ‡Wan *et al.* (2012).

879 §Xu & Qiang (2018).

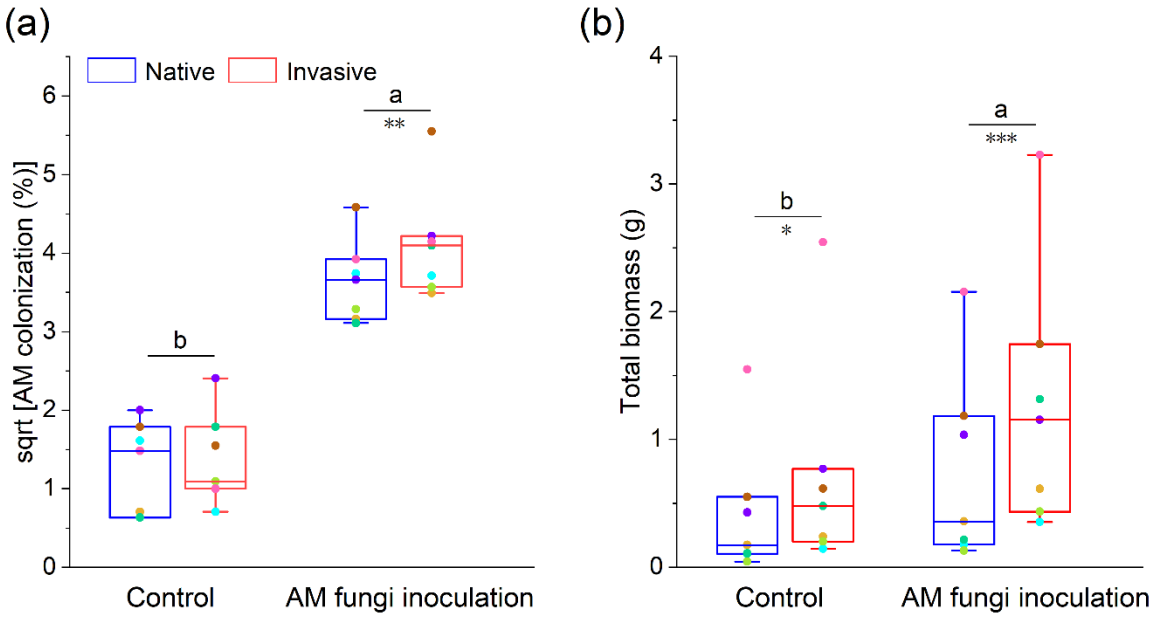
880

881 **Fig. 1**


(a)
Native
Invasive
log [Total biomass (g)]
n=19
*
n=19
***
n=18
***
30 days
60 days
90 days
(b)
sqrt [AM colonization (%)]
n=19
***
n=19
***
n=18
*
30 days
60 days
90 days
(c)
Quercetin (µg ml-1)
n=14
**
n=10
*
n=7
30 days
60 days
90 days
(d)
GR24-referenced strigolactones (ng g-1 FW)
n=13
*
Native
Invasive

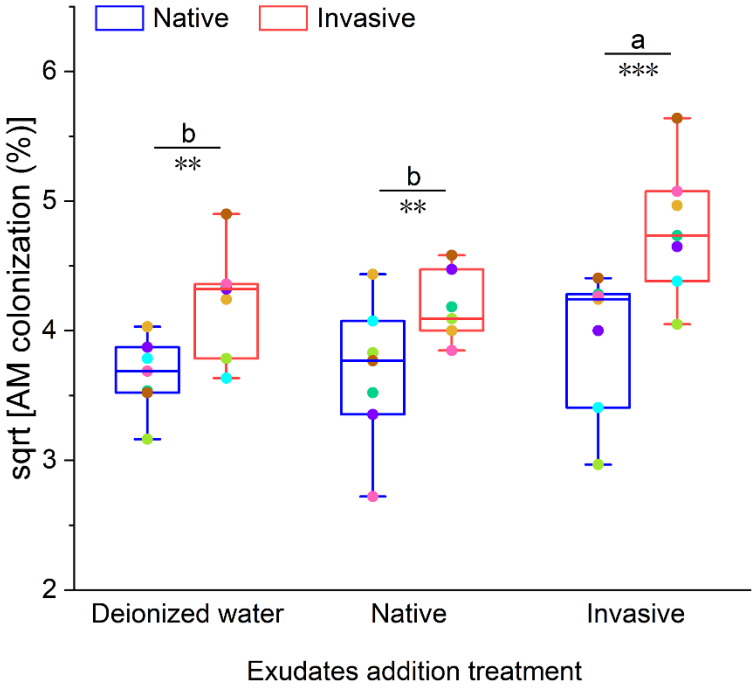
882

883 **Fig. 2**


(a)
Native
Invasive
a
**
b
sqrt [AM colonization (%)]
0
1
2
3
4
5
6
Control
AM fungi inoculation
(b)
b
*
a
***
Total biomass (g)
0
1
2
3
4
Control
AM fungi inoculation

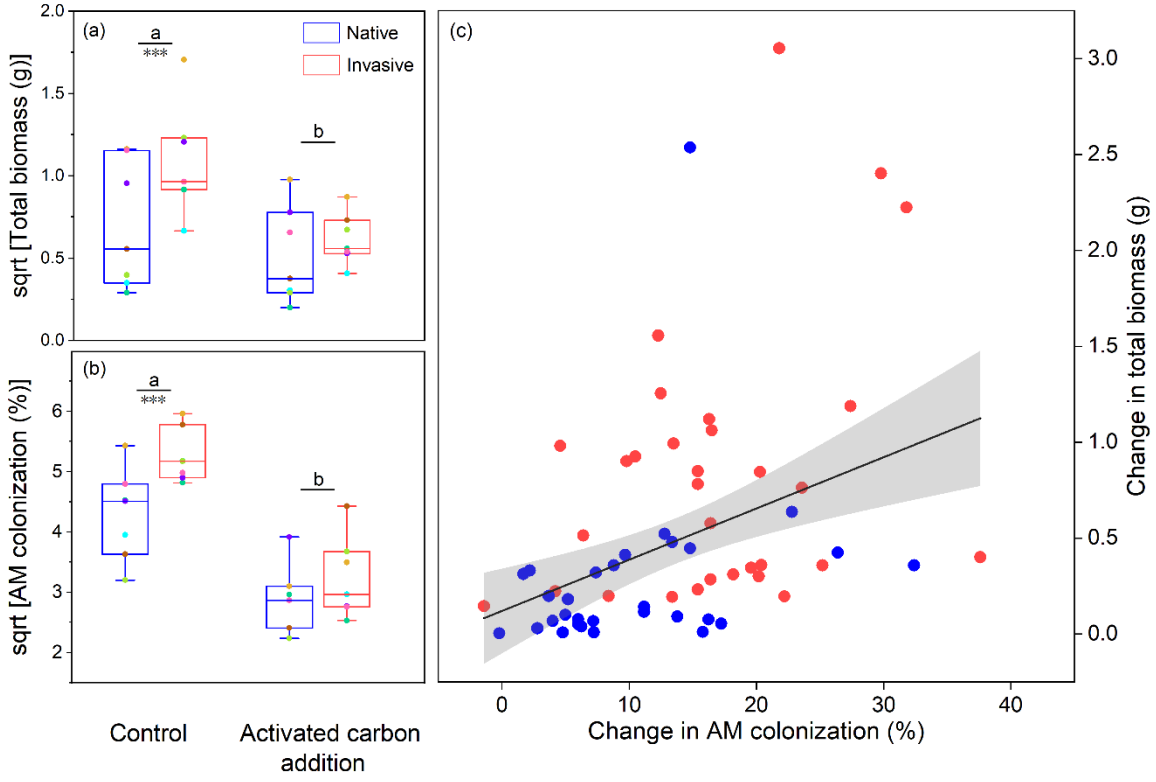
884

885 **Fig. 3**


Native
Invasive
sqrt [AM colonization (%)]
6
5
4
3
2
b
**
b
**
a
***
Deionized water
Native
Invasive
Exudates addition treatment

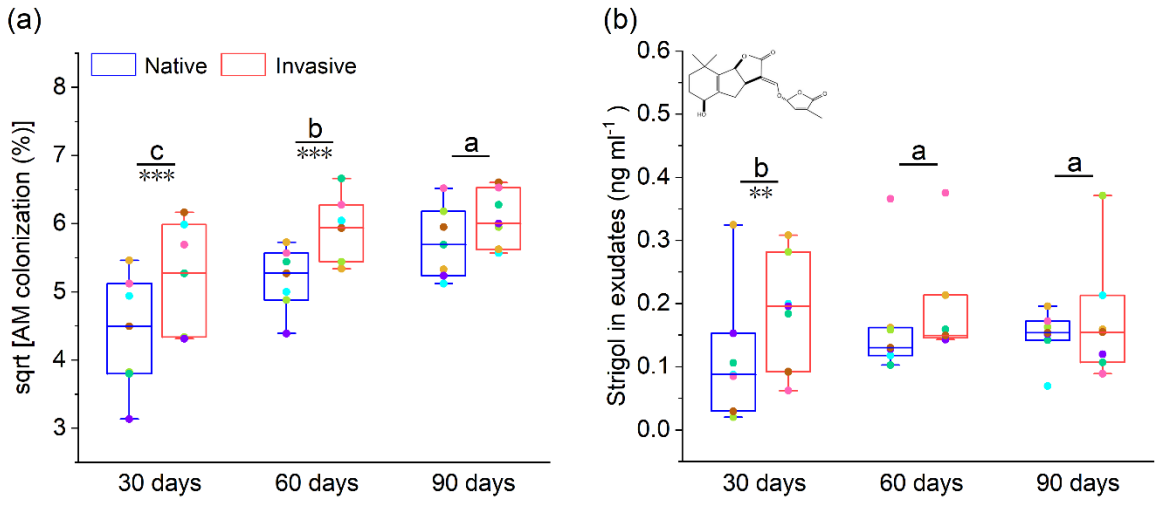
886

887 **Fig. 4**

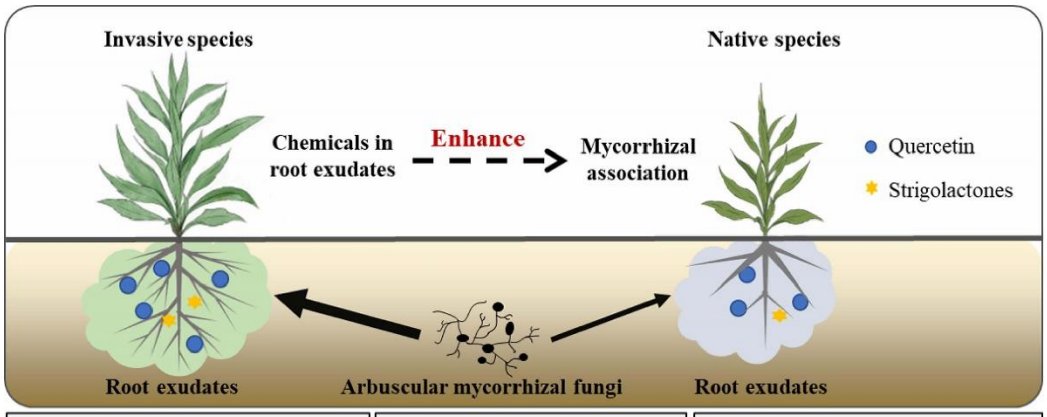

(a)
a
***
Native
Invasive
b
sqrt [Total biomass (g)]
2.0
1.5
1.0
0.5
0.0
(b)
a
***
b
sqrt [AM colonization (%)]
6
5
4
3
2
Control
Activated carbon addition
(c)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Change in total biomass (g)
0
10
20
30
40
Change in AM colonization (%)

888

Fig. 5


(a)
Native
Invasive
sqrt [AM colonization (%)]
8
7
6
5
4
3
c
***
b
***
a
30 days
60 days
90 days
(b)
Strigol in exudates (ng ml-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
b
**
a
a
30 days
60 days
90 days

891 **Fig. 6**



Experiment 1 (n = 19)	Experiment 2 (n = 7)	Experiment 4 (n = 7)
☐ Plant performance, AM colonization and root exudate chemicals: ● **Total biomass:** Invaders > natives ● **AM colonization:** Invaders > natives ● **Quercetin:** Invaders > natives ● **GR24-referenced strigolactones in roots:** Invaders > natives	☐ Inoculating AM fungi promoted growth of both natives and invaders, and this positive effect was stronger for invasive plants than for native plants.	☐ Application of activated carbon reduced AM colonization, and this negative effect was stronger for invasive plants than for native plants.
	Experiment 3 (n = 7)	Experiment 5 (n = 7)
	☐ Adding root exudates from invasive species rather than from native species promote AM colonization.	☐ Strigol in root exudates was positively correlated with AM colonization, and invasive plants tended to have greater concentration of strigol than native plants.

Conclusion: root exudate chemicals of invasive species can enhance AM colonization, playing key roles in plant invasions by regulating interactions with soil mutualists.

892