

Differences in interactions of aboveground and belowground herbivores on the invasive plant *Alternanthera philoxeroides* and native host *A. sessilis*

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Received: 29 January 2016 / Accepted: 12 July 2016 / Published online: 19 July 2016
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Abstract Plant invasions may result in novel plant-herbivore interactions. However, we know little about whether and how invasive plants can mediate native above- and belowground herbivore interactions. In this study, we conducted greenhouse experiments to examine the interaction between a native defoliating beetle, *Cassida piperata*, and a native root-knot nematode, *Meloidogyne incognita*, on the invasive alligator weed, *Alternanthera philoxeroides*. We also included their native host *A. sessilis* in the experiments to examine whether the patterns of above- and belowground herbivore interaction vary with host plants (invasive vs. native). We analyzed total carbon and nitrogen in leaves and roots attacked by *M. incognita* and *C. piperata*. *M. incognita* slightly negatively affected feeding by *C. piperata* on *A. philoxeroides*, and the leaf area damaged decreased as the number of *M. incognita* increased. *M. incognita* had a negative impact on total leaf nitrogen, but had no

impact on total leaf carbon. *M. incognita* egg production on *A. philoxeroides* roots decreased as the amount of damage caused by *C. piperata* increased. Herbivory by *C. piperata* did not affect total root carbon or nitrogen. *M. incognita* and *C. piperata* did not affect each other on the native plant *A. sessilis*. These results suggest that invasive plants can mediate native above- and belowground herbivore interactions. The knowledge of how invasive plants affect those interactions is crucial for better understanding the impacts of biological invasions on native above- and belowground organisms.

Keywords Above- and belowground interaction · Novel interactions · *Meloidogyne incognita* · *Cassida piperata* · Alligator weed

Electronic supplementary material The online version of this article (doi:10.1007/s10530-016-1234-y) contains supplementary material, which is available to authorized users.

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Introduction

Many studies have indicated that while invasive plants leave behind their co-evolved enemies, mutualists, and competitors from their native regions (Keane and Crawley 2002; Nuñez and Dickie 2014), they inevitably establish novel associations with herbivores from introduced regions (Colautti et al. 2004). Agrawal et al. (2005) suggested that invasive plants can be easily attacked by generalist herbivores as well as consumers of plants in exotic regions phylogenetically related to invasive plants, including

aboveground herbivores and soil organisms. Although the effects of those generalists on invasive plants have received a lot of attentions (see Levine et al. 2004; Chun et al. 2010; Cushman et al. 2011), we have little knowledge about whether invasive plants can affect interactions between root and shoot herbivores from invaded ranges.

Plants can mediate above- and belowground herbivore interactions (Wardle et al. 2004; van der Putten et al. 2009; Johnson et al. 2012). Foliar herbivory may induce primary metabolites allocation to root for tissue re-growth or storage of substrates for biosynthesis of root defensive compounds (Schwachtje and Baldwin 2008; Orians et al. 2011), which may in turn affect root feeding herbivores. For example, foliar herbivory increased sugars allocating to root in *Nicotiana attenuata* L. (Solanaceae) (Schwachtje et al. 2006). Soler et al. (2007) reported shoot damage by *Pieris brassicae* L. (Pieridae) increased indole glucosinolates concentrations in root, resulting in a negative impact on root herbivores. On the other hand, damage caused by root-feeding organisms can lead to a similar response as drought stress (Masters et al. 1993), which causes an increase of soluble nitrogen and carbon in plant shoots, increasing plant nutritional quality for aboveground herbivore (Masters and Brown 1997). Also, root-feeding herbivores may disrupt synthesis of secondary metabolites that are transported between roots and leaves, leading to decreasing resistance to aboveground herbivores (Erb et al. 2009). Kaplan et al. (2008) demonstrated that a root-feeding nematode, *Meloidogyne incognita* (Kofoid and White 1919) Chitwood (1949) (Heteroderidae) inhibited foliar nicotine dynamics, resulting in positive effects on aboveground herbivores. Therefore, host plant traits which can be systematically induced by herbivores play an important role in the linkage of above- and belowground herbivores (Bezemer and van Dam 2005).

Plant chemistry is highly species-specific and diverse among species. Invasive plants may possess unique phytochemicals differing from native plants (Cappuccino and Arnason 2006), which can affect native above- and belowground herbivores, soil pathogens and decomposer organisms (Callaway et al. 2008; Schaffner et al. 2011). A recent study reported that a group of invasive Asteraceae plants had more species—unique metabolites and higher total number of metabolites than native congeners,

resulting in poor performance of the generalist herbivore [*Mamestra brassicae* L. (Noctuidae)] on most of invasive species (*Senecio inaequidens* D.C., *Solidago gigantea* Ait and *Artemisia biennis* Willd.) compared to their native congeners (Macel et al. 2014). Moreover, invasive plants may evolve and allocate resource from defense to growth due to enemy release (see Blossey and Notzold 1995; Siemann and Rogers 2003; Feng et al. 2009), which may affect both above- and belowground herbivores (Huang et al. 2012). Thus, above- and belowground interactions may vary between invasive and native hosts.

Native to South America, *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae) is a noxious weed that can now be found invasive in North America, Asia and Australia (Julien et al. 1995). *A. sessilis* (L.) R.Br. ex DC. (Amaranthaceae) is the only native congener of *A. philoxeroides* in China, and as such it has often been used for comparison with *A. philoxeroides* because they share the same phylogenetic history, and sympatrically distributed in terrestrial, semi-aquatic, and aquatic habitats (Li 1998; Geng et al. 2006; Pan et al. 2006). In China, several native generalist defoliators were found on *A. philoxeroides*, including *Prodenia litura* (Fabricius) (Noctuidae) and *Spoladea recurvalis* (Fabricius) (Crambidae) (Lin et al. 1990), as well as a native oligophagous beetle, *Cassida piperata* Hope (Chrysomelidae) which mainly occurs on the native species *Chenopodium album* L. (Chenopodiaceae) and *A. sessilis* (Lin et al. 1990). A recent study found that *C. piperata* has been expanding its host range to *A. philoxeroides* (Dai et al. 2014). A native root-knot nematode *M. incognita* was broadly found to cause damage to the roots of both *A. philoxeroides* and *A. sessilis* in China (Mao et al. 2011). Previous studies found that shoot removal increased soluble carbon concentration and decreased nitrogen concentration in roots of *A. philoxeroides* (Schooler et al. 2007). *A. philoxeroides* possessed higher leaf nitrogen, lignin and cellulose concentrations than *A. sessilis*, but cellulose and polyphenolic concentrations were higher in *A. sessilis* leaves than in *A. philoxeroides* leaves after defoliating by a native generalist herbivore (Fan et al. 2013).

In this study, we investigated whether *A. philoxeroides* could mediate the interaction between the native defoliator *C. piperata* and the root-knot nematode *M. incognita*. We predicted that nutrients

allocated to *A. philoxeroides* root may decrease with increasing aboveground herbivory, resulting in negative impact on root-knot nematode, and vice versa. We also included the native congener *A. sessilis* to examine whether the patterns of above- and belowground herbivores interaction vary with host plants (invasive vs. native).

Materials and methods

Study species

Alternanthera philoxeroides is a perennial and stoloniferous herbaceous plant. In its invaded region, *A. philoxeroides* can reproduce asexually, usually from apical stem buds and axillary stem or root buds, and can form dense monocultures in terrestrial, semi-aquatic, and aquatic habitats (Julien et al. 1995). Its native range includes Brazil, Paraguay, and Argentina (Vogt et al. 1979). *Alternanthera philoxeroides* was first introduced into China as forage in the later 1930s, and then widely dispersed in southern and central China in the middle of 1970s (Fan et al. 2013). The invasion of *A. philoxeroides* always results in the loss of native species and large economic losses in agriculture and aquaculture (Pan et al. 2007). Co-occurring with *A. philoxeroides* in many habitats but native to China, *A. sessilis* is also a stoloniferous and amphibious perennial herbaceous plant with sexual or clonal growth (Li 1998).

Cassida piperata is an oligophagous defoliator that feeds on some plants in the Amaranthaceae and Chenopodiaceae (Lin et al. 1990). The beetle naturally occurs in Siberia, Korea, Japan, Vietnam, Philippines, as well as a large area of China (Tan 1994). *Cassida piperata* has two to four generations per year and overwinters as an adult (Lin et al. 1990). Both larvae and adults feed on leaves of *A. philoxeroides* and *A. sessilis*, leaving transparent membranes or large holes.

Meloidogyne incognita is a world-widely distributed root-knot nematode in agriculture and semi-natural systems (Castagnone-Sereno et al. 2013). *Meloidogyne incognita* is capable of reproducing in >2000 species of plants (Sasser 1980). It needs 3–4 weeks to complete its life cycle at the optimum temperature range of 20–30 °C (Liu 2000). Infective second-stage juveniles can penetrate epidermis cells and migrate intracellularly toward the vascular

cylinder (Wyss and Zunke 1986). Giant cells that are metabolically active are induced at the feeding sites, forming a strong nutrient sink with highly upregulated transport processes (McClure 1977).

For this study, *C. piperata* larvae were collected on *A. philoxeroides* in fields near the Wuhan Botanical Garden of the Chinese Academy of Sciences (30°32'44.5" N, 114°24'45.6"E) in Hubei Province, China. Larvae were reared on potted *A. philoxeroides* or *A. sessilis* in the laboratory, and the offspring of these colonies were used for tests on *A. philoxeroides* or *A. sessilis* in our experiments. Meanwhile, the eggs of *M. incognita* were collected from infected roots of native tomato plants [*Lycopersicon esculentum* Mill. (Solanaceae)] from a farm in the suburban of Wuhan. They were extracted from the cyst using 0.5–1 % sodium hypochlorite solution which can destroy the cyst and allow obtaining eggs (Barker 1985).

Experimental design

Experiment 1. The impact of belowground herbivore on aboveground herbivore To assess the effects of root-knot nematode (*M. incognita*) on defoliator (*C. piperata*) with *A. philoxeroides* and *A. sessilis* as host plants, we conducted a greenhouse experiment at the Wuhan Botanical Garden from June to August in 2013, with the temperature around 26–28 °C. We changed the position of the pots every 15 days to minimize the impacts of light and temperature. In early June, stems of *A. philoxeroides* and *A. sessilis* collected near Wuhan Botanical Garden were cut to a similar size (4–5 cm lengths, each with one node) and planted in seedling trays with sterilized soil in a greenhouse. Three weeks later, when seedlings of *A. philoxeroides* and *A. sessilis* were 15 and 10 cm lengths respectively, we transplanted them singly into 500 ml pots containing a growing medium and all the pots were immediately covered with nylon cages (70 cm high). The growing medium was composed with 25 % sphagnum peat moss, as well as 75 % field soil collected from a wasteland in Wuhan Botanical Garden where *A. philoxeroides* and *A. sessilis* are common. Soils were sieved and large root fragments were removed. Soils were then homogenized and autoclaved at 0.15 m pa, 120 °C for 1.5 h to kill root-knot nematodes with an autoclave sterilizer (YXQ.WY21.600, Zhengzhou Nanbeiyi instrument and equipment Co. Ltd, China). Plants were

supplemented with nutrients weekly using Hoagland's nutrient solution (the proportion of nutrient: nitrogen: 117 mg/L and phosphorus: 24 mg/L). Seedlings of each species were randomly assigned to different nematode egg densities with *c.* 0, 2000, 5000, 10,000, or 20,000 eggs. Each treatment (species \times nematode egg density) was repeated ten times and 50 seedlings were used for each *Alternanthera* species. The seedlings of each species that did not receive eggs served as controls.

Three weeks after *M. incognita*-inoculation, six newly hatched *C. piperata* larvae from a laboratory colony were applied to each control and *M. incognita* infested plant. We used similar-sized larvae (ANOVA, $F_{1,40} = 0.63$, $P = 0.6438$), excluding any individuals that were unusually large or small. At the end of the experiment, when all the *C. piperata* larvae pupated 11 days after larval release, we estimated the amount of damage the plant experienced by scanning foliage on a piece of 10 \times 10 grid graph paper with a flatbed scanner. We also measured the leaf area removed by *C. piperata* on each seedling using Adobe Photoshop CS 8.0.1 (Adobe System Incorporate, US). Percent of leaf damage was calculated as the ratio between leaf area consumed by larvae and total leaf area. In addition, to measure larval performance, we weighed (nearest 0.00001 g) and counted the number of the larvae and pupae, and then calculated the survival and pupation rates.

Plant leaves were collected individually, dried (5 days at 40 °C), weighed (nearest 0.0001 g) and ground up with a mortar for analyzing total carbon and nitrogen. To obtain sufficient samples for total carbon and nitrogen analysis, we mixed the powder from the same treatment (0.5–1 g for each sample). Then they were analyzed for total carbon and nitrogen in an elemental auto analyzer (Vario MAX CN, Elementar, GmbH, Hanau, Germany). Shoots were clipped, dried (48 h at 80 °C) and weighed. Roots were dug up and washed. All roots were stained and decolorized individually with a modified sodium hypochlorite (NaOCl)-acid fuchsin-glycerin technique (Byrd Jr et al. 1983; Liu 2000). The number of nematodes were dissected and counted under an anatomical lens (adult nematodes were stained red) after removing the excessive solutions by rinsing in running water. We collected all dissected roots and nematodes individually, dried (48 h at 80 °C) and weighed.

Experiment 2. The impact of aboveground herbivore on belowground herbivore To investigate the impact of defoliation (*C. piperata*) on *M. incognita* on *A. philoxeroides* and *A. sessilis*, we conducted a greenhouse experiment from August to October in 2013. In mid-August, we prepared plant seedlings and soils as described in Experiment 1. Four weeks later, the roots of all plants were inoculated with *c.* 3500 *M. incognita* eggs obtained from a laboratory culture. This egg density is well within the range of infected-root of *A. philoxeroides* and *A. sessilis* observed in the field (Wei et al. unpublished data). At the same time, seedlings of each species used in treatments received 0, 1, 2, 3 or 5 newly hatched larvae of *C. piperata*, while seedlings of each species received no insects as controls. Each treatment (species \times larvae defoliation) was repeated ten times and 50 seedlings were used for each *Alternanthera* species. After 2 weeks, we removed the *C. piperata* larvae because a previous study showed that the leaf nitrogen, lignin, cellulose and polyphenolic concentrations in both *A. philoxeroides* and *A. sessilis* were changed after 19 days of defoliation (Fan et al. 2013).

At the end of the experiment, when *M. incognita* completed one generation in two *Alternanthera* plants (30 days after nematode inoculation according to pilot study), we collected the damaged leaves of each species and scanned them on a piece of 10 \times 10 grid graph paper with a flatbed scanner, measuring the leaf area on each plant that had been removed by *C. piperata* using Adobe Photoshop CS 8.0.1. The aboveground was clipped, dried and weighed. The roots were dug and washed. The number of root knots was counted firstly, then eggs were extracted from all roots and counted (as per Experiment 1). The roots were randomly divided into two subsamples for each *Alternanthera* species. One was used to count the number of nematodes using the method mentioned in Experiment 1. The roots were dried (48 h at 80 °C) and weighed. To exam the impact of *C. piperata* larval density on root nutrient concentrations, the other subsamples were rinsed to remove NaOCl solution, dried (5 days at 40 °C), weighed and ground up with a mortar for analyzing carbon and nitrogen (after Experiment 1). The number of nematode of the subsamples were estimated based on the relationship between the number of root knots (NNO) and nematodes (NNE) for each *Alternanthera* species

Table 1 Summary of GLMs on the effect of host plant species (Species), root feeding nematode *Meloidogyne incognita* (Nematode) and their interaction on *Cassida piperata* larval performances, as well as plant growth and nutrient concentration at the end of Experiment 1

Response variable	Source of variance	df.	F-value	P
Plant mass	Species (S)	1,86	45.75	<.0001***
	Nematode (N)	4,86	3.48	0.01**
	S × N	4,86	1.75	0.15
Root-to-shoot mass ratio	Species (S)	1,86	2	0.16
	Nematode (N)	4,86	10.51	<.0001***
	S × N	4,86	8.28	<.0001***
Leaf carbon	Species (S)	1,14	55.75	<.0001***
	Nematode (N)	4,14	0.32	0.86
	S × N	4,14	0.79	0.55
Leaf nitrogen	Species (S)	1,14	24.82	0.0002***
	Nematode (N)	4,14	1.44	0.27
	S × N	4,14	4.22	0.02*
Carbon-to-nitrogen ratio	Species (S)	1,13	33.91	<.0001***
	Nematode (N)	4,13	0.54	0.71
	S × N	4,13	2.94	0.06
Larval mass	Species (S)	1,90	27.69	<.0001***
	Nematode (N)	4,90	1.48	0.21
	S × N	4,90	1.24	0.3
Larvae survival	Species (S)	1,90	1.27	0.26
	Nematode (N)	4,90	1.11	0.35
	S × N	4,90	0.87	0.48
Pupation rate	Species (S)	1,90	3.28	0.07
	Nematode (N)	4,90	0.66	0.6
	S × N	4,90	0.63	0.64
No. adult nematode g ⁻¹ root	Species (S)	1,90	78.2	<.0001***
	Nematode (N)	4,90	48.4	<.0001***
	S × N	4,90	6.8	<.0001***

*P < 0.05, **P < 0.01, ***P < 0.001

[*A. philoxeroides*: NNE = 1.641 + 1.106 NNO (r² = 0.795, P < 0.0001), and *A. sessilis*: NNE = 1.610 NNO - 12.317 (r² = 0.801, P < 0.0001)].

Statistical analysis

In Experiment 1, we conducted General Linear Model (GLM) to test the impact of *M. incognita* egg density and host plant species on larval mass, larval survival and pupation rate and number of adult nematodes in roots. Larval mortality and pupation rate were arcsine transformed while the number of adult nematodes was square root transformed to meet the assumption of normality. We also employed GLM to exam the impact of *M. incognita* egg density and host plant species on plant mass and the ratio of root to shoot

mass (RtoS) and leaf carbon, nitrogen and the ratio of carbon to nitrogen (C/N). Pearson product-moment correlation was used to determine the relationship between the final overall number of *M. incognita* in the roots and the percent of leaf damage from *C. piperata* for the two host species respectively, where the number of *M. incognita* were square root transformed to meet assumption of normality.

In Experiment 2, we ran GLM to test the impact of *C. piperata* larval density and host plant species on the number of adult *M. incognita* and *M. incognita* egg production. The data were square-root transformed to improve normality. We also conducted GLM to test the impact of *C. piperata* larval density and host plant species on plant mass and RtoS, as well as total carbon, nitrogen and C/N

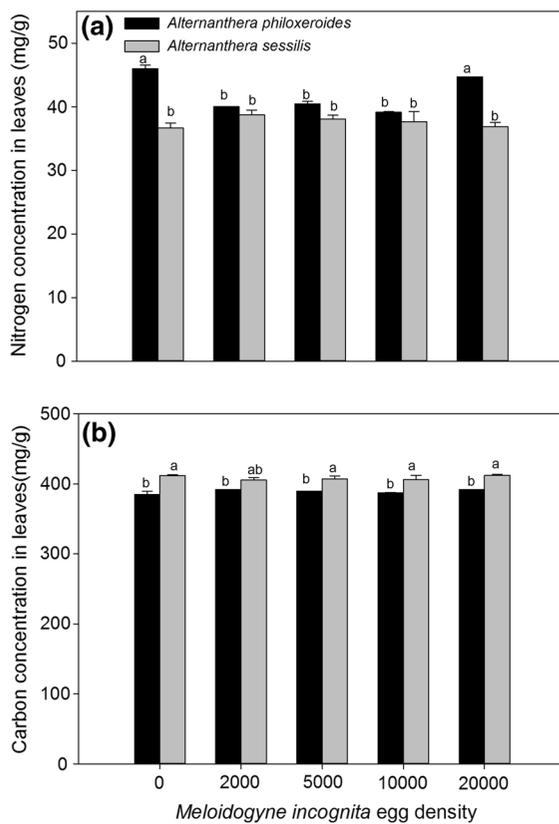


Fig. 1 The impact of native root feeding nematode *M. incognita* on plant nitrogen (a) and carbon (b) concentration of the invasive plant (*Alternanthera philoxeroides*, black bar) and native plant (*Alternanthera sessilis*, grey bar) at the end of Experiment 1. The values are means (\pm SE) of plant nitrogen and carbon concentrations. See Table 1 for the associated analysis of variances. Means sharing same letters indicate no significant difference ($P < 0.05$)

in root. In addition, we used Pearson product-moment correlation to test the relationship between overall damage area (mm^2 of leaf tissue removed) and the number of adult *M. incognita*, as well as its relationship with *M. incognita* egg production at the end of the experiment. Damaged area was square root transformed and the number of adult *M. incognita* and fecundity were \log_{10} transformed to meet the assumption of normality. We conducted adjusted means partial difference ($P < 0.05$) to examine the differences among treatments for significant interaction terms. All data analyses were performed using SAS 8.1 (SAS Institute Inc, NC, USA).

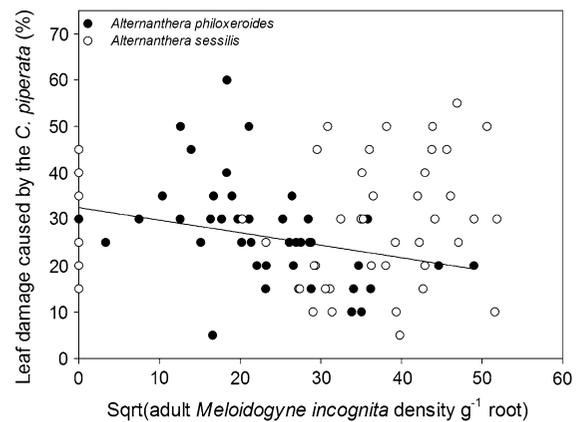


Fig. 2 Pearson's product correlation between number of native root feeding nematode (*Meloidogyne incognita*) and the percent of leaf damage from native leaf feeding larval beetle (*Cassida piperata*) on the invasive (*Alternanthera philoxeroides*, filled circle) and native (*Alternanthera sessilis*, open circle) host plants. Data were collected 32 days after *M. incognita* egg inoculation in Experiment 1. The trend line indicate a significantly negative relationship between *M. incognita* and *C. piperata* hosted on *A. philoxeroides*: $y = -0.27x + 32.51$, $r^2 = 0.1$, $P = 0.025$, where y is the percent of leaf damage from larval *C. piperata* and x is the number of adult *M. incognita*

Results

The impact of belowground herbivore on aboveground herbivore

Host plant species and *M. incognita* egg density significantly affected plant growth, but not their interaction (Table 1). *Meloidogyne incognita* egg density greatly increased RtoS, especially in *A. sessilis* (Table S 1). Additionally, *A. philoxeroides* leaf nitrogen concentration significantly decreased when the *M. incognita* egg densities were at *c.* 2000, 5000, 10,000 (but no change at the highest egg density), while *M. incognita* egg density did not affect leaf nitrogen in *A. sessilis* (Fig. 1a). Leaf nitrogen concentration was higher in *A. philoxeroides* than in *A. sessilis*, while *A. sessilis* had higher leaf carbon concentration than *A. philoxeroides* (Fig. 1b).

In this respect, the percent of leaf area consumed by larval *C. piperata* negatively correlated with final overall number of *M. incognita* eggs on *A. philoxeroides*, but not on *A. sessilis* ($r^2 = 0.007$, $P = 0.56$; Fig. 2). Host plant species significantly affected larval mass (Fig. 3a), as well as adult nematode number in roots (Fig. 3b), but not larval mortality and pupation

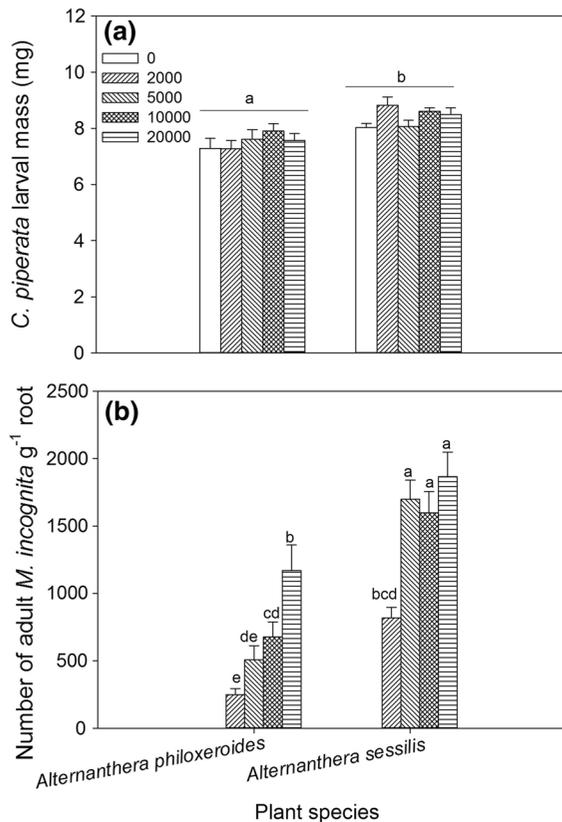


Fig. 3 Impact of native root feeding nematode (*Meloidogyne incognita*) density (c. 0, unfilled bar; c. 2000, left diagonal filled; c. 5000, right diagonal filled; c. 10,000, cross filled; c. 20,000, line filled) on native defoliator (*Cassida piperata*) (a) and nematode abundance in roots (b) hosted on *Alternanthera philoxeroides* and *Alternanthera sessilis* at the end of Experiment 1. Values are means (\pm SE) larval mass of *C. piperata* (a) and number of adult nematodes (b) hosted on two *Alternanthera* species. See Table 1 for the associated analysis of variances. Means sharing same letters indicate no significant difference ($P < 0.05$)

rate (Table 1). *Meloidogyne incognita* egg density had no significant impact on larval mass (Fig. 3a), larval mortality and pupation rate (Table 1), but significantly influenced adult nematode number in roots (Fig. 3b).

The impact of aboveground herbivore on belowground herbivore

Cassida piperata larval defoliation significantly decreased plant mass with the larval density increased (Table S 2). *Alternanthera sessilis* with no larval had higher RtoS compared to herbivory treatment (Table S 2).

Alternanthera philoxeroides had significantly higher root C/N than *A. sessilis* (mean \pm SE: 48.1 ± 0.58 vs. 37.1 ± 1.06). RtoS, root carbon and nitrogen were not affected by *C. piperata* larval density, host plant species and their interaction (Table 2). In this respect, *M. incognita* egg production decreased significantly as the amount of damage from larvae increased for *A. philoxeroides*, but not for *A. sessilis* ($r^2 = 0.04$, $P = 0.16$) (Fig. 4a), while there was no correlation between leaf damage area from the beetle and number of adult *M. incognita*, on *A. philoxeroides* ($r^2 = 0.03$, $P = 0.24$) and *A. sessilis* ($r^2 = 0.02$, $P = 0.32$) (Fig. 4b). Also, the number of adult *M. incognita* and egg production were significantly affected by host plant species, but not by *C. piperata* larval density (Table 2).

Discussion

Although the effects of invasive plants on insect herbivores have recently received much attention (Bezemer et al. 2014), there is little direct experimental evidence that invasive plants can affect the interaction of native aboveground and belowground herbivores. In this study, we found that the native root-knot nematode *M. incognita* decreased the damage level to the invasive plant *A. philoxeroides* caused by the native defoliator *C. piperata*, while *C. piperata* decreased *M. incognita* fecundity on *A. philoxeroides*. However, these above- and belowground herbivores did not affect each other in these ways on the native plant *A. sessilis*.

In response to herbivory by nematodes, plants can reduce the transport of nutrients from roots to shoots (Brueske and Bergeson 1972; Bird and Loveys 1975; Carneiro et al. 2002). In this case, nitrogen in *A. philoxeroides* leaves was significantly reduced when plants were attacked by *M. incognita* at low and medium densities. Nitrogen, being involved in fundamental plant physiological processes, may have a large impact on insect performance (Berenbaum 1995). A growing body of evidences suggests that aboveground herbivores preferentially consume species with high leaf nitrogen (Perez-Harguindeguy et al. 2003). Thus our finding that nitrogen decreased in leaves infected by *M. incognita* may explain the decrease in leaf damage by *C. piperata* on *M. incognita*-infected plants.

Table 2 Summary of GLMs on the impact of host plant species (Species), larval of leaf feeding beetle *Cassida piperata* (Beetle) and their interaction on plant and root feeding nematode *Meloidogyne incognita* performances at the end of Experiment 2

Response variable	Source of variance	<i>d.f.</i>	F-value	<i>P</i>
Plant mass	Species (S)	1,84	0.2	0.65
	Beetle (B)	4,84	3.94	0.006**
	S × B	4,84	0.22	0.93
Root-to-shoot mass ratio	Species (S)	1,84	0.04	0.84
	Beetle (B)	4,84	0.52	0.72
	S × B	4,84	2.5	0.05*
Root carbon	Species (S)	1,8	0.91	0.37
	Beetle (B)	4,8	0.63	0.65
	S × B	3,8	0.72	0.57
Root nitrogen	Species (S)	1,8	2.48	0.15
	Beetle (B)	4,8	0.72	0.6
	S × B	3,8	0.7	0.58
Carbon-to-nitrogen ratio	Species (S)	1,7	34.98	0.0006***
	Beetle (B)	4,7	0.85	0.53
	S × B	3,7	0.51	0.69
Egg density	Species (S)	1,84	8.63	0.004**
	Beetle (B)	4,84	1	0.41
	S × B	4,84	1.21	0.31
Nematode density	Species (S)	1,84	150.57	<.0001***
	Beetle (B)	4,84	0.81	0.52
	S × B	4,84	0.76	0.55

P* < 0.05, *P* < 0.01,

****P* < 0.001

Insect defoliation can induce root resistance (Erb et al. 2011) or nutrient reallocation to different tissues (Orians and Jones 2001), resulting in an indirect impact of aboveground herbivores on belowground herbivores. In this case, we found that herbivory by *C. piperata* did negatively affect *M. incognita* egg production. This might be because nutrient concentrations decrease in the roots after herbivory by the foliar herbivore, resulting in poor growth and low fecundity of *M. incognita*. Mao et al. (2011) found that root water-soluble carbohydrate content decreased after herbivory by *C. piperata*. Carbohydrate is one of the key resources that affect nematode development (Spiegel and McClure 1995; Hofmann et al. 2008), while it is also the main composition of nematode oocysts (Bird 1956). Thus, low levels of water-soluble carbohydrate after herbivory by *C. piperata* may negatively affect *M. incognita* development, resulting in lower fecundity.

Several studies have demonstrated that invasive plants may have specific traits which did not historically co-occur with native herbivores, and may affect native above- and belowground herbivores (Schaffner et al. 2011; Pearse and Altermatt 2013; Macel et al.

2014). In this study, we found above- and belowground herbivores did not affect each other on the native species *A. sessilis* as they did on the invasive plant *A. philoxeroides*. However, we found *M. incognita* increased RtoS for *A. sessilis*, while *C. piperata* decreased RtoS of *A. sessilis*, but both of them had little impact on the growth of *A. philoxeroides*. The possible cause may be that nutrients or defensive chemicals elicited by *M. incognita* or *C. piperata* were different between the two plants, in turn resulting in altered native above- and belowground herbivores interaction. In this case, we found leaf nitrogen concentration had no change in *A. sessilis* after herbivory by *M. incognita* as *A. philoxeroides* did, which may be the cause that *M. incognita* did not affect *C. piperata* on *A. sessilis*. The possible reason is that there is a trade-off between tolerance (e.g. water-soluble carbohydrate concentration) and resistance (e.g. lignin and cellulose concentration) (Leimu and Koricheva 2006), while the total carbon or nitrogen concentration did not change after being fed upon herbivores. Mao et al. (2011) found that water-soluble carbohydrate concentration in *A. sessilis* root did not change after herbivory by *C. piperata* as they did on

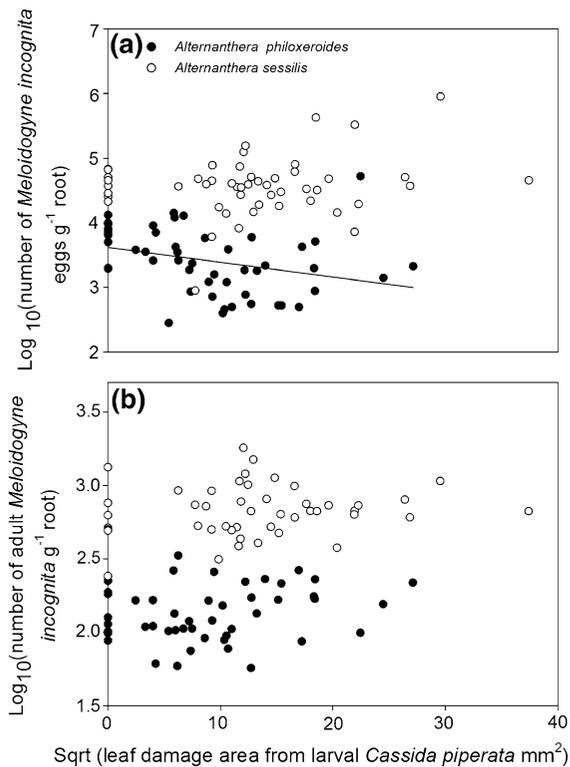


Fig. 4 Effect of native leaf feeding beetle (*Cassida piperata*) on the population growth and fecundity of native root feeding nematode (*Meloidogyne incognita*) on invasive (*Alternanthera philoxeroides*, filled circle) and native (*Alternanthera sessilis*, open circle) host plants at the end of Experiment 2 (30 days after nematode inoculation). **a** number of adult *M. incognita* and **b** number of *M. incognita* eggs. The trend line indicates negative impact of *C. piperata* of *M. incognita* hosted on *A. philoxeroides*: $y = -0.02x + 3.62$, $r^2 = 0.1$, $P = 0.025$, where y is leaf damage area and x is number of *M. incognita* eggs

A. philoxeroides, which may explain why *C. piperata* had no impact on *M. incognita*.

In summary we found that native above- and belowground herbivores affected each other on the invasive plant host *A. philoxeroides*, but had no interactive effect on native host *A. sessilis*, suggesting that the invasive plant can mediate and alter the interactions of native above- and belowground herbivores. We predict that invasion by *A. philoxeroides* may affect both above- and belowground herbivore communities but further field investigations are needed. Because many invasive plants, such as *A. philoxeroides*, have close relatives in their new ranges, the altered interactions between native above- and belowground herbivores could affect both the native

host plants and the novel invasive hosts. In this study, we only included one pair of invasive and native hosts, but future work needs to study multiple pairs to improve our understanding of how invasive plants, above- and belowground herbivores and native hosts interact with each other. This knowledge is crucial for better understanding the impacts of biological invasions on native above- and belowground organisms.

Acknowledgments We thank Evan Siemann for the suggestions on data analysis. We also thank Xu Shao and Jingzhong Lu for their field and laboratory assistance. We also appreciate Professor Roy van Driesche and Dr. Tilley Luke and for the language edits and anonymous reviewers' comments on the early version of this manuscript. This work was funded by the National Science Foundation of China (Grant No. 331100302).

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