Aboveground herbivory increases soil nematode abundance of an invasive plant

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Abstract

Aims Plant invasions have the potential to affect the community structure of soil nematodes, but little is known about whether such effects are mediated by aboveground herbivores since invasive plants are not completely released from herbivores in the introduced range. In this study, we explored how aboveground insect herbivores mediated the effect of invasive plant *Alternanthera philoxeroides* on soil nematodes and examined the temporal variations of such an herbivory-elicited effect.

Methods We conducted a greenhouse experiment by applying different herbivory treatments (no insect herbivores, specialist *Agasicles hygrophila* and generalist *Cassida piperata*) to potted *A. philoxeroides*, and then measured the community compositions of soil nematodes in corresponding pots on the 1st, 10th and 20th day after removal of all herbivores. In addition, the carbon content of roots and root exudate of *A. philoxeroides* were also measured.

Important Findings Our results showed that aboveground herbivory significantly increased the abundance of soil nematodes of *A. philoxeroides*, likely plant feeder nematodes, after insect herbivores were removed immediately (1st day). However, such impacts waned with time and there was no significant difference at later stages (10th and 20th days). Furthermore, the effects of specialist *A. hygrophila* and generalist *C. piperata* herbivory were consistent on the abundance of soil nematodes. Overall, our results suggest that aboveground insect herbivores have the potential to alter the effects of plant invasions on soil nematodes, but such impacts are transient. Furthermore, our study highlighted the importance of integrating the effects of above- and belowground organisms when evaluating the impacts of plant invasions.

Keywords: aboveground insect herbivores, soil nematodes, trophic groups, plant invasions, temporal change

摘要：入侵植物往往可以影响土壤线虫的群落结构。然而，入侵植物在入侵地并未完全逃逸地上植食性昆虫的取食危害，而我们对昆虫取食是否会调节入侵植物对土壤线虫群落的影响还知之甚少。在本研究中，我们探讨了地上植食性昆虫取食危害如何调节入侵植物空心莲子草(*Alternanthera philoxeroides*)对土壤线虫群落的影响，并研究了昆虫调节作用的时间效应。我们开展温室实验，对盆栽的空心莲子草施加三种不同的昆虫取食处理[无昆虫取食、专食性昆虫莲草直胸跳甲(*Agasicles hygrophila*)取食、广食性昆虫虾钳菜披龟甲(*Cassida piperata*)取食]，并在移除所有植食性昆虫后的第1、10和20天测定土壤线虫的群落结构。此外，我们还测定了空心莲子草根系及根系分泌物的碳含量。研究发现，在植食性昆虫被移除的第1天，昆虫取食处理显著提高了空心莲子草根围土壤线虫的丰度，特别是植食性线虫。然而，随着时间推移，这种影响逐渐减退，并在后期(第10和20天)完全消失。此外，专食性昆虫莲草直胸跳甲和广食性昆虫虾钳菜披龟甲的取食危害对土壤线虫丰度的影响并无显著性差异。综上所述，本研究表明地上植食性昆虫的取食危害可以改变入侵植物对土壤线虫群落的影响，但昆虫的调节作用是短暂的。此外，我们的研究强调了在评估入侵植物的影响时，需要同时考虑地上和地下生物群落。

关键词：地上植食性昆虫，土壤线虫，功能群，植物入侵，时间效应

INTRODUCTION

Plant invasions can have harmful, even devastating, influences on the local biotic communities and ecosystems, and as such they are considered a major threat to native biodiversity around the world (Charles and Dukes 2007; Vilà *et al.* 2011). Numerous studies have focused on the effects of invasive plants on aboveground biotic components, such as plants, insects, pollinators and even herbivores at higher trophic levels (Bartomeus *et al.* 2008; Bezemer *et al.* 2014; Hejda *et al.* 2009; Maerz *et al.* 2005). However, how invasive plants affect the soil biota is poorly understood, particularly for soil nematodes, which are ubiquitous in the soil environment and are an important part of soil ecosystems (Bongers and Bongers 1998; Ferris *et al.* 2001; Zhao *et al.* 2015). Studies on the impacts of plant invasions on soil nematodes may provide a novel perspective from belowground communities for better understanding and exploring the mechanisms of plant invasions (Lu *et al.* 2018; Wolfe and Klironomos 2005), however, results of the existing limited studies are inconsistent, with either promoting (Čerevková *et al.* 2019; Qin *et al.* 2019) or inhibiting effects (Belnap *et al.* 2005; Lazzaro *et al.* 2018).

The effects of plants on soil nematodes are often regulated by aboveground herbivores in the natural environment (De Deyn *et al.* 2007) through regulation of plant carbon allocation, induction of root exudates and alteration of plant litter quality (Bardgett *et al.* 1998; Wondafrash *et al.* 2013). However, it is so far unknown about the effects of aboveground herbivores on soil nematode communities of invasive plants. Actually, invasive plants are also attacked by aboveground herbivores, either by specialists introduced into invaded sites as biological control agents (Lu *et al.* 2013) or by native generalists. Thus, it is crucial to take into consideration the effects of such herbivores when studying the effects of invasive plants on soil nematode communities (Bardgett and Wardle 2003). For example, Zhou *et al.* (2017) found that herbivory of the native moth *Laelia coenosa* on the invasive *Spartina alterniflora* significantly increased nematode abundance compared with the no-herbivory treatment. Since it was reported that the effects of plants on soil nematodes could decrease with the trophic level of nematodes in the soil food web (Morrien *et al.* 2012), the plant feeder (PF) nematodes were expected to be most affected by aboveground herbivory. Furthermore, many studies have demonstrated that the regulations and inductions of specialists and generalists on physiological indicators and chemical defenses of invasive plants are different (Abhilasha and Joshi 2009; Ali and Agrawal 2012; Hull-Sanders *et al.* 2007), which may result in different effects on soil nematode communities through plant mediations. However, little is known about how aboveground specialists and generalists mediate the effects of invasive plants on soil nematode communities.

The effects of herbivores on plants are generally time dependent, with different response times and durations, something which has been widely demonstrated in various plant systems (Bergelson *et al.* 1986; Edwards *et al.* 1985; Karban 2011). Specifically, there is a time delay from the onset of herbivory attack to the induction of plant defenses, and these inductions are often unstable (Karban and Myers 1989). Moreover, plant defenses induced by herbivores are generally evident or strong only in the initial response, ranging from dozens of minutes to several weeks, and then decline with time (Carroll and Hoffman 1980; Karban and Baldwin 1997). For example, Underwood (1998) showed that the systemic induced responses of soybeans (*Glycine max*: Fabaceae) to Mexican bean beetle (*Epilachna varivestis*: Coccinelidae) herbivory became evident 3 days after damage and then declined by 15 days. However, it remains unknown whether the effects of invasive plants mediated by insect herbivores on soil nematode communities exhibit such a temporal change. On the basis of the hypothesis that the impacts of aboveground herbivory on invasive plants can cascade through to soil nematodes, we expected that the herbivore-induced effects of invasive plants on soil nematodes would decrease with time.

In this study, we examined how aboveground insect herbivores mediated the effects of invasive plants on soil nematode communities, using the invasive plant *Alternanthera philoxeroides* (Amaranthaceae), as well as the specialist beetle *Agasicles hygrophila* (Coleoptera: Chrysomelidae) and generalist beetle *Cassida piperata* (Coleoptera: Cassididae) as the study system. *Alternanthera philoxeroides*, native to south America, was introduced into China in 1930s as horse fodder (Wu *et al.* 2019). In China, *A. philoxeroides* expanded its invaded area rapidly due to its strong adaptive capacity and reproduction (Lu *et al.* 2013; Ma 2001). For efficient biological control of *A. philoxeroides*, the specialist *A. hygrophila*, which is native to Argentina, South America (Sainty *et al.* 1997; Wang *et al.* 2018), was introduced into China in 1986 and released in many provinces (Wang *et al.* 2019). The native tortoise beetle *C. piperata*, which is a generalist insect herbivore feeding on Chenopodiaceae and Amaranthaceae (Chujo 1961), also feeds on *A. philoxeroides* because the beetle has expanded its realized host range since the introduction of *A. philoxeroides* (Dai *et al.* 2013). Thus, *A. philoxeroides* was faced herbivory pressure from both specialist and generalist in the introduced range. In this study, we addressed the following questions: (i) how do the effects of invasive *A. philoxeroides* on soil nematode communities change under the herbivory of the aboveground specialist *A. hygrophila* or the generalist *C. piperata*? (ii) how do these herbivore-induced effects of *A. philoxeroides* on soil nematode communities change with time after the removal of insect herbivores?

MATERIALS AND METHODS

Study organisms

Alternanthera philoxeroides is a perennial, amphibious herb, the stolon of which can grow up to 10 m long via clonal propagation (Dong *et al.* 2012; Sainty *et al.* 1997). In this study, *A. philoxeroides* was collected from local populations in terrestrial environments in Wuhan, China (30°32′ N, 114°25′ E). Plant stems were then cut into a number of segments, each with one internode, which were 3–5 mm in diameter and 5–8 cm in length, for the following experiments.

Agasicles hygrophila is a specialist insect on *A. philoxeroides*, with a generation time of about 25 days, allowing for eight generations per year (Ma 2001). *Cassida piperata* is a generalist insect native to China, with a generation time of about 32 days, allowing for four generations per year (Tang 1994). Except for the explosive phase, the leaf damage level of *A. philoxeroides* caused by the two insect herbivores is generally about 20–30%. Larvae and adults of *A. hygrophila* and *C. piperata* were collected from the field in Wuhan and brought back to the greenhouse. They were separately reared in $1 \times 1 \times 1$ m³ mesh cages with five pots of *A. philoxeroides*. Active and healthy adults of the two insects were used for the experiments.

Greenhouse experiment

A greenhouse experiment was conducted at the Wuhan Botanical Garden, Chinese Academy of Sciences (30°32′36″ N, 114°25′11″ E) from May to August 2018. The experiment was established as a 3 × 3 fully factorial design incorporating three herbivory treatments (no insect herbivores, *A. hygrophila* and *C. piperata*) and three sampling dates (1st, 10th and 20th day after removing insects). There were six replicates for each combination and a total of 54 pots.

The cut stems of *A. philoxeroides* were separately transplanted into cylindrical pots (16 cm in diameter, 15 cm in height) with one stem per pot, filled with a homogenized mixture of 1/2 seedling substrate (Klasmann-Deilmann, Germany) and 1/2 top-soil. The top-soil was

collected from a local field where *A. philoxeroides* existed in order to ensure similar soil nematode communities in experimental pot soils, and the residual plant roots were removed. After three months of growth, plants were placed in $20 \times 20 \times 50$ cm³ mesh cages and insect herbivores were released inside cages. To obtain the intended 20–30% damage level for both plants caused by *A. hygrophila* and *C. piperata*, four to five adults of *A. hygrophila* or three to four adults of *C. piperata* were released on *A. philoxeroides*, and all the insects were removed after 3 days of feeding. The potting soils of each herbivory treatment were then sampled in three batches on the designated sampling dates, making sure that each pot was sampled only once. For each sampling, 200 g of fresh samples of the top 10 cm of soil in pots around the plant roots were collected and placed into a refrigerator (4°C) and held for nematode measurement. The plant individuals were also collected and taken back to the lab immediately for root exudate extraction, as well as the carbon content measurement of roots and root exudate.

Soil nematode measurement

To extract soil nematodes, subsamples of 100 g of fresh soil were manipulated using the shallow dish method (Mao *et al.* 2004). Briefly, for this procedure, a stainless-steel sieve tray (10 mesh) was custom-built and placed on a matching shallow dish. On the sieve tray, we put two layers of gauze and another layer of nematode filter paper. A 100 g subsample was spread evenly over the filter paper, and water was added into the shallow dish until the soil sample was submerged. After being placed at room temperature for 2 days, the water in the shallow dish was collected and then filtered through two stacked-500 mesh (30 µm) sieves. Most nematodes left on the surface of the sieves because they were too large to pass through these sieves. Then we washed them into a plastic petri dish with parallel horizontal lines at the bottom (convenient for counting). Using a dissecting microscope (BJP-200, Batuo, China), all the soil nematodes in the petri dish were counted.

To further measure the composition of the soil nematode trophic groups, we randomly collected 100 soil nematodes from every petri dish after nematode counting and placing them on slide mounts. For samples with fewer than 100 soil nematodes, all of the nematodes were processed in this manner. Using an optical microscope (CX31, Olympus, America), soil nematodes in the slides were identified under 100–400× magnification. Primarily, most of soil nematodes were identified to order level or some common families according to the distinct features of the head or mouth, esophageal bulb and tail. Then, these nematode were classified at the level of trophic groups— bacterivore (BF), fungivore (FF), PF and omnivore-predator (OP) (Yeates *et al.* 1993). For the remaining nematodes, the features of head (such as bristle or lip) or mouth (such as mouth cavity or tooth needle) were further identified for the classification of trophic groups. The abundance of each trophic group was expressed as the number of individuals of the trophic group per 100 g soil (nematode abundance × relative proportion of the trophic group).

To analyze the structure of the soil nematode community, the three ecological indices were used, including F/B, which is the ratio of fungivores to bacterivores; WI (Wasilewska index), which is the ratio of (bacterivores + fungivores) to plant feeders; and TD (trophic diversity), $TD = 1/\sum Pi^2$, where Pi is the relative proportion of each of the four trophic groups (Freckman and Ettema 1993; Wasilewska 1994).

Roots and root exudate measurement

Because roots and root exudate were reported to have potential effects on soil nematode communities (Bais *et al.* 2006; Bardgett *et al.* 1998), we determined the carbon content of roots and root exudate and examined whether they played an important role in herbivore-induced effects of plants on soil nematode communities.

Root exudate was collected according to the methods of Liu *et al.* (2013) and Chen *et al.* (2018). Before extracting root exudate, plant individuals collected in the greenhouse experiment were washed with tap water in order to wipe off residual soils attached to roots. Then, the roots of plant individuals were rinsed three times with distilled water. After cleaning the roots, individual plants were put in the 250 ml beakers, in which 200 ml distilled water were added to submerge the roots. After being placed at room temperature for 2 days, the distilled water in beakers were collected as extracting solutions of root exudate and sieved through a 9 mm filter paper using vacuum filtration (filter device, JTFA0203, Jinteng, China; vacuum pump, SHB-III, Greatwall, China). The filtered extracting solutions were then condensed to 10 ml using rotary evaporator (R-1001VN, Greatwall, China) and stored in the biomedical freezer (−20°C) for carbon determination. Total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC) of concentrated solutions were determined using TOC analyzer (Vario TOC, Elementar, Germany). Based on the concentration ratio, the measured concentrations of TC, TOC and TIC were converted into original concentrations.

After root exudate extraction, the plant roots were cut down, air-dried and weighed. Then, the plant roots were ground into powder using plant tissue ball mill instrument (Scientz-192, Xinzhi, China). TC of plant roots was determined using TOC analyzer (Vario TOC, Elementar, Germany).

Statistical analysis

To examine the effects of invasive *A. philoxeroides* under different herbivory treatments on soil nematode communities and how these effects changed with time, the abundance of soil nematodes and nematode trophic groups were analyzed using a generalized linear model with a negative binomial distribution, and ecological indices were analyzed using a linear model (LM). To examine the effects of different herbivory treatments on roots and root exudate of *A. philoxeroides* and how these effects changed with time, root dry weight and root TC, as well as TC, TOC and TIC of root exudate, were analyzed using LM. For all the analyses, the models included herbivory treatments (no insect herbivores, *C. piperata* and *A. hygrophila*) and sampling dates (1st, 10th and 20th day) as fixed factors. Then, two-way analysis of variance was conducted for each fitting model, followed by multiple comparisons using least square mean *post hoc* tests when there was a significant interactive effect, and *P*-values were adjusted using the false discovery rate (Benjamini and Hochberg 1995). All data analyses were performed with the statistical analysis software R 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) using 'lme4', 'car' and 'lsmeans' packages (Bates *et al.* 2015; Fox and Weisberg 2019; Lenth 2016).

RESULTS

Response of nematode abundance to herbivory treatment and sampling date

Variation of nematode abundance of *A. philoxeroides* was significantly affected by sampling date ($\chi^2 = 8.18$, $P = 0.017$) and the interaction of sampling date × herbivory treatment ($\chi^2 = 17.14$, $P = 0.002$), but not by herbivory treatment ($\chi^2 = 0.88$, $P = 0.645$). Specifically, in the absence of insect herbivores, the nematode abundance of *A. philoxeroides* increased with time (Fig. 1). Compared with the control treatment, nematode abundance was significantly increased on the 1st day when insect herbivores were present (Fig. 1). However, on the 10th and 20th day there was no significant difference in nematode abundance between the control and herbivory treatments. In addition, specialist *A. hygrophila* and generalist *C. piperata* always exhibited similar effects on nematode abundance of *A. philoxeroides* (Fig. 1).

Response of nematode trophic group abundance to herbivory treatment and sampling date

Abundance of BF and FF of *A. philoxeroides* depended on sampling date (χ^2 = 17.44, P < 0.001, Fig. 2a; χ^2 = 10.84, P = 0.004, Fig. 2b)
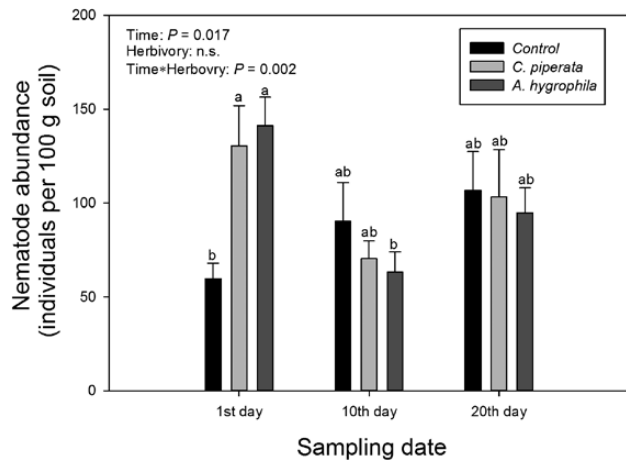

Figure 1: Variations of nematode abundance of *Alternanthera philoxeroides* under different herbivory treatments with time after removal of herbivores. All the results are the means ± SE (n = 6). The letters indicate the significant differences among all the combinations (sampling date × herbivory treatment).

and herbivory treatment (χ^2 = 8.58, P = 0.014, Fig. 2a; χ^2 = 6.29, P = 0.043, Fig. 2b), but were not affected by the interaction of sampling date × herbivory treatment. In contrast, abundance of PF of *A. philoxeroides* were significantly affected by sampling date (χ^2 = 8.18, P = 0.017, Fig. 2c) and the interaction of sampling date × herbivory treatment (χ^2 = 21.41, P < 0.001, Fig. 2c), but not by herbivory treatment. In addition, the abundance of OP responded only to herbivory treatment (χ^2 = 6.16, P = 0.046, Fig. 2d). With time, abundance of BF, FF and PF mainly exhibited decreasing trends. Moreover, the abundance of BF and FF under *A. hygrophila* herbivory treatment was higher than that under *C. piperata* herbivory treatment, whereas no significant difference was found for the abundance of OP between these two herbivory treatments. However, aboveground insect herbivores, both *A. hygrophila* and *C. piperata*, significantly increased the abundance of PF on the 1st day while they did not significantly affect the abundance of PF on the 10th and 20th day. Furthermore, the abundance of PF under the three herbivory treatments exhibited similar time-dependent trends with nematode abundance.

Response of nematode ecological indices to herbivory treatment and sampling date

F/B values responded to none of the factors (Fig. 3a), while WI values were significant affected by sampling date ($F_{2,45}$ = 6.36, P = 0.004), herbivory treatment ($F_{2,45}$ = 4.17, P = 0.022) and their interaction ($F_{2,45}$ = 3.53, P = 0.014) (Fig. 3b). In contrast, TD values were significantly affected by sampling date ($F_{2,45}$ = 3.69, P = 0.033)
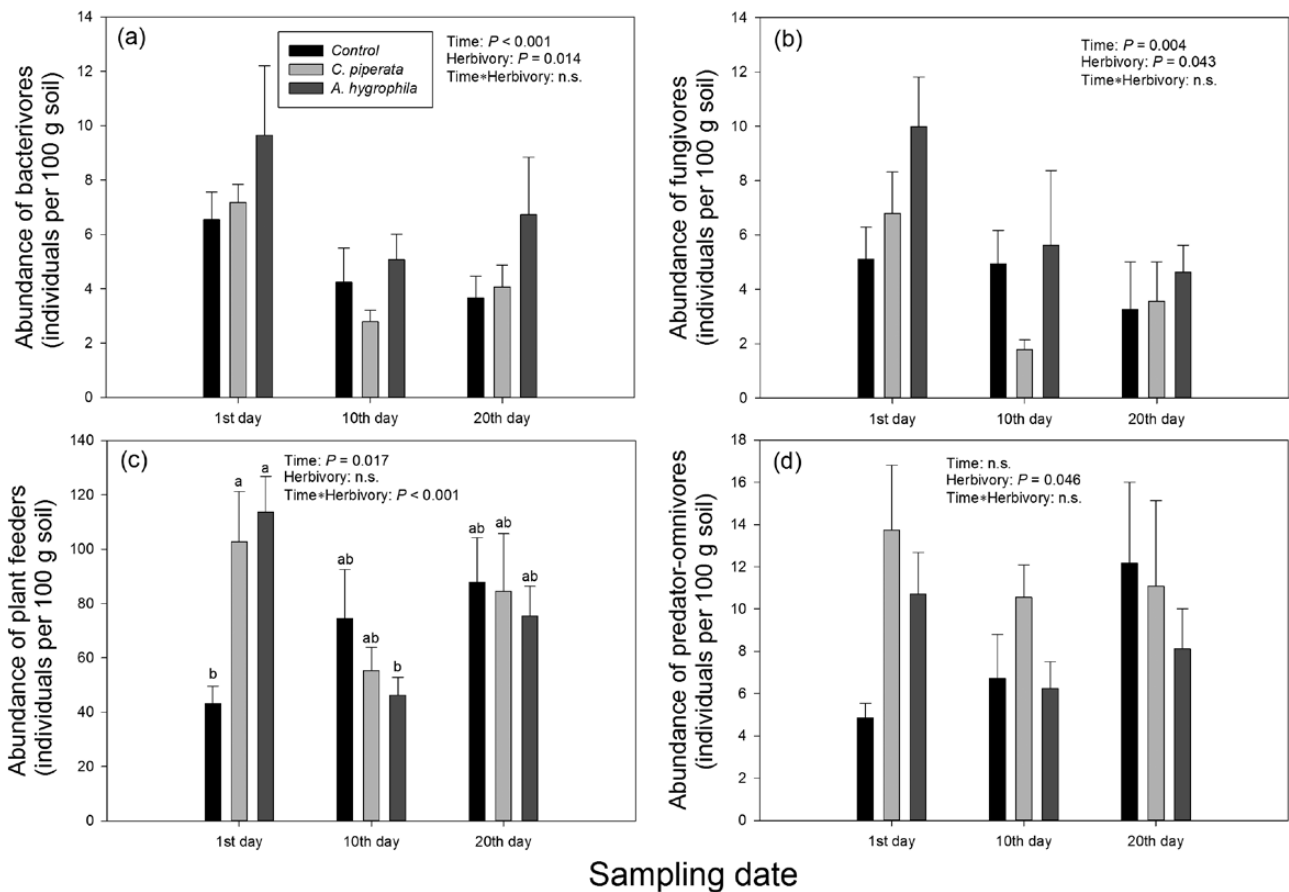

Figure 2: Variations of nematode trophic group abundance of *Alternanthera philoxeroides* under different herbivory treatments with time after removal of herbivores, including (**a**) bacterivores, (**b**) fungivores, (**c**) PFs and (**d**) OPs; n.s., non-significant. All the results are the means ± SE (n = 6). The letters indicate the significant differences among all the combinations (sampling date × herbivory treatment).

and the interaction of sampling date × herbivory treatment ($F_{2,45}$ = 3.93, P = 0.008), but not herbivory treatment (Fig. 3c). WI values mediated by the specialist were significantly higher than those mediated by the generalist. Moreover, both WI and TD values mainly exhibited decreasing trends with time. However, there was no significant difference for WI and TD values between different herbivory treatments on the same sampling date.
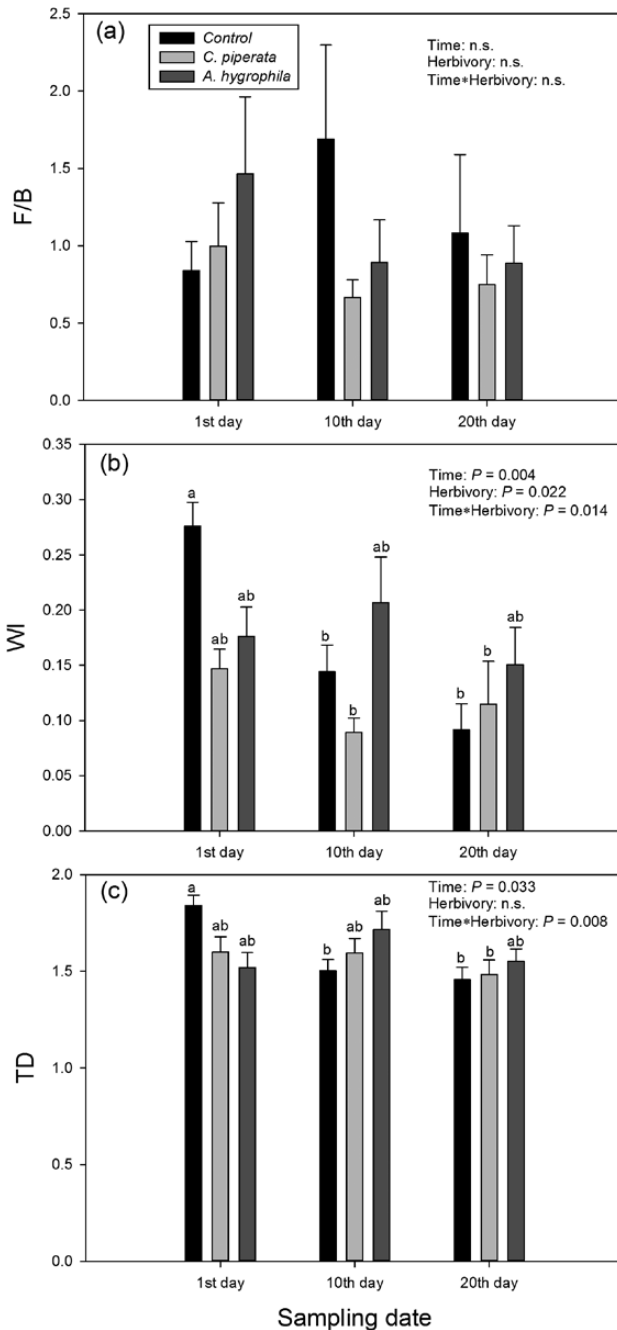
Response of carbon content of roots and root exudate to herbivory treatment and sampling date

The concentration of TC ($F_{2,45}$ = 3.22, P = 0.049), TOC ($F_{2,45}$ = 6.67, P = 0.003) and TIC ($F_{2,45}$ = 4.18, P = 0.022) of root exudate responded only to sampling date (Fig. 4). With time, the concentration of TC and TOC mainly exhibited decreasing trends, whereas the concentration of TIC mainly exhibited increasing trends.
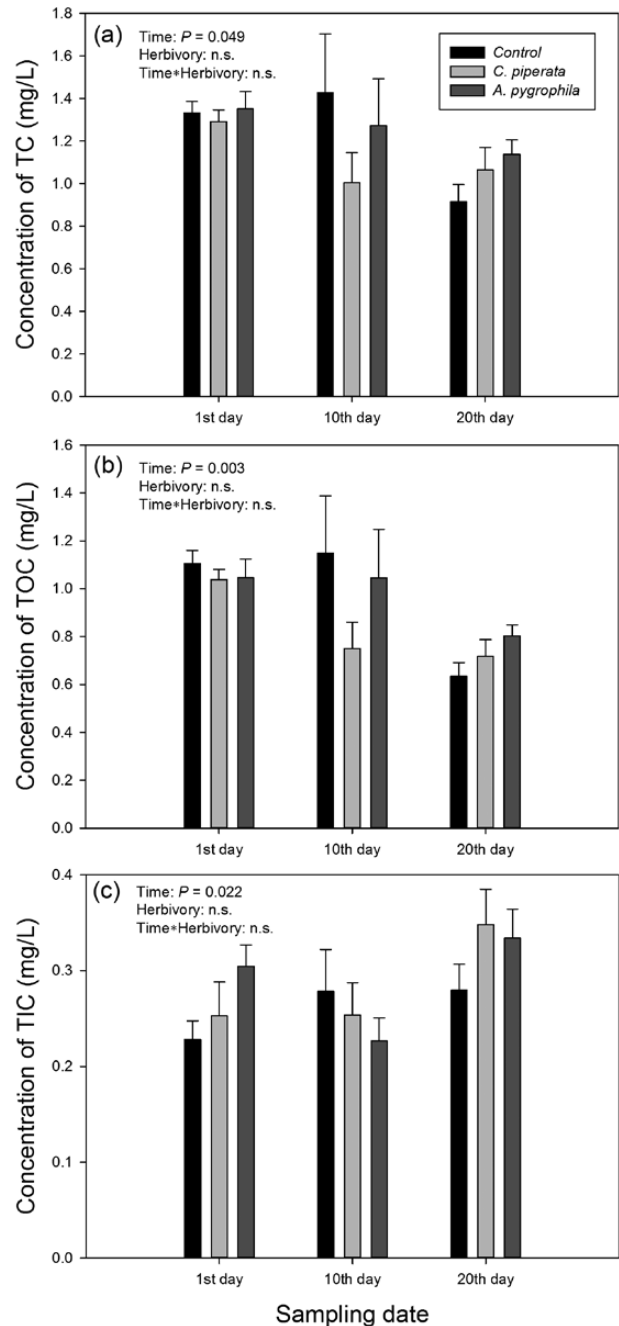

Figure 3: Variations of nematode ecological indices of *Alternanthera philoxeroides* under different herbivory treatments with time after removal of herbivores, including (**a**) F/B, (**b**) WI and (**c**) TD; n.s., non-significant. All the results are the means ± SE (*n* = 6). The letters indicate the significant differences among all the combinations (sampling date × herbivory treatment).

Figure 4: Variations of carbon content of *Alternanthera philoxeroides* root exudate under different herbivory treatments with time after removal of herbivores, including (**a**) TC, (**b**) TOC and (**c**) TIC; n.s., non-significant. All the results are the means ± SE (*n* = 6).
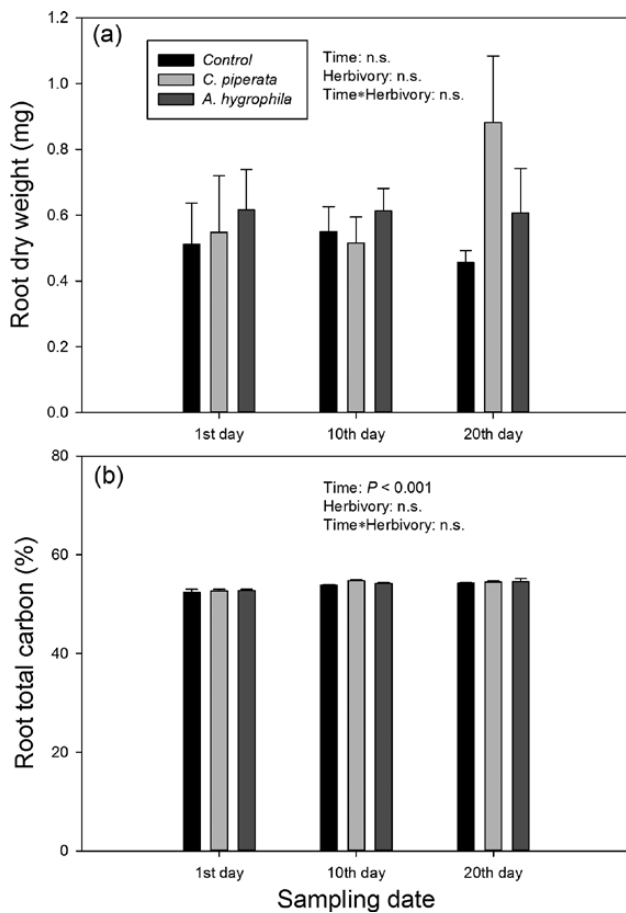
Figure 5: Variations of dry weight (**a**) and TC (**b**) of *Alternanthera philoxeroides* roots under different herbivory treatments with time after removal of herbivores; n.s., non-significant. All the results are the means ± SE (*n* = 6).

Root dry weight responded to none of the factors (Fig. 5a), while the TC of roots responded only to sampling date ($F_{2,45}$ = 24.69, $P < 0.001$) (Fig. 5b). With time, TC of roots mainly exhibited increasing trends.

DISCUSSION

Invasive plants have been reported to have the potential to alter soil nematode communities (Liang *et al.* 2007; Porazinska *et al.* 2014), and the interactions between plants and soil organisms are often regulated by aboveground herbivores (Bardgett and Wardle 2003; Bardgett *et al.* 1998). However, little is known about whether and how aboveground herbivores mediate the effects of invasive plants on soil nematode communities. Here, our results indicated that aboveground insect herbivores might have remarkable influences on the effects of *A. philoxeroides* on soil nematode communities, among which PFs might be most affected. In addition, these herbivore-elicited effects of *A. philoxeroides* on soil nematode communities decreased with time after the removal of herbivores.

The effects of *A. philoxeroides* on soil nematodes were significantly affected by aboveground insect herbivores, which corresponded with previously observed regulatory influences of herbivores on the interaction between plants and soil nematodes (Fu *et al.* 2001; Veen *et al.* 2010; Wardle *et al.* 2005). In our study, we found that nematode abundance was significantly increased in the first sample. This increase might be a result of reproduction and/or aggregation triggered by

herbivory-induced changes in plant roots or root exudates. Previous study showed that some soil nematodes may reproduce in 3 days (Liu and Zhang 2013; Moens and Vincx 2000). Alternatively, as a result of the aboveground herbivory, more soil nematodes might migrate to the top-soil layer with larger soil pore space for nematode aggregation (Briar *et al.* 2011). In addition, specialist *A. hygrophila* and the generalist *C. piperata* had similar regulatory influences. This result was also supported by Kaplan *et al.* (2008), who found that effects of generalist and specialist caterpillar species on the fecundity of nematodes under tobacco (*Nicotiana tabacum*) were equally strong. Many studies documented that the effects of induced defense in plants on nematodes were generally related to the specific metabolic substance—jasmonate (Cooper *et al.* 2005; Fujimoto *et al.* 2011; Hu *et al.* 2017; Nahar *et al.* 2011). Moreover, the specialist and generalist used in this study are chewing herbivores, most of which elicited plant resistance through jasmonic acid (JA) signaling pathway (Ali and Agrawal 2012). The effects of *A. hygrophila* and *C. piperata* on the induction of JA might be similar, which in turn led to the same impacts on soil nematodes. Therefore, inconsistent with our original assumption, our results suggested that the regulatory influences of aboveground insect herbivores cascading onto soil nematodes might be not dependent on insect host range, but other potential aspects. For instance, insect feeding guild was found to be an important factor for interactions between insects and nematodes mediated by plants (Kaplan *et al.* 2009).

Among nematode trophic groups, only PFs might be significantly affected by herbivory treatments on the 1st day, which suggests that herbivore-induced effects of plants on nematode trophic groups might depend on the trophic levels of nematodes, and the effects often waned with increasing trophic levels (De Deyn *et al.* 2007). Furthermore, both *A. hygrophila* and *C. piperata* herbivory treatments may enhance the abundance of PFs, which might explain the increase in nematode abundance of *A. philoxeroides* under herbivory. For example, Wardle *et al.* (2004) found that aphid species treatments had significant effects on the abundance of specific nematode trophic groups, thereby affecting total nematode abundance. Therefore, our results suggest that aboveground insect herbivores had potential effects in stimulating the enhancement of root-feeding nematodes of *A. philoxeroides*, which might lead to a cosuppression for *A. philoxeroides* through top-down control (Shao *et al.* 2017). Although our study did not focus on biocontrol of invasive plants, this result may indicate that the aboveground biocontrol insects could affect root-feeding nematodes to some extent. We acknowledge that, the approach of the classification of soil nematode trophic groups in this study was relatively limited as of uncertainties about the trophic group classification of some nematodes, particularly for some fungivores and PFs. Future studies identifying the soil nematodes at genus and even species level could help to provide more accurate classification of trophic groups and explicitly reveal the patterns of nematode abundance.

Aboveground insect herbivores resulted in significant variation in nematode abundance of invasive *A. philoxeroides* immediately (the 1st day), whereas this effect of insect herbivores, on both nematode abundance and the four nematode trophic groups, could finally vanish on the 10th and 20th day. These results indicate that the timing effect, which is found for effects of aboveground herbivores on plants (Carroll and Hoffman 1980; Karban and Baldwin 1997), also occurred for the herbivore-induced effects of invasive plants on soil nematode communities. More importantly, the decline of herbivore-induced effects on plants after the removal of herbivores might be the main factor causing the decrease over time of herbivore-induced effect of invasive plants on soil nematodes. In the absence of insect herbivores, *A. philoxeroides* stimulated increases in the abundance of nematodes with time. However, for *A. philoxeroides* which had experienced herbivory treatments, the enhanced nematode abundance reduced nematode abundance by the 10th day and then

increased it again on the 20th day, which suggests that *A. philoxeroides* might stimulate increasing abundance of nematodes more rapidly after herbivory. Except for OPs, the variation of the other three trophic groups might respond to sampling date. Moreover, the decrease in abundance of microbial-feeding nematodes and the increase in abundance of PF nematodes might lead to the decrease in the WI value, which also resulted partly in the decline of nematode TD. Among nematode trophic groups, the potential dominant PFs, which may exhibit similar variation trend with nematode abundance, might be the main contributor to variation in nematode abundance over time.

Roots and root exudate, which linked plants and soil microbe, were reported to play an important role in the interactions between plants and soil microbe (Bais *et al.* 2006; Meier *et al.* 2017; Sasse *et al.* 2018). This also provided the possibility for the effects of herbivory on plants cascading to soil nematodes. As Holland *et al.* (1996) argued, foliar herbivory might lead to the allocation of plant nutrient resources from aboveground to belowground, thereby making more resources available to soil microbial communities. In our results, however, no significant difference was found for carbon content of roots and root exudate of *A. philoxeroides* among different herbivory treatments on the 1st day. Therefore, we speculate that the secondary metabolites of roots and root exudate induced by aboveground insect herbivores might be possible factors influencing soil nematode communities, which also needs more in-depth studies in the future. Nevertheless, the variations in nematode trophic groups with time might correspond with the variations in carbon content of roots and root exudate. Specifically, the decrease in abundance of BF and FF with time might be associated with the concentrations of TC and TOC of root exudate, because root exudate could provide essential nutrients for bacteria and fungi which were also food sources of BF and FF (Meixia *et al.* 2017). In contrast, the increase of PF with time might be associated with the TC concentration of roots, because PF basically fed on roots.

In conclusion, our results indicate that aboveground insect herbivores exhibit significant effects on soil nematodes of the invasive plant *A. philoxeroides*, significantly increasing the abundance of soil nematodes, likely the PF nematodes. Moreover, the regulatory influences of specialist *A. hygrophila* and generalist *C. piperata* are similar. In addition, herbivory-elicited effects of *A. philoxeroides* on soil nematodes will fall into a decline with time. These results highlight the importance of aboveground herbivores in examining the effects of invasive plants on belowground biotic communities. Despite no evidence in our results showing that carbon content of roots and root exudate play an important role in the process of insect herbivores mediating the effects of plants on soil nematodes, variations in abundance of soil nematode communities with time maybe partly related to carbon content of roots and root exudate. However, further in-depth studies exploring the involved mechanisms through analyzing secondary metabolites of plant roots and root exudate are needed.

Authors' contribution

J.D. and J.L. conceived the idea. J.L. conducted the greenhouse experiment and performed lab measurements. J.L. and W.H. analyzed the data. J.L. wrote the manuscript. All authors revised and approved the manuscript.

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