

## LETTER

# Climate warming increases biological control agent impact on a non-target species

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### Abstract

Climate change may shift interactions of invasive plants, herbivorous insects and native plants, potentially affecting biological control efficacy and non-target effects on native species. Here, we show how climate warming affects impacts of a multivoltine introduced biocontrol beetle on the non-target native plant *Alternanthera sessilis* in China. In field surveys across a latitudinal gradient covering their full distributions, we found beetle damage on *A. sessilis* increased with rising temperature and plant life history changed from perennial to annual. Experiments showed that elevated temperature changed plant life history and increased insect overwintering, damage and impacts on seedling recruitment. These results suggest that warming can shift phenologies, increase non-target effect magnitude and increase non-target effect occurrence by beetle range expansion to additional areas where *A. sessilis* occurs. This study highlights the importance of understanding how climate change affects species interactions for future biological control of invasive species and conservation of native species.

### Keywords

Biological control, climate change, exotic insect, life history, native plant, non-target effect, range expansion, warming.

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## INTRODUCTION

Global climate change may facilitate alien species invasions, causing greater damage to natural ecosystems, agriculture and human society (Stachowicz *et al.* 2002; Walther *et al.* 2009; Chown *et al.* 2012; Sandel & Dangremond 2012). Thus, mitigating damage from biological invasions effectively with an environmentally friendly approach is essential for current and future biodiversity conservation. Introduction of coevolved natural enemies from an invasive species' home range (classical biological control) has been one of the key methods for suppressing invasive species (McFadyen 1998; Moran *et al.* 2005; Messing & Wright 2006). Although host specificity tests of natural enemies are required worldwide before release, insects introduced for biocontrol of invasive plants may negatively affect native plants due to potential host shifts and/or host range expansion (Louda *et al.* 1997, 2003; Van Klinken & Edwards 2002; Messing & Wright 2006). For example, a Eurasian weevil, *Rhinocyllus conicus* (Frölich) deliberately introduced to control invasive *Carduus* spp. was found to heavily attack native thistles in North America (Louda *et al.* 1997). Moreover, these deliberate introductions of exotic insects may subsequently lead to direct or indirect cascading effects on native food webs and ecosystems (Henneman & Memmott 2001; Carvalheiro *et al.* 2008) and even affect human health (Pearson & Callaway 2006). Given climate change can shift interactions of invasive plants, herbivorous

insects and native species, studies of risks to non-target species from biocontrol agents under climate change are critical for future management of invasive species and conservation of native species (Simberloff 2012). Furthermore, such an 'invasive plant-introduced insect-native plant' system provides a valuable model to predict how climate change and novel biotic exchange from species range shifts and/or expansions will impact insect host use and plant interactions.

As one of the key factors affecting plants, insects and their interactions, climate has long been considered critical in the theory and application of biocontrol of invasive species. For example, when selecting locations for field surveys of specialist insects in their native ranges, climate similarity with the range where insects are to be introduced for biocontrol is a key criterion (Sands & Harley 1980; Cameron *et al.* 1993). Moreover, insect and invasive plant phenology must also be considered, since success in biocontrol requires good synchrony between herbivory and plant growth (or reproduction) (Raghu *et al.* 2006). On the other hand, when assessing potential non-target effects of insects to native plants, mismatches in phenology and geographical distribution between biocontrol agents and potentially at-risk native plants may allow the insect to be introduced (Raghu *et al.* 2006; Pratt & Center 2012), as risk of contact in the field would be judged to be low. Therefore, climate variables, such as temperature, can influence both efficacy of the biocontrol agent (Ortega *et al.* 2012; Lu *et al.* 2013) and its safety to native species. Because

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range expansion to higher latitudes or elevations depending on a species' dispersal ability will be a key adaptive response of many species to global warming (Parmesan & Yohe 2003; Chen *et al.* 2011), warming may increase contact between biocontrol insects and populations of non-target plants previously isolated by high latitude or elevation. Also, since plant and insect phenology and life history are largely driven by temperature (Root *et al.* 2003; Cleland *et al.* 2007; Mitton & Ferrenberg 2012), change induced by global warming may shift interactions between insects and plants, potentially increasing risks of herbivory to native non-target species. To date, however, tests of these predictions and evidence from field studies are rare.

Since plant and insect species can undergo range shifts to higher latitudes in response to global warming (Buse *et al.* 2013; Cavanaugh *et al.* 2014), shifting interactions of insects and plants across latitudes may reflect the effects of temperature on both geographical ranges and interactions. Therefore, field data from latitudinal surveys, together with evidence from experimental temperature treatments are needed to unveil the impact of climate warming on host use of biocontrol insects on invasive and native plants. Here, we report impacts of climate warming on abundance and damage of an introduced beetle *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) on a non-target native host plant *Alternanthera sessilis* (L.) DC. (Amaranthaceae) in China. We conducted extensive field surveys across a latitudinal gradient from southern to northern China (Fig. 1) to assess the effects of climate on *A. hygrophila*'s occurrence and damage to *A. sessilis*. To predict responses of *A. sessilis*, *A. hygrophila*, and their interaction to elevated temperature, we conducted a 2-year field experiment near the northern limit of the beetle's distribution.

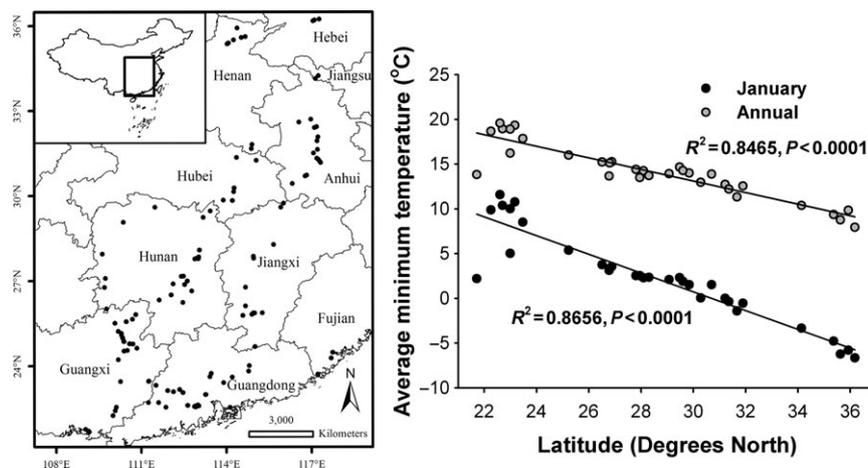
## MATERIALS AND METHODS

### Study species

Native to South America, *Alternanthera philoxeroides* is an amphibious herbaceous perennial that has invaded about 30 countries and it is predicted that its range will continue expanding in North America, Asia and Africa (Julien *et al.* 1995). Since its introduction into China in the 1930s, its range has rapidly expanded northward (Lu *et al.* 2013).

*Alternanthera sessilis*, an annual or perennial herb native to Asia, is found throughout the Old World tropics, tropical Africa, south-eastern Asia, south-eastern Australia and south-eastern USA (<http://www.issg.org>). It propagates from seeds or stem buds, and grows in both terrestrial and aquatic habitats, although *A. sessilis* rarely occurs in aquatic habitats in China.

*Agasicles hygrophila* has been widely used as a biocontrol agent for the management of *A. philoxeroides* in North America, Australia and Asia since late 1960s (Coulson 1977; Sainty *et al.* 1997; Ma 2001). Host specificity tests show that *A. hygrophila* is a specialist with a narrow host range in the genus *Alternanthera* (Wu *et al.* 1994). While it uses *A. philoxeroides* as its major host, laboratory tests and recent field surveys indicate that *A. sessilis* may also be able to physiologically support beetle development (Lu *et al.* 2010). Beetle adults and larvae feed on *A. philoxeroides* and *A. sessilis* leaves and stems, and third instar larvae pupate in stems (Fig. S1). It overwinters in living host stems as larvae, pupae and adults or as adults in living stems of host plant or the underlying soil surface (Liu *et al.* 2010). It passes through eight to nine generations per year in south China. It suppresses *A. philoxeroides* in aquatic habitats in China, USA and Australia but not in terrestrial habitats (Coulson 1977;



**Figure 1** Locations of the sites for the field surveys across China and temperatures along latitude transect. The field surveys carried out in April 2013 covered 33 sites and 313 quadrats and the August/September 2013 surveys covered 61 sites and 619 quadrats, and the July/August 2014 surveys covered 51 sites and 421 quadrats. Quadrat size:  $0.5 \times 0.5$  m. Both minimum temperature in January and average annual minimum temperature in 1960s–2010s decreased significantly with latitude (January,  $R^2 = 0.8611$ ,  $P < 0.0001$ ; annual,  $R^2 = 0.8413$ ,  $P < 0.0001$ ). Meteorological data around the sites were obtained from the official website of The National Meteorological Center of China (<http://www.nmc.gov.cn/>).

Sainty *et al.* 1997; Ma 2001). As the only biocontrol insect introduced into China, *A. hygrophila* was first released in 1987 in Kunming (24°23' N–26°22' N), Fuzhou (25°15' N–26°39' N), Changsha (27°51' N–28°40' N), Nanchang (28°09' N–29°11' N), Chongqing (28°10' N–32°13' N) and Yangzhou (32°15' N–33°25' N) (Ma 2001). But subsequent field surveys found that *A. hygrophila* could not establish populations in Changsha and northern regions due to cold winters in natural ecosystems, while artificial warming enabled it to establish populations in Changsha (Li *et al.* 1994), suggesting that its range is restricted by cold temperature at high latitudes in China as it is in the USA (Coulson 1977). Similarly, based on the beetle's distribution in its native South America, Julien *et al.* (1995) used CLIMEX and predicted that *A. hygrophila* could not establish in Changsha. However, a survey in 2012 showed the northern limit of its distribution in China has expanded from about 28° N to 31.8° N (Lu *et al.* 2013), coinciding with increased temperatures in the past decade. Since the 1980s China has experienced an increase in temperature of 0.352 °C and 0.548 °C per decade for maximum and minimum temperatures respectively (Zhou *et al.* 2004).

### Field surveys

To identify distributions of the focal species and the beetle's non-target attack, we conducted two field surveys along latitudinal gradients in terrestrial ecosystems in China in 2013 (13 to 28 April; 19 August to 28 September) and one field survey in 2014 (24 July to 5 August). We also conducted surveys in aquatic habitats in areas > 31° N in the second and third surveys to identify the beetle's northern limit.

We conducted the April 2013 survey at 34 random locations along a latitudinal transect from 22.5° N to 33.3° N (Fig. 1). We observed no herbivores other than *A. hygrophila* on defoliated *A. philoxeroides* or *A. sessilis*, suggesting that foliar damage was caused solely by *A. hygrophila*.

We conducted the August/September 2013 survey at 60 random locations from 21.6° N to 36.6° N (Fig. 1). During summer 2013, China experienced a heat wave. Some southern sites lacked beetles which might reflect this heat wave since the beetle is sensitive to heat shock (Zhao *et al.* 2009). We observed native insects, including *Cassida piperata* (Coleoptera: Cassididae) and *Hymenia curvalis* (Lepidoptera: Pyralidae), defoliating both plant species. We could not distinguish damage by *C. piperata* and *A. hygrophila*. We conducted another survey in July/August 2014 from 21.6° N to 32.7° N, covering the full range of the beetle. We surveyed all sites in August/September 2013 within this range.

Our field survey sites span from tropical to temperate regions. To investigate whether climate at these sites gets colder along the latitude transect from south to north, we obtained meteorological data for the past 50 years from the database accessed through the official website of The National Meteorological Center of China (<http://www.nmc.gov.cn/>). The minimum temperature in January and annual average minimum temperature around these sites decreased by 1.04 and 0.65 °C per degree of latitude (Fig. 1). Altitudes ranged from –2 m to 273 m, with 69% of sites below 100 m. Nearly

all the sites were located in open fields and received full sunlight.

### Survey data collection

We measured cover of *A. philoxeroides* and *A. sessilis* in both 2013 surveys. We counted *A. hygrophila* and other insects on and damage to each plant species separately in all three surveys. In each location, we chose 10 to 15 quadrats (0.5 m × 0.5 m, 2 m apart) along two or three 10 m transects (spaced > 3 m apart). For each quadrat, we counted *A. hygrophila* adults, larvae and eggs and other insects on each plant species and visually estimated plant cover (%) and defoliation (% leaf area removed) of the entire quadrat. Results generated by collecting and scanning leaves from subsamples showed that our visual defoliation estimates accurately represent damage levels in the field (for details see Fig. S2). In the July/August 2014 survey, we also counted the number of *A. hygrophila* emergence holes on *A. sessilis* stems. We collected undamaged leaves in each site for each plant species if available in the August/September 2013 survey. We dried leaves and measured nitrogen and carbon content with a C/N analyser (Vario MAX CN, Elementar Analysensysteme GmbH, Hanau, Germany).

### Survey analyses

We regressed *A. hygrophila*, *A. sessilis* and *A. philoxeroides* abundances and defoliation (plant species and surveys separately) against latitude. We regressed defoliation of *A. sessilis* (July/August 2014 survey) against minimum temperature in January and annual average minimum temperature (two simple regressions). We also regressed defoliation against plant abundance to test whether defoliation was affected by abundance. We did not analyse August/September 2013 defoliation data because there was indistinguishable damage from *C. piperata*. We used an ANCOVA including plant species (fixed factor) and latitude (covariate) to compare N and C/N ratio of leaves.

### Field experiment

To determine whether and how climate change might influence non-target effects at higher latitudes, we conducted a 2-year experiment in the field used by Lu *et al.* (2013) in Wuhan, China (30°32'44.5" N, 114°24'45.6" E), where the beetle usually cannot overwinter. By conducting the field experiment near the northern distribution boundary of the beetle, we could test effects of climate on both beetle occurrence and interactions among *A. hygrophila*, *A. sessilis* and *A. philoxeroides*. We mowed and hand-weeded the field and established twelve 3 m × 4 m plots (3 m apart) in May 2012.

The experiment was a split-plot design with warming as whole-plot factor, and plant composition (*A. philoxeroides*, *A. sessilis*, *A. philoxeroides* + *A. sessilis*) and herbivore treatments (beetles present or absent) as split-plot factors. We heated warming plots with MSR-2420 infrared radiators (1.65 m in length, Kalglo electronics, Bethlehem, PA, USA) for 24 h per day from 27 June 2012 to the end of the

experiment. We set heaters at a radiation output of 2000 W and suspended them at the height of 2.25 m at which the heater could warm an area of 3 m × 4 m evenly (Wan *et al.* 2009). In control plots, we used dummy heaters to control for shading effects. From July to September 2013, Wuhan experienced a heat wave, and we turned off the heaters. We monitored soil temperature and moisture at 10 cm depth in the centre of one randomly selected subplot during the trial as Lu *et al.* (2013). We recorded soil temperature in eight plots (four ambient and four warming) automatically with a data logger (YM-04, Handan Yimeng Electricity Company, Handan, China) every 10 min. Data indicate that heaters increased soil temperature at 10 cm depth by 2.50 °C in winter (December–February) and 1.10 °C in summer (June–August). Predicted temperature increases for Wuhan are 1.5 to 3 °C in winter and 1.5 to 2 °C in summer in 2065 (IPCC 2013). The increases in temperature were highly significant (repeated measures ANOVA controlling for plot:  $F_{1,6} = 59.03$ ,  $P = 0.0003$ ) but did not affect soil moisture (treatment:  $F_{1,4} = 0.08$ ,  $P = 0.7880$ ; treatment × time:  $F_{37,148} = 0.74$ ,  $P = 0.8557$ ) in the period when the heaters were turned on.

We established four 1.0 m × 1.0 m subplots (0.5 m apart) in each plot. We buried plastic plates (0.5 cm thick, 35 cm deep) to delineate subplots and exclude neighbouring plants. We randomly assigned each subplot to one of six treatment combinations: (1) undamaged control, *A. philoxeroides*; (2) herbivory, *A. philoxeroides*; (3) undamaged control, *A. sessilis*; (4) herbivory, *A. sessilis*; (5) undamaged control, *A. philoxeroides* + *A. sessilis* and (6) herbivory, *A. philoxeroides* + *A. sessilis*. Each plot received four of the six split-plot treatment combinations which caused the design to be unbalanced. We established a 0.5 m × 0.5 m quadrat in the centre of each subplot in which data were collected. We collected *A. sessilis* seeds in December 2011 and cut stems of *A. philoxeroides* in May 2012 in Wuhan. We propagated both species in a naturally lit, unheated greenhouse. For monocultures, we planted four similar-sized seedlings of *A. sessilis* or *A. philoxeroides* in quadrat corners on 27th June 2012; for mixed cultures, we planted two similar-sized seedlings of each species in opposing quadrat corners. We caged all subplots immediately with 1 m<sup>3</sup> nylon mesh cages. On 24th August 2012, we released two pairs of *A. hygrophila* into each subplot assigned to an herbivory treatment. These beetles were offspring of field-collected individuals from local populations. One week later, we found live beetles in each herbivory subplot, indicating that populations were established.

### Experiment data collection

In November 2012, we counted flowers on half of *A. sessilis* individuals in each subplot and counted seeds of 10 flowers per subplot. We returned seeds to subplots. We marked half of the individuals of each plant species in each subplot in January 2013 and counted total internodes (alive and dead). We counted dead internodes in March (early spring) for each marked individual to estimate internode winter survival rate. To test whether elevated temperature altered phenology and life history of *A. sessilis*, we counted germinated stem buds and seed-germinated seedlings of *A. sessilis* from 8 March to 28 May.

To estimate the impact of warming and plant species on insect populations, we counted beetle emergence holes on each plant species for each herbivory treatment subplot in late December 2012, when beetles had entered diapause. Then, we monitored beetle emergence from the first day we observed beetles (15 March) to 28 May, when we counted beetles on each plant species. In late November 2013, we counted emergence holes on each plant species again. These emergence holes were mostly on new shoots formed in 2013, as 2012 emergence holes had disappeared when old shoots decomposed.

### Experimental data analyses

We used repeated measures ANOVAs to test dependence of number of seedlings (four categorical time periods) and germinated buds (five time periods) of *A. sessilis* on warming (whole-plot), plant composition (in pure or mixed stands) (split-plot), herbivore presence (split-plot) and their interactions (split-plot). We square-root transformed number of seedlings to improve normality and reduce heterogeneity of variances. We conducted additional follow-up ANOVAs to examine herbivore effect on the number of seedlings in April 2013 per starting plant within a warming and plant composition treatment combination.

We used ANOVAs to test dependence of winter survival rates of internodes (# living internodes in March 2013 divided by total internodes in January 2013) on warming, plant composition, plant species nested in plant composition (split-plot), herbivore presence and their interactions. When significant interactive effects occurred, we examined differences among treatment combinations using adjusted means partial difference tests ( $P < 0.05$ ).

We used ANOVAs to test dependence of flower and seed number per plant of *A. sessilis* at the end of 2012 on warming, herbivore presence and their interaction with data for *A. sessilis* in pure stands.

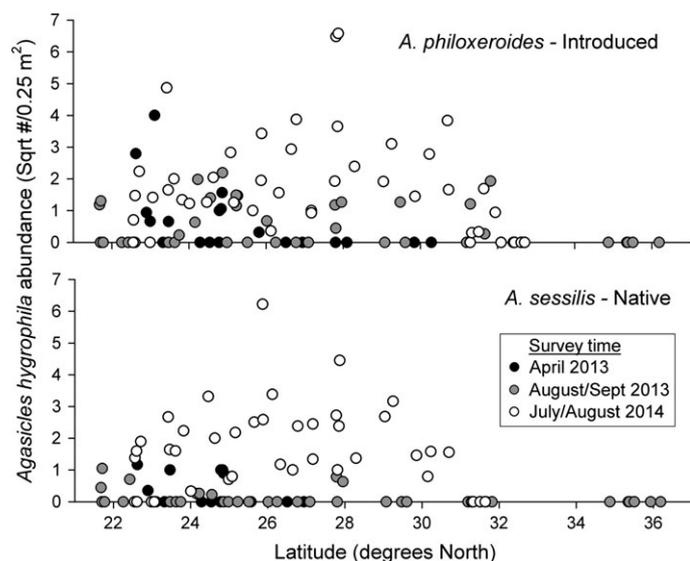
We used ANOVAs to test dependence of number of emergence holes per coverage area at the end of each growing season and used repeated measures ANOVA to test dependence of number of beetles (six time periods) on warming, plant composition, plant species and their interactions (A more detailed explanation of methods is available in Supporting information).

## RESULTS

### Field survey

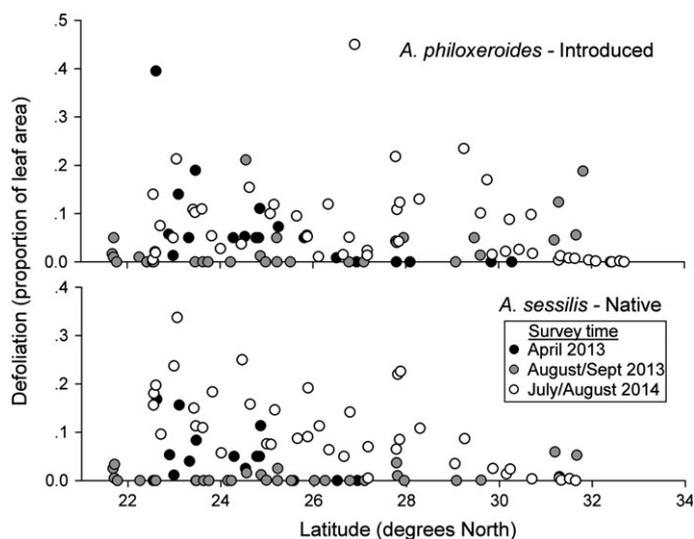
In April 2013, ranges of *A. hygrophila*, *A. sessilis* and *A. philoxeroides* overlapped up to 26° N beyond which no beetles were found (Figs 1 and 2). *Agasicles hygrophila* was the only species that defoliated both plant species. Beetle defoliation and plant relative abundance were positively related for *A. sessilis* ( $R^2 = 0.20$ ,  $P = 0.0104$ ), but not for *A. philoxeroides* ( $R^2 < 0.01$ ,  $P = 0.7663$ ). Between 25.8° N and 26.5° N, life history of *A. sessilis* changed from perennial to annual.

In August/September 2013, geographical ranges of *A. hygrophila*, *A. sessilis* and *A. philoxeroides* overlapped up to



**Figure 2** The abundance of the beetle *Agasicles hygrophila* on the target invasive plant *Alternanthera philoxeroides* and the non-target native plant *Alternanthera sessilis* during each survey.

31.4° N, and occurrence and feeding of *A. hygrophila* on *A. sessilis* were only detected below 27.9° N, north of which (up to 31.8° N) the beetle only fed on *A. philoxeroides* (Figs 1, 2 and 3). Geographical ranges of *A. sessilis* and *A. philoxeroides* overlapped up to 36.6° N (Fig. S3) where relative abundance of *A. sessilis* fit a U-curve ( $R^2 = 0.2847$ ,  $P = 0.0004$ ), with the lowest value between 27.2° N and 29.7° N (Fig. S4). Leaf N (species:  $F_{1,50} = 0.99$ ,  $P = 0.3248$ ; latitude:  $F_{1,50} = 3.52$ ,  $P = 0.0666$ ; species  $\times$  latitude:  $F_{1,50} = 0.80$ ,  $P = 0.3768$ ) and C/N (species:  $F_{1,50} = 0.66$ ,  $P = 0.4192$ ; lati-



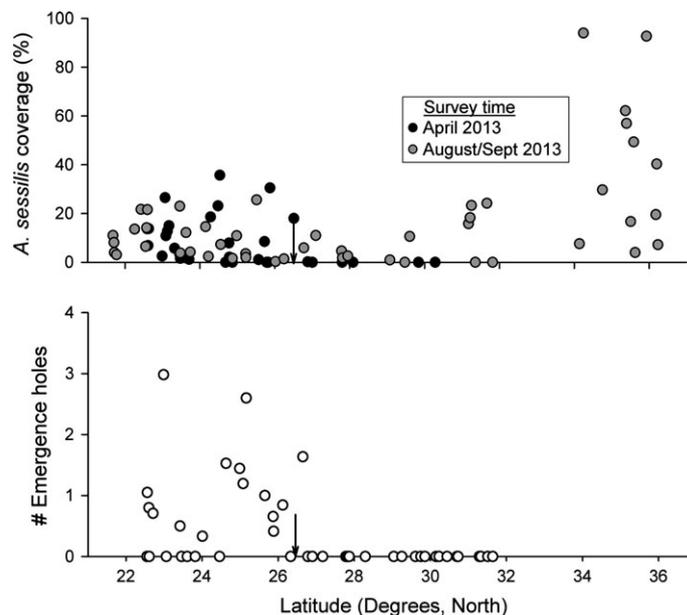
**Figure 3** Defoliation of the target invasive plant *Alternanthera philoxeroides* and the non-target native plant *Alternanthera sessilis* during each survey. During April 2013 and July/August 2014 field surveys, we only observed *Agasicles hygrophila* feeding and causing damage to either plant species; while in the August/September 2013 survey, the native insect species *Cassida piperata* and *Hymenia recurvalis* also attacked the two plant species.

tude:  $F_{1,50} = 2.70$ ,  $P = 0.1063$ ; species  $\times$  latitude:  $F_{1,50} = 0.43$ ,  $P = 0.5133$ ) did not differ between plant species along latitudes (Fig. S5). Other insects, such as *Hymenia recurvalis* and *Cassida piperata* also defoliated *A. sessilis* and *A. philoxeroides* in this survey.

In July/August 2014, geographical ranges of *A. hygrophila*, *A. sessilis* and *A. philoxeroides* overlapped up to 31.5° N, and occurrence and feeding of *A. hygrophila* on *A. sessilis* were only detected in the area below 30.7° N, north of which (up to 31.6° N) the beetle only occurred on *A. philoxeroides* (Figs 1, 2 and 3). We observed beetle emergence holes on *A. sessilis* stems up to 26.7° N (Fig. 4). Defoliation on *A. sessilis* decreased linearly with latitude ( $R^2 = 0.4438$ ,  $P < 0.0001$ ), and this pattern existed, regardless of *A. philoxeroides* presence in quadrats (Fig. S6). Defoliation on *A. sessilis* increased linearly with January minimum temperature (defoliation =  $4.49 + 1.08 \times \text{°C}$ ,  $R^2 = 0.26$ ,  $P = 0.0069$ ) and average annual minimum temperature (defoliation =  $-29.17 + 2.50 \times \text{°C}$ ,  $R^2 = 0.51$ ,  $P < 0.0001$ ). Beetle defoliation and plant abundance (coverage) were positively correlated for *A. sessilis* ( $R^2 = 0.18$ ,  $P = 0.0019$ ), but not for *A. philoxeroides* ( $R^2 = 0.03$ ,  $P = 0.1125$ ). Other herbivorous insects were rarely observed in this survey.

#### Field experiment

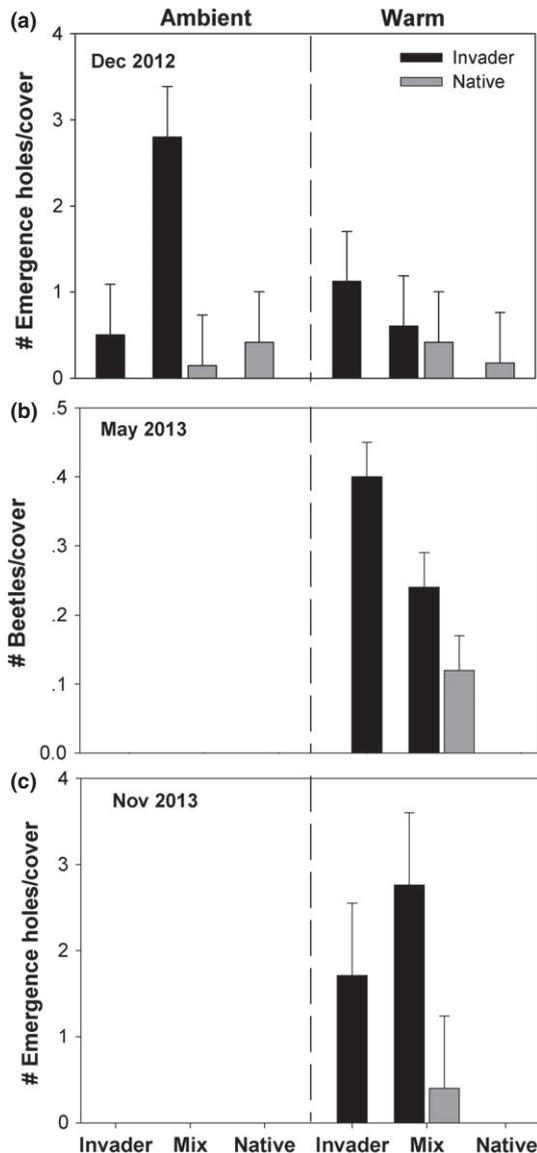
Warming increased beetle abundance on *A. sessilis* in mixed stands in both years (warming  $\times$  plant  $\times$  species,  $F_{1,14} = 49.90$ ,  $P < 0.0001$ , warming  $\times$  plant  $\times$  species  $\times$  time,  $F_{1,14} = 0.28$ ,  $P = 0.6045$ ). At the end of year 1, there were more emergence holes per area coverage on *A. philoxeroides* than on *A. sessilis* ( $F_{1,14} = 5.48$ ,  $P = 0.0346$ ) but there were no other



**Figure 4** Plant coverage of the native plant *Alternanthera sessilis* across latitudes during surveys conducted in 2013 and the number of *Agasicles hygrophila* beetle emergence holes in *A. sessilis* stems in the 2014 survey (square root number/0.25 m<sup>2</sup>). The arrow indicates the lowest latitudes at which *A. sessilis* populations with no perennial individuals were found.

significant effects (Fig. 5a). In year 2, beetles only emerged in warm plots with *A. philoxeroides*, indicating that elevated temperature improved its overwintering and *A. sessilis* was not a suitable overwintering host (Fig. 5b, c). From March to May 2013, beetle abundance did not differ on the two species (species,  $F_{1,6} = 0.30$ ,  $P = 0.6015$ , species  $\times$  time,  $F_{5,30} = 0.07$ ,  $P = 0.9956$ ) (Fig. S7).

Warming and herbivory interacted with plant species to affect internode survival rate (warming  $\times$  species:  $F_{1,38} = 37.22$ ,  $P < 0.0001$ ; herbivory  $\times$  species:  $F_{1,37} = 5.37$ ,  $P = 0.0260$ ; warming  $\times$  herbivory  $\times$  species:  $F_{1,38} = 3.95$ ,  $P = 0.0541$ ). Neither warming nor herbivory affected inter-



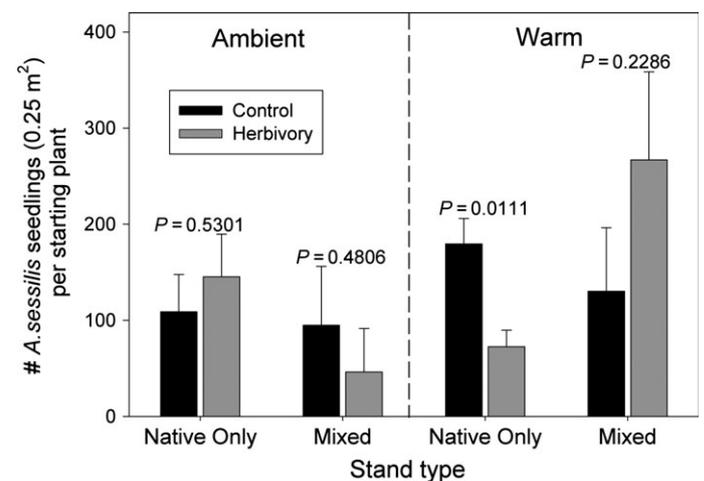
**Figure 5** *Agasicles hygrophila* beetle abundance on the non-target native plant *Alternanthera sessilis* and the target invader *Alternanthera philoxeroides* in mixed and pure stands under ambient and warming temperatures in the field experiment. (a) Number of emergence holes per cover (where 1 denotes 1 hole/0.25 m<sup>2</sup> at 100% coverage) in December 2012; (b) beetle (adults and larvae) abundance per cover on both plant species on 28 May 2013; (c) Number of emergence holes per cover in November 2013. Adjusted means + 1 SE.

node survival rate of *A. sessilis*; meanwhile, warming increased (mean  $\pm$  SE: 72.69  $\pm$  8.16% vs. 16.80  $\pm$  2.11%), but herbivory decreased (33.93  $\pm$  8.56% vs. 56.62  $\pm$  9.49%), internode survival rate of *A. philoxeroides* (Fig. S8). Warming changed *A. sessilis* life history from annual to perennial, corresponding to germinated overwintered buds only in warm plots in the spring (bud number, warming:  $F_{1,10} = 28.62$ ,  $P = 0.0003$ ; Fig. S9). In contrast, over the course of the experiment, warming had no significant effects on seedling number ( $F_{1,10} = 4.22$ ,  $P = 0.0670$ ) or seed germination time (warming  $\times$  time,  $F_{3,8} = 1.95$ ,  $P = 0.1288$ ; Fig. S10).

Warming increased *A. sessilis* flower production by 48.0% ( $F_{1,7} = 8.88$ ,  $P = 0.0205$ ), but did not affect seed number ( $F_{1,7} = 2.65$ ,  $P = 0.1473$ ) at the end of 2012. Number of flowers (314.9  $\pm$  53.4 vs. 472.7  $\pm$  83.6,  $F_{1,15} = 5.05$ ,  $P = 0.0744$ ) and seeds (2695.4  $\pm$  596.4 vs. 5516.0  $\pm$  1250.2,  $F_{1,14} = 5.33$ ,  $P = 0.0822$ ) per plant were lower on average with herbivory in pure stands at the end of the first year. Herbivory decreased native plant seedling recruitment in pure stands in the following spring under warming conditions, but had no impacts under other conditions (Fig. 6).

## DISCUSSION

Climate change can affect plant and insect fitness, phenology and geographical ranges and shift species interactions (Hegland *et al.* 2009; Yang & Rudolf 2009), potentially influencing the non-target effects of introduced biocontrol insects on native species and ecosystems (Simberloff 2012). To our knowledge, this work is the first study combining field surveys and experiments to examine how climate change impacts non-target effects of biocontrol introductions. In the field surveys, we found *A. hygrophila* damage on non-target *A. sessilis* increased along latitudes with rising temperature. In these regions with non-target effects, *A. hygrophila* and *A. sessilis* co-occurred with the target plant *A. philoxeroides*, while at



**Figure 6** Effect of the introduced *Agasicles hygrophila* beetle on number of seedlings (0.25 m<sup>2</sup>) in spring 2013 per starting plant of the non-target native species *Alternanthera sessilis* in different warming (warm or ambient temperatures) and plant composition (in pure or mixed stands) treatments in the field experiment. Adjusted means + 1 SE.  $P$ -values  $< 0.05$  shown above paired bars indicate a significant herbivory effects.

some higher latitudes both plant species existed but no beetles occurred, showing a spatial gap between the beetle and non-target host. Our warming experiment showed that elevated temperature allowed *A. hygrophila* to maintain populations by improving overwintering and increased beetle impacts on non-target seedling recruitment. Together, these results suggest that global warming may increase the magnitude of non-target effects of an introduced biocontrol agent on a native plant and expand these non-target effects to areas where at present it is too cold for the insect to overwinter.

Our study shows that herbivory on non-target plants in biocontrol may be intensified by global warming. Previous studies found insect population and damage on plants are enhanced by increases in temperature (Currano *et al.* 2008; Lemoine *et al.* 2013). Our recent study reported increasing *A. hygrophila* populations under warming conditions (Lu *et al.* 2013). In this study, we found increasing defoliation on *A. sessilis* along latitudes as climate was warmer, while the warming experiment showed *A. hygrophila* significantly reduced seedling recruitment in elevated temperatures, together indicating a negative impact of the insect will likely be higher under warming conditions. We expect that reproduction and population size of the native non-target plant may be impacted by this insect in the future especially for populations that do not co-occur closely with *A. philoxeroides* and so do not benefit from suppression of the host plant. Our results are perhaps the clearest evidence to date that warming temperatures have the potential to increase biocontrol impacts on non-target hosts.

Our study also suggests that warming-induced geographical range expansion of biocontrol insects will likely increase contact between the insect and the non-target plant. Our previous studies (Lu *et al.* 2013) and the results obtained from the warming experiment in this study show that elevated temperatures allow *A. hygrophila* to sustain populations in areas where at present it is too cold on average to overwinter. This indicates that with warming temperatures, exotic insects released as biocontrol agents, such as *A. hygrophila* may expand their geographical range to higher latitudes or altitudes where non-target plants currently grow, thus potentially increasing the chance of contact to native plant populations. Such warming-induced filling of geographical gap may lead to more native plant exposure to biocontrol agents and consequently increase risk of non-target effects as a result of host range expansion.

Like insects, many plants also respond to climate warming by shifting their geographical range and changing their life history and growth strategy (Parmesan & Yohe 2003; Root *et al.* 2003; Cleland *et al.* 2007), thus potentially affecting non-target effects of biocontrol agents. *A. sessilis* reproduces solely via seeds in areas  $> 26^{\circ}$  N in China; however, it is perennial at lower latitudes, with both seed-based and vegetative reproduction. The warming experiment indicated that plant nodes bearing dormant buds could overwinter and produce new shoots and plants in the following spring, suggesting that global warming will change its life history and reproduction mode. These responses may bridge the insect–plant geographical gap and potentially increase its exposure to the biocontrol insects. Moreover, the high number of adult emergence holes at low latitudes ( $< 26^{\circ}$  N; Fig. 4) indicates that

*A. sessilis* is a suitable host that meets requirements for pupation in these warmer areas likely due to *A. sessilis* being a perennial. In fact, plant nutritional quality of the two hosts seems similar as leaf nitrogen content and C/N did not differ between the two plant species or vary with latitude. Thus, in accordance with the prediction that climate can determine insect host range by influencing insect behaviours and plant availability and palatability (Peters *et al.* 2006; Braschler & Hill 2007) and shifting synchrony (Liu *et al.* 2011), host suitability of non-target native plants in biocontrol is also affected by climate.

In addition to these direct effects, climate warming may also indirectly influence insect impact on native species. Our experiment (Fig. 5) showed that *A. sessilis* is not a suitable overwintering host for *A. hygrophila* in some climate conditions which can sustain populations on *A. sessilis* only when it grows alongside *A. philoxeroides*. Thus, *A. philoxeroides* acts as an overwintering bridge host for the insect to spread onto *A. sessilis*. Since global warming could expand the range of *A. philoxeroides* and *A. hygrophila*, this ‘bridge species’ effect on *A. sessilis* may occur over larger areas, thus likely enlarging non-target effects in the future. However, the ‘net effect’ of warming in locations where *A. sessilis* and *A. philoxeroides* grow close enough to each other to compete will depend on insect use of the non-target native plant vs. the invasive plant as well as the direct effects of warming on each plant species. Under some scenarios, native plants may benefit from the decreasing competition from the invaders even while being attacked by the insects. However, studies at multiple sites under climate manipulations may be required to accurately predict the net effects of warming on non-target plants.

Climate change and biological invasions are two major components of global change, significantly affecting species range, abundance and shaping biotic interactions. Many previous studies on shifting species interactions as a result of climate change and novel biotic exchange were model based (Yang & Rudolf 2009; Gilman *et al.* 2010), providing theoretical predictions. Our study, combining large-scale field data across latitudinal gradients and warming experimental results, together with our previous findings (Lu *et al.* 2013), explicitly shows that climate warming can expand the geographical range of the invasive plant, native congener and the introduced insect and affect insect host use. As the rates of their range expansion may differ in response to climate warming and the insect host use of the invasive and native plants varies across different climatic ranges, such shifts in the synchrony of species interactions could result in different effects on plant invasions and native species.

Our results also have significant implications for biocontrol theory and application. Since invasive species will continue to threaten native ecosystems, even more so under climate change (Walther *et al.* 2009; Chown *et al.* 2012; Sandel & Dangremond 2012), classical biocontrol is expected to play a more important role in fighting invasive species. Mismatches in phenology and in geographical range between natural enemies and non-target species have been considered to be important criteria when screening and introducing exotic biocontrol agents to reduce risk of damage to non-target species (Frick 1974; Sands & Harley 1980; Cameron *et al.* 1993; Pratt &

Center 2012). Range expansion of the introduced biocontrol insect and more native plant exposure induced by climate warming in this study indicate this classical methodology needs to be reformed under global climate change. Considering such changes in plants and insects and shifting interactions among these species will be necessary for screening future biocontrol agents and assessing their efficacy, lest the introduced natural enemies become new invasive species themselves. For biocontrol insects causing non-target effects, such as *R. conicus* (on thistles *Cirsium* spp.) (Louda *et al.* 1997), *Larinus planus* (Fabricius) (on thistles *Cirsium* spp.) (Louda & O'Brien 2002) and *Tyria jacobaeae* (on ragwort *Senecio* spp.) (McEvoy *et al.* 2012) that have been widely expanding their geographical ranges, investigation into the potential role of climate change may help unveil the current patterns and predict their future effects. In addition to invasive plants, the implications of our work may expand to other types of biocontrol programs, for example, introductions of predators or parasitoids to control insect pests. While the effects of these biocontrol introductions on communities and ecosystems have recently been reported (Henneman & Memmott 2001; Carvalheiro *et al.* 2008), potential shifting interactions induced by climate change and the cascading non-target effects should also be considered for those systems.

In summary, this study shows that in response to warming, biocontrol insects could expand their geographical range to impact more native host plants and also increase their negative effects on native plants. Furthermore, global warming may change the life history of native plants from annual to perennial, potentially leading to more exposure to biocontrol insects. Warming may directly alter interactions among invasive and native plants, and indirectly change impacts of herbivory and interactions of introduced biocontrol agents and native herbivores. These direct and indirect interactions of invasive and native host plants and insects will make predicting biocontrol efficacy and ecological effects more complicated and difficult under climate change. Thus, improving our understanding of how invasive plants, biocontrol agents and associated native species respond to climate change is critical for informing future management of biological invasions and conservation of native species.

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#### AUTHORSHIP

X.M.L and J.Q.D designed research; X.M.L., M.Y.H., H.W and X.S performed research; X.M.L, E.S and J.Q.D analysed data and X.M.L., J.Q.D. and E.S wrote the manuscript.

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