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# Effects of vegetative propagule pressure on the establishment of an introduced clonal plant, *Hydrocotyle vulgaris*

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Some introduced clonal plants spread mainly by vegetative (clonal) propagules due to the absence of sexual reproduction in the introduced range. Propagule pressure (i.e. total number of propagules) may affect the establishment and thus invasion success of introduced clonal plants, and such effects may also depend on habitat conditions. A greenhouse experiment with an introduced plant, *Hydrocotyle vulgaris* was conducted to investigate the role of propagule pressure on its invasion process. High (five ramets) or low (one ramet) propagule pressure was established either in bare soil or in an experimental plant community consisting of four grassland species. *H. vulgaris* produced more total biomass under high than under low propagule pressure in both habitat conditions. Interestingly, the size of the *H. vulgaris* individuals was smaller under high than under low propagule pressure in bare soil, whereas it did not differ between the two propagule pressure treatments in the grassland community. The results indicated that high propagule pressure can ensure the successful invasion in either the grass community or bare soil, and the shift in the intraspecific interaction of *H. vulgaris* from competition in the bare soil to facilitation in the grassland community may be a potential mechanism.

Some introduced plants have the potential to spread over large areas<sup>1</sup>, representing a risk to biodiversity<sup>2,3</sup> and the integrity of natural systems throughout the world<sup>4</sup>. Ideally, the risk of a species becoming invasive should be assessed before the species is introduced and released in a new region. For introduced plants that are released without a priori assessment, an evaluation of their risk of becoming invasive is also helpful to avoid future economic and ecological damage<sup>5</sup>.

Propagule pressure is the total number of individuals introduced at a given location<sup>6,7</sup>, and is one of the factors that affect plant invasion<sup>8–10</sup>. It has been proposed that with a sufficient propagule supply, few communities are likely to be free of invasion<sup>8,9</sup>. Increasing the availability of propagules of introduced plants raises the chances for the species to establish itself, persist, naturalize, spread and invade<sup>11,12</sup>. Such a positive relationship between propagule pressure and invasion success of introduced plants was theoretically predicted by Richter-Dyn<sup>13</sup> and proved by findings of several experimental studies<sup>11,14,15</sup>. In a meta-analytical study, Colautti *et al.* (2006) showed that, for both analyses of invasiveness and invasibility, the majority of studies showed a positive association between propagule pressure and invasion success<sup>14</sup>. Therefore, assessing the intensity of the propagule pressure for a given species is a critically important step to evaluate the invasion risk of introduced exotic species<sup>16,17</sup>.

The effects of propagule pressure on establishment success of introduced plants may depend on habitat conditions<sup>11,12,18</sup>. In habitats where vegetation is dense and interspecific competition intensity is high, introduced species need a large amount of propagules to ensure establishment success in the local region. In contrast, in habitats where vegetation is scarce and much space is available, introduced species may need only a few propagules to maintain the same performance as that in the habitats with dense vegetation<sup>12</sup>. However, relatively few studies have addressed how habitat conditions affect the role of propagule pressure on invasion success of introduced species<sup>18–20</sup>.

*Hydrocotyle vulgaris* was introduced in China in the 1990s as an ornamental plant and now considered to be of potential invasiveness<sup>21</sup>. The species can grow in both aquatic and terrestrial habitats and spread quickly by clonal growth. To test the effects of habitat conditions and propagule pressure on its invasion success, we conducted a greenhouse experiment in which either one ramet (low vegetative propagule pressure) or five ramets (high vegetative propagule pressure) of the introduced clonal plant *H. vulgaris* were grown in either bare soil (no

Table 1 | Effects of propagule pressure and habitat conditions on growth measures of *H. vulgaris* at the container level

Source of variation	Biomass <sup>1</sup>			Number of ramets			Leaf area <sup>1</sup>			Stolon length <sup>1</sup>		
	SS	$F_{1,16}$	<i>P</i>	SS	$F_{1,16}$	<i>P</i>	SS	$F_{1,16}$	<i>P</i>	SS	$F_{1,16}$	<i>P</i>
Propagule pressure (P)	8.4	40.9	<0.001	45505.8	22.7	<0.001	8.1	21.1	<0.001	7.0	62.1	<0.001
Habitat (H)	34.1	166.7	<0.001	264960.2	132.3	<0.001	60.5	157	<0.001	36.0	317.7	<0.001
P × H	2.8	13.7	0.002	15456.8	7.7	0.013	4.4	11.4	0.004	1.1	10	0.006
Error	3.3			32046			6.2			1.8		

<sup>1</sup>Ln transformed.

vegetation) or dense communities consisting of four grassland plant species that were *Agrostis stolonifera* L., *Festuca elata* K., *Lolium perenne* L. and *Poa pratensis* L. We also set up an additional control treatment, i.e. grassland communities that were not invaded by *H. vulgaris*. Using this model (microcosm) system, we specially addressed the following questions: (1) Does propagule pressure affect the invasion success of *H. vulgaris*? (2) Do habitat conditions affect the role of propagule pressure?

## Results

**The growth of *H. vulgaris*.** Both vegetative (clonal) propagule pressure and habitat conditions significantly affected total biomass, ramet number, leaf area and stolon length of *H. vulgaris* at the population (container) level (Table 1). The four growth measures were 48.1–95.7% higher when propagule pressure was high than when it was low (Figure 1; all  $P < 0.001$ ; explaining 10.2–17.3% of total variation). They were also 7.1–17.9 times higher in the bare soil than in the grassland community (Figure 1; all  $P < 0.001$ ; explaining 70.2–78.4% of total variation). There were significant interaction effects of propagule pressure by habitat conditions (Table 1,  $P = 0.002$ – $0.013$ ; explaining 2.3–5.8% of total variation), and the effect of propagule pressure on the growth of *H. vulgaris* was more significant in the grassland community than in the bare soil (Figure 1).

**Relative competition intensity (RCI) of *H. vulgaris*.** The values of intraspecific RCI of biomass, ramet number, leaf area and stolon

length were significantly lower in the grassland community than in the bare soil (Figure 2;  $P = 0.01$ – $0.001$ ). Notably, in the grassland community, the values of the intraspecific RCI were negative (Figure 2), suggesting that the intraspecific interactions between *H. vulgaris* individuals tended to be facilitation. The values of interspecific RCI of biomass, ramet number, stolon length and leaf area were significantly lower when propagule pressure was high than when it was low (Figure 3; All  $P < 0.01$ ), suggesting that interspecific competition between *H. vulgaris* and the grassland species became weaker when propagule pressure was higher.

**Responses of grassland communities and each species.** Total mass, ramet number or Shannon's diversity index of the grassland communities was not affected by the invasion of *H. vulgaris* (Figure 4; All  $P > 0.05$ ). Similarly, biomass or ramet number of *A. stolonifera*, *F. elata*, or *L. perenne* was not affected by the invasion of *H. vulgaris* (Figure 5A–C, E–G; All  $P > 0.05$ ). The invasion by *H. vulgaris*, regardless of propagule pressure, significantly decreased biomass and number of ramets of *P. pratensis* (Figure 5D, H; All  $P < 0.001$ ).

## Discussion

Propagule pressure determines the chance for introduced species to be released in new habitats. When propagules of introduced species have a high survival probability, propagule pressure may further determine the establishment success of these species, and thus become the primary control parameter for preventing invasions<sup>7,10</sup>.

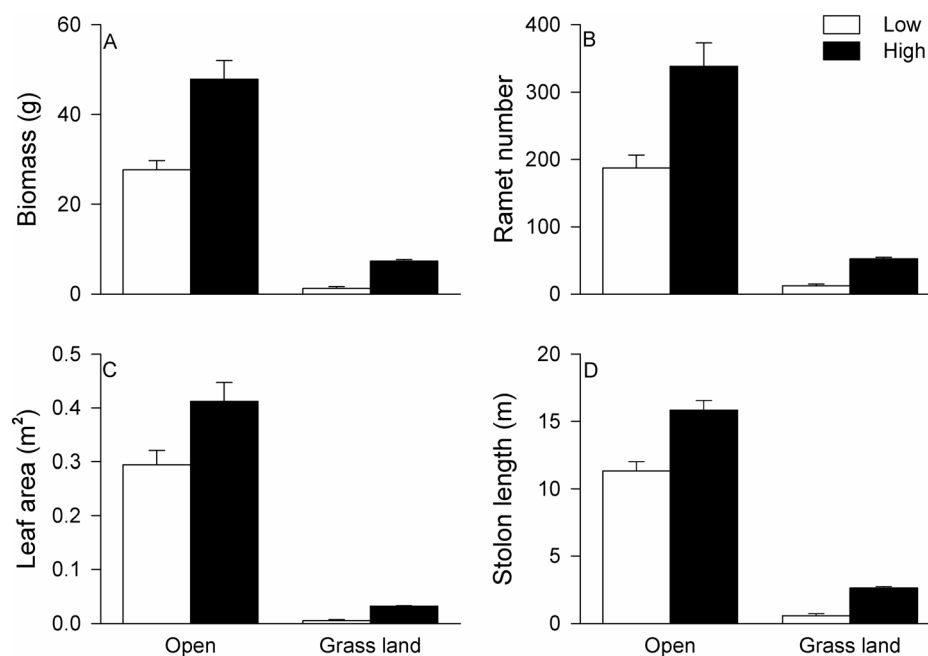
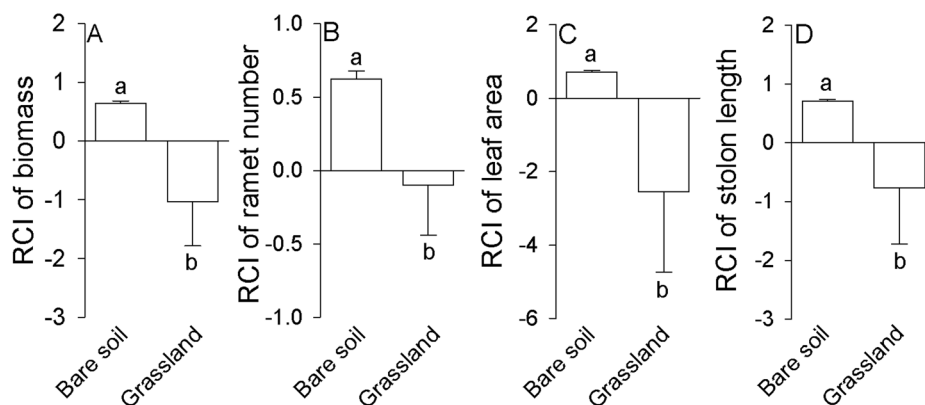


Figure 1 | Total biomass (A), ramet number (B), leaf area (C) and stolon length (D) of *H. vulgaris* with low (one ramet, open bars) or high propagule pressure (five ramets, filled bars) in bare soil and grassland community. Means + 1 SE are given.



**Figure 2** | Effects of habitat conditions on the intraspecific relative competition intensity of biomass (A), ramet number (B), leaf area (C) and stolon length (D). Bars having the same letter are not significantly different. Means + 1 SE are given.

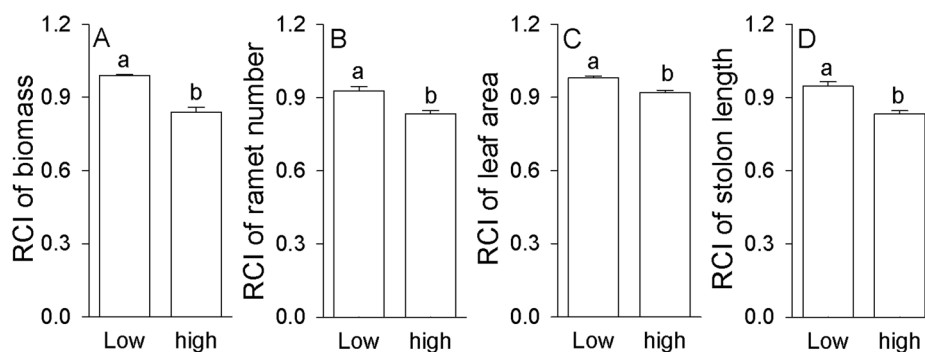
Our study showed that *H. vulgaris* with high propagule pressure grew better and thus had a higher probability to establish itself and invade than that with low propagule pressure in both the bare soil and the grassland communities. In the bare soil, high propagule pressure resulted in increased biomass and ramet production of the whole *H. vulgaris* population at the expense of the reduced growth of individual plants (Appendix figure 1) due to increased intraspecific competition (Figure 2). Interestingly, however, when *H. vulgaris* invaded the grassland community, high propagule pressure resulted in the enhanced growth of the whole *H. vulgaris* population (Figure 1) without sacrificing the growth of individual plants (Appendix figure 1). As a result, the role of propagule pressure in the invasion success of the whole *H. vulgaris* population seems to be more important in the grassland community than in the bare soil.

In the bare soil, there were only intraspecific interactions and no interspecific interactions. Under such a habitat condition, the intraspecific interaction of *H. vulgaris* was competition, as demonstrated by the positive values of relative competition intensity (RCI; Figure 2). In contrast, in the grassland community there were both intra- and inter-specific interactions (see, for example, Mangla, *et al.* 2011<sup>22</sup>). In such a habitat condition, the interspecific interaction on *H. vulgaris* was competition (Figure 3), whereas the intraspecific interaction tended to be facilitation, as indicated by the negative values of RCI (Figure 2). The shift in the intraspecific interaction of *H. vulgaris* from competition in the bare soil to facilitation in the grassland community was due to the presence of strong interspecific competition by the grassland species (Figure 3, Appendix fig 1), as shown by the large, positive values of RCI (0.8–1.0)<sup>23</sup>.

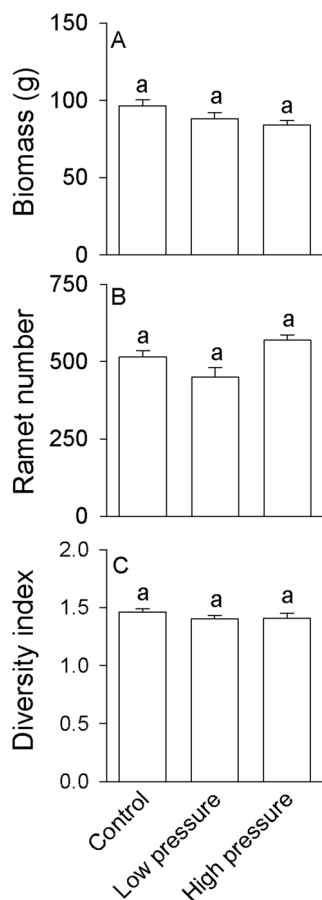
The different impact of propagule pressure in the two different habitats may be closely associated with the relative importance of intra- and interspecific interactions of *H. vulgaris*. When an intro-

duced plant species invades a grassland community, if the relative effect of intraspecific competition exceeds that of interspecific competition, then the benefit from high propagule pressure may be counteracted by the negative effect of intraspecific competition. Conversely, if the relative effect of intraspecific competition is similar to or lower than that of interspecific competition, or if the intraspecific interaction shifts to facilitation, then high propagule pressure will facilitate the invasion of the introduced species, as shown in the present study. With high propagule pressure, individuals of *H. vulgaris* may benefit from the suppressive effect of the whole *H. vulgaris* population posed on the grassland species, so that individual performance was better and the competitive effect from the grassland species was smaller than that with low propagule pressure (Figure 3, Appendix figure 1).

The performance of *H. vulgaris* was severely inhibited when it invaded the grassland community. Although one of the grassland species (*P. pratensis*) was negatively affected by the invasion of *H. vulgaris*, the overall growth or species diversity of the grassland community was not affected. These findings suggest that the invasion by *H. vulgaris* under the propagule pressure examined in this experiment may not greatly impact the local grassland communities. The population size of *H. vulgaris* did not reach or exceed a constant level, indicating that the presence of introduced plants may not cause more suppressive effects on the native plants, and thus *H. vulgaris* needed more time to accumulate enough propagules to establish a stable population after being introduced into a native community<sup>7,10</sup>. Another possible reason is that in the experiment the unbalanced competition design (i.e. the total initial number of grassland species in the community is prominently more than the initial number of *H. vulgaris*) could reduce the performance of *H. vulgaris*.



**Figure 3** | Effects of propagule pressure on the interspecific relative competition intensity of biomass (A), ramet number (B), leaf area (C) and stolon length (D). Bars having the same letter are not significantly different. Means + 1 SE are given.



**Figure 4** | Biomass (A), ramet number (B) and Shannon's diversity index (C) of the grassland communities not invaded by *H. vulgaris* (control), and invaded by *H. vulgaris* with low or high propagule pressure. Bars sharing the same letters are not significantly different. Means + 1 SE are given.

## Conclusions

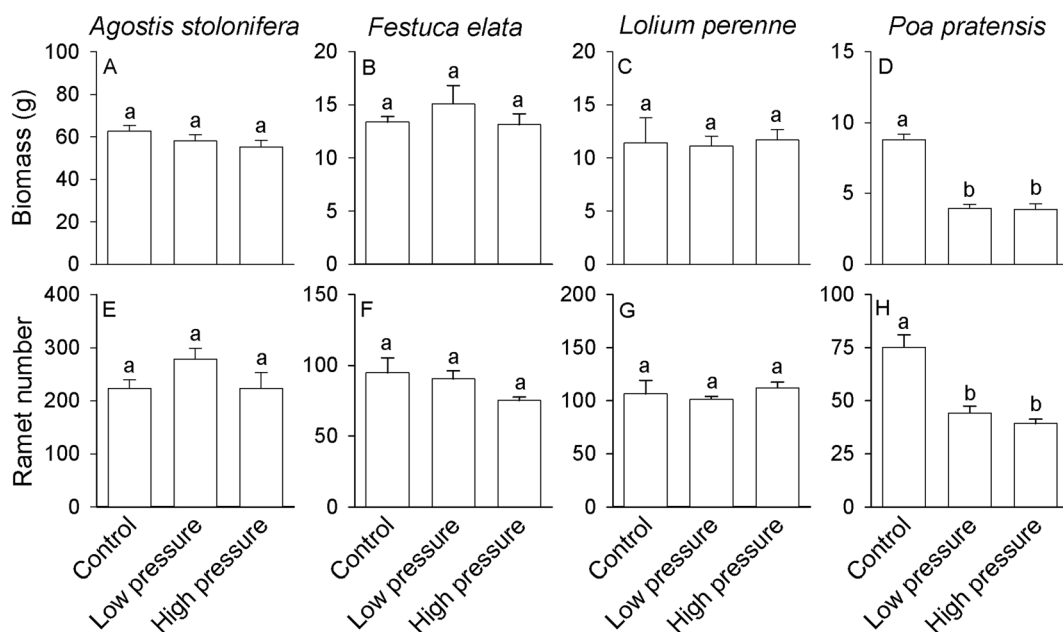
Using a model system, we shows that even a small difference in the number of vegetative (clonal) propagules may greatly impact the invasion success of introduced clonal plants, and such an effect also depends on habitat conditions. Besides the number of clonal propagules, the size of clonal propagules may also play an important role, which unfortunately was not assessed in this study. Therefore, further studies could be designed to explore the roles of propagule size and the interaction of propagule number and size to fully understand how vegetative (clonal) propagule pressure affects the invasion process of introduced species.

## Methods

**Ethics statement.** The experimental plants were obtained from a wasteland, so no permission was requested for the collection. The experiment was conducted in a greenhouse that was established by the research team, and thus no special permission was requested for the experiment. The experiment did not involve any endangered or protected species.

**The species.** *Hydrocotyle vulgaris* L. is a perennial herb of the Araliaceae family, and originates from Europe, where it is commonly distributed in moist habitats<sup>24</sup>. It can reproduce both sexually and clonally. *H. vulgaris* produces plagiotropic stems that grow either beneath or above the soil surface, elongating its petioles and positioning its leaves in better-lit places<sup>25</sup>. In this experiment, all stems were produced aboveground, so that they were referred as stolons. Each node along a stolon has the capacity of producing one petiolate leaf and adventitious roots<sup>26,27</sup>. Although *H. vulgaris* is in flower from March to October and produces plenty of seeds in the field, the species mainly relies on asexual reproduction via stolon fragments for spreading<sup>21</sup>. In China, the coverage of *H. vulgaris* enlarges recently<sup>21</sup>. Being an ornamental plant, *H. vulgaris* has already escaped from the aquarium trade and expanded widely into native plant communities by vegetative propagation.

**The experiment.** In the experiment, one (low propagule pressure) or five (high propagule pressure) ramets of *H. vulgaris* were grown in two habitat conditions (bare soil without plants or communities consisting of four grassland species). In the low pressure treatment, *H. vulgaris* was planted centrally in the pot. In the high pressure treatment, one ramet was planted centrally with the other four evenly distributed in a circle of 12.5 cm radius. There was also an additional treatment, which had a plant community that was not invaded by *H. vulgaris*. The four grassland species used were *Agrostis stolonifera* L., *Festuca elata* K., *Lolium perenne* L. and *Poa pratensis* L., and each grassland community was constructed in a pot (30 cm in height and 25 cm in diameter) that was initially planted with three plants of each of the four grassland species. The 12 individuals of the four grasses were evenly interspersed in each pot so that consistency between pots was ensured. All the four grassland species are native and widely distributed in



**Figure 5** | Biomass (A–D) and ramet number (E–H) of each of the four species in the grassland communities not invaded by *H. vulgaris* (control), and invaded by *H. vulgaris* with low or high propagule pressure. Bars sharing the same letters are not significantly different. Means + 1 SE are given.





China<sup>28</sup>. They are also components of natural or man-made grassland communities, which can be potentially invaded by *H. vulgaris*.

On 19 May 2011, plants of *H. vulgaris* were collected from a wasteland in Hangzhou, Zhejiang province, China, and then cultivated in a greenhouse at Forest Science Co. Ltd. of Beijing Forestry University.

On 16 November 2011, about 300 seeds of each of the four grass species were sown in planting trays filled with peat (Pindstrup Seeding; Pindstrup Mosebrug A/S, Pindstrup, Denmark). On 21 December 2011, three plants of each grass were transplanted in the experimental pots filled to a depth of 20 cm by a 1:1 (v:v) mixture of sand and peat, plus 2 g/l slow-release fertilizer (15N-11P-13K, Osmocote, the Scotts Company, USA). The initial heights of *A. stolonifera*, *F. elata*, *L. perenne* and *P. pratensis* were  $14.75 \pm 0.73$  cm,  $16.08 \pm 0.28$  cm,  $16.25 \pm 0.80$  cm and  $12.50 \pm 0.66$  cm (Mean  $\pm$  SE,  $N=6$ ), respectively.

On 28 December 2011, 60 *H. vulgaris* ramets were selected and used for the experiment; each had one node, one petiole leaf ( $16.10 \pm 0.12$  cm in petiole length, Mean  $\pm$  SE,  $N=6$ ) and some adventitious roots. Of the 60 ramets, half were grown in the bare soil and the other half in the communities.

The experiment was a block design and each block had five replicates. The experiment was conducted in a greenhouse in the Research Center for Eco-environmental Science of the Chinese Academy of Science in Beijing. It lasted for 15 weeks, from 28 December 2011 to 15 April 2012. Considering the day length changes during the experiment period, the natural light was supplemented by sodium light (3000 lux) to ensure a photoperiod of 14:10 h (day: night). The mean temperature and mean relative humidity were  $16.37 \pm 0.19^\circ\text{C}$  and  $53.01 \pm 1.14\%$  (Mean  $\pm$  SE). They were measured hourly by two Hygrochron temperature loggers (iButton DS1923; Maxim Integrated Products, USA). Tap water was supplied every three days to satisfy the demand of plant growth.

**Measurements.** At harvest, ramet number was counted and total stolon length and leaf area (obtained by WinFOLIA Pro 2004a, Regent Instruments, Inc., Québec, Canada) of each *H. vulgaris* plant were measured. Each plant was divided into petiole, leaf, stolon and root, and then weighed after drying at  $70^\circ\text{C}$  for 72 h. Biomass and ramet number of each of the four grass species in the community were measured as well.

**Data analysis.** Before analysis, we calculated total biomass, ramet number, stolon length and leaf area of *H. vulgaris* at the population (container) level. Total biomass, stolon length and leaf area were then logarithmically transformed to improve homogeneity of variance and normality. We also calculated total biomass, ramet number and Shannon diversity index of the grassland communities, and also calculated total biomass and ramet number of each of the four grassland species, respectively.

The intraspecific relative competition intensity (RCI) was calculated as intraspecific RCI =  $(B_{\text{low}} - B_{\text{high}})/B_{\text{low}}$ , where  $B_{\text{low}}$  is the mean growth measure of *H. vulgaris* in low propagule pressure and  $B_{\text{high}}$  is the mean growth measure of *H. vulgaris* in high propagule pressure<sup>29</sup>. The index was calculated for each block and averaged for comparison between the two habitat conditions (bare soil vs. grassland community) using t-tests. The interspecific RCI was calculated as interspecific RCI =  $(B_{\text{bare}} - B_{\text{grassland}})/B_{\text{bare}}$ , where  $B_{\text{bare}}$  is the mean growth measure of *H. vulgaris* in the bare soil and  $B_{\text{grassland}}$  is the mean growth measure of *H. vulgaris* in the grassland community<sup>29</sup>. T-tests were used to compare the differences between the low and high propagule pressure treatments. A positive value of RCI suggests competition and a negative one indicates facilitation<sup>30</sup>.

We used two-way ANOVA to test effects of propagule pressure and habitat conditions on the growth measures of *H. vulgaris*. We used one-way ANOVA followed by Student-Newman-Keuls tests to examine effects of the *H. vulgaris* invasion (control vs. low vs. high) on total biomass, ramet number and Shannon diversity index of the grassland communities, as well as total biomass and ramet number of each grassland species. We also employed non-parametric tests to compare the differences of intraspecific RCI between the two habitat conditions, and t-tests to compare the differences of interspecific RCI between the two propagule pressure treatments. All analyses were conducted using SPSS 16.0 (SPSS, Chicago, IL, USA). Effects were considered significant at  $P < 0.05$ .

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## Author contributions

R.H.L. and Q.W.C. devised the original concept, designed the experiment, discussed the interpretation of results and co-wrote the paper. B.C.D. and R.H.L. performed the experiments. R.H.L. and F.H.Y. analysed the data. All authors reviewed the manuscript.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

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