Soil heterogeneity affects ramet placement of *Hydrocotyle vulgaris*

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**Abstract**

**Aims**

Soil heterogeneity is common in natural habitats. It may trigger foraging responses (placing more ramets and/or roots in nutrient-rich patches than in nutrient-poor patches) and further affect the growth of plants. However, the impact of soil heterogeneity on competitive interactions has been little tested.

**Methods**

We conducted a greenhouse experiment to investigate the effects of soil heterogeneity on intraspecific competition with a stoloniferous herb *Hydrocotyle vulgaris*. We grew one (without competition) or nine ramets (with competition) of *H. vulgaris* under a homogeneous environment and two heterogeneous environments differing in patch size (large or small patches). In the heterogeneous treatment, the soil consisted of the same number of nutrient-rich and nutrient-poor patches arranged in a chessboard manner, and in the homogeneous treatment, the soil was an even mixture of the same amount of the nutrient-rich and the nutrient-poor soil.

**Important Findings**

Irrespective of intraspecific competition, *H. vulgaris* showed foraging responses to soil heterogeneity in the large patch treatment, e.g. it produced significantly more biomass, ramets, aboveground mass and root mass in the nutrient-rich patches than in the nutrient-poor patches. In the small patch treatment, foraging responses were observed when intraspecific competition was present, but responses were not observed when there was no competition. However, we find a significant effect of soil heterogeneity on neither overall growth nor competitive intensity of *H. vulgaris*. Our results suggest that foraging responses to soil heterogeneity may not necessarily be adaptive and intraspecific competition may not be influenced by soil heterogeneity.

**Keywords:** clonal plant, competition, environmental heterogeneity, foraging response, patch size, physiological integration

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**INTRODUCTION**

Spatial heterogeneity in soil nutrients potentially creates difficulty for plants to exploit soil resources (Gross et al. 1995; Jackson and Caldwell 1993a, 1993b; Lechowicz and Bell 1991). To efficiently capture heterogeneously distributed soil resources, clonal plants can show foraging responses, e.g. placing more nutrient-absorbing organs (e.g. roots or ramets) in high-quality patches than in low-quality ones (de Kroon et al. 2005; Gao et al. 2012; Hodge 2004; Hutchings and de Kroon 1994; Hutchings and John 2004; Peng et al. 2013). Meanwhile, physiological connection via stolons or rhizomes allows the transport of carbohydrates, water and minimal nutrients from ramets growing in high-quality patches to those in low-quality ones (Alpert and Stuefer 1997; He et al. 2010, 2011; Price and Marshall 1999; Stuefer 1996). A number of studies have revealed that physiological integration can contribute to the fitness of ramets growing in low-quality patches and further enhance the development of the whole plant (Hutchings and Wijesinghe 1997, 2008; see Song et al. 2013 for a review).

Soil heterogeneity is also an important factor influencing the outcome of competition between plants (Fransen et al. 2001; Janecek et al. 2004; Mommer et al. 2012; van der Waal et al. 2011). In heterogeneous environments, preferential ramet or root placements can cause overgrowth of ramets in high-quality patches and little growth of ramets in low-quality ones. In high-quality patches, competitive intensity
between ramets may be aggravated due to densely packed ramets and roots, and the reverse is the case in low-quality patches (Casper et al. 2000). Therefore, we predict that in heterogeneous environments intraspecific competitive interactions between the whole plants (genets) will be determined by interactions of ramets in both high- and low-quality patches (Bliss et al. 2002; Casper et al. 2000; Schwinning and Weiner 1998). Because the majority of biomass, ramets and roots may be produced in high-quality patches, we predict further that soil heterogeneity will intensify intraspecific competition of whole plants.

The spatial scale of soil heterogeneity can influence foraging responses of clonal plants, individual performance and outcome of competition (Day et al. 2003a; Fransen et al. 2001). If patch size is too small, clonal plants may be hard to sense soil heterogeneity and no effect will be shown (Hutchings and John 2004; Wijesinghe and Hutchings 1997, 1999). If patch size is too large, then plants in most cases will meet only one type of patches and effects of soil heterogeneity will be small (Hutchings et al. 2003). Only at some scales, preferential ramet and root placements can happen, and fitness and competitive interactions of clonal plants can be affected (Day et al. 2003a; Fransen et al. 2001).

To investigate the responses of clonal plants to soil heterogeneity and intraspecific competition, we conducted a greenhouse experiment with a stoloniferous, clonal plant Hydrocotyle vulgaris. We grew one (without intraspecific competition) or nine ramets (with competition) of H. vulgaris under a homogenous environment or two heterogeneous environments differing in patch scale (large patch and small patch). We predicted that (i) in heterogeneous environments, like many clonal plants, H. vulgaris can show foraging responses, i.e. it will place more ramets and biomass in the nutrient-rich patches than in the nutrient-poor ones, (ii) such foraging responses will depend on patch scale, (iii) soil heterogeneity will intensify intraspecific competition of H. vulgaris and (iv) competitive intensity will be affected by patch size.

MATERIALS AND METHODS

The species

Hydrocotyle vulgaris L. (Araliaceae), a perennial clonal herb, is widely distributed in tropical and temperate regions such as Southeast Asia, Europe and North America (Murphy et al. 1990). The species is introduced in China as a garden species in the 1990s and recently considered potentially invasive in China (Miao et al. 2011; Murphy et al. 1990). It can grow in a broad range of habitats, from semi-moist to wet conditions (Haslam 1987), and form large clones by producing plagiotropic stems, i.e. stolons (Miao et al. 2011). Each node along stolons has the potential to produce an independent ramet that consists of a simple leaf and adventitious roots. Hydrocotyle vulgaris produces flowers and fruits from May to August (Lacey and Herr 2005; Miao et al. 2011).

Plants of H. vulgaris were collected from a wetland (30°16′N; 120°05′E) in the suburb of Hangzhou in Zhejiang Province, China. They were propagated vegetatively in a greenhouse at Forest Science Co. Ltd of Beijing Forestry University in Beijing. We selected 320 ramets of H. vulgaris, each having a node, a leaf and a few roots. Of them, 150 ramets were used for the experiment and 20 ramets for the initial measurement. The average initial petiole length was 15.95 ± 0.97 cm (mean ± standard error, n = 20).

Experimental design

The experiment was a 2 × 3 factorial design, consisting of two intraspecific competition treatments (without or with intraspecific competition, i.e. each container was planted with one or nine ramets of H. vulgaris) and three heterogeneous treatments (homogeneous, heterogeneous with large patches or heterogeneous with small patches; Fig. 1). There were five replicates of containers (32 cm long × 32 cm wide × 18 cm deep) for each of the six treatments and thus 30 containers in total. Of the 30 containers, 10 (five with and five without competition) assigned to the large patch treatment were each divided into four large patches (each measuring 16 cm × 16 cm), 10 assigned to the small patch treatment were each divided into 16 small patches (each measuring 8 cm × 8 cm) and the remaining 10 were assigned to the homogeneous treatment. For the two heterogeneous treatments, each container had two types of patches arranged in a checkerboard pattern. In each container, half patches were filled to a depth of 15 cm with a mixture of sand and commercial compost (Meishimei Bio-Tech Co. Ltd, Beijing, China) at a 1:9 volume ratio (termed ‘nutrient-rich patches’; 8.70 mg total N g⁻¹ and 4.64 mg total P g⁻¹) and the other half with a mixture of sand and commercial compost at a 9:1 ratio (termed ‘nutrient-poor patches’; 0.79 mg total N g⁻¹ and 1.03 mg total P g⁻¹). For the homogeneous treatment, each container was filled with an even mixture of sand and commercial compost at a 1:1 volume ratio. The total amount of soil nutrients was the same in all treatments. There were no physical barriers between patches, and thus, plant roots could grow through different patches.

For the treatments without intraspecific competition, one ramet of H. vulgaris was grown in the center of each container (Fig. 1). For the treatments with intraspecific competition, nine ramets were grown in each container, positioned at the corners of nutrient patches in the heterogeneous, small patch treatment, and the corresponding places in the homogeneous treatment and in the heterogeneous, large patch treatment (Fig. 1).

The experiment was conducted from 1 August to 1 November 2012 in the greenhouse. During the experiment, the mean temperature and mean relative humidity in the greenhouse were 22.0°C and 73.9%, respectively (measured by iButton DS1923; Maxim Integrated Products, Sunnyvale, CA, USA). Water was added to the containers when the surface soil became dry. To avoid mass flow of water and thus soil nutrients between adjacent patches,
water was added slowly to the soil. During the experiment, the containers were randomly repositioned three times to avoid potential effects of environment patchiness in the greenhouse.

Measurements

At the end of the experiment, for the two heterogeneous treatments, we counted total number of ramets of *H. vulgaris* in nutrient-rich patches and nutrient-poor patches separately. The plants in the homogeneous treatment were harvested in a similar fashion, i.e. plants in the imagined, nutrient-rich patches (hereafter also referred to as ‘nutrient-rich patches’) and the imagined, nutrient-poor patches (hereafter also referred to as ‘nutrient-poor patches’) were harvested separately. The plants were then separated into petioles, leaf blades, stolons and roots, dried at 70°C for 72h and weighed. Total biomass was the sum of dry mass of petioles, leaf blades, stolons and roots; aboveground mass was the sum of dry mass of petioles, leaf blades and stolons.

Data analysis

We calculated biomass, number of ramets, aboveground mass and root mass per initial ramet of *H. vulgaris* in each container. We also calculated biomass, number of ramets, aboveground mass and root mass per initial ramet in nutrient-rich patches and nutrient-poor patches separately. These data (thereafter we omitted ‘per initial ramet’ for simplicity) were used in the following analyses.

We used two-way analysis of variance (ANOVA) to test the effects of soil heterogeneity (homogeneous vs. heterogeneous with large patches vs. heterogeneous with small patches) and intraspecific competition (with vs. without competition) on biomass, number of ramets, aboveground mass and root mass of *H. vulgaris* at the whole plant (container) level. We also employed ANOVA with repeated measures to test the effects of patch type (nutrient-rich patches vs. nutrient-poor patches), heterogeneity and intraspecific competition on the variables at the patch level (Roiloa and Retuerto 2006). In this model, patch type was treated as a repeated variable because the nutrient-rich and the nutrient-poor patches in each container were not independent (Roiloa and Retuerto 2006). Differences between nutrient-rich patches and nutrient-poor patches within each treatment were tested by paired *t*-tests and differences between soil heterogeneity within competition treatments at the patch level were tested by *post hoc* Tukey honest significant difference tests.

To measure the intraspecific competitive intensity, we calculated the log response ratio (LnRR) as $\text{LnRR} = \ln(B_o/B_w)$, where $B_o$ is the mean value of a growth measure (biomass, number of ramets, aboveground mass and root mass) per initial ramet without competition across the five replicate containers, and $B_w$ is the value of the corresponding growth measure per initial ramet with competition in each replicate container. Values of LnRR are symmetrical around zero (Armas et al. 2004; Hedges et al. 1999). Positive values indicate competition, negative values indicate facilitation and zero indicates neutral. We used one-way ANOVA to test the effect of soil heterogeneity on LnRR at the patch level and at the whole plant level, respectively. All analyses were conducted using SPSS 16.0 (SPSS, Chicago, IL, USA).
Table 1: ANOVA results for effects of soil heterogeneity (homogeneous vs. large patch vs. small patch) and intraspecific competition (without vs. with competition) on growth measures of *H. vulgaris* at the whole plant level

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Biomass</th>
<th>Number of ramets</th>
<th>Aboveground mass</th>
<th>Root mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>2.24</td>
<td>2.1*</td>
<td>2.2*</td>
<td>2.2*</td>
<td>0.6*</td>
</tr>
<tr>
<td>C</td>
<td>1.24</td>
<td>474.2*****</td>
<td>482.7****</td>
<td>472.0*****</td>
<td>202.1*****</td>
</tr>
<tr>
<td>H × C</td>
<td>2.24</td>
<td>1.4*</td>
<td>1.5*</td>
<td>1.5*</td>
<td>0.6*</td>
</tr>
</tbody>
</table>

Abbreviations: C = competition, H = heterogeneity. Significance levels: *****P < 0.0001 and *P ≥ 0.1 (not significant).

**RESULTS**

**Effects at the whole plant level**

We found a significant effect of soil heterogeneity on none of the four growth measures (biomass, number of ramets, aboveground mass or root mass) of *H. vulgaris* (Table 1, Fig. 2). Intraspecific competition dramatically decreased all growth measures of *H. vulgaris* (Table 1, Fig. 2). There were significant interactive effects between intraspecific competition and soil heterogeneity on none of the four growth measures (Table 1), and we did not find a significant effect of soil heterogeneity on the LnRR (Fig. 3). These results suggest that there is little effect of soil heterogeneity on intraspecific competition of *H. vulgaris* at the whole plant level.

**Effects at the patch level**

Patch type significantly affected biomass, aboveground mass and root mass (Table 2). There were interactive effects of patch type by soil heterogeneity on all the four measures (Table 2; significant effects of patch type × heterogeneity). Without competition, biomass, number of ramets and aboveground mass were greater in the nutrient-rich patches than in the nutrient-poor patches in the large patch treatment, but not in the homogeneous or the small patch treatment (Fig. 4a, c and e). With competition, biomass, number of ramets and aboveground mass were greater (P < 0.05) or tended to be greater (P < 0.1) in the nutrient-rich patches than in the nutrient-poor patches in both the small patch and the large patch treatments, but not in the homogeneous treatment (Fig. 4b, d and f). With competition, root mass was also significantly greater in the nutrient-rich than in the nutrient-poor patches in the small patch treatment (Fig. 4h).

Irrespective of competition, none of the four growth measures in the nutrient-rich patches in the large and small patch treatments differed significantly from that in the corresponding areas in the homogeneous treatment (Fig. 4). Similarly, there was a significant effect of soil heterogeneity on none of the growth measures in the nutrient-poor patches, except number of ramets (Fig. 4). Number of ramets in the nutrient-poor patches in the large patch treatment was significantly smaller than that in the corresponding areas in the homogeneous treatment (Fig. 4c and d).

There were significant effects of patch type × soil heterogeneity × intraspecific competition on none of the four growth measures except number of ramets (Table 2). Also, there was no significant effect of soil heterogeneity on the LnRR of ramets growing in nutrient-rich patches or in nutrient-poor patches (Fig. 5). These results suggest that there is little effect of soil heterogeneity on intraspecific competitive intensity of *H. vulgaris* at the patch level.

**DISCUSSION**

Irrespective of intraspecific competition, *H. vulgaris* showed foraging responses in the heterogeneous environment with large patches, i.e. it produced higher total mass, number of ramets and aboveground mass in the nutrient-rich patches than in the nutrient-poor ones. Also, such foraging responses existed in the heterogeneous, small patch treatment when intraspecific competition was present, but did not when there was no competition. Thus, our results partly support the first and second predictions, and suggest that foraging responses of *H. vulgaris* may depend on the spatial scale of soil heterogeneity (Wijesinghe and Hutchings 1997, 1999). These results also indicate that foraging responses of *H. vulgaris* in the small patch treatment can be triggered by the presence of intraspecific neighbors. The plausible reason can be the existence of a negative correlation between plant growth rate and foraging precision (Cahill et al. 2010; Campbell et al. 1991; Wijesinghe et al. 2001). If *H. vulgaris* grows alone, high ramet production rate (~62.6 ramets per week) may decrease its foraging precision. Because of such high ramet production rate, when growing alone, *H. vulgaris* may ignore the heterogeneous resource distribution in the small patch environment. On the other hand, if *H. vulgaris* grows with intraspecific neighbors, low ramet production rate (~8.0 ramets per week) caused by intraspecific competition may enable *H. vulgaris* to have a higher foraging precision in response to nutritious cues in the small patch environment (Cahill et al. 2010; Campbell et al. 1991; Wijesinghe et al. 2001).

Previous studies have shown that preferential ramet and root placements in nutrient-rich patches may greatly increase the efficiency and amount of resource capture of these ramets and further increase their local growth (Roloa and Retuerto 2006; Wang et al. 2012, 2013). Furthermore, the efficiency of resource capture by these ramets in nutrient-rich patches can also benefit growth of the whole plant, partly due to physiological integration (He et al. 2011; Song et al. 2013; Zhou et al. 2012). However, foraging responses of *H. vulgaris* to soil
nutrient heterogeneity did not increase the growth of ramets growing in nutrient-rich patches compared with that in the homogeneous treatment. Moreover, the growth of ramets growing in nutrient-poor patches was also little affected by soil heterogeneity. Consequently, the growth of the whole plants was statistically the same in the homogeneous and the heterogeneous treatments.

The positive effect of soil heterogeneity on plant growth may be transitory, and such effect may be eliminated if resource becomes limited (Casper and Cahill 1996; Day et al. 2003a; Peng et al. 2013; Roiloa and Retuerto 2006; Zhang and He 2009). For example, Day et al. (2003a, 2003b) reported that soil nutrient heterogeneity could increase the yield of the Cardamine hirsute population at the early stage of growth.
(in ~31 days), but not in the long run (in ~60 days). In this study, the density of newly produced ramets of *H. vulgaris* reached up to ~85.4 ramets dm$^{-2}$ at harvest, and in the container, nearly all space was overloaded. Due to the continuous increase of new ramets, the nutrient-rich patches could not always maintain equal suitability and might gradually decline to the same level of suitability as the nutrient-poor patches. As a result, foraging responses could not eventually increase to the growth of *H. vulgaris* in the heterogeneous environments.

Intraspecific competition strongly inhibited the growth of *H. vulgaris* (e.g. biomass declined by 85% and ramet number by 86%). However, we did not find a significant effect of soil heterogeneity on competitive interactions of *H. vulgaris* and such an effect also did not depended on patch scale. Previous studies have shown that soil heterogeneity increased intraspecific competition of *Briza media*, but it did not affect that of *Festuca rubra* or *Alternanthera philoxeroides* (Day et al. 2003c; Zhou et al. 2012). It has also been shown that light heterogeneity increased intraspecific competition of *Duchesnea indica* and such an effect occurred at both large and small patch scale (Wang et al. 2012). It has been suggested that a significant effect of soil heterogeneity on competition may be caused by the differences between plants in their ability to concentrate ramets and/or roots where nutrient levels are high (Bliss et al. 2002; Fransen et al. 2001; Zhou et al. 2012). In this study, although *H. vulgaris* showed the ability to concentrate ramets

**Table 2:** repeated-measure ANOVA results for effects of patch type (nutrient-rich vs. nutrient-poor patches), soil heterogeneity (homogeneous vs. large patch vs. small patch) and intraspecific competition (without vs. with competition) on growth measures of *H. vulgaris* at the patch level

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Biomass</th>
<th>Number of ramets</th>
<th>Aboveground mass</th>
<th>Root mass</th>
</tr>
</thead>
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<tr>
<td>Between-subject</td>
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<tr>
<td>H</td>
<td>2.24</td>
<td>2.1*</td>
<td>2.2*</td>
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<td>C</td>
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</tr>
<tr>
<td>H × C</td>
<td>2.24</td>
<td>1.4*</td>
<td>1.5*</td>
<td>1.5*</td>
<td>0.6*</td>
</tr>
<tr>
<td>Within-subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt</td>
<td>1.24</td>
<td>9.3****</td>
<td>2.4*</td>
<td>8.1****</td>
<td>5.2***</td>
</tr>
<tr>
<td>Pt × H</td>
<td>2.24</td>
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<td>3.4***</td>
</tr>
<tr>
<td>Pt × C</td>
<td>1.24</td>
<td>2.4*</td>
<td>0.08*</td>
<td>2.0*</td>
<td>1.7*</td>
</tr>
<tr>
<td>Pt × H × C</td>
<td>2.24</td>
<td>2.9**</td>
<td>9.5****</td>
<td>2.8**</td>
<td>0.9*</td>
</tr>
</tbody>
</table>

Abbreviations: C = competition, H = heterogeneity, Pt = patch type.
Significance levels: ****P < 0.001, ***P < 0.01, **P < 0.05, *P < 0.1 and P ≥ 0.1 (not significant).
and root mass in nutrient-rich patches, the differences in the ability to respond to soil nutrient heterogeneity between the *H. vulgaris* individuals may be little. Consequently, a significant effect soil heterogeneity on the intraspecific interactions was not observed.

Another possible reason is that physiological integration may weaken the influence of soil heterogeneity on plant competition (Fransen *et al.* 2001; Rajaniemi and Reynolds 2004).

Physiological integration may alleviate the severe competition between ramets in nutrient-rich patches by allowing internal re-distribution of resources acquired by ramets in the nutrient-rich patches to support spreading of new ramets in the nutrient-poor patches, avoiding overgrowth of ramets in the nutrient-rich patches (Novoplansky 2009).

We conclude that there is little effect of soil heterogeneity on intraspecific competition of *H. vulgaris*. We hypothesize that

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**Figure 4:** effects of patch type (nutrient-rich vs. nutrient-poor patches), soil heterogeneity (homogeneous vs. large patch vs. small patch) and intraspecific competition (without vs. with competition) on biomass (a and b), number of ramets (c and d), aboveground mass (e and f) and root mass (g and h) of *H. vulgaris* at the patch level. Error bars show +1 SE. Letters at ends of bars show which means differed between heterogeneity treatments within patch-type treatments (Tukey tests, \( P = 0.05 \)); symbols at ends of bars show which means differed between patch-type treatments (paired \( t \)-tests): no symbol, \( P \geq 0.1 \); \# \( P = 0.05–0.1 \); * \( P = 0.01–0.05 \); ** \( P = 0.001–0.01 \).
effects of intraspecific neighbors on foraging responses of *H. vulgaris* to soil heterogeneity at small scales may be closely related to the trade-off between ramet production rate and foraging precision. Our results suggest that foraging responses to soil heterogeneity may not necessarily be adaptive and intraspecific competition may not be influenced by soil heterogeneity.

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