RESEARCH PAPER

Combined effects of resource heterogeneity and simulated herbivory on plasticity of clonal integration in a rhizomatous perennial herb

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ABSTRACT

Previous lines of investigation assuming potential advantage of clonal integration generally have neglected its plasticity in complex heterogeneous environments. Clonal plants adaptively respond to abiotic heterogeneity (patchy resource distribution) and herbivory-induced heterogeneity (within-clone heterogeneity in ramet performance), but to date little is known about how resource heterogeneity and simulated herbivory jointly affect the overall performance of clones. Partial damage within a clone caused by herbivory might create herbivory-induced heterogeneity in a resource-homogeneous environment, and might also decrease or increase the extent of heterogeneity under resource-heterogeneous conditions. We conducted a greenhouse experiment in which target-ramets of *Leymus chinensis* segments within homogeneous or heterogeneous nutrient treatments were subject to clipping (0% or 75% shoot removal). In homogeneous environments with high (9:9) nutrient availability, ramet biomass of *L. chinensis* with intact or severed rhizomes is 0.70 or 0.69 g. Conversely, target-ramet biomass with intact rhizomes is obviously lower than that of the severed target-ramets in the homogeneous environments with medium (5:5) and low (1:1) nutrient availability. High resource availability and the presence of herbivory can alleviate negative effects of rhizome connection under homogeneous conditions, by providing copious resource or creating herbivory-induced heterogeneity respectively. Herbivory tolerance of clonal fragments with connected rhizomes was higher than that of fragments with severed rhizomes under heterogeneous conditions. These findings confirmed the unconditional advantage of clonal integration on reproduction under the combined influence of resource heterogeneity and simulated herbivory. Moreover, our results made clear the synergistically interactive effects of resource heterogeneity and simulated herbivory on costs and benefits of clonal integration. This will undoubtedly advance our understanding on the plasticity of clonal integration under complex environmental conditions.

INTRODUCTION

Clonal integration is a ubiquitous life history trait of clonal plants, which has been extensively studied during the past two decades (Petelka & Ashmun 1985; Abrahamson *et al.* 1991; Stuefer *et al.* 1994; Dong & Alaten 1999; Liu *et al.* 2008). The benefits of clonal integration are generally thought to overweigh its costs in heterogeneous conditions, which might be one of the underlying mechanisms of the widespread distribution and dominance of perennial herbaceous species. Essential resources are patchily distributed on a fine scale (Farley & Fitter 2001), so that different ramets of the same genet or different modules of the same individual will experience heterogeneity of microhabitats. Ramets in fertile patches can share acquired resources with and provide support to the other ramets in unfavourable conditions (Alpert 1999; Saitoh *et al.* 2006). The study in the stoloniferous plant provides convincing evidence that physiological support to offspring ramets of *Fragaria vesca*

brings about an enhancement in the photosynthetic efficiency of parents, especially when offspring ramets grow in unfavourable conditions (Roiloa & Retuerto 2007). Even when the whole clonal plant grows in unfertile patches, connected organs can elongate into resource-rich patches to establish ramets, which can support the whole plant (Abrahamson *et al.* 1991). Thus, clonal integration is generally thought to improve plant's ability to cope with heterogeneous environments, which is classically defined as 'support' (Salzman & Parker 1985; Chapman *et al.* 1992; Landa *et al.* 1992; Dong & Alaten 1999).

Further, when ramets within a same clone are experiencing different levels of stress (*e.g.* salt or alkali) or disturbance (*e.g.* herbivory or mowing), clonal integration is also expected to increase plant tolerance (Abrahamson *et al.* 1991; Chapman *et al.* 1992; Hellström *et al.* 2006; Liu *et al.* 2007). When some ramets are subject to herbivory and the others escape herbivore attacks, the undamaged ramets may aid damaged ones by translocating resources, or by mobilizing shared reserves

(Schmid *et al.* 1988; Chapman *et al.* 1992; Olson & Wallander 1999; Wang *et al.* 2004; Liu *et al.* 2007). When part of a clone is consumed by herbivores, the damaged ramets can recover by compensatory growth or the support from the undamaged ramets (Gao *et al.* 2008; Liu *et al.* 2012). Compared with the classical 'beneficial' effects especially under heterogeneous and stressful conditions, the plasticity of clonal integration has been generally neglected in previous empirical and theoretical studies. Partial damage can change the source-sink relationship within a clonal fragment, and alter the competitive interaction between ramets within the same clone (Hellström *et al.* 2006; Janeček *et al.* 2008). Thus, it is very necessary to make clear the effects of heterogeneity extent on the plasticity of clonal integration, and the combined effects of resource heterogeneity and herbivory.

A relatively large number of studies have reported advantages of clonal integration, while costs of physiological integration are less studied (Stuefer *et al.* 1994; Chen *et al.* 2010). Costs of clonal integration for some clone parts are usually overweighed by benefits from other clone parts. Therefore, this results in net benefits in overall performance and fitness of the same clones and populations (Li *et al.* 2012). However, physical connections between ramets do not imply the performance of the connected ramets will be equalized in the two contrasting habitats (Salzman & Parker 1985). Ramets in a resource-poor patch do not always obtain the support from ramets in a rich patch under certain conditions or for some species (Klimeš & Klimešová 1999; Hay & Kelly 2008). Therefore, it is still necessary to clarify the balance between costs and benefits of clonal integration in complicated environments.

Abiotic heterogeneity especially caused by patchy resource distribution is one of the basic characteristics of natural environments (Kolasa *et al.* 1991). Additionally, herbivory-induced heterogeneity was created by damaging one ramet within the same clonal fragment (Orians & Jones 2001). More attention should be paid to the plasticity of clonal integration in heterogeneous conditions, especially under the interactions of resource heterogeneity and herbivory-induced heterogeneity. However, few studies have attempted to examine how their interaction affects costs and benefits of clonal integration. Further studies on the effects of their interactions are needed to explain the advantage of clonal plant species in coping with herbivory disturbance under the influence of several factors (Wilsey 2002; Li *et al.* 2004).

We conducted a pot experiment in controlled greenhouse to explore the plasticity of clonal integration in environments with patchy nutrient distribution and simulated herbivory. The central objectives of this study were to confirm the balance between costs and benefits of clonal integration under different nutrient conditions, and to determine how clonal integration responds to the combined effects of nutrient heterogeneity and simulated herbivory. Firstly, we hypothesized that herbivory-induced heterogeneity, like resource heterogeneity, would enhance the advantage of clonal integration due to the support from undamaged to damaged ramets. Herbivory disturbance has been proved to directly affect plant performance, and result in the difference within a same clone (Gao *et al.* 2008). Consequently, we presumed that the clones with partial herbivory damage under the nutrient-homogeneous conditions would do better than the clones with a pair of undamaged ramets in the same nutrient treatment.

When resource conditions are heterogeneous, both the extent of abiotic heterogeneity and the presence/absence of herbivory-induced heterogeneity are expected to influence the plasticity of clonal integration. We aimed to ascertain whether the effects of abiotic and herbivory-induced heterogeneity were synergistic or antagonistic. When target-ramets grew in the nutrient-poor soil of heterogeneous patches, the addition of herbivory to target-ramets was considered to enlarge heterogeneity extent. Under this condition, our second hypothesis was that the interaction between resource heterogeneity and herbivory-induced heterogeneity would be 'synergistic' (increasing heterogeneity extent) and increase benefits of clonal integration. On the contrary, the third hypothesis was that the 'antagonistic' effects of herbivory would decrease heterogeneity extent and might decrease costs of clonal integration when target-ramets were in a higher nutrient environment than neighbour-ramets.

MATERIAL AND METHODS

Study site and plant species

The experiment was carried out in the greenhouse at the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, P. R. China (44°45′ N, 123°45′ E). The station is located in the southern part of the Songnen Plains in the eastern Eurasian steppe region. The mean temperature of the study area is 5.0–6.0 °C, varying from −16 °C in January to 25 °C in July. The annual precipitation is about 350 mm, approximately 70% of which falls in June, July and August.

Leymus chinensis is a perennial dominant species in arid and semi-arid steppes in northern China and eastern Mongolia, with extensive plasticity in morphological and physiological characteristics. Rhizome growth of this species plays a major role in its spatial expansion and colonization. The rhizomes lie horizontally about 5–15 cm under the ground surface, and rhizome length between two adjacent ramets is appropriated 2–6 cm under natural field conditions (Zhu 2004; Wang & Ba 2008). Rhizome buds can be dormant, or grow upward to form new ramets, or grow horizontally to form new rhizomes (Zhu 2004). We selected *L. chinensis* as the study species for this experiment because it is a typical rhizome clonal plant, and is often exposed to grazing and mowing due to its good palatability for livestock. Consequently, it is always subject to nutrient heterogeneity and herbivory-induced heterogeneity. There have been reports on the inherent ability of *L. chinensis* to recover from grazing via compensatory growth, and to regrow rapidly after grazing or mowing early in the growing season (van Staalduinen & Anten 2005; Gao *et al.* 2008; Liu *et al.* 2012).

Experimental and treatment design

The pot experiment was conducted to test effects of clonal integration between ramets on grazing tolerance in heterogeneous environments in a factorial design involving nutrient (homogeneity and heterogeneity environments), simulated herbivory (clipping versus unclipping) and rhizome connection (intact versus severed) treatments. The space within each plastic pot (20 cm in diameter and 15.5 cm deep) were divided into two parts (side A or B) by placing a 1 mm thick plastic partition along the pot midline. Each partition had five round holes of 3 mm in diameter at 6 cm from the bottom, which allowed rhi-

zomes of clonal fragments (described below) to pass. The humus and sands were sieved through a mesh to remove coarse aggregates. Organic matter content was 14% and the pH was 5.1 in the humus. Different proportions of humus and sand were used to creat three types of soil mixture which were 90% humus (High), 50% humus (Medium), and10% humus (Low), respectively. Both side A and B of each pot were respectively filled with the thoroughly mixed soils (1000 g). There were three nutrient levels at side A of the pot (High-A_H, Medium-A_M, and Low-A_L), and three nutrient levels at side B (B_H, B_M, and B_L). The ratio of nutrient content in side A to that in side B was used to indicate resource heterogeneity. Thus nine nutrient treatments were created, including three homogeneity (A_HB_H-9:9, A_MB_M-5:5 and A_LB_L-1:1), three high-low heterogeneous treatments (A_HB_L-9:1, A_HB_M-9:5 and A_MB_L-5:1) and three low-high heterogeneous treatments (A_LB_H-1:9, A_LB_M-1:5 and A_MB_H-5:9).

The clonal fragments of *L. chinensis* were selected and carefully excavated from natural populations in the study area on April 25, 2010. Each clonal fragment consisted of two ramets interconnected by a rhizome fragment of about 5 cm long. One ramet was assigned as 'target-ramet', and would be transplanted into side A of a pot and subsequently would be exposed to clipping treatments (clipping/unclipping); The other ramet was assigned as 'neighbour-ramet', and would be transplanted into side B of the same pot. Five clonal fragments were transplanted into a same plastic pot (Fig. 1). The intact or severed rhizome connections were put through the holes on the partition on the transplanting day. On 20 days after being transplanted, target-ramets were subject to the two clipping treatments: clipping (75% shoot removal) and unclipping (0% shoot removal). Initially, the clonal fragments were irrigated with 300 ml water per pot daily for 1 month, and then with 500 ml per pot once every 2 days. The mean temperature (the highest/the lowest) during the experimental period was 22.9 °C/10.1 °C in May, 30.4 °C/18.1 °C in June, 28.9 °C/18.7 °C in July, 28.6 °C/18.2 °C in August, and 24.3 °C/12.2 °C in September.

Totally, the experimental design included nine nutrient treatments as mentioned above, two clipping treatments (clipping vs unclipping) and two rhizome-severing treatments (intact vs severed). These 36 treatments (9 nutrient × 2 clipping × 2 rhizome connection) were each replicated four times. All the 144 pots were randomly rearranged every week to reduce shading effects.

Sampling and measurements

Plants were harvested at the end of growing season (170 days after being transplanted), and the shoot, rhizome and fibrous root of each ramet were separated, cleaned and measured. Plant height, tiller number, and bud number were recorded separately at ramet level. Biomass of culm, leaf, rhizome, fibrous root, tillering node and bud was measured after being dried at 70 °C for 24 h.

Tolerance calculation and cost-benefit analysis

Fitness is a parameter reflecting the expected contribution of individual plants to future generations of plant population (Winkler & Fischer 1999). Plant tolerance to herbivory is generally defined as the ability of plants to maintain the same level of fitness after experiencing herbivory (Rosenthal & Kotanen 1994; Strauss & Agrawal 1999), which is quantified by the slope of the regression line of fitness on damage level. Actually, seed number and seed mass are most common fitness estimates in many reported studies. However, bud biomass (reproductive biomass of *L. chinensis*) was the preferred fitness estimate compared to the other variables (such as, total biomass, seed number and/or biomass) in our study, because this species depends mainly on vegetative propagation to expand its population, and had almost no sexual reproduction during our experiments. Here, tolerance scores were calculated by dividing fitness (reproductive biomass) of damaged plants by that of undamaged plants in the same rhizome condition and the same nutrient treatment.

To analyze the balance between costs and benefits of clonal integration, we defined cost as the difference in final biomass between target-ramets in high-low (9:1 or 9:5) and high-high (9:9) treatments or between target-ramets in 5:1 and 5:5 nutrient treatments. Benefit was estimated as the increased performance of target-ramets between low-high (1:9 or 1:5) and low-low (1:1) treatments, or the difference in target-ramet biomass between 5:9 and 5:5 nutrient treatments.

Fig. 1. Distribution of five clonal fragments in the pot for greenhouse experiments. The pot was partitioned into two parts (side A and B) by a plastic barrier. Different nutrient level of soil would be filled into the two sides, illustration the heterogeneous treatment design. Circles indicate ramets of clonal fragments with or without rhizome connection. The ramets in side A were considered as 'Target-ramet (T-ramet)', and those ramets in side B were 'Neighbour-ramet (N-ramet)'. T-ramets would be further subject to clipping treatments.

Statistical analysis

To avoid the damage of harvest, relative growth rate (RGR) of each tiller was calculated as the change in plant height during the 15 days after clipping divided by the initial plant height. To estimate the relative investment of plants into vegetative growth
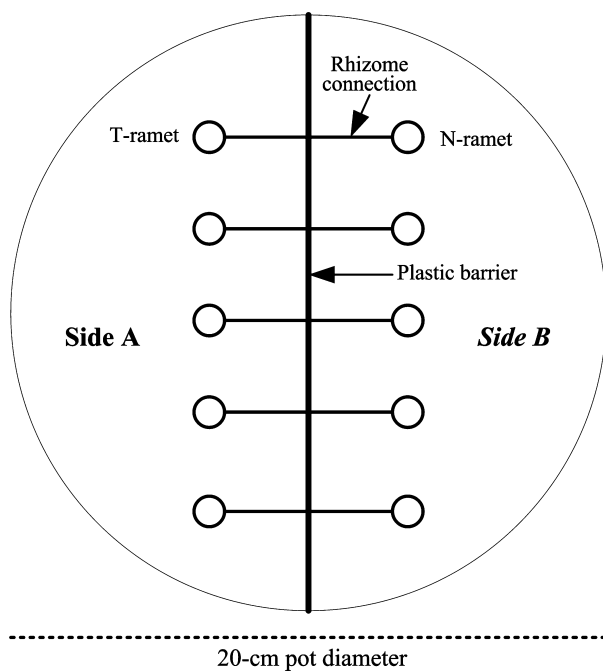
and reproduction, we measured vegetative biomass (VB) and reproductive biomass (RB) separately. Then we used three-way ANOVA to test the effects of clonal integration (integrated vs. severed), clipping (clipping vs. unclipping), nutrient treatments, and the interactive effects on plant growth and reproduction. The response variables include vegetative biomass, reproductive biomass, reproduction potential (RP, the total number of tillers and vegetative buds per pot), and root-to-shoot ratio (R/S). Data were further analysed using one-way ANOVA within each nutrient, clipping or rhizome connection treatment if the interaction between the treatments was significant. Tukey-Kramer test was followed to examine the difference within nutrient treatments. Bonferroni correction-test was carried out to compare the difference between clipping and unclipping treatments, or between treatments with intact and with severed rhizome at each nutrient level, in which the 'P' value for each test was equal to alpha divided by the number of test (Liu *et al.* 2012). All statistical analyses were performed with SAS 8.2 statistical package. Significant level was set at $P < 0.05$.

RESULTS

Ramet biomass and relative growth rate of target-ramets

Within unclipping treatments, average ramet biomass of target-ramets with intact rhizome was significantly higher than that with severed rhizome under resource heterogeneous conditions (Fig. 2a–c). On the contrary, ramet biomass with intact rhizome was obviously lower than that of the severed ramets in the homogeneous environments with medium (5:5) and low (1:1) nutrient availability (Fig. 2b and c). With clipping treatments, no difference in ramet biomass between with and with-

out rhizome connections was found in 9:5, 5:5, 1:1, 5:1 nutrient treatments (Fig. 2d–f). In other nutrient treatments, average ramet biomass in intact treatments was higher than that in severed treatments (Fig. 2d–f).

Within the same nutrient and unclipping treatments, there was almost no difference in relative growth rate (RGR) between target-ramets with and without rhizome connection. Clipping treatments significantly stimulated the increase of RGR of target-ramets in all nutrient treatments (Fig. 3). When target-ramets were growing in the environments with the highest nutrient availability, their RGR along nutrient gradient was kept unchanged from 9:9, 9:5 to 9:1 (Fig. 3a–c). On the contrary, the changing rate of RGR slowed down gradually along the 5:9, 5:5 and 5:1 gradient (Fig. 3d–f), and the 1:9, 1:5 and 1:1 gradient (Fig. 3g–i), indicating the decreased clonal integration due to the decrease of nutrient level available to the neighbour-ramets. However, clipping addition resulted in the advantage of clonal integration in 5:9, 1:9 and 1:5 nutrient treatments (low-high heterogeneous treatments), reflecting the support from neighbour-ramets in nutrient-rich environments through clonal integration (Fig. 3d, g and h). Within high-low heterogeneous treatments (9:5, 9:1 and 5:1), RGR of clipped ramets with intact rhizome was wondrously lower than that of severed ramets (Fig. 3b, c and f). In 5:5 homogeneous nutrient treatments, RGR of clipped ramets with intact rhizome was higher than that of clipped ramets with severed rhizome (Fig. 3e).

Fitness and tolerance score

Reproductive biomass, an estimate of fitness in our study, was significantly influenced by nutrient, the interaction of integration and nutrient, and the interaction of integration, clipping
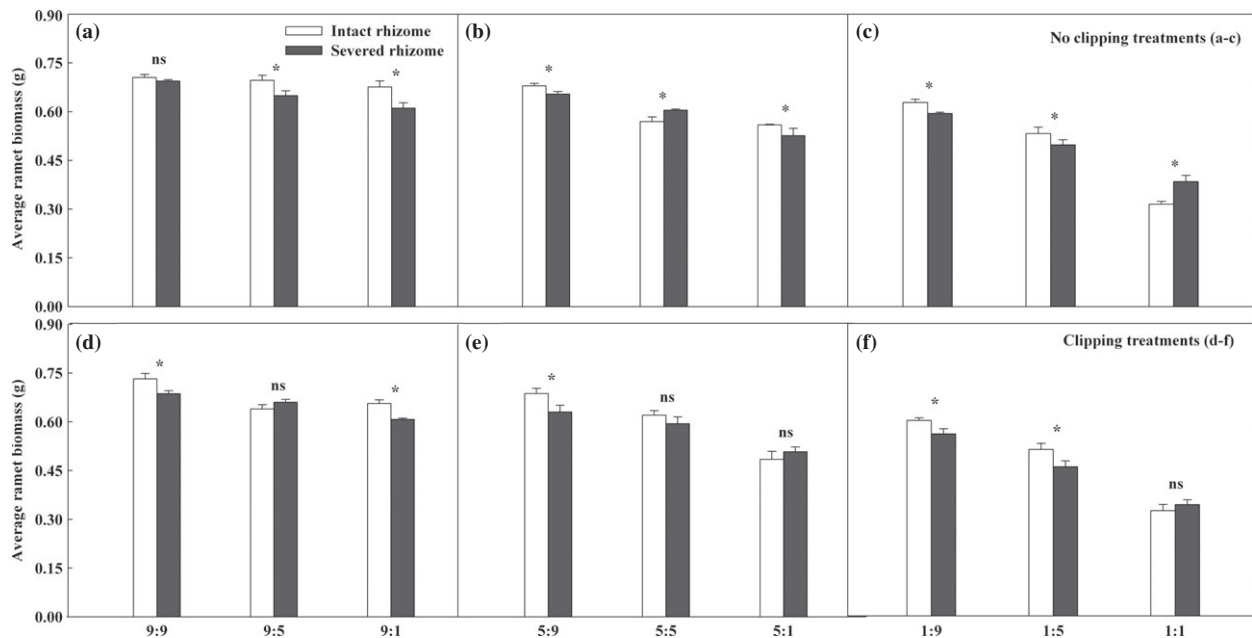

Fig. 2. Average ramet biomass of *Leymus chinensis* with intact or severed rhizomes under the conditions of (a–c) unclipping and (d–f) clipping treatments. There are three gradients of nutrient treatments as 9:9, 9:5, 9:1, and 5:9, 5:5, 1:5, and 1:9, 1:5, 1:1, respectively. Considering the structure and spatial scale of genets, nine nutrient treatments in this experiment include three levels of homogeneous nutrient availability (9:9, 5:5, and 1:1), three high-low (9:5, 9:1 and 5:1) and three low-high (1:9, 1:5 and 5:9) heterogeneous treatments. Values represent means ± SE. The bars with * within a nutrient treatment with are significantly different at $P < 0.05$.
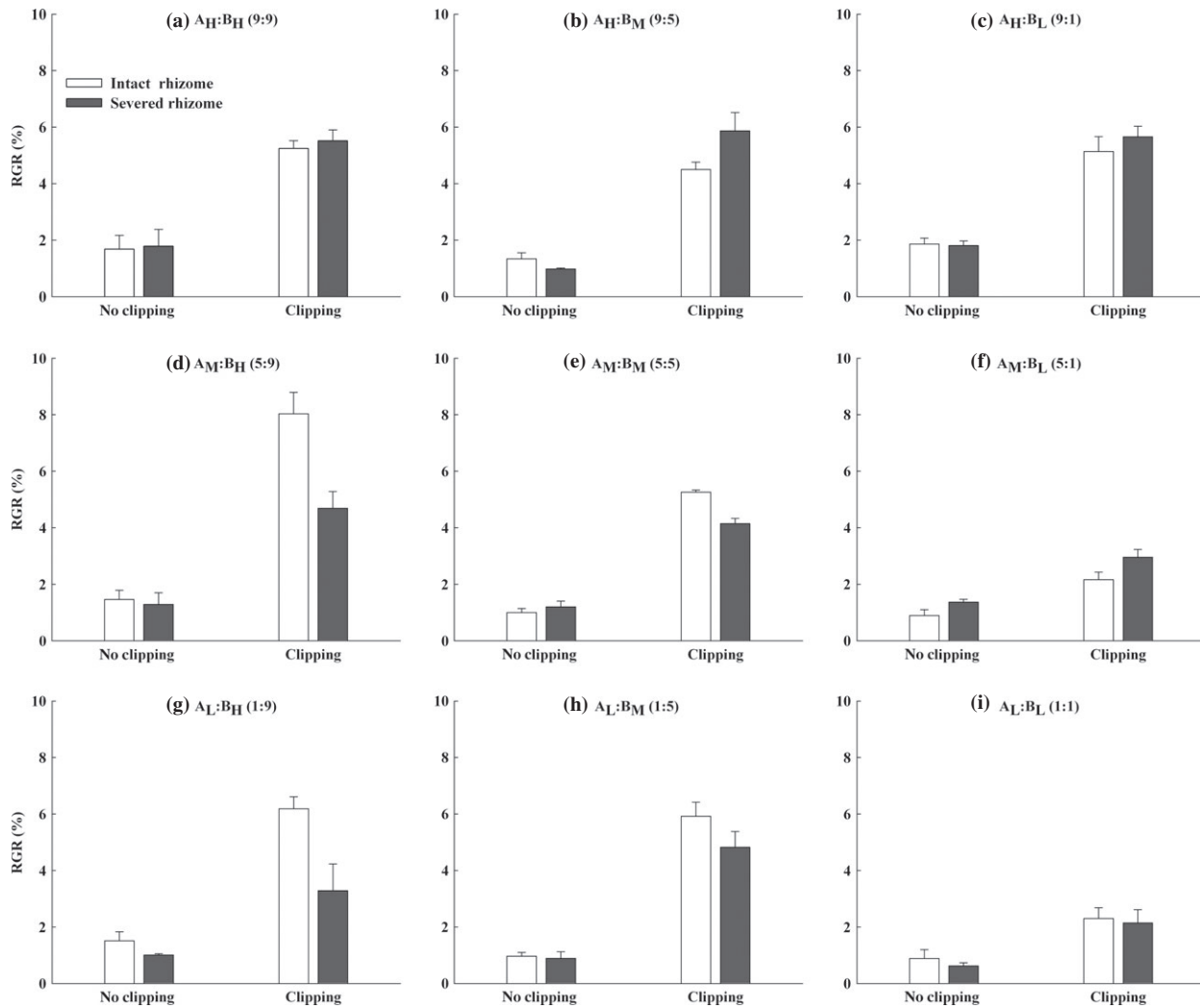
Fig. 3. The relative growth rate (RGR) of target-ramets in *Leymus chinensis* 15 days after clipping treatments in nine nutrient treatments. There were three nutrient levels at side A of the pot for target-ramet (A_H-high, A_M-medium, and A_L-low), and three nutrient levels side B for neighbour-ramet (B_H, B_M, and B_L). Three gradients of nutrient treatments were (a–c) the gradient of 9:9, 9:5, and 9:1, (d–f) the gradient of 5:9, 5:5, and 5:1, and (g–i) the gradient of 1:9, 1:5, and 1:1. Values represent means $\pm$ SE.

and nutrient (Table 1). Within 9:9, 5:5 and 1:1 homogeneous nutrient treatment, there was no significant difference in tolerance score of intact and severed population. Within six heterogeneous nutrient treatments, grazing tolerance of *L. chinensis* with intact rhizomes was higher than that with severed rhizomes (Fig. 4). In rhizome connected treatment, tolerance score of *L. chinensis* varied from 0.80 (minimum, in 5:1 nutrient treatment) to 1.21 (maximum, in 9:9 nutrient treatment). In rhizome severed treatment, the range of tolerance score ranged from 0.52 in 5:1 nutrient treatment to 1.09 in 9:9 nutrient treatment.

Root-shoot ratio and biomass allocation

Rhizome connection and nutrient treatments showed significant effects on root-shoot ratio (R/S; $P < 0.05$), but the effects of clipping and all the interactions were not statistically significant (Table 1). Clipping showed no effect on biomass allocation between plant aboveground and below-ground plant organs. However, vegetative biomass and reproductive biomass were significantly affected by clipping, integration, nutrient and their interactions (Table 1).

Costs and benefits of clonal integration

The extent of nutrient heterogeneity and nutrient availability significantly affected costs and benefits of clonal integration in unclipping treatments. Specifically, cost of clonal integration was higher in 9:1 and 5:1 nutrient treatments than in 9:5 nutrient treatment. Its benefit was the highest in 1:9 nutrient treatment, and the lowest in 5:9 nutrient treatment. Clipping significantly increased costs and benefits of clonal integration under heterogeneous nutrient conditions, except for 5:1 nutrient treatment. Clipping resulted in higher costs in 9:5 than in 5:1 nutrient treatments (Fig. 5).

Table 1. ANOVAS for the effects of clonal integration, clipping, nutrient treatment on vegetative biomass (VB), reproductive biomass (rhizome bud biomass, RB), reproduction potential (RP), root-to-shoot ratio (R/S) of *Leymus chinensis*.

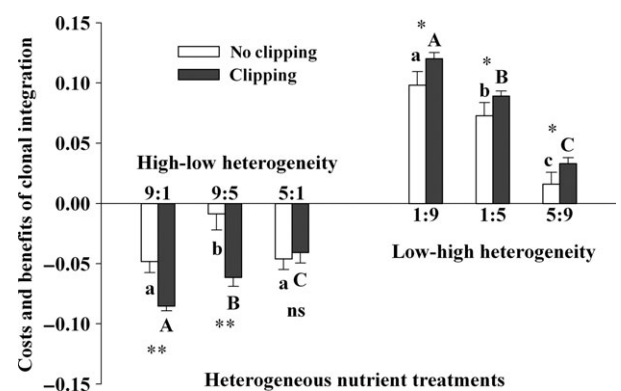| treatment	df	VB(g)		RB(g)		RP		R/S(g·g⁻¹)	
		F	P	F	P	F	P	F	P
integration (I)	1	2306.01	<0.001	4.44	0.038	7.00	0.009	23.10	<0.001
clipping (C)	1	2375.33	<0.001	3.19	0.042	0.74	0.391ns	0.05	0.825ns
nutrient (N)	8	487.04	<0.001	8.44	<0.001	12.62	<0.001	2.97	0.005
I × C	1	143.85	<0.001	0.02	0.902ns	0.01	0.938ns	0.36	0.550ns
I × N	8	767.37	<0.001	3.48	0.001	2.45	0.018	1.58	0.140ns
C × N	8	566.05	<0.001	0.68	0.704ns	1.06	0.397ns	0.36	0.938ns
I × C × N	8	264.59	<0.001	2.48	0.016	1.53	0.155ns	0.26	0.979ns

ns-not significant.
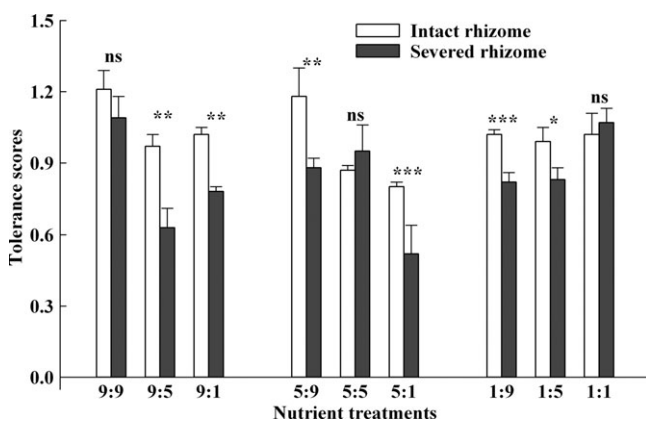
Fig. 4. Tolerance scores of *Leymus chinensis* as affected by clonal integration and nutrient availability. Tolerance score was calculated as the ratio of fitness (reproductive biomass) for clipped plants divided by the mean value of unclipped control in the same nutrient treatment. The *X*-axis represents the ratio of nutrient availability of side A (target-ramet) to that of side B (neighbour-ramet). There are three gradients of nutrient treatments, which were the gradient of 9:9, 9:5, and 9:1, the gradient of 5:9, 5:5, and 1:5, and the gradient of 1:9, 1:5, and 1:1. Values represent means ± SE. The bars with * within a nutrient treatment are significantly different at *P* < 0.05.

Fig. 5. Costs and benefits of clonal integration in *Leymus chinensis*. The *X*-axis represents the ratio of nutrient availability of side A (target-ramet) to that of side B (neighbour-ramet). There are three high-low (9:5, 9:1 and 5:1) and three low-high (1:9, 1:5 and 5:9) heterogeneous treatments. Values are means ± SE. Within unclipping treatments, means with the same lower case letters within three high-low or three low-high heterogeneous patches were not significantly different. Within clipping treatments, means with the same capital letters at three high-low or three low-high heterogeneous treatments were not significantly different at *P* < 0.05. The bars with * within a nutrient treatment are significantly different at *P* < 0.05.

DISCUSSION

Herbivory-induced heterogeneity and high resource availability reduce costs of rhizome connection under uniform soil nutrient

Our study clearly showed costs of maintaining rhizome connection under homogeneous conditions, and this negative response was alleviated by high nutrient availability and herbivory. Benefits from physiological integration in heterogeneous environments have been extensively described in several clonal species (Hartnett & Bazzaz 1983; Bullock *et al.* 1994; Březina *et al.* 2006; Wang *et al.* 2008), but costs in the maintenance of energetically costly inter-ramet connections are always neglected, especially when resource distribution is uniform. The costs of maintaining rhizome connection was detected in homogeneous environments with medium and low nutrient (5:5 and 1:1 nutrient treatments), rather than with high nutrient level (9:9 nutrient treatment). This fact accords with the

prediction of allocation theory that costs of clonal integration should be more apparent in low-resource environments than under optimal growth conditions (van Dam & Baldwin 1998). We did not find high costs of maintaining rhizome connection in homogeneous environments with high-resource availability, indicating that enhanced resource acquisition can easily compensate for resource investment on rhizome connection of clonal species in homogeneous environments. This is the consequence of high resource availability by providing copious resources for plant growth and reproduction, and thus the costs of plants on a certain trait (physiological integration) can be considerably alleviated by high resource availability.

Additionally, clonal plants can spread across considerable areas by elongating rhizomes, and therefore may easily be subject to uneven animal grazing in natural grassland. Previous studies have demonstrated that clonal integration enhances plant tolerance to herbivory, sand burial, flood, drought and some other disturbance, mainly due to the support from old to young ramets, from the ramets in hospitable microsites to the

ramets in stressful microsites, or from undamaged to damaged ramets (Abrahamson *et al.* 1991; Li *et al.* 2004; Liu *et al.* 2007; Chen *et al.* 2010). When nutrient distribution was uniform and clipping was applied to target-ramets in our experiment, *L. chinensis* grew more in condition with intact than with severed rhizomes within 9:9 homogeneous nutrient treatment, but no difference was found in 5:5 and 1:1 nutrient treatments (Fig. 2d–f). In other words, clipping addition ameliorated the negative effects of rhizome connection and increased its benefits under uniform soil nutrient. The alleviation of herbivory disturbance under homogeneous nutrient conditions can be attributed to herbivory-induced heterogeneity and compensatory effects. Herbivory-induced heterogeneity further reinforces within-clone heterogeneity and offsets the relative costs of rhizome connection under the resource-homogeneous conditions.

Resource heterogeneity and simulated herbivory jointly affect costs and benefits of clonal integration

Theoretical study predicts that physiological integration can be enhanced under higher level of heterogeneity contrast in environments (Caraco & Kelly 1991). Drought-stressed ramets of *Fragaria orientalis* obtain more benefits in high-contrast than in low-contrast patches, which provide experimental evidence for the positive correlation between heterogeneity contrast and the strength of clonal integration (Zhang *et al.* 2009; Wang *et al.* 2013). The extent of resource-heterogeneity, estimated as the difference between patches or between a patch and its surrounding matrix (Kotliar & Wiens 1990), is believed to be the main external drive for clonal integration. Resource transportation in clonal plants commonly occurs between ramets in resource heterogeneous environments, and between ramets under herbivory-induced heterogeneous conditions. Simulated herbivory changed the performance of target-ramets and thereby might alter the relationship between source and sink ramets in clonal species (Orians & Jones 2001).

Physical connection between ramets allows resources to be transported between connected ramets. Its maintenance has commonly been interpreted as costs of clonal integration for the ramets in nutrient-rich environments, but it confers benefits for the ramets growing under low nutrient conditions (Stuefer *et al.* 1994; Hellström *et al.* 2006; Janeček *et al.* 2008). In this study, *L. chinensis* gained more benefits in high- (1:9) than in low- (1:5) contrast patches, and engendered more costs in high- (9:1) than in low- (9:5) contrast patches, implying that costs and benefits were sensitive to the extent of resource heterogeneity. Moreover, we found costs and benefits of clonal integration also depended upon resource availability. For example, in the absence of herbivory, cost of clonal integration in 9:5 treatments was lower than that in 5:1 treatments. Again our study confirmed that high resource availability can partly offset the costs of clonal integration in clonal species. Higher costs and benefits in low nutrient (5:1) than in high nutrient (9:5) patch support the conclusion from other study cases that effects of clonal integration are easier to be detected when part of clones is growing in the environments with limiting resource (Hellström *et al.* 2006; Xu *et al.* 2012).

Clipping significantly increased benefits of clonal integration in clonal species (at least in *L. chinensis*), which also provide evidence for our second hypothesis. Surprisingly, the same 'synergistic' effects of herbivory on costs of clonal integration did not coincide with our third hypothesis. It is most likely that simulated herbivory induces over-compensatory growth of target-ramets under high nutrient conditions (Gao *et al.* 2008; Liu *et al.* 2012). The overcompensation after herbivory enhanced the difference between target-ramets and neighbour-ramets at high-low patch heterogeneity, and increased costs of clonal integration.

Unconditional advantage of clonal integration on reproduction in heterogeneous nutrient environments, rather than on vegetative growth

Clonal integration has been proved to benefit clonal plants when their ramets are growing in discrete patches differing in quality (Wijesinghe & Handel 1994; Wijesinghe & Hutchings 1997; Pennings & Callaway 2000). A straightforward approach to testing whether a trait is advantageous is the plants with this trait perform more better than those without the trait. Our study revealed positive effects of clonal integration on asexual reproduction (bud number) and herbivory tolerance both in low-high and in high-low patches, but such positive effects on vegetative growth (RGR) were found only in low-high nutrient patches.

Clonal integration may confer clonal plant networks with considerable benefits in terms of plant growth (biomass and/or survival) in heterogeneous environments (Dong & Alaten 1999). Clipping applied to target-ramets in low-high heterogeneous treatment increased the extent of clonal integration, demonstrating the synergistic interactions between resource heterogeneity and herbivory-induced heterogeneity. The synergistic interaction increased the heterogeneity extent and beneficial effects on vegetative growth at low-high patch heterogeneity. On the contrary, clipping applied on target-ramets in high-low heterogeneous treatments reduced the extent of clonal integration. Therefore, the interactive effects of resource heterogeneity and herbivory-inducded heterogeneity could increase, and also could decrease the strength of clonal integration on growth-related traits, depending on patch type where damaged target-ramets are growing.

Bud number and bud biomass are among the key traits of clonal plants, indicating the potential to produce offspring ramets (Gao *et al.* 2008). Clonal organs (rhizomes) can store not only carbohydrates but also meristems (buds) (Suzuki & Stuefer 1999), which confers clonal plants notable advantages in coping with herbivory. For instance, energy and meristems stored in belowground rhizomes can effectively avoid being damaged by livestock and can be ultilized by plants to recover from the damage of above-ground parts (Iwasa & Kubo 1997; Lehtilä 2000). Nutrient heterogeneity and simulated herbivory can enhance the clonal integration and thus improve plant tolerance to herbivory and increase reproductive biomass allocation (rhizome bud production) in almost all treatments, including low-high and high-low heterogeneous treatments.

Biomass of *L. chinensis* did not change during the experimental period despite that it was found to be influenced by longer-term exposure to herbivory. Plasticity in vegetative and reproductive biomass allocation and unconditional advantage of clonal integration on reproduction might at least partly account for adaptation of *L. chinensis* to the complex environments.

Our findings indicate that high resource availability can provide copious resources and compensate for costs of clonal integration. Herbivory-induced heterogeneity within a clone also can alleviate negative effects of rhizome connection under homogeneous conditions. In heterogeneous resource environments, herbivory increases the benefits by enlarging heterogeneity extent at low-high heterogeneity treatments, and also increased the costs by over-compensatory growth of target-ramets in high-low heterogeneity treatments. Taken together, these findings add significantly to our understanding about the plasticity of clonal integration under combined effects of resource heterogeneity and herbivory. The long-term balance of costs and benefits of clonal integration may strongly depend on natural habitats of plants and selective pressures from herbivory. How the plasticity of clonal integration contributes to the performance of clonal plants in natural ecosystems is more complicated than results from the pot experiments and the interactions between resource heterogeneity and herbivory-induced heterogeneity should be studies under field conditions in the future.

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