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Effects of soil heterogeneity and clonal integration on *Alternanthera philoxeroides* populations with a radial ramet aggregation

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Abstract

Some clonal plants can spread their ramet populations radially, and soil heterogeneity and clonal integration may greatly affect the establishment of these types of populations. We constructed *Alternanthera philoxeroides* populations with a radial ramet aggregation, allowing old ramets of clonal fragments to concentrate in central pots and younger ramets to root in peripheral pots. The peripheral pots were supplemented either with three different levels (high, medium and low) of soil nutrients to simulate a heterogeneous soil environment, or only one medium level of soil nutrients to simulate a homogeneous environment. Stolon connections between the central older ramets and the peripheral younger ramets were left intact or severed to test the effect of clonal integration. The maintenance of stolon connection could induce the division of labor between different-aged ramets, by increasing the root investment in central ramets and the above-ground growth in peripheral ramets. The maintenance of stolon connection could improve the growth of the central and peripheral ramets, clonal fragments and even the whole population. However, the positive consequence in peripheral ramets and whole fragments was only detected in the high-nutrient patch of heterogeneous treatment. In sum, in the population with the radial ramet aggregation, clonal integration can play a key role in the rapid recruitment of young ramets of *A. philoxeroides* fragments, as well as the expansion of the whole population. The magnitude of clonal integration also became more obvious in the peripheral young ramets and whole fragments that experienced high-nutrient patches.

Keywords: Alternanthera philoxeroides, clonal growth, labor division, physiological integration, radial structure, soil heterogeneity.

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Introduction

In heterogeneous soil environments, different modules of one plant may simultaneously experience sites with contrasting levels of nutrients (Jackson & Caldwell 1993; Farley & Fitter 1999; Cain *et al*. 1999). As plants that produce genetically identical but physiologically interconnected modules (i.e. ramets), clonal species often show flexible morphological and/or physiological plasticity in response to soil heterogeneity (Hutchings & Wijesinghe 1997; de Kroon *et al*. 2005). To efficiently explore heterogeneous distributed resources, many clonal species such as *Brachypodium plnnatum* and *Slavonia molesta* can exhibit the preferential placement of roots and/or ramets in patches with relatively higher resources, by shortening stolon or rhizome spacers or increasing branch frequency (referred to as 'foraging behavior', Hutchings & de Kroon 1994). Meanwhile, physiological connections between ramets also allow the sharing of resources within one clone, thereby potentially alleviating unfavorable habitats for ramets grown in low-resource or high-stress patches (referred to as 'clonal integration', You *et al*. 2013; Luo *et al*. 2014). These clonal

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traits may contribute to the finial fitness of the whole clone even when the majority of ramets are located in unfavorable patches (de Kroon *et al*. 2009).

Clonal integration is considered to be one of the most distinguishing features in clonal species (Hartnett & Bazzaz 1983; Slade & Hutchings 1987). One general prediction is that clonal integration will remarkably improve the performance of recipient ramets in unfavorable patches at limited costs to the fitness of donor ramets in favorable patches, which to some degree brings predictable benefits in clonal fragments (Janecek *et al*. 2008; Song *et al*. 2012; Zhou *et al*. 2014). For instance, the maintenance of stolon or rhizome connections can significantly improve the survival and growth of *Alternanthera philoxeroides* (Luo *et al*. 2014; You *et al*. 2014) and *Carpobrotus edulis* (Roiloa *et al*. 2010), the photosynthetic efficiency of *Myriophyllum aquaticum* (You *et al*. 2013) and the sexual reproduction of *S. alterniflora* (Xiao *et al*. 2011).

Clonal integration has been also reported to influence the intraspecific relationship (Holzapfel & Alpert 2003) and the productivity of a population (Pennings & Callaway 2000; Oborny *et al*. 2000; Fransen *et al*. 2001). One possible prediction is that when the inter-ramet connection is absent, selective placement of ramets generally causes overgrowth in favorable patches and limited growth in unfavorable patches (Casper *et al*. 2000; Hutchings *et al*. 2003; Dong *et al*. 2014). Correspondingly, intraspecific competition will be aggravated in favorable patches and alleviated in unfavorable patches. However, when the inter-ramet connection is kept intact, clonal integration may reduce local competitive intensity in favorable patches (Price & Hutchings 1996; Yu *et al*. 2009; Roiloa *et al*. 2010), overcompensate for the limited growth in unfavorable patches (He *et al*. 2010; Xu *et al*. 2010; Gao *et al*. 2014) and eventually increase the population productivity in the heterogeneous environment. Until now, how clonal integration affects population productivity has been poorly known.

Many clonal plants in nature can spread ramet populations quickly and most ramets are aggregated rather than randomly scattered, because of the short-distance dispersal of clonal propagates (Zobel *et al*. 2010; Dong *et al*. 2013). The population structure thus tends to be a radially spreading structure, where old ramets concentrate in the central areas that vegetative propagates initially established, and newly recruited ramets scatter in the peripheral open areas (Roiloa & Retuerto 2006; Gao *et al*. 2012). One example of a radially structured population is structured by individuals or ramets all connected to each other, and the resource sharing will possibly occur within the entire population (clone). Previous studies have documented that clonal integration in such populations remarkably contributes to the ability of peripheral ramets to respond to environmental variability (e.g. light availability, soil nutrients and defloration) (Roiloa & Retuerto 2006; Xu *et al*. 2010; Cornelissen *et al*. 2014; You *et al*. 2014). The other type of population is structured by clonal fragments separated from each other, and the resource sharing between ramets will be limited in the intact clonal fragments. For instance, *A. philoxeroides* grown in gravel dunes often germinates from perennial dense tubers in the spring, produces creeping stems radially and establishes a ramet population with a circular growth pattern (Pan *et al*. 2006; Jia *et al*. 2009). For the second type of population, the potential consequence of soil heterogeneity and clonal integration at the individual and population scales has been rarely investigated.

We thus constructed a radially structured population with clonal fragments of the stoloniferous clonal herb *A. philoxeroides*, by allowing old ramets of clonal fragments to concentrate in the central pot and younger ramets to root separately in six peripheral pots. Stolon connections between the central and peripheral ramets in the same stolon fragment were left intact or severed to investigate the effect of clonal integration. The peripheral pots were separately supplemented with three different levels of soil nutrients (i.e. high, medium and low) to simulate a heterogeneous soil environment or supplemented only with the medium level of soil nutrients to simulate a homogeneous environment. We proposed five specific hypotheses. (i) The maintenance of stolen connections will improve the performance of young (i.e. peripheral) ramets in clonal fragments at the cost of decreased fitness of older (i.e. central) ramets. (ii) Both the benefits of stolon connections to peripheral ramets and the costs to central ramets will be influenced by the patch-type of the peripheral areas. Particularly, the costs will become less when younger ramets are grown in peripheral high-nutrient patches. (iii) Stolon connections will trigger the functional specialization (i.e. division of labor) of different-aged ramets (e.g. the increased root investment in central ramets and above-ground growth in peripheral ramets). (iv) The consequence of stolon connections will be positive at the whole-fragment scale, provided that the benefits to peripheral ramets outweigh the costs to central ramets. (v) The maintenance of stolon connections will enhance the productivity of the population in the heterogeneous soil treatment than in the homogeneous treatment, provided that the stolon connection could increase the utilization efficiency of the intact clonal fragments when responding to heterogeneous distributed resources.

Materials and methods

Plant material

Alternanthera philoxeroides (Mart.) Griseb. is an amphibious, stoloniferous clonal herb of the Amaranthaceae family, native to South America (Holm *et al*. 1977; Sainty *et al*. 1998; Julien *et al*. 2012). The species is listed as one of the worst invasive weeds in China and many other countries, including the USA, Australia, New Zealand and India (Holm *et al*. 1977; Julien *et al*. 2012). *A. philoxeroides* in China has extremely low genetic diversity and may be derived from a single clone (Xu *et al*. 2003; Geng *et al*. 2006; Gao *et al*. 2010). *A. philoxeroides* can establish extensive clonal networks during a single growing season by producing creeping stems with ramets that root at each node (Dong *et al*. 2010, 2012). Clonal integration is considered to be an important trait of *A. philoxeroides* that enables it to cope with disturbance and environmental stress (Yu *et al*. 2009; Xu *et al*. 2010; Luo *et al*. 2014). Also, as a typical amphibious species, *A. philoxeroides* is widespread in aquatic and adjacent terrestrial habitats, including lakes, ponds, crop fields and irrigation ditches (Ma & Wang 2005; Pan *et al*. 2006).

Experimental design

The experiment included two soil nutrient treatments (homogeneous vs. heterogeneous) crossed with two stolon connection treatments (intact vs. severed) (Fig. 1). Each replicate of each treatment had a set of seven same-size polyvinyl chloride pots (10 cm diameter and 13 cm high). Of seven pots in each replicate, one was fixed in the center and the other six were regularly placed around its circumference. All pots used for the experiment were filled with a 1:1 volume ratio of sand and a peat-based substrate (Pindstrup Seeding, Pindstrup Mosebrug A/S, Ryomgaard, Denmark), but they were separately supplemented with different amounts of slow-release fertilizer (16N-9P-12K-2Mg; Osmocote 301, Scotts, Marysville, OH, USA), according to the treatment set-up. For the heterogeneous treatment, each central pot had 2 g/L slow-release fertilizer added; two of the six peripheral pots per replicate had 3.5 g/L fertilizer added (hereinafter referred to as 'high-nutrient patches'), two had 2 g/L fertilizer added (referred to as 'medium-nutrient patches') and two had 0.5 g/L fertilizer added (referred to as 'low-nutrient patches'). For the homogenous treatment, all central and peripheral pots had 2 g/L slow-release fertilizer added (respectively referred to as 'uniform central patches' and 'uniform peripheral patches'). Total amounts of soil nutrients were the same in both soil treatments.

There were in total 168 similar-size stolon fragments of *A. philoxeroides* used in the experiment, each consisting of one stolon approximately 20 cm long with four ramets and one apex. Each of the six stolon fragments were randomly assigned to one replicate of each of four crossed treatments, giving seven replicates. For each stolon fragment, the most basal ramet rooted in the central pot, the second basal ramet rooted in one of the six peripheral pots and the two most apical ramets naturally curved upward and did not root (Fig. 1). In half of the replicates of the stolon connection treatment, stolon connections between the central and peripheral ramets were severed (i.e. the absence of clonal integration), and in the other half they were kept intact (i.e. the presence of clonal integration). The experimental set-up might unavoidably cause unequal initial ramet density between central and peripheral pots, and ultimately result in the difference in the biomass and biomass allocation between central and peripheral ramets. However, we think such an experimental set-up did not affect the final results, because all other environmental factors excluding four treatments are kept same.

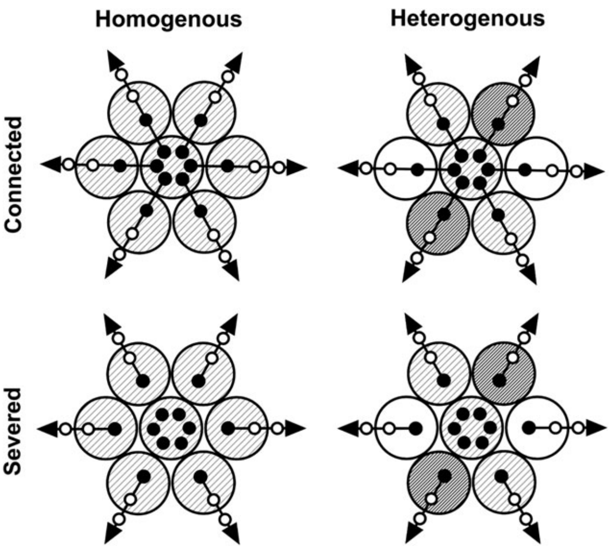

Fig. 1 Experimental design. Each treatment included a set of seven polyvinyl chloride pots of the same size, of which one was fixed in the center and the other six were regularly placed around its circumference. Darkly shaded, lightly shaded and unshaded pots represented high-, medium- and low-nutrient patches. Total amounts of soil nutrients were the same in both soil treatments. A population of *A. philoxeroides* with a radially symmetrical structure was constructed, allowing the oldest ramets to concentrate in the central pot, the second oldest ramets to root in peripheral isolated pots, and the other two younger ramets naturally to curve upright and not to root. Filled and open circles mark positions of rooted and unrooted ramets along a stolon (line) with an apex (arrow). Stolon connections between the central and peripheral ramets are either left intact or severed to test the effect of clonal integration.

To simplify terms in the *Results* and *Discussion* sections, we will refer to each of the initial one-stolon, four-ramet units plus its new ramets and stolons as one 'fragment', and six fragments of each replicate as one 'population'. Also, each fragment was also classified into two parts, namely the peripheral part, which comprised the three youngest ramets and one apex, and the central part, which comprised the oldest ramet. Eight additional fragments were selected for the initial measurement and the average dry mass of fragments was 0.49 ± 0.03 g (mean ± SE, $N = 8$).

On 18 and 19 May 2011, stolon fragments of *A. philoxeroides* were collected from several isolated populations in a riparian agricultural area (28.87°N, 121.01°E) in Zhejiang Province, China. The materials were then randomly mixed and cultivated vegetatively for 2 years in a heated glasshouse at Forest Science Co., Ltd., Beijing Forestry University, Beijing, China. The experiment started on 7 June 2013 and ended on 2 August 2013. The mean temperature in the glasshouse during the experiment was 28.6 ± 0.2°C and the relative humidity was 62.4 ± 0.7% (iButton DS1923; Maxim Integrated Products, Sunnyvale, CA, USA). Enough tap water was supplied every 3 days to keep the soil moist.

Measurements and analyses

At harvest, we counted the number of new nodes separately produced by the central and peripheral parts of each fragment. Each part of the stolon fragments was divided into roots, stems and leaves, dried at 70°C for 48 h and then weighed. Before analysis, we calculated the total biomass, number of new nodes, root to shoot ratio, stem mass, leaf mass and root mass of the whole population. For the heterogeneous treatment, we calculated the growth measures and root to shoot ratio of central parts, peripheral parts and whole fragments, by averaging the corresponding values of two plants grown in the same type of patch (high-, medium- or low- nutrient patches, Fig. 1) of one central-peripheral pot. For the homogeneous treatment, we calculated the above-mentioned measures of central parts, peripheral parts and whole fragments, by averaging the corresponding values of six plants grown in the uniform nutrient patches of one central-peripheral pot (Fig. 1).

We used two-way ANOVAS to test the effects of patch type (uniform patches of homogeneous treatments, high-, medium- and low-nutrient patches of heterogeneous treatment; random effect) and stolon connection (intact and severed; fixed effect), with the replicate as a block effect, on the total biomass, number of ramets, stem mass, leaf mass and root mass of the central and peripheral parts of fragments of *A. philoxeroides*, as well as of the whole fragments. We also used two-way ANOVAS to examine the effects of soil heterogeneity (homogeneous vs. heterogeneous; fixed factor) and stolon connection (intact vs. severed; fixed effect), with the replicate as a block effect, on the total biomass, number of ramets, root to shoot ratio, stem mass, leaf mass and root mass of the whole population. Two-way ANCOVAS were used to test the patch type (or heterogeneous) and stolon connection effects on the root to shoot ratio at the ramet, whole fragment and population scales, with the replicate as a block effect and total mass as covariate. When there were significant effects detected, linear contrasts were employed to examine the difference between individual means of connection treatments within each specific nutrient patch-type (or heterogeneous) treatment, and the difference between individual means of patch-type or heterogeneous treatments within each specific connection treatment, using LMATRIX subcommands in the SPSS syntax (see IBM SPSS statistic 22.0 command syntax reference). All data met the assumption of homogeneity of variances and thus did not need any data transformations before analysis. All analyses were conducted using SPPS 22.0 (SPSS, Chicago, IL, USA).

Results

Performance of the central parts

No growth measures of the central parts in *A. philoxeroides* fragments were affected by the local resource availability of peripheral patches, even when the stolon connection was kept intact (Table 1a). The above-ground growth as measured by stem and leaf mass was not affected by stolon connection (Fig. 2d,e), but the total mass and root mass increased and the number of nodes tended to decrease ($P < 0.1$) when the stolon connection was kept intact (Table 1a; Fig. 2a,b,f). The root to shoot ratio was only affected by stolon connection (Table 3a), showing that the maintenance of stolon connection could increase the root to shoot mass ratio of central parts, but the positive effect appeared to be more obvious for the central parts attached to the peripheral parts that rooted in the high-nutrient patches of the heterogeneous treatment (Table 1a; Fig. 2c).

Performance of the peripheral parts

There were significant interaction effects of patch type by stolon connection detected in total mass, stem mass and leaf mass, but not in the number of nodes and root mass (significant P × C effect, Table 1b; Fig. 3b,f). The total mass, leaf mass and stem mass were significantly greater in the high- and medium-nutrient patches of the heterogeneous treatment than in the corresponding low-nutrient patch and uniform patches of the homogeneous treatment (Table 1b; Fig. 3a,d,e). For the peripheral parts grown in high-nutrient patches, the total mass, stem mass and leaf mass were significantly greater when the stolon connection was intact than when it was severed (Table 1b; Fig. 3a,d,e). The root to shoot ratio became lower when the stolon connection was intact than when it was severed, especially in the low-nutrient patches (Table 3; Fig. 3c).

Table 1 Two-way ANOVA results for effects of patch type (uniform patch in the homogeneous treatment, high-, medium- *and* low-nutrient patches in the heterogeneous treatment) and stolon connection (intact *vs.* severed) on the growth of the central part, the peripheral part and the whole fragment of *Alternanthera philoxeroides*, with the replicate as a block effect

Effects	d.f.	Total mass	Number of nodes	Stem mass	Leaf mass	Root mass
(a) Central part						
Replicate	6	1.4^{ns}	2.9^{*}	1.8^{ns}	0.5^{ns}	1.9^{ns}
Patch (P)	3	0.9^{ns}	$<0.01^{ns}$	0.9^{ns}	0.5^{ns}	3.9^{ns}
Connection (C)	1	10.9^{*}	$8.6^{\#}$	0.3^{ns}	1.1^{ns}	$<0.01^{***}$
P × C	3	0.4^{ns}	1.2^{ns}	0.5^{ns}	0.9^{ns}	0.4^{ns}
Error	42	—	—	—	—	—
(b) Peripheral part						
Replicate	6	10.5^{***}	26.7^{***}	10.6^{***}	11.1^{***}	3^{*}
Patch (P)	3	2.1^{ns}	3.7^{ns}	1.9^{ns}	3.9^{ns}	1.1^{ns}
Connection (C)	1	1.9^{ns}	5.3^{ns}	2.4^{ns}	3.1^{ns}	$<0.01^{ns}$
P × C	3	3.5^{*}	2.2^{ns}	2.9^{*}	3.7^{*}	2^{ns}
Error	42	—	—	—	—	—
(c) Whole fragment						
Replicate	6	9.7^{***}	27.3^{***}	10.7^{***}	9.4^{***}	3.3^{**}
Patch (P)	3	2.3^{ns}	3.4^{ns}	2.2^{ns}	3.8^{ns}	1.4^{ns}
Connection (C)	1	2.7^{ns}	1.8^{ns}	2.7^{ns}	2.4^{ns}	$<0.01^{ns}$
P × C	3	$2.7^{\#}$	1.9^{ns}	$2.4^{\#}$	3^{*}	1.6^{ns}
Error	42	—	—	—	—	—

Degree of freedom (d.f.) and F values are given. Symbols give P: *** < 0.001; ** < 0.01; * < 0.05; $^{\#}$ < 0.1; ^{ns} ≥ 0.1.

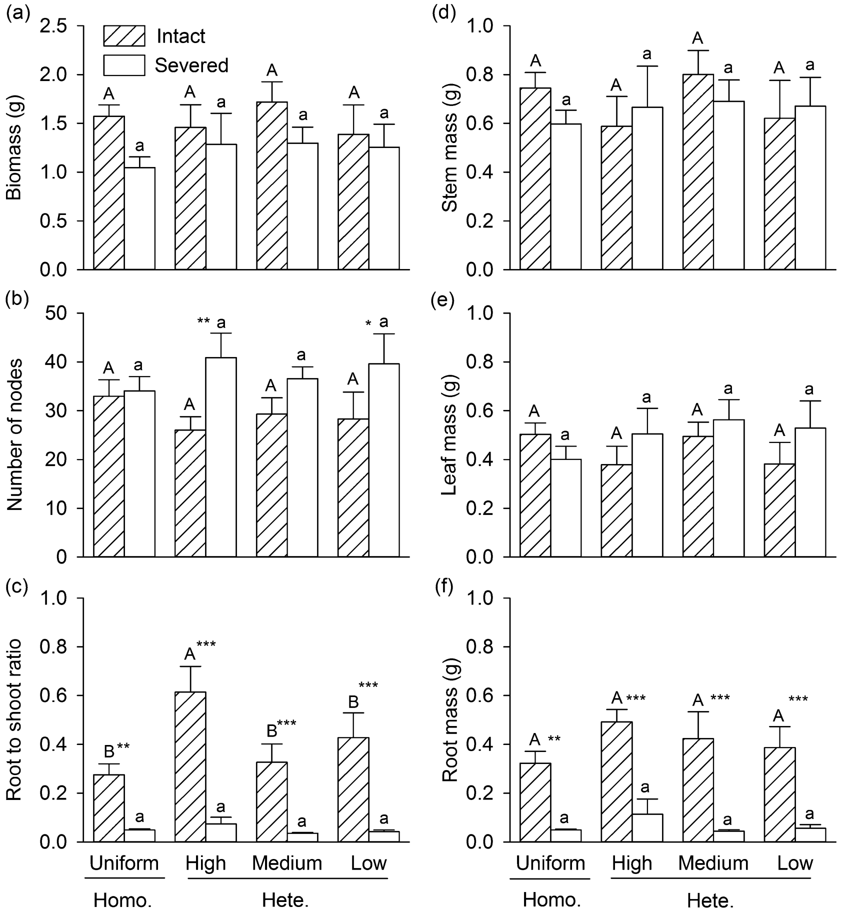

Fig. 2 Effects of patch type (uniform, high-, medium- and low-nutrient patches) and stolon connection (intact vs. severed) on (a) total biomass, (b) number of nodes, (c) root to shoot ratio, (d) stem mass, (e) leaf mass and (f) root mass in the central parts of *Alternanthera philoxeroides*. Error bars show +1 SE. Letters show which means differed between patch-type treatments within stolon connection treatments; symbols show which means differed between stolon connection treatments within patch-type treatments; no symbol means P > 0.05; *P = 0.01–0.05; **P = 0.001–0.01; ***P < 0.001.

Performance of the whole fragment

In the patch-type treatment, the growth pattern of the whole fragment was the same as that of the peripheral parts (Table 1b,c). The total mass, leaf mass and stem mass in the whole fragment were significantly greater when the peripheral parts of the stolon fragments were rooted in the medium- and high-nutrient patches. Meanwhile, only for clonal fragments attached to the peripheral parts grown in high-nutrient patches, were the total mass, stem mass and leaf mass significantly greater when the stolon connection was intact than when it was severed (Table 1; Fig. 4a,d,e). On the other hand, the number of nodes and the root mass were not affected by the patch-type treatment (Table 1c; Fig. 4b,f). The root to shoot ratio was only affected by the patch-type treatment, but not by the stolon connection treatment or their interaction effect, and the root to shoot ratio was greater in the low-nutrient patches than in the other three patch types (Table 3a; Fig. 4c).

Performance of the whole population

The main effects of soil heterogeneity and stolon connection were detected in most of the growth measures (Table 2), but the root to shoot ratio was not affected by any treatment effects (Table 3b). The total mass, stem mass, leaf mass and root mass were significantly greater in the heterogeneous treatment than in the homogeneous treatment (Fig. 5). Furthermore, all of the growth measures were significantly or tended to ($P < 0.1$) be greater when the stolon fragments were kept intact than when they were severed (Table 2, Fig. 5).

Discussion

The peripheral parts of *A. philoxeroides* fragments could accumulate greater total and above-ground biomass in the high-nutrient patches than in the other two patch types of the heterogeneous treatment and the uniform patches of the homogeneous treatment, especially when the stolon connection was kept intact. Meanwhile, the root to shoot ratio in peripheral parts became greater in the low-nutrient patches, especially when the stolon connection was severed. On the contrary, the central parts did not exhibit any observed responses to different patch quality of the peripheral areas. This result basically supported the classic theory

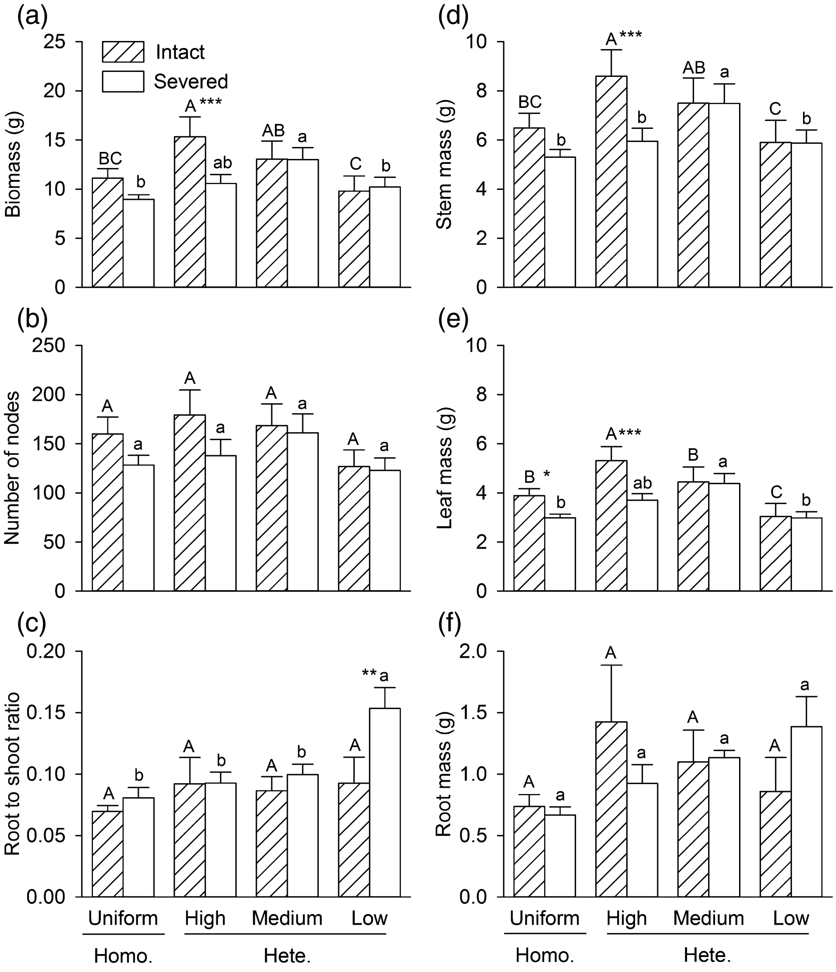

Fig. 3 Effects of patch type (uniform, high-, medium- and low-nutrient patches) and stolon connection (intact vs. severed) on (a) total biomass, (b) number of nodes, (c) root to shoot ratio, (d) stem mass, (e) leaf mass and (f) root mass in the peripheral parts of *Alternanthera philoxeroides*. Error bars show +1 SE. Letters show which means differed between patch-type treatments within stolon connection treatments; symbols show which means differed between stolon connection treatments within patch-type treatments; no symbol means P > 0.05; *P = 0.01–0.05; **P = 0.001–0.01; ***P < 0.001.

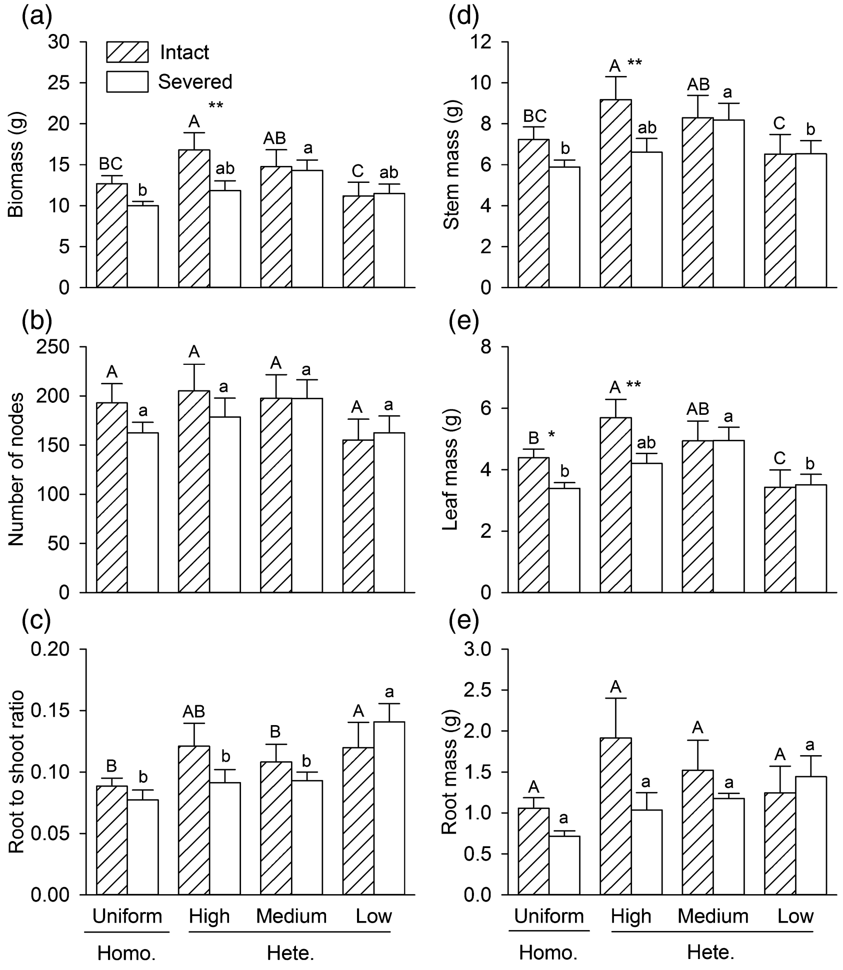

Fig. 4 Effects of patch type (uniform, high-, medium- and low-nutrient patches) and stolon connection (intact vs. severed) on (a) total biomass, (b) number of nodes, (c) root to shoot ratio, (d) stem mass, (e) leaf mass and (f) root mass of *Alternanthera philoxeroides* at the whole-fragment level. Error bars show +1 SE. Letters show which means differed between patch-type treatments within stolon connection treatments; symbols show which means differed between stolon connection treatments within patch-type treatments; no symbol means P > 0.05; *P = 0.01–0.05; **P = 0.001–0.01; ***P < 0.001.

of biomass allocation (i.e. the increase of the proportional biomass allocated to produce the structures that have to acquire the limiting resource, Thornley 1972; Bloom *et al.* 1985), and also reflected the presence of nutrient effects even when the ramets under the low-nutrient conditions did not exhibit an apparent nutrient scarcity.

The maintenance of stolon connection remarkably improved the biomass assimilation of the central ramets in *A. philoxeroides* fragments, as well as that of peripheral ramets grown in high-nutrient patches. This result was partly consistent with the first two hypotheses, suggesting that the effect of the stolon connection in peripheral ramets strongly depended upon patch type, and that the maintenance of stolon connection would become a 'win-win' strategy for both young and older ramets in *A. philoxeroides* fragments that experienced high-nutrient patches. In another work on the amphibious species *Ipomoea aquatica*, clonal integration also provided similar benefits to both young and older ramets if young ramets experienced an aquatic habitat with high nitrogen concentration (Liu *et al.* 2016). One plausible reason for this is that the increased favorability of the

Table 2 Two-way ANOVA results for effects of soil heterogeneity (heterogeneous *vs.* homogeneous) and stolon connection (intact *vs.* severed) on the growth of the whole population of *Alternanthera philoxeroides*, with the replicate as a block effect

	d.f.	Total mass	Number of nodes	Stem mass	Leaf mass	Root mass
Replicate	6	4.8**	19.8***	5.8**	4.7**	1.7^{ns}
Heterogeneity (H)	1	5.8*	0.5^{ns}	4.7*	4.5*	<0.01^{ns}
Connection (C)	1	6.6*	6*	5.9*	7.7*	3.5$^{\#}$
H x C	1	0.3^{ns}	2.5^{ns}	0.2^{ns}	1^{ns}	<0.01^{ns}
Error	18	—	—	—	—	—

Degree of freedom (d.f.) and F values are given. Symbols give P: *** < 0.001; ** < 0.01; * < 0.05; $^{\#}$ < 0.1; ^{ns} ≥ 0.1.

Table 3 Two-way ANCOVA results for (a) effects of patch type and stolon connection on the root to shoot ratio of the central part, the peripheral part and the whole fragment of *Alternanthera philoxeroides*, and (b) effects of soil heterogeneity and stolon connection on the root to shoot ratio of the whole population, with the replicate as a block effect and total mass as the covariate

(a)	d.f.	Central part	Peripheral part	Whole fragment	(b)	d.f.	Whole population
Total mass	1	1.2^{ns}	11.1^{**}	14.5^{***}	Total mass	1	10.3^{**}
Replicate	6	1.3^{ns}	1^{ns}	1.5^{ns}	Replicate	6	1.6^{ns}
Patch (P)	3	1.6^{ns}	$6.4^{\#}$	12.1^{*}	Heterogeneity (H)	1	0.2^{ns}
Connection (C)	1	33.1^{**}	11^{**}	0.3^{ns}	Connection (C)	1	2.1^{ns}
P × C	3	2.2^{ns}	1.1^{ns}	0.6^{ns}	H × C	1	0.1^{ns}
Error	41	—	—	—	Error	17	—

Degree of freedom (d.f.) and F values are given. Symbols give P: $^{***} < 0.001$; $^{**} < 0.01$; $^{*} < 0.05$; $^{\#} < 0.1$; $^{ns} \geq 0.1$.

peripheral habitat could accelerate the development and maturation of young ramets, and thus reduce the costs from clonal integration to older ramets. The other plausible explanation is that young ramets under high-nutrient conditions will have a higher probability of being new donors because they take over the role of older ramets at the late developmental stage, although the initial direction of clonal integration is often acropetal from older to younger ramets (Tomasko & Dawes 1989; Xiao *et al*. 2007; Liu *et al*. 2016).

The result was also consistent with the third hypothesis, showing that the maintenance of stolon connection could further trigger an observed division of labor between the central and peripheral ramets (Wang *et al*. 2009; Luo *et al*. 2014; Dong *et al*. 2015), with the increased above-ground growth (measured by stem and leaf mass) of peripheral ramets grown in high-nutrient patches and increased root investment (measured by root to shoot ratio and root mass) of central parts. For central parts, the ramet specialization derived from a developmentally programmed division of

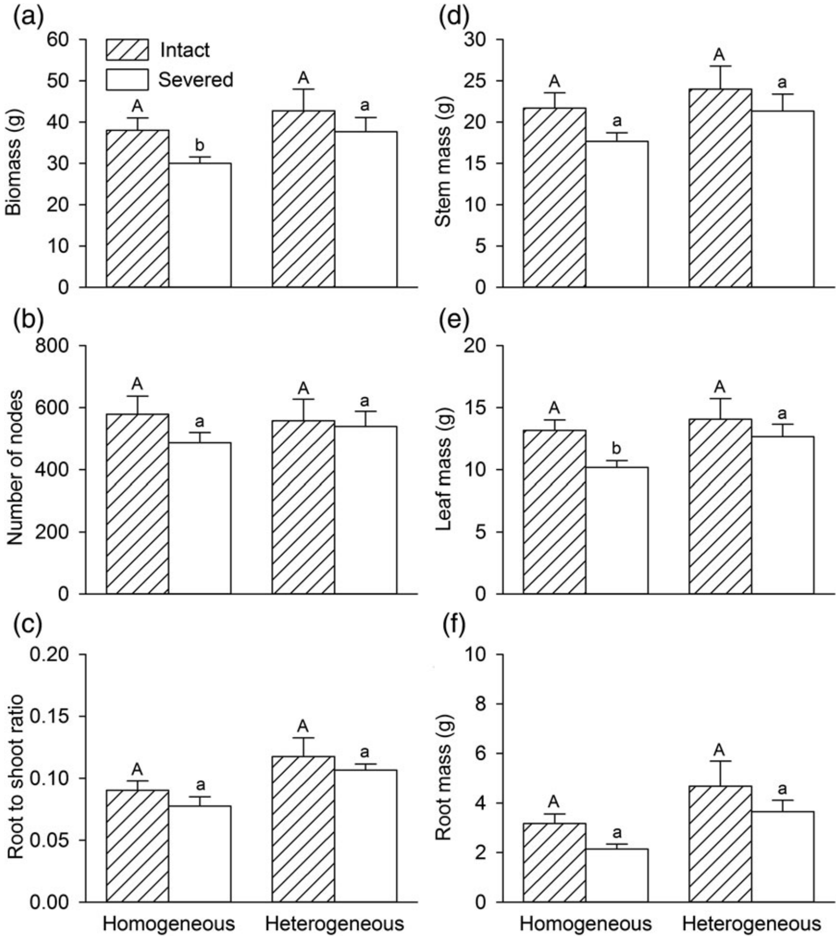

Fig. 5 Effects of soil nutrient heterogeneity (homogeneous and heterogeneous) and stolon connection (intact and severed) on (a) total biomass, (b) number of nodes, (c) root to shoot ratio, (d) stem mass, (e) leaf mass and (f) root mass of *Alternanthera philoxeroides* at the whole-population level. Error bars show +1 SE. Letters show which means differed between heterogeneous treatments within stolon connection treatments; symbols show which means differed between stolon connection treatments within heterogeneous treatments; no symbol means P > 0.05; *P = 0.01–0.05; **P = 0.001–0.01; ***P < 0.001.

labor characterized by some hormones (e.g. indole-3-acetic acid) regulating the ontogeny of the ramets, as detected by the significant main effect of stolon connection in Table 1(a) (Julien & Bourne 1986; Stuefer 1998; Alpert *et al.* 2002; Schwarzschild & Zieman 2008). For peripheral parts, the ramet specialization would be further modified by nutrient variability in the peripheral patches, as detected by the significant effect of clonal integration related to patch type in Table 1(b) (Alpert & Stuefer 1997; Hutchings & Wijesinghe 1997; Stuefer 1998; Roiloa *et al.* 2013). To some extent, the developmentally programmed division of labor could provide *A. philoxeroides* with the ability, in terms of the plasticity of biomass allocation, to respond to soil heterogeneity.

The positive consequence of clonal integration related to patch type (measured by total mass and above-ground mass) was also detected in the whole fragment. The result was the same as that in peripheral ramets rather than central ramets. The result was consistent with the fourth hypothesis, showing that the benefits of clonal integration to peripheral ramets outweighed the costs to central ramets, and also that the peripheral ramets made a greater contribution to the finial fitness of the whole fragment compared with the central ramets, possibly because of its body size, which is approx. 7.6–9.3 times bigger in biomass than the central parts. Otherwise, if there was similar body size between different-aged ramets, the outcome of clonal integration in the whole fragment would be determined together by all parts, as shown in some previous studies (Wang *et al.* 2009). For example, for the ramet pair of *A. philoxeroides* with similar size, clonal integration can contribute to the expansion of *A. philoxeroides* from terrestrial to aquatic habitats by increasing the growth of young ramets, but did not bring the net benefits at the whole clone scale, because the costs to older ramets counterbalanced the benefits to younger ramets (Wang *et al.* 2009).

Because high-nutrient patches could stimulate biomass assimilation in whole fragments, especially for fragments with an intact stolon connection, and low-nutrient patches did not cause any limiting effects, the positive consequences of either soil heterogeneity or stolon connection were also detected at the population scale. The results suggested that the individual performance of *A. philoxeroides* at small scales could be extrapolated to the productivity of the whole population, at least in the present experiment. However, some previous researchers also provided contrasting results; for example, the increased growth of the ramet population in *Fragaria vesca* and the population yield in *Cardamine hirsute* in the heterogeneous soil environment might be terminated after a long growth period (Day *et al.* 2003a; Roiloa & Retuerto 2006). A possible reason for these opposite results is that the continuing nutrient consumption of individuals in high-nutrient patches degraded the favorability of the habitat (Casper & Cahill 1996; Day *et al.* 2003a; Roiloa & Retuerto 2006; Dong *et al.* 2014), or the dramatically increased density of newly produced ramets might also weaken the benefits from morphological or physiological plasticity in heterogeneous environments (Hartnett & Bazzaz 1985; Schmid & Bazzaz 1987; Oborny & Kun 2001; Dong *et al.* 2014). Nevertheless, at least in the early developmental period, the maintenance of stolon connection and soil heterogeneity will both determine the productivity and size of *A. philoxeroides* populations.

Conclusions

Stolon connection could trigger a developmentally programmed division of labor between different-aged ramets of *A. philoxeroides*, by increasing root mass in the central parts and above-ground growth in the peripheral parts. The maintenance of stolon connection also became a double-win strategy for *A. philoxeroides* when responding to heterogeneous distributed nutrients, which contributed to the fitness of either the older or younger parts that experienced peripheral high-nutrient patches. The effect of stolon connection also brought benefits to the productivity of the whole population. Overall, the maintenance of stolon connection (i.e. clonal integration) is an important life-history trait for *A. philoxeroides* that enhances both the offspring recruitment in clonal fragments and the rapid expansion of the whole population.

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