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POPULATION ECOLOGY - ORIGINAL RESEARCH

Clonal integration in homogeneous environments increases performance of *Alternanthera philoxeroides*

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Abstract Physiological integration between connected ramets can increase the performance of clonal plants when ramets experience contrasting levels of resource availabilities in heterogeneous environments. It has generally been shown or assumed that clonal integration has little effect on clonal performance in homogeneous environments. However, a conceptual model suggests that integration could increase performance in a homogeneous environment when connected ramets differ in uptake ability and external resource supply is high. We tested this hypothesis in a greenhouse experiment with the amphibious plant *Alternanthera philoxeroides*. Ramets in clonal fragments containing three rooted and two unrooted ramets were either left connected or divided into a basal part with two rooted ramets and an apical part with the other ramets. To simulate realistic, homogeneous environments of the species with different levels of resource supply, plants were grown at 0, 20, or 40 cm of water depth. Water depth had a positive effect on most measures of growth, indicating that resource supply increased with depth. Connection had negative to neutral effects on total growth of fragments at a water depth of 0 cm, and neutral to positive effects at 20- and 40-cm depths; effects on the apical part were generally positive and larger at greater depth; effects on the basal part were generally negative and smaller at greater depth. Results largely supported the hypothesis and further suggest that clonal integration of allocation and reproduction may modify benefits of resource sharing in homogeneous environments.

Keywords Allocation · Clonal plant · Developmental stage · Physiological integration · Water depth

Introduction

Clonal plants are those that produce new potentially physiologically independent units, called ramets, along stems or roots such that new ramets stay connected to the parent at least until they are established, forming sets of connected ramets, or clonal fragments (de Kroon and van Groenendael 1997). Clonal integration, the movement of resources or signals between connected ramets within clonal fragments, has been repeatedly shown to increase the performance of clonal plants in heterogeneous environments where connected ramets experience different levels of resource availability (e.g., Alpert and Mooney 1986; Hutchings and Wijesinghe 1997; Xu et al. 2010; Zhang et al. 2012; Song et al. 2013; Touchette et al. 2013). By resource, we mean something whose use by one plant precludes its simultaneous use by another, such as light, water, mineral nutrients, CO_2, or O_2 taken up by plant organs; or physical space used for attachment.

Clonal integration between ramets with similar ability to take up and use resources has not been shown to increase their combined performance, i.e., clonal performance, in homogeneous environments (e.g., Evans and Whitney 1992; Alpert 1999; Yu et al. 2002). Performance of clonal fragments in homogeneous environments has, accordingly, often been used as an experimental control for effects of

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clonal integration (e.g., Poor et al. 2005; He et al. 2011; Song et al. 2013), based on the assumption that integration has no effect when resource availability is spatially uniform. However, clonal fragments often contain ramets that are in different stages of development and differ in ability to take up resources (e.g., Alpert 1996; Roiloa et al. 2013). In this case, connected ramets might take up different amounts of resources even when exposed to the same external levels of resource supply, and resource sharing between ramets might increase clonal performance even in an homogeneous environment. Although many natural habitats are probably heterogeneous on the scale of connected ramets within clones, other natural and some anthropogenic habitats are likely relatively homogeneous (e.g., Alpert 1999; Dutilleul 2011; Caldwell and Pearce 2012), such as some shallow wetlands where movement of water can homogenize fine-scale conditions and anthropogenic habitats such as crop fields and irrigation ditches that are intended to be uniform. However, no previous study appears to have tested the hypothesis that clonal integration increases performance on the level of the clone when clonal fragments contain ramets that differ in uptake capacity.

To test this hypothesis, we conducted an empirical test expanding upon an earlier conceptual model of clonal performance, clonal integration, and external resource supply (Alpert 1999) by including ramets with different resource uptake ability (Fig. 1). We considered the case in which ramet performance first increases linearly with an increased external resource supply and then levels off with a further increase in resource supply, as could be the case when a different resource then limits performance. This type of relationship between plant performance and resource supply is frequently observed (Alpert 1991; Vermeulen et al. 2009; Lobo et al. 2013), although modifications such as zero performance at a very low resource supply or a decrease in performance at a very high resource supply are also common (Jackson 2000; Lambers et al. 2008).

A second condition was that ramets be either unestablished and have zero uptake or be fully established such that uptake ability does not limit internal supply. This was to some extent a heuristic simplification, since natural clonal fragments of many clonal plant species can contain ramets in intermediate stages of establishment (de Kroon and van Groenendael 1997). However, natural clonal fragments can consist of rooted and unrooted ramets that can approximate this condition with regard to resources such as water or mineral nutrients in some wetland and terrestrial habitats (e.g., Roiloa and Hutchings 2013). For instance, *Fragaria chiloensis* in California tends to produce new ramets during a dry season that precludes rooting, so that many fragments consist only of well rooted and unrooted ramets during much of the year (Alpert 1996). We assumed that resource sharing between connected ramets equalizes their internal resource supply, as expected if sharing is controlled by source–sink relationships (e.g., Alpert 1999). We also assumed that transport between connected ramets has no significant metabolic cost, since no such cost appears to have been documented so far.

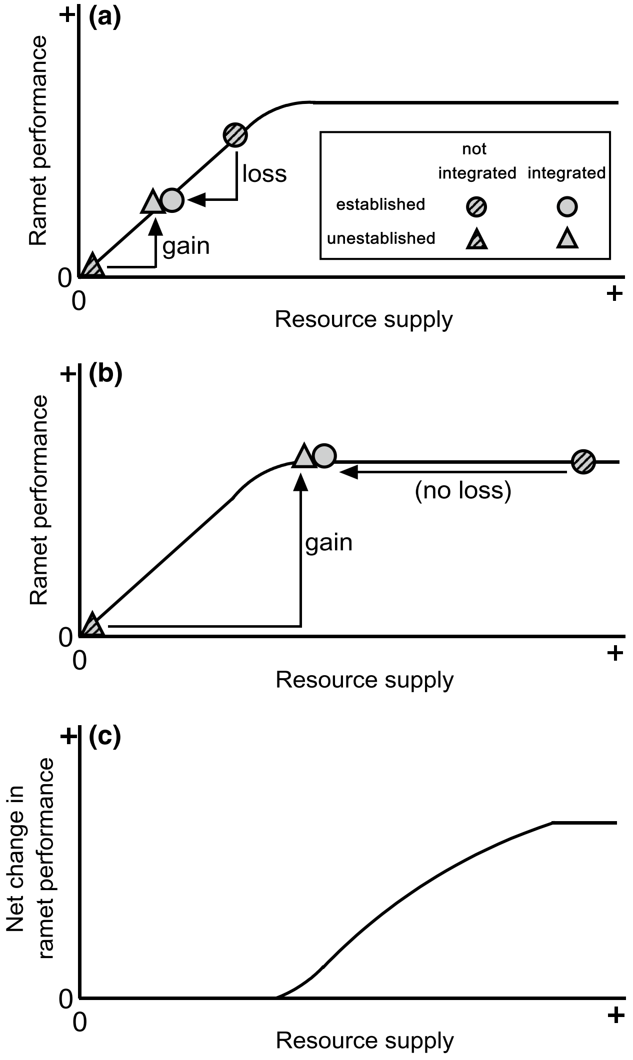

Fig. 1 Expected effects of clonal integration, establishment of ramets, and external resource supply on performance of **a** individual ramets when resource supply is low, **b** individual ramets when resource supply is high, and **c** clones. See text for additional explanation

At rates of external resource supply where performance increases linearly with supply, the increase in performance of an unestablished ramet due to import of the resource from an established ramet should then equal the decrease in performance of the established ramet due to export and produce no net change in clonal performance (Fig. 1a). The effect of integration should reach a maximum when supply is at least twice as high as the level at which performance of a fully established ramet reaches a maximum (Fig. 1b).

Net change in clonal performance due to resource sharing should thus show an S-shaped relationship to resource supply (Fig. 1c). The effect of clonal integration on clonal performance should be zero below the point at which the increase in performance of an established ramet with an increase in supply starts to depart from linearity. The effect of clonal integration should be maximal when supply is at least twice as high as the level at which performance of a fully established ramet is maximal.

This suggests three general predictions about the effects of clonal integration on performance of clones in homogeneous environments even when the exact relationship between external resource supply and ramet performance is not known. First, the net effect of integration on clonal performance should be more positive at high than at low levels of spatially uniform resource supply. This follows from the above assumptions that importing ramets are limited by uptake capacity rather than external resource availability and thus benefit equally from import when resource levels are low to at least moderately high, and that exporting ramets are less limited by external resource availability when resource levels are higher and thus experience less reduction in performance due to export of resource when external levels are higher. Second, the effect of integration on ramets with relatively low uptake capacity should be positive and greater at high than at low levels of supply. Third, the effect of integration on ramets with relatively high uptake capacity should be negative but smaller at high than at low levels of supply. We tested these predictions in an experiment designed to be relevant to clonal fragments of the well-studied, amphibious, clonal plant *Alternanthera philoxeroides* under conditions in many habitats where it occurs in China.

Materials and methods

Species and propagation

Alternanthera philoxeroides (Mart.) Griseb. (Amaranthaceae) is an amphibious, perennial herb native to South America (Julien et al. 1995; Holm et al. 1997). The decumbent stems vary in orientation from prostrate to erect and can bear opposite leaves, new stems, and roots at each node, which thus corresponds to a ramet. In China, *A. philoxeroides* has spread widely in moist, terrestrial to aquatic habitats including irrigation ditches and riparian crop fields where humans have likely reduced fine-scale heterogeneity (Ma and Wang 2005; Pan et al. 2007; Wang et al. 2008, 2009; Yu et al. 2009). The species rarely produces viable seeds and has very low genetic diversity in China (Ye et al. 2003; Li and Ye 2006; Geng et al. 2007), suggesting that it spreads mainly vegetatively. Clonal integration can increase the performance of *A. philoxeroides* in heterogeneous environments (Yu et al. 2009; Xu et al. 2010).

Plants of *A. philoxeroides* were collected from a riparian agricultural area in Taizhou (28.87°N, 121.01°E) on 18–19 May 2011 in Zhejiang Province, China, and vegetatively propagated at ambient temperature and light in a greenhouse operated by Forest Science Co., Ltd., for Beijing Forestry University in Beijing. It is very likely that all plants belonged to the same genotype because only one genotype has been found in southern China (Ye et al. 2003).

On 16 July 2011, 36 clonal fragments each consisting of a single stem about 25 cm long with five nodes and an apex were selected for experimental use. *A. philoxeroides* often occurs naturally in fragments at least this big (Liu and Yu 2009).

Six additional, similar fragments not used in the experiment were dried to constant mass to give a measure of initial dry mass, which was 444 ± 21 mg [mean ± standard error of the mean (SE)].

The selected fragments were placed on a 1:1 (v:v) mixture of soil collected from a riverbank near Beijing (0.99 g total N kg^{-1} dry mass of soil and 0.63 g total P kg^{-1}) and peat (Pindstrup Seeding; Pindstrup Mosebrug A/S, Pindstrup, Denmark); the peat was added to increase porosity of the clayey riverbank soil. The three most basal nodes contacted the soil and rooted. The natural, upward curvature of the stem held the two most apical nodes above the soil so that they did not root; the initial height above of the soil of the apices of fragments was about 10 cm. On 22 July, fragments were transplanted into nine plastic containers that were each physically divided by plastic partitions into eight cells that were each 20 cm wide × 20 cm long × 60 cm deep. Cells were filled to a depth of 15 cm with the soil mixture described above. The container walls and partitions above the soil level were transparent (70 % transmission of photosynthetically active radiation, measured using a LI-250A light meter, Li-Cor, Lincoln, NE, USA) to reduce shading.

Treatments and measurements

The experiment was conducted in the same greenhouse as propagation and used a full factorial design with three water depth treatments (0, 20 and 40 cm above the soil surface) crossed with two severance treatments [stem of *A. philoxeroides* left intact or severed into an apical part comprising the 2 unrooted ramets and 1 rooted ramet and a basal part comprising the remaining 2 rooted ramets (Fig. 2a)]. There were six replicates of each treatment combination. We varied water depth to simulate natural environments of *A. philoxeroides* that are likely to be relatively homogenous but different in resource supply. Use of water depth provided realism because water depth varies over time and

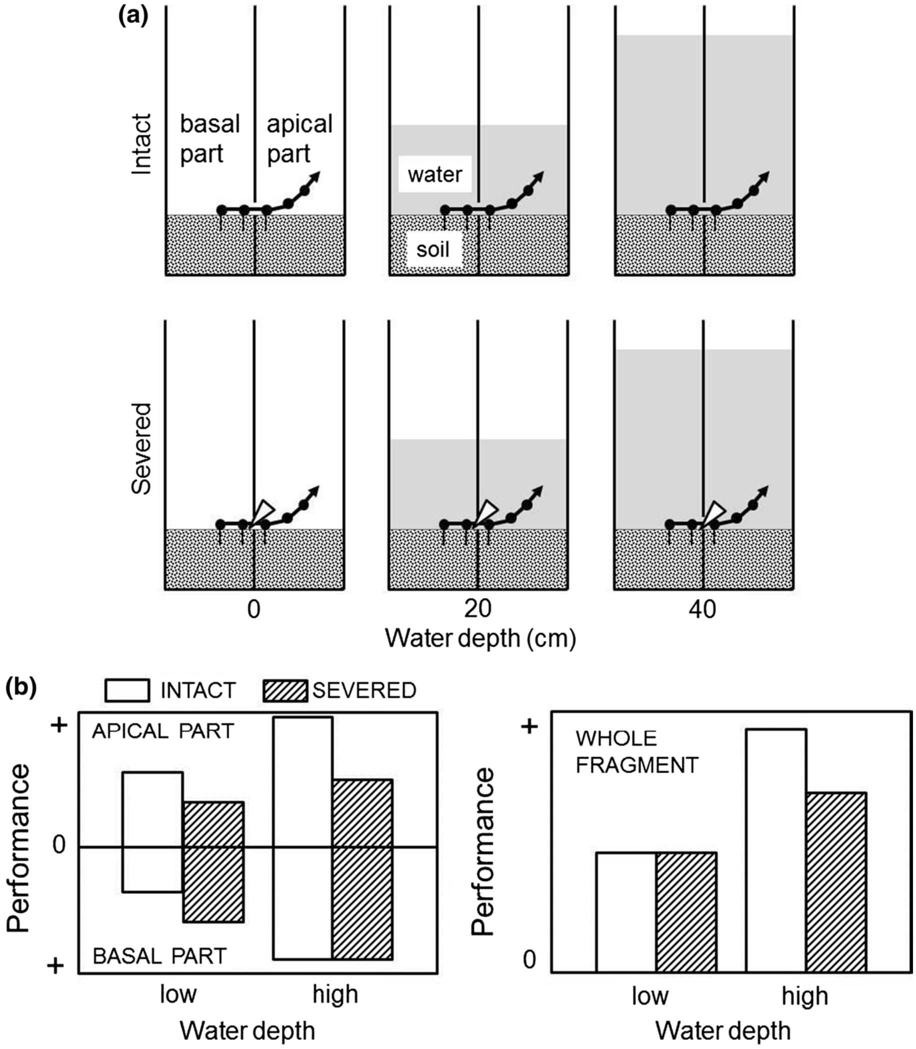

Fig. 2 Experimental design: **a** clonal fragment and severance treatment; **b** predicted outcomes. Outcomes depicted for low and high water depth match cases in Fig. 1a and b, respectively. See text for additional explanation

between habitats of the species but is often spatially uniform on the scale of clonal fragments (Liu and Yu 2009). Moreover, submergence to moderate depths increases performance of *A. philoxeroides* (Schooler et al. 2010), suggesting that supply of a limiting resource increases with depth, and we likewise found that the total final dry mass of intact fragments was higher at greater water depth (see "Results"). Although manipulation of water depth could affect supply of multiple resources and did not identify the specific resources limiting growth, it provided an initial screen across resources for the validation of the model and a test with clear relevance to growth in natural habitats. We discuss how the observed effects of water depth might be interpreted in terms of resources in the discussion below.

We used severance to prevent clonal integration. To test the effect of clonal integration between ramets with different uptake capacities, we chose the severance point within a fragment so as to create apical and basal parts with different proportions of unrooted ramets and, consequently, different overall uptake capacities. The apical part was mostly unestablished, and the basal part entirely established. The severance point was also chosen so that each part would have more than one ramet and would have at least one rooted ramet and, as such, be more likely to survive after severance.

Given that clonal integration between apical and basal parts occurred in the intact but not in the severed treatment, and that the apical part of a fragment had less uptake capacity than the basal part, then the three general predictions introduced above, respectively, led to three expected outcomes (Fig. 2b). Severance would: (1) decrease performance of whole fragments (i.e., the apical and basal parts combined) more at greater water depth; (2) decrease the performance of the apical part of a fragment and more so at greater water depth; and (3) increase the performance of the basal part of a fragment but less so at greater water depth. To support the requirement that water depth serves as a proxy for resource supply, it was also expected that the performance of whole fragments and of their apical and basal parts individually would be greater at greater water depth within each severance treatment.

The apical and basal parts of each fragment were transplanted into adjacent cells in a container as described above. In the intact treatment, the partition between the two cells was pierced with a 3-cm-diameter hole at the level of the soil surface to allow passage of the intact, connecting stolon between parts. Putting different parts in different cells facilitated separate harvest of the roots of each part at the end of the experiment. Fragments were randomly assigned to pairs of cells, and treatments were assigned to pairs of cells in a regular pattern such that each treatment occupied nearly the same mean position. Containers were placed close together to minimize environmental differences across the array.

Plants were watered with tap water. In the 0-cm water depth treatment, the soil was kept saturated with water so that only the water depth above the soil differed between water depth treatments. Air temperature during the experiment was 23.5 ± 0.4 °C, measured hourly with a Hygrochron temperature logger (iButton DS1923; Maxim Integrated Products, CA, USA). During the experiment, almost all nodes in contact with the soil produced new stems, which were decumbent like the original stems. All plants in the 20- and 40-cm treatments were fully submerged at the start of the treatments and produced some emergent growth after approximately 6 weeks of treatment.

After 12 weeks, on 15 October 2011, the apical and basal parts of each fragment were measured for total stem length, number of nodes, and leaf area (WinFOLIA Pro 2004a, Regent Instruments, Inc., QC, Canada), divided into roots, stems and leaves, and dried at 70 °C for 48 h and weighed.

Data were analyzed using two-way analysis of variance (ANOVA) in Systat 12 (Systat Software, Inc., Chicago, IL, USA). All data met the assumptions of homogeneity of variances and normality, and, thus, did not need transformation before analysis. The model had water depth and severance as fixed factors and total dry mass, shoot mass, root mass, leaf area, number of stem nodes, or length of stems as the dependent variables. Differences between individual means were tested with Tukey tests and orthogonal comparisons; because we tested a limited number of planned comparisons rather than all possible pair-wise comparisons, we reported comparisons even when the corresponding, overall effects were non-significant (i.e., $P > 0.05$; Maxwell and Delaney 2004; Rutherford 2012). Separate analyses were run for apical and basal parts and for whole fragments. In each case, we used the relevant mass from the whole part or fragment as the raw data, rather than the mean of the ramets within a part or fragment.

Results

Interaction between effects of connection and water depth

Severance of the connection between the apical and basal parts of a fragment was expected to decrease performance of the apical part and more so at greater water depth. Aboveground growth largely met this expectation. Severance significantly affected final total mass, shoot mass, leaf area, number of nodes, and total length of stems of apical parts (Table 1). These measures were all greater when the connection between the apical and basal part of a fragment was intact than when it was severed (Fig. 3). The differences between individual means for apical parts in intact and severed fragments tended to be more significant at greater depth for all measures of aboveground size except for number of nodes (Fig. 3), though the interactive effects of severance and water depth were not significant (Table 1). Severance did not significantly affect the final root mass of apical parts.

Severance of the connection between the apical and basal parts of a fragment was expected to increase performance of the basal part but less so at greater depth. Consistent with this expectation (Table 1; Fig. 3), final total mass and shoot mass of basal parts were greater in severed than in intact fragments at 0 but not at 20 or 40 cm of depth, effect of severance on root mass of basal parts was more positive at a lower water depth, and effects of severance on final leaf area, number of nodes, and length of stems were positive at low water depth. Contrary to expectations, the effect of severance on root mass was negative at high water depth, and effects of severance on leaf area, nodes, and stem length did not decrease with greater water depth.

Severance was expected to decrease performance of whole fragments more at greater water depths. Consistent with this expectation, severance had little effect on leaf area at a depth of 0 cm and decreased it at depths of 20 and 40 cm (Table 1; Fig. 4). Total mass, shoot mass, and root mass of whole fragments likewise showed more negative effects of severance at greater water depths, but the interactive effects of severance and water depth were very marginally significant (Table 1; $P = 0.1$–0.15). Effects of severance and of the interaction between severance and water depth on the final number of nodes and total stem length of whole fragments were relatively small (Table 1; Fig. 4). Severance increased allocation of mass to roots slightly in apical parts (Fig. 5; $F_{1,30} = 19.1$, $P < 0.001$) and decreased it greatly in basal parts ($F_{1,30} = 84.3$; $P < 0.001$); there was no interaction between effects of severance and water depth on allocation to roots in either apical ($F_{2,30} = 0.2$, $P = 0.8$) or basal parts ($F_{2,30} = 0.6$, $P = 0.6$).

Table 1 ANOVAs of effects of severance and water depth on final mass, size, and morphology of fragments of *Alternanthera philoxeroides*

Effect	*df*	Total mass		Shoot mass		Root mass		Leaf area		Nodes		Stem length	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Apical part													
Severance (*S*)	1,30	*7.1*	*0.013*	**9.7**	**0.004**	0.5	0.5	**42.4**	**<0.001**	**11.4**	**0.002**	**10.6**	**0.003**
Water (*W*)	2,30	2.7	0.08	**6.4**	**0.005**	**12.2**	**<0.001**	**5.5**	**0.009**	**11.0**	**<0.001**	**13.2**	**<0.001**
S × *W*	2,30	0.6	0.6	0.8	0.5	0.4	0.7	0.3	0.8	0.2	0.8	0.2	0.8
Basal part													
Severance (*S*)	1,30	**11.0**	**0.002**	**17.8**	**<0.001**	1.6	0.2	**70.1**	**<0.001**	**62.9**	**<0.001**	**71.0**	**<0.001**
Water (*W*)	2,30	1.4	0.3	0.8	0.5	*4.9*	*0.015*	0.06	0.9	3.1	0.06	1.2	0.3
S × *W*	2,30	*4.5*	*0.019*	*4.0*	*0.03*	**5.5**	**0.009**	1.8	0.19	1.2	0.3	1.6	0.2
Whole fragment													
Severance (*S*)	1,30	0.3	0.6	0.4	0.5	<0.1	0.9	**7.7**	**0.009**	0.6	0.4	0.8	0.4
Water (*W*)	2,30	0.9	0.4	3.0	0.07	**14.5**	**<0.001**	*4.2*	*0.024*	**12.2**	**<0.001**	**10.8**	**<0.001**
S × *W*	2,30	2.4	0.11	2.3	0.12	2.1	0.14	0.9	0.4	0.4	0.7	0.6	0.6

Values are in italics where $P < 0.05$ and in bold where $P < 0.01$. See figures for data

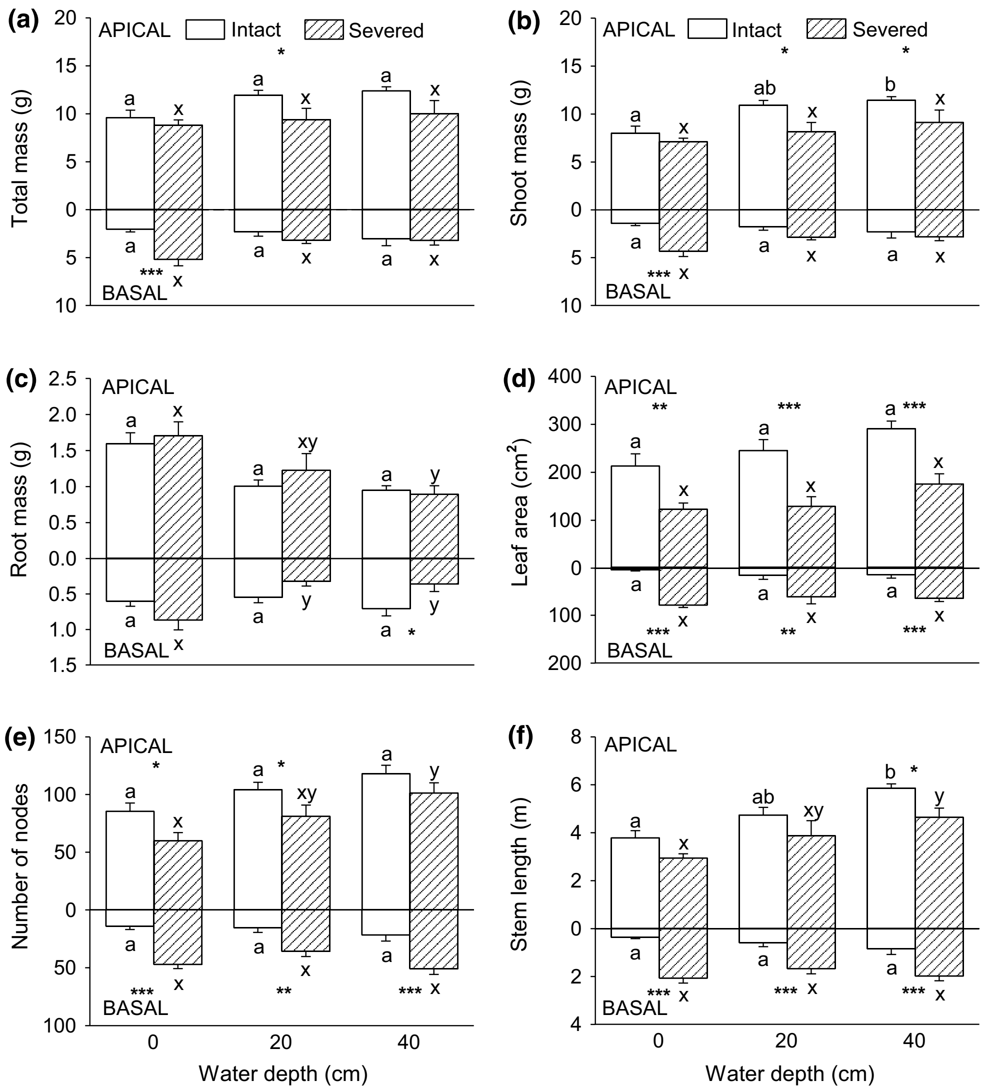

Fig. 3 Effects of water depth and severance on **a** total mass, **b** shoot mass, **c** root mass, **d** leaf area, **e** number of nodes, and **f** stem length of apical parts (*top bars*) and basal parts (*bottom bars*) of fragments of *Alternanthera philoxeroides*. *Bars* show mean + SE. *Letters* show which means differed between water depth treatments within severance treatments (Tukey tests, $P = 0.05$); *symbols* show which means differed between severance treatments within water depth treatments: no symbol means $P > 0.05$; $*P = 0.01–0.05$; $**P = 0.001–0.01$; $***P < 0.001$. See Table 1 for ANOVAs

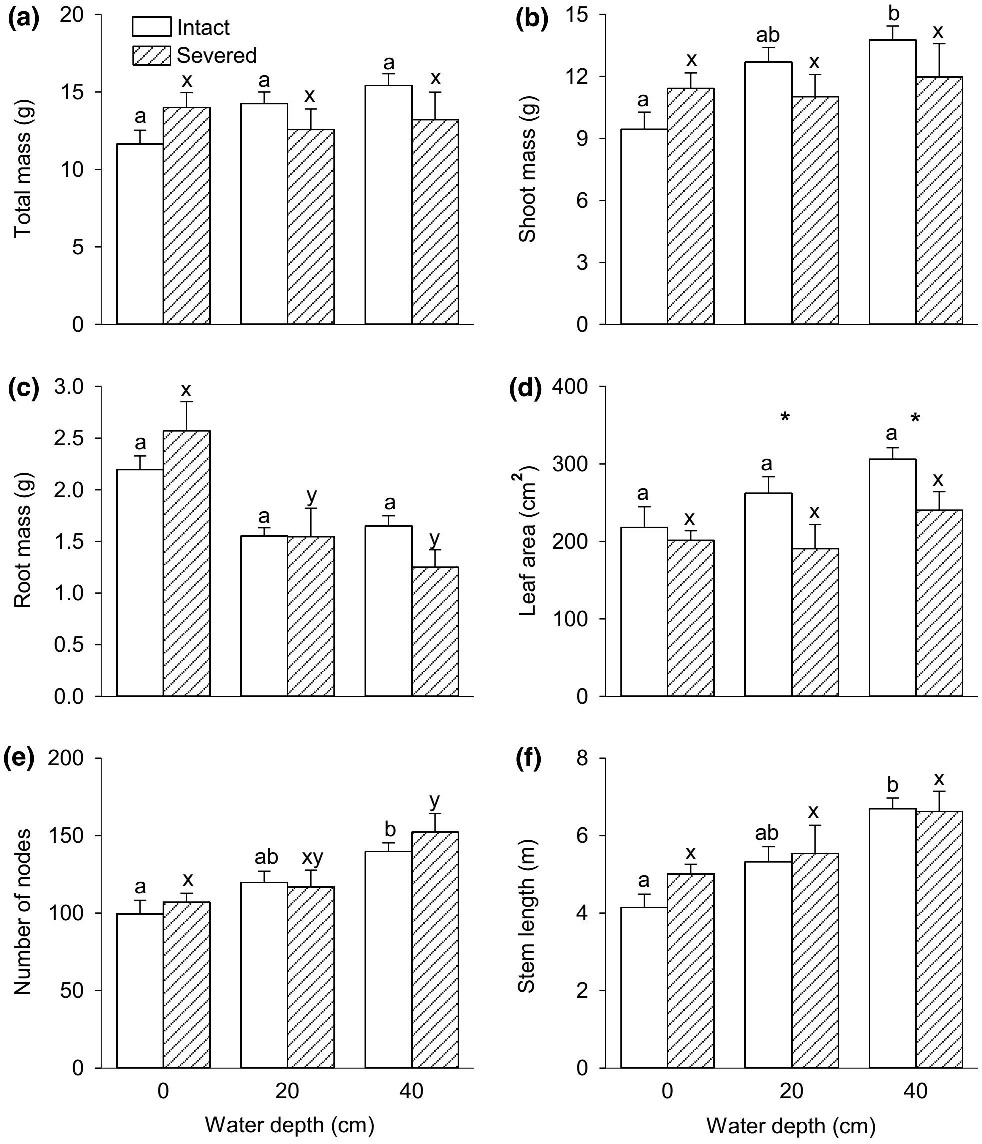

Fig. 4 Effects of water depth and severance on **a** total mass, **b** shoot mass, **c** root mass, **d** leaf area, **e** number of nodes, and **f** stem length of whole fragments of *Alternanthera philoxeroides*. *Bars* show mean + SE. *Letters* show which means differed between water depth treatments within severance treatments (Tukey tests, $P = 0.05$); *symbols* show which means differed between severance treatments within water depth treatments: no symbol means $P > 0.05$; $^*P = 0.01$–0.05; $^{**}P = 0.001$–0.01; $^{***}P < 0.001$. See Table 1 for ANOVAs

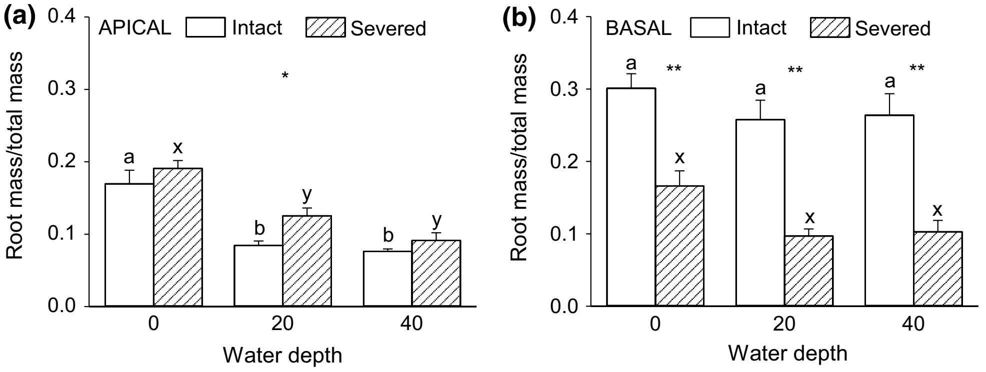

Fig. 5 Effects of water depth and severance on proportion of mass allocated to roots in apical and basal parts of fragments of *Alternanthera philoxeroides*. *Bars* show mean + SE. *Letters* show which means differed between water depth treatments within severance treatments (Tukey tests, $P = 0.05$); *symbols* show which means differed between severance treatments within water depth treatments: no symbol means $P > 0.05$; $^*P = 0.01$–0.05; $^{**}P = 0.001$–0.01; $^{***}P < 0.001$. See text for ANOVAs

Main effect of water depth

In accordance with the use of water depth to control resource supply, the performance of *A. philoxeroides* was generally higher at greater water depth. Across severance treatments in both apical and basal parts of clonal fragments, water depth had neutral to positive effects on accumulation of aboveground mass, vegetative reproduction, and clonal spread, though effect on accumulation of root mass was negative. In apical parts, water depth had highly significant ($P < 0.01$) effects on final shoot mass, root mass, leaf area, number of nodes, and total length of stems (Table 1). Shoot mass, leaf area, number of nodes, and length of stems were about 15–35 % higher at a depth of 40 cm than at a water depth of 0 cm (Fig. 3); root mass was about 50 % lower. In basal parts, the main effects of water depth were less pronounced, but the effects that were significant (Table 1; $P < 0.05$) or marginally significant ($P = 0.05$–0.1) were similar to those on apical parts: the number of nodes was about 25 % higher and root mass was about 40 % lower at a 40-cm depth than at a 0-cm water depth (Fig. 3).

Across severance treatments in whole fragments (i.e., apical and basal parts combined), water depth significantly affected final root mass, leaf area, number of nodes, and stem length, and marginally affected final shoot mass (Table 1). Measures of aboveground size were about 25–50 % higher at 40 cm than at 0 cm of water depth, while final root mass was about 40 % lower (Fig. 4). Apical parts allocated about 50 % less mass to roots at 20 and 40 cm than at 0 cm of water depth (Fig. 5; effect of water: $F_{2,30} = 3.8$, $P = 0.033$); basal parts showed a parallel but less pronounced and only marginally significant pattern ($F_{2,30} = 2.6$, $P = 0.090$).

Discussion

Effects of water depth and severance on clonal fragments of *A. philoxeroides* were largely consistent with general predictions based on the initial model of clonal integration, resource supply, resource uptake capacity, and plant performance introduced above. Based on the general prediction that the net effect of integration on clonal performance should be more positive at high than at low levels of spatially uniform resource supply, it was expected that severance would decrease the final mass and size of whole fragments more at greater water depth. This was true for accumulation of mass and leaf area, though effects on mass were statistically weak. It was not true for measures of clonal reproduction or spread, which may reflect effects of clonal integration other than resource sharing, as discussed below. The most relevant recently published work is probably that of Gao et al. (2014), who incidentally showed by testing a different prediction that the effect of clonal integration in homogeneous environments on mass per ramet of pairs of ramets of the grass *Leymus chinensis* was negative at a low level of nutrients but not at a high level. Though these ramets were not intended to differ in uptake capacity and integration did not have a positive effect at any level of nutrients, this result is consistent with the present study in showing an interactive effect of resource availability and integration on clonal performance in homogeneous environments.

Based on the general prediction that the effect of integration on ramets with relatively low uptake capacity should be positive and greater at high than at low levels of resource supply, it was expected that severance would decrease the final mass and size of apical parts and more so at greater water depth. This was true for measures of aboveground mass and size, except that severance decreased the number of nodes less at greater water depth. At least two previous studies have likewise reported the negative effects of severance on younger portions of clonal fragments given high, uniform resource levels (Wang et al. 2008; Wolfer and Straile 2012).

Based on the general prediction that the effect of integration on ramets with relatively high uptake capacity should be negative and smaller at high than at low levels of resource supply, it was expected that severance would increase the final mass and size of basal parts but less so at greater water depth. This was true for shoot and total mass. It was only partly true for leaf area, number of nodes, and total stem length, which were greater in severed than in intact basal parts, but did not show smaller effects of severance at greater depth.

The last two of these measures, which can be more generically termed number of ramets and length of spacers connecting ramets, are measures of clonal reproduction and spread, and there is considerable evidence that clonal reproduction depends not just on resource sharing but also on other aspects of clonal integration. Severance can release more distal ramets from apical dominance by more proximal ramets in various species (Evans and Whitney 1992; Balestri and Lardicci 2013; Gao et al. 2013). In *A. philoxeroides*, severance from proximal ramets can induce distal ramets to produce more offspring with no change in the total mass of clonal fragments (Dong et al. 2012). Chen et al. (2014) recently found that interactive effects of severance and environment on growth in mass and on production of new ramets differed in the grass *Phalaris arundinacea*, and clonal reproduction and total accumulation of mass show different effects of nutrient availability in the aquatic clonal plant *Eichhornia crassipes* (You et al. 2014). Measures of reproduction and spread may thus require different treatment than measures of mass in models of effects of clonal integration in homogeneous environments.

Effects of water depth on root mass ran counter to effects on other measures. First, water depth had a negative effect on final root mass, both in whole fragments and in their apical and basal parts. Second, final root mass in basal parts at high water depth was lower in severed than in intact fragments, unlike any other measure of performance. Severed basal parts had lower final root mass at 20- or 40-cm water depths than at 0 cm, while basal parts in intact fragments maintained nearly the same root mass at all depths.

One explanation for a negative effect of water depth on root mass is that demand for water was less when shoots were submerged than when they were not, and that plants accordingly allocated a lower proportion of mass to roots at greater water depth. An alternative explanation is that submergence induced shoot growth, drawing resources away from growth of roots. The former explanation appears to be more consistent with the effect of severance, if distal, unrooted ramets with relatively high demand for water in the 0-cm water depth treatment signaled proximal, rooted ramets to increase allocation to roots. For example, Roiloa et al. (2013) observed a similar "spatial division of labour" between younger and older ramets of *Carpobrotus edulis* in a homogeneous environment with high resource availabilities; younger ramets allocated more mass to shoots and older ramets allocated more mass to roots when ramets were connected than when they were severed.

Although the use of water depth as the independent environmental variable had the advantage of relating results to an important factor in the growth and demography of many aquatic plants (e.g., da Cunha et al. 2014), it had the disadvantage of not isolating the effects of a single resource. Water depth can directly affect availabilities of light, O_2, and CO_2 (e.g., Vretare Strand and Weisner 2002; Vervuren et al. 2003). However, availabilities of these resources are likely to have been lower rather than higher at greater water depth and so cannot explain the generally positive effect of depth on growth of *A. philoxeroides*. Moreover, Luo et al. (2014) found that fragments of *A. philoxeroides* did not accumulate more mass in 45-cm water depth than in 0-cm water depth if deeply shaded. This is inconsistent with greater availability of light as the mechanism for the positive effect of water depth, since making light the most limiting resource eliminated the effect.

In this study, water depth seems unlikely to have directly affected availability of two other major resources, nutrients and water itself. Nutrient levels in the tap water used to vary depth were probably very low compared to levels in the soil, so that adding water added little nutrients. Assuming that plants took up water mainly through roots, submerging soil should have affected water availability very little because soils were kept saturated in all treatments.

Instead of directly increasing resource availability, greater water depth might have ameliorated a condition that differentially limited the growth of established and unestablished ramets. By condition, we mean something not used by a plant in such a way as to preclude its simultaneous use by another plant. For example, submergence in water could provide physical support that allows the shoots of ramets to spread into a larger volume and avoid self-shading. Another possibility is that submergence prevented transpiration and allowed shoots to maintain higher water potential. This seems likely to differentially benefit unestablished ramets, which lack direct water uptake. This effect could also have increased, not just between 0 and 20 cm, but also between 20 and 40 cm, since all plants produced emergent shoots, and the proportion of submerged leaves is likely to have been greater at a depth of 40 cm than at a 20-cm water depth. Higher shoot water potential at greater water depth is also consistent with reduced allocation to roots at greater depth, discussed above. If water depth did affect growth via differences in a condition rather than through direct effect on a resource, this could be accommodated in the conceptual model introduced above by allowing the independent factor to be either a condition or a resource.

The generality of benefits of clonal integration in relatively homogeneous aquatic or terrestrial environments remains to be explored, and some recent work suggests interesting directions. A review by Si et al. (2014) concluded that plants from sunny habitats had more clonal traits than those from shady habitats. It would be interesting to test whether high light availability increases benefits of integration under uniform conditions in some of these species. Engelhardt et al. (2014) reported a negative, genetically based association between clonal size and plant height in the clonal, aquatic plant *Vallisneria americana*, suggesting that genetic constraints might need to be considered in exploring the adaptiveness of integration in homogeneous habitats. In Japan, the introduced, clonal, aquatic plant *Egeria densa* is more abundant in canals that have been lined with concrete and thus made relatively homogeneous than in more natural canals (Hara et al. 2015). The possible role of clonal integration in this effect of anthropogenic habitat change is apparently not yet tested.

In sum, results from this study suggest that physiological integration can increase the performance of clonal plants in homogeneous environments when clones contain ramets that differ in uptake ability and when resource supply is high. However, results also suggest that the initial conceptual model introduced here would be made more predictive by incorporating effects of clonal integration other than just resource sharing, such as coordination of allocation and vegetative reproduction in connected ramets. Development and testing of such models may help explain the dominance of some clonal plant species in habitats that appear to have relatively little fine-scale spatial patchiness.

Author contribution statement BCD, PA, and FHY conceived and designed the experiment, analyzed the results, and wrote the manuscript. BCD and QZ performed the experiment.

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