Research Article

Clonal integration affects growth and sediment properties of the first ramet generation, but not later ramet generations under severe light stress

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Abstract

Clonal integration benefits clonal plants by buffering environmental stress and increasing resource extraction efficiency. However, the number of connected ramet generations that benefit from clonal integration in a clonal system has received relatively little attention. A pot experiment was conducted to evaluate the extent of physiological integration within the clonal system of *Vallisneria natans* consisting of a mother ramet and three sequentially connected offspring ramets. Mother ramets were grown in full sunlight, and offspring ramets were heavily shaded with limited light availability. Stolons between mother ramets and offspring ramets were severed or connected, but connection among the three offspring ramets remained. The photosynthetic ability of unshaded mother ramets of *V. natans* was significantly enhanced, but their biomass accumulation was greatly reduced when connected to shaded offspring ramets. Clonal integration significantly increased biomass accumulation, C and N availabilities, extracellular enzyme activities and microbial biomass of the first ramet generation (adjacent ramet), but not later ramet generations. Our results indicate that support from the mother ramet of *V. natans* may be limited to the adjacent offspring ramet in a clonal system under severe light stress, implying an effect of ramet generation. Our results contribute to a better understanding of the hierarchy and segmentation of clonal plants. These findings suggest that the extent of clonal integration plays a vital role in ecological interactions of the ramet population.

Keywords clonal integration, ramet generation, clonal system, sediment properties, *Vallisneria natans*

克隆整合影响严重光胁迫下第一分株世代的生长和沉积物特征但不影响后续分株世代的生长和沉积物特征

摘要：克隆整合通过缓冲环境压力和提高资源获取效率使克隆植物受益。然而，在一个克隆系统中，受益于克隆整合的连接分株世代的数量很少受到关注。我们进行了一个盆栽实验来评估沉水植物苦草(*Vallisneria natans*)克隆系统内的生理整合程度，该克隆系统由一个母株和3个依次连接的后代分株组成。母株生长在正常光照下，而后代分株被严重遮荫。母株与后代分株间的葡匐茎被切断或保持连接，但3个后代分株之间的连接仍然存在。与遮荫的后代分株连接时，苦草未遮荫的母株的光合能力显著增强，但其生物量积累大大减少。克隆整合显著增加了第一分株世代(相邻分株)的生物量积累和土壤的碳氮可用性、胞外酶活性和微生物生物量，但没有增加后续分株世代的这些特征。我们的结果表明，在严重光

胁迫下，来自苦草母株的支持可能仅限于克隆系统中相邻的后代分株，这暗示着一个分株世代的效应。我们的结果有助于更好地理解克隆植物的层次结构和分段化。这些发现表明克隆整合程度在分株种群的生态相互作用中起着至关重要的作用。

关键词： 克隆整合，分株世代，克隆系统，沉积物特性，苦草(*Vallisneria natans*)

Graphical Abstract
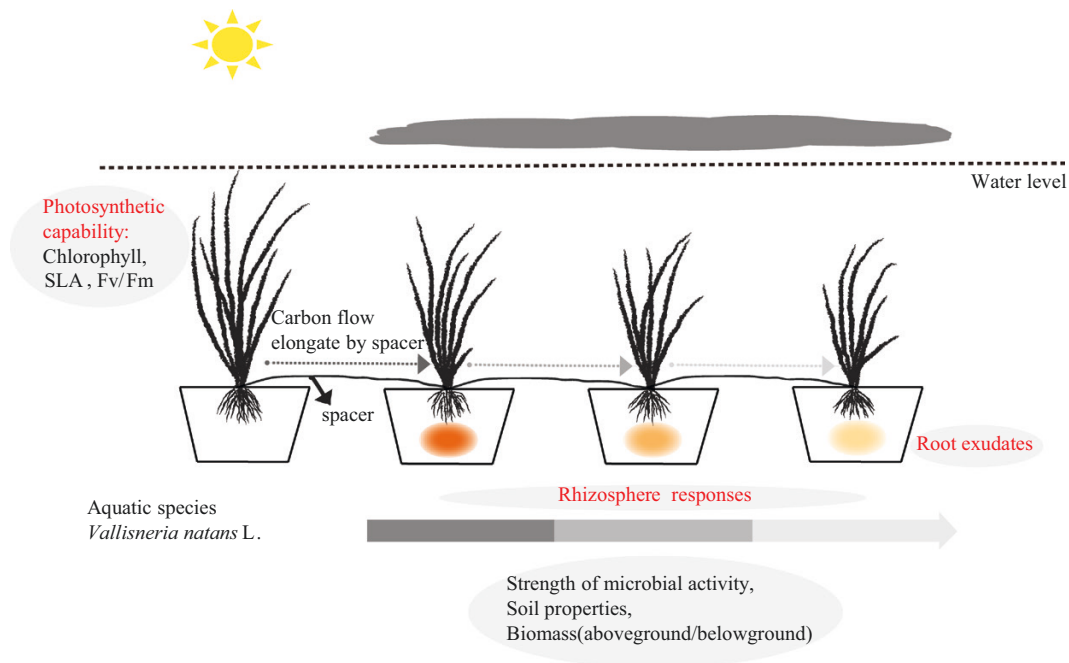
INTRODUCTION

The various resources required for plant growth and reproduction, such as light, water and mineral nutrients, are usually heterogeneous in time and space, even at the plant-neighbourhood scale (Chen *et al*. 2002; Roiloa and Retuerto 2007). Under natural conditions, the common configuration of clonal plants is one parental ramet bearing a series of offspring ramets due to rapid clonal reproduction (Wolfer and Straile 2004). Therefore, clonal plants often experience small-scale spatial heterogeneity because of large clonal systems (van Kleunen *et al*. 2000). For example, some ramets of a clone may grow in a dense patch of vegetation with low light intensity, while the connected ramets may grow in a patch with high light intensity (Dong 1993; Stuefer *et al*. 1994).

In heterogeneous environments, clonal integration can facilitate the internal transport of resources such as water, carbohydrates and mineral nutrients between connected ramets of a clonal network (Marbà *et al*. 2002; Song *et al*. 2013; Stuefer *et al*. 2004; Wang *et al*. 2008; Zhang *et al*. 2016). Thus, the performance (e.g.

biomass accumulation, production of new ramets or competitive ability) of ramets growing in resource-poor or stressful environments can be improved by clonal integration (Dong *et al*. 2015; Wang *et al*. 2008, 2016a; You *et al*. 2013). Studies have shown that clonal integration had no or little cost in terms of biomass to mother ramets in heterogeneous environments (Duchoslavová and Jansa 2018; Wang *et al*. 2021). And the photosynthetic performance of mother ramets were enhanced in response to the assimilate demand of light-stressed or drought-stressed offspring ramets (Chen *et al*. 2015; Roiloa and Retuerto 2007; Xu *et al*. 2010). However, when there are multiple offspring ramets in a clonal system that are in a disadvantageous habitat, how the mother ramet responds has rarely been investigated.

A small clone may be completely integrated, but as it matures and acquires more structural complexity, resource transport becomes sectorial (Wijesinghe 1994). A mature ramet may subsidise only a fraction of the ramets comprising a large clonal system, while other fractions are not integrated (Vuorisalo and Hutchings 1996). Such units of resource sharing are

termed 'integrated physiological units', which may change with increases in plant size and structural complexity (Marshall 1989; Watson 1986). In a clonal network with a hierarchical structure, the first-formed ramets may command a preferential supply of resources compared with later-formed ramets (Wareing and Phillips 1981). The increased spacer length of later generation, greater energy requirements for resource translocation and spacer growth maintenance lead to fewer resources being shared among ramets (Hu *et al.* 2015). Therefore, the extent of clonal integration may be related to the order of ramet generation (D'hertefeldt and Jónsdóttir 1999; Price *et al.* 1992). Furthermore, resource translocation to offspring ramets depends on the habitat quality of the individual ramets. Ramets in inferior situation are not integrated when support for other ramets provides higher benefits for the genet (Wolfer and Straile 2012). A large proportion of aquatic plant species are clonal and spread over a relatively large area by producing connected offspring ramets along rhizomes or stolons to form long-lived clonal systems (Wolfer and Straile 2004; Zhang *et al.* 2019). We propose that in clonal systems of aquatic plants under stressful conditions, the adjacent offspring ramet receives the most support from the mother ramet, while further successive ramets receive much less support.

It is widely known that ramets can transport photosynthates to shaded, connected ramets (Duchoslavová and Jansa 2018; Qureshi and Spanner 1973; Xu *et al.* 2010). Such photosynthates supply caused by clonal integration could compensate for the reduced belowground carbon allocation of shaded ramets (Kaiser *et al.* 2010; Li *et al.* 2018). Photosynthates released into soil by plant roots in the form of rhizodeposition are a major source of growth substrates, structural materials and labile carbon for soil microbes (Rajaniemi and Allison 2009). Further, microorganisms in the plant rhizosphere may regulate nutrient availability through a series of decomposition processes (Lei *et al.* 2014). Researches have shown that clonal integration may significantly alter photosynthate supply and microbial community composition and positively influence extracellular enzyme activities and N turnover in the rhizosphere of shaded offspring ramets (Chen *et al.* 2015; Lei *et al.* 2014; Li *et al.* 2018). However, whether these significant effects of clonal integration are extensive in the clonal system of clonal plants is unknown.

To address these unknowns, a pot experiment was conducted with the clonal fragment of *Vallisneria natans* consisting of four sequentially connected ramets where the mother ramet was grown in full sunlight and three offspring ramets were heavily shaded with limited light available. The stolon between the mother ramet and offspring ramets was severed or connected. The following hypotheses were addressed: (i) the photosynthetic capability of mother ramets will be enhanced by clonal integration; (ii) stolon connection will enhance C and N availabilities, extracellular enzyme activities and microbial biomass in the rhizosphere of shaded offspring ramets and will increase their biomass accumulation; and (iii) such effects of clonal integration will progressively decrease along the stolon.

MATERIALS AND METHODS

Experimental materials

Vallisneria natans, a submerged macrophyte with a wide geographical range, is a dominant native species in many freshwater habitats in China (Zhou *et al.* 2019). It usually spreads horizontally aboveground by producing stolons and forming many clonal ramets. These ramets are usually interconnected and form a large clonal system (Xiao *et al.* 2006). Studies have shown that clonal integration can enhance the growth of *V. natans* in heterogeneous light environments and facilitate its invasion of vegetated habitats (Xiao *et al.* 2007, 2011). In mid-June 2018, established individual plants of *V. natans* were collected from one population of this species in Liangzi Lake (30°05′–30°18′ N, 114°21′–114°39′ E) in Hubei Province, China. The plants (regarded as mother ramets here) were propagated in three aquariums (length: 100 cm, width: 50 cm, height: 60 cm) filled with 10 cm of sediment from Liangzi Lake and 200 L of lake water. After 3 weeks, the mother plants grew into many clonal fragments including one mother ramets and two to four offspring ramets. As similar in size as possible, 10 clonal fragments consisted of one mother ramet and three connected offspring ramets were selected as test plants, in which the length of clonal fragments were 33 ± 3 cm (mean ± SE, $n = 5$). The heights of the mother ramet, first ramet, second ramet and third ramet were 30 ± 2, 26 ± 2, 22 ± 3 and 20 ± 3 cm (mean ± SE, $n = 5$), respectively.

Experimental design

The experiment was conducted at The National Field Station of the Freshwater Ecosystem of Liangzi Lake (30°05′–30°18′ N, 114°21′–114°39′ E), Hubei Province, China. On 7 July 2018, the four small pots (11 cm in diameter, 6 cm in height) were arranged

one after another in a large container (63 cm in diameter, 44 cm in height). Then, for each clonal fragment, four ramets including one mother ramet and three offspring ramets were planted in four adjacent plastic pots in order (Fig. 1). The pots were fully filled with lake clay containing 32.76 mg g^{-1} total organic carbon (TOC) and 4.64 mg g^{-1} total nitrogen (TN). The large containers were fully filled with lake water [0.71 mg L^{-1} TN, 0.03 mg L^{-1} total phosphorus (TP), pH 8.0]. The TN and TP of the lake water were measured using a flow injection analyser (QC8500, LACHAT, USA) and a TP analyser (IL500P, HACH, USA), respectively. After 1 week of growth, the offspring ramets of 10 clonal fragments were subjected to a heavy shade imposed with a three-layered shading cloth, and mother ramets received full sunlight. The full sunlight intensity at the water surface was 1608.85 ± 64.1 µmol m^{-2} s^{-1} (mean ± SE, $n = 6$), and the light intensity under shading was 0.53 ± 0.06 µmol m^{-2} s^{-1} (mean ± SE, $n = 6$) at 12:00 on sunny days. This extreme resource contrast was implemented to determine the maximum potential resource supply from the mother ramet to the offspring. When performing shading treatment, the stolon between the mother ramet and offspring ramets was severed or connected, but connection among the three offspring ramets remained (Fig. 1). The cutting point was the midpoint of the stolon between the mother ramet and first ramet. Each treatment was replicated five times. Newly developing stolons were removed immediately as they appeared during
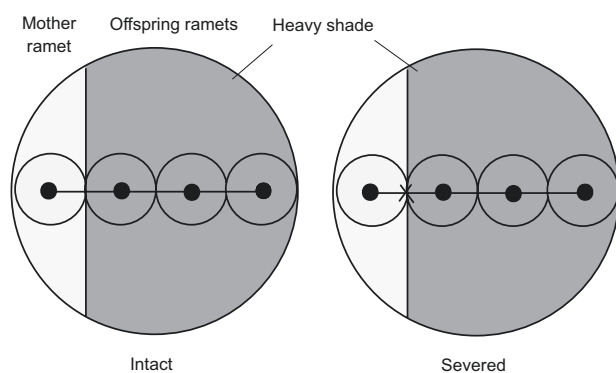

Figure 1: Schematic diagram of the experimental design. Each clonal fragment consisted of a mother ramet and three offspring ramets. The heavy shade treatment was applied to offspring ramets, and the mother ramets were exposed to full sunlight. Stolons between mother ramets and offspring ramets were intact or severed. Each treatment was replicated five times.

the experiment. Day temperatures were 27–37 °C during the experimental period. The plants were harvested on August 21, and the rhizosphere soil of shaded ramets were collected for measurements of extracellular enzyme activities, soil properties and microbial biomass. Rhizosphere soil was defined as soil directly attached to the root (Yanai *et al.* 2003).

Photosynthetic parameters

The *F*v/*F*m characterizes the photosynthetic process related with electron transport (light reactions), and provides an evaluation of the efficiency of excitation energy capture by open photosystem II (PSII) reaction centres (Butler and Kitajima 1975). To assess the effect of clonal integration on photosynthetic performance of mother ramets, before harvest, *F*v/*F*m was measured with three fully developed and healthy leaves of each mother ramet after dark adaptation for 2 h using a portable chlorophyll fluorometer (DIVING-PAM-II, Germany). Then, the leaves were used to measure the chlorophyll content with the dimethyl sulfoxide (DMSO) chlorophyll extraction method (Richardson *et al.* 2002). The leaf areas were measured using a leaf area meter (LI-COR, LI-3100 AREA METER, USA), and the leaves were oven-dried and weighed to calculate the specific leaf area (SLA). SLA reflects the ability of plant leaves to obtain light and other resources and is therefore proportional to photosynthetic capacity (Wright *et al.* 2004).

Biomass

After harvest, the mother ramets and offspring ramets were separated into roots and leaves. The stolon was not counted as a part of any ramet. Then, the ramets were oven-dried to a constant mass at 70 °C for 72 h and weighed with an analytical balance to determine the biomasses. Then root/leaf ratios were calculated.

Extracellular enzyme activities

Urease activity was measured by colorimetric determination of ammonium with short-term incubation (Kandeler *et al.* 1988). The activity of *β*-1, 4-*N*-acetylglucosaminidase (NAGase) was measured with an improved colorimetric determination of the intensity of the yellow colour produced by *p*-nitrophenyl (*p*NP) release (Ekenler and Tabatabai 2002). Phenol oxidase (POXase) activity was assayed using a spectrophotometric determination of the red compound developed from the reaction of POXase activity and

proline (Perucci *et al.* 2000). These three enzymes are the soil enzymes that play important roles in the C and N cycling of rhizosphere soil (Zou *et al.* 2018).

Soil properties and microbial biomass

Soil TOC and TN were measured with a FLASH 2000 Organic Elemental Analyser (Thermo Fisher Scientific Inc., USA). A newly developed chloroform-fumigation extraction method with fumigation at atmospheric pressure (CFAP) was used to determine microbial biomass C and N (Witt *et al.* 2000). Ten grams of fumigated or unfumigated (without adding chloroform) soil samples were mixed with 40 mL of 0.5 mol L^{-1} K$_2$SO$_4$ at a ratio of 1:4 (w/v), shaken for 60 min on a shaker and filtered through Whatman No. 42 filter paper. Then the extracts were used to analyse dissolved organic carbon (DOC) with a TOC analyser (TOC-L CPN CN200/Lachat Instruments) and dissolved organic nitrogen (DON) with a flow injection analyser (QC8500, LACHAT). Microbial biomass carbon (MBC) and nitrogen (MBN) were calculated according to the equation MBC $(N) = 2.22*E_B$, where E_B is the difference between DOC/DON from fumigated and nonfumigated soil (Wu *et al.* 1990). NH$_4^+$-N and NO$_3^-$-N concentrations were determined by spectrophotometry using the ammonium indophenol blue method and the correction factor method, respectively (Ivančič and Degobbis 1984; Song *et al.* 2007).

Statistical analysis

One-way multivariate analysis of variance (MANOVA) was used to test the effects of stolon connection on response variables (photosynthetic parameters and biomass parameters) of mother ramets. Corresponding univariate analyses were also conducted. For multivariate analysis of offspring ramets, according to the contributions of response variables to the first axes (PC1), 8 response variables were filtered from 12 response variables of offspring ramets using principle component analysis (PCA) (Supplementary Fig. S1). PERMANOVA was used to test whether the variation of offspring ramets could be explained by connection and ramet generation; significance was based on a permutation test (999 permutations). Because these ramets in the clonal fragment consisted of offspring ramets were not independent on each other, response variables (growth parameters, enzyme activities and soil properties) of offspring ramets were analysed with linear mixed models. In these models, we included connection, ramet generation and their two-way

interaction as fixed factors. Clonal fragment was included as random factor. We further conducted linear contrasts to test the effect of clonal integration at each ramet generation. In addition, the relationships between response variables of offspring ramets were examined using Pearson's correlation. The total biomass and DON of offspring ramets were log$_{10}$ transformed before analysis to meet the assumptions of homoscedasticity and normality. Due to the loss of DOC samples from fumigated soil around the third ramets in the severed treatment, the analysis of MBC was not included in multivariate statistics.

The PCA was performed using the *prcomp* function. PERMANOVA were conducted using the *adonis* function in the *vegan* package (version 2.5-6). Linear mixed models were implemented using R package *nlme* (Pinheiro *et al.* 2012). Above analyses were performed using R version 3.6.2 and other data analyses were performed with SPSS 20.0 (IBM, Armonk, NY, USA).

RESULTS

Growth performance of mother ramets

Clonal integration significantly affected the performance of mother ramets (Table 1). The *F*v/*F*m, chlorophyll content and SLA of mother ramets

Table 1: Summary of MANOVA for effects of stolon connection on the response variables (*F*v/*F*m, chlorophyll content, SLA, total biomass and root/leaf ratio) of mother ramets

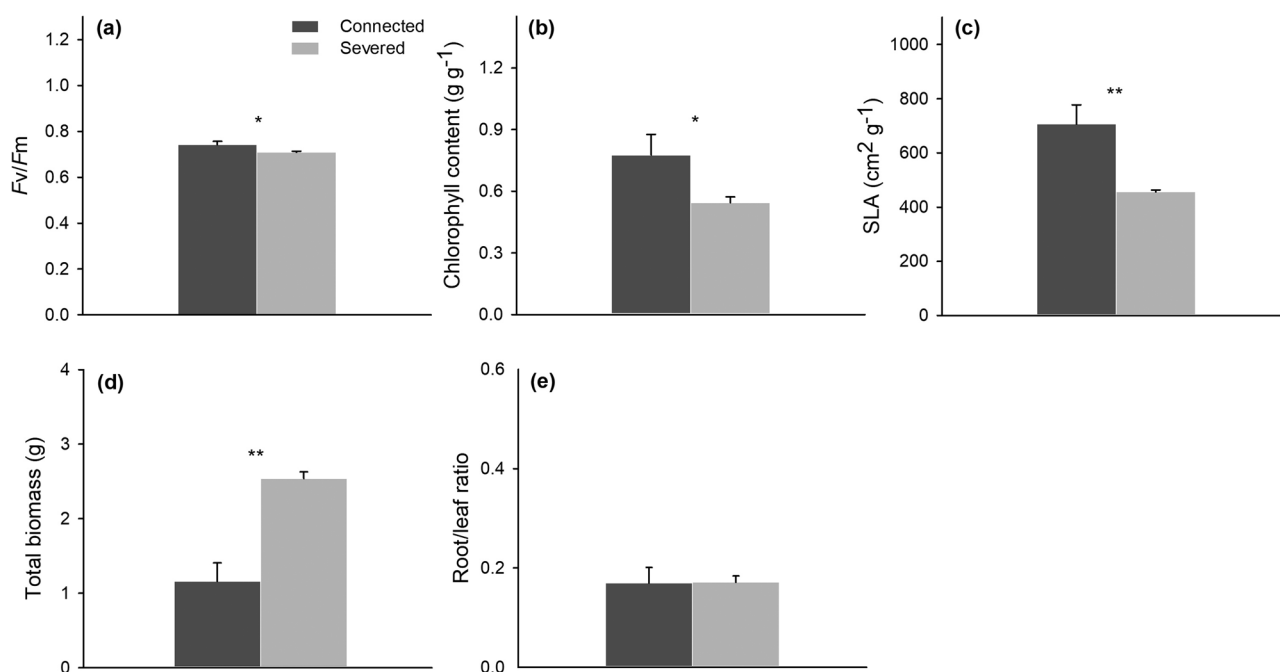| Multivariate test statistics			
	Response variables		
Effect	**Wilk's lambda**	*F*	*P*
Connection	0.100	7.233	**0.039**
Univariate test statistics			
	Connection		
Variables	$F_{1,8}$	*P*	
*F*v/*F*m	5.464	**0.048**	
Chlorophyll content	7.932	**0.023**	
SLA	12.494	**0.008**	
Total biomass	27.714	**0.001**	
Root/leaf ratio	0.111	0.747	

P values <0.05 are in bold.

Figure 2: Fv/Fm (**a**), chlorophyll content (**b**), SLA (**c**), total biomass (**d**) and root/leaf ratio (**e**) of mother ramets in the connected and severed treatments. The data are shown as the mean ± SE. Significant differences at $P < 0.05$, indicated with asterisk (*): *$P < 0.05$, **$P < 0.01$.

were significantly increased by stolon connection, while the total biomass was significantly decreased (Fig. 2a–d). Connection had no significant effects on the root/leaf ratio of mother ramets (Table 1; Fig. 2e).

Growth performance of offspring ramets

The samples collected from the first ramets in connected treatments (FC) were clearly separated from other groups by the first axis of the PCA plot (Supplementary Fig. S1). Results of PERMANOVA showed that responses of offspring ramets were significantly influenced by connection ($P = 0.001$), ramet generation ($P = 0.002$) and their interaction ($P = 0.001$, Supplementary Table S1).

The total biomass of offspring ramets were significantly affected by connection, ramet generation and their interaction (Table 2). The total biomass of the first ramet generation was significantly increased by stolon connection, whereas the second and third ramet generations were not affected (Fig. 3a).

Extracellular enzyme activities in the rhizosphere of offspring ramets

Stolon connection, ramet generation and their interactive effects significantly influenced NAGase in the rhizosphere of offspring ramets (Table 2).

The activity of NAGase in the rhizosphere of the first ramet generation was greatly increased by clonal integration, while significant differences were not observed in the rhizosphere of the second and third ramet generations (Fig. 3c). Although the activity of urease in the rhizosphere of first connected ramet generation was higher than other ramet generations, no significant effect was found (Table 2; Fig. 3b).

Soil properties in the rhizosphere of offspring ramets

TOC, MBN and NH_4^+-N among the soil properties were significantly affected by the interactive effects of connection and ramet generation (Table 2). TN, TOC and DON were strongly affected by stolon connection, while TOC, DON and NH_4^+-N were strongly affected by ramet generation (Table 2). Significantly higher TN, TOC, NH_4^+-N and MBN and lower DON were observed in the connected treatment for the first ramet generation, whereas there were no significant differences between connected and severed treatments in other ramet generations (Fig. 4a–e).

DISCUSSION

Plant's photosynthetic efficiency can be estimated by measuring Fv/Fm, chlorophyll content and SLA

Table 2: Results of linear mixed models for effects of stolon connection, ramet generation and their interaction on the response variables (total biomass, urease, NAGase, TN, TOC, MBN, DON and NH$_4^+$-N) of offspring ramets

Effect	df	Total biomass		Urease		NAGase		TN		TOC		MBN		DON		NH$_4^+$-N	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Fixed factor																	
Connection (C)	1	8.33	**0.020**	0.10	0.759	35.40	**<0.001**	14.84	**0.005**	25.81	**0.001**	4.01	0.080	8.84	**0.018**	2.46	0.156
Ramet generation (R)	2	12.87	**<0.001**	3.56	0.053	30.14	**<0.001**	1.75	0.205	4.28	**0.032**	2.87	0.086	3.83	**0.044**	9.10	**0.002**
C × R	2	4.76	**0.024**	1.46	0.262	44.08	**<0.001**	3.24	0.066	4.33	**0.032**	5.22	**0.018**	1.39	0.278	21.20	**<0.001**
Random factor		SD		SD		SD		SD		SD		SD		SD		SD	
Clonal fragment	10	0.06		<0.01		9.37		<0.01		<0.01		0.96		<0.01		5.16	
Residual		0.24		1.74		20.82		0.17		0.62		2.06		0.12		15.10	

P values <0.05 are in bold.

(Cornelissen *et al.* 2003). In our study, higher Fv/Fm, chlorophyll content and SLA of mother ramets in connected treatments were observed, which indicates enhanced photosynthetic capability of mother ramets and further reflects a strong demand for carbon created by connected shaded ramets (Pitelka and Ashmun 1985; Xiao *et al.* 2007). Previous studies also found that photosynthetic capability of unshaded ramets would enhance when connected to shaded ramets (Hartnett and Bazzaz 1983; Roiloa and Retuerto 2007). Biomass allocation reflects the relationship between the optimal allocation of resources and the environment (Chapin *et al.* 1987). This study found that clonal integration reduced the biomass accumulation of mother ramets when connected to shaded offspring ramets, while it had no effect on the root/leaf ratio of mother ramets. The reason for this result may be that the mother ramet grew under optimal environment with unlimited nutrient and light, but incurred cost in the process of supporting the offspring.

It is well known that clonal integration can increase the growth of shaded offspring ramets (Liu *et al.* 2009; Wang *et al.* 2017). In our study, growth performance of offspring ramets showed that such support from mother ramets may only exist in the first ramet generation. A possible reason of this result is that, under severe light stress, the first connected ramet generation may only maintain its growth and have no additional resources to share with other connected offspring ramets although it may receive some subsidy from the mother ramet through clonal integration. In addition, according to the result of correlation analysis (Supplementary Fig. S2), increased resources availability in rhizosphere of the first ramet generation may be the reason for its increased growth. Similar phenomena appeared in the clonal systems of some perennial herbs. For example, a ^{14}C labelling field experiment showed that C resource sharing in *Phalaris aquatica* was restricted to three growing tiller generations (primary, secondary and tertiary tillers) (Cullen *et al.* 2005). In addition, physiological integration in single ramet sequences of the caespitose graminoid *Schizachyrium scoparium* was limited to three connected ramet generations instead of all ramets within the clone (Welker and Briske 1992; Welker *et al.* 1991). However, in the growth of *Carex arenaria* (D'hertefeldt and Jónsdóttir 1999) and *Glechoma hederacea* (Price *et al.* 1992), assimilated carbon was found to be translocated towards the growing apex. The extent of physiological integration of clonal plants may vary from species to species and
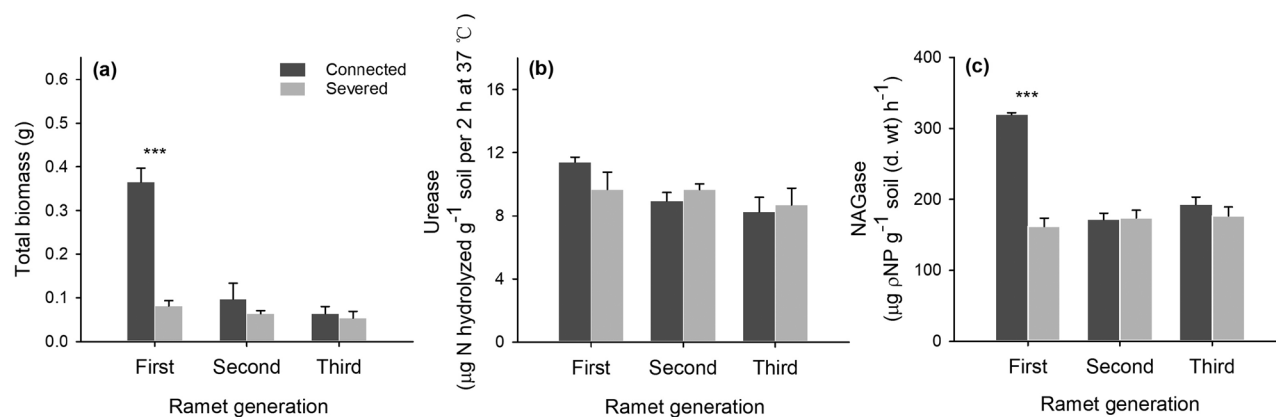
Figure 3: Total biomass (**a**), rhizosphere urease (**b**) and NAGase (**c**) of offspring ramets compared between connected and severed treatments under each ramet generations. The data are shown as the mean ± SE. Significant differences at $P < 0.05$, indicated with asterisk (*): ***$P < 0.001$.

depend on environmental conditions. Although clonal integration significantly increased the total biomass of the three offspring ramets, it did not improve the biomass of the whole clonal fragment (Supplementary Fig. S3), which is inconsistent with previous studies (Lin *et al.* 2018; Zhang and Zhang 2013). These results indicated that the benefits of clonal integration of *V. natans* could not balance the energy required for adaptation to the severe light stress.

Significant effects of connection and ramet generation were observed on the sediment properties of shaded ramets. A large proportion of assimilable C derived from photosynthates produced by plants is an available C source for microorganisms and affects their abundance, composition and activity (Grayston *et al.* 1997). In this study, increased C availability in rhizosphere of the shaded first ramet generation ramets as a result of clonal integration improves microbial biomass and further enhances extracellular enzyme activities, which is in line with previous studies (Kuzyakov 2002; Li *et al.* 2018). Extracellular enzyme activities can be regarded as potential indicators of soil nutrient cycling process (Lei *et al.* 2014). Increased NAGase activity of the first ramet generation may stimulate the degradation of recalcitrant SOM and N mineralization, which may be the reason for the decrease in DON and the increase in N availability (TN and NH_4^+-N) (Supplementary Fig. S2) (Chen *et al.* 2015). Our results provide new support for the mechanisms of nutrient recycling mediated by clonal integration (Li *et al.* 2018). These phenomena in sediment properties were observed only in the first ramet generation, but not in the second and third ramet generations.

The results indicate that the extent of resource sharing may depend on ramet generation along the spacer in a clonal system, and the effects of clonal integration may only exist in the adjacent (first) offspring ramet. Although numerous data clearly demonstrate that stressed ramets can benefit from resource sharing, this benefit of clonal integration may not involve all ramets within a clonal genet (Hu *et al.* 2015; Welker *et al.* 1991). The effect of clonal integration on the growth and sediment properties of shaded offspring ramets that we observed confirm this conclusion.

In addition to ramet generation, there may be other reasons that drive the extent of clonal integration. For example, basipetal transport has also been observed in some studies (Duchoslavová and Jansa 2018; Li *et al.* 2018), although the direction of resource translocation is usually predominantly acropetal (Price *et al.* 1992; Zou *et al.* 2018). Whether the extent of resource sharing in basipetal transport is different from that in acropetal transport remains to be explored. Moreover, resources transported from donor ramets to recipient ramets generally increase with patch contrast (Wang *et al.* 2016b; Zhang and He 2009). In this study, we imposed a maximum patch contrast, so this pattern of resource sharing may represent the maximum supply of resources from the mother ramet to the offspring ramets. Changing the level of light limitation may generate different results by adding complex factors of resource translocation between descendants and thus deserves further, more detailed exploration.
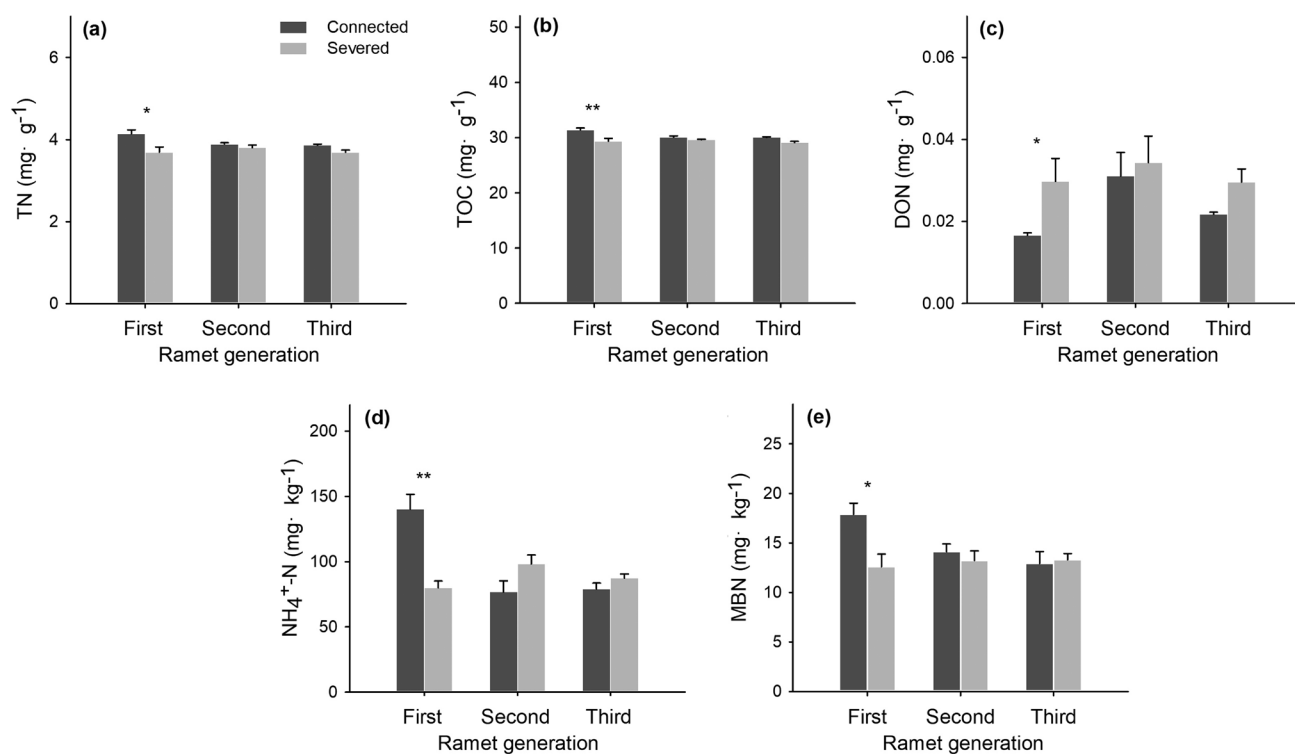
Figure 4: TN (**a**), TOC (**b**), DON (**c**), NH_4^+ -N (**d**) and MBN (**e**) in the rhizosphere of offspring ramets compared between connected and severed treatments under each ramet generations. The data are shown as the mean ± SE. Significant differences at $P < 0.05$, indicated with asterisk (*): *$P < 0.05$, **$P < 0.01$.

CONCLUSIONS

Clonal integration significantly affected growth and sediment properties of the first ramet generation, but not second or third ramet generations, which suggests that clonal integration among ramets of *V. natans* may be limited to the adjacent ramet in a clonal system under severe stress, and the extent of clonal integration may be related to ramet generation. This study facilitates our understanding of the hierarchy and segmentation of clonal plants and suggests that the extent of clonal integration plays a vital role in the responses of clonal plants to biotic or abiotic environmental conditions at the ramet population level. Therefore, when scaling conclusions from the individual to community level, the extent (or limitation) of clonal integration should be thoroughly considered.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.
Table S1: Effects of connection, ramet generation and their interaction on overall responses of offspring ramets.

Figure S1: Principal components analyses (PCA) of response variables of offspring ramets and contribution of response variables to PC1.
Figure S2: Relationships between response variables of offspring ramets.
Figure S3: The biomass of the whole clonal fragments (**a**) and the biomass of all offspring ramets (**b**) in the connected and severed treatments.

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