# Clonal integration facilitates invasiveness of a non-native plant under heterogeneous water availability in China

| | |
|---|---|
| Journal: | *Oecologia* |
| Manuscript ID: | OEC-CWO-2012-0493 |
| Category of manuscript: | Physiological ecology- Original research |
| Date Submitted by the Author: | 27-Apr-2012 |
| Complete List of Authors: | You, Wenhua; The National Field Station of Lake Ecosystem of Liangzi Lake, College of Life Sciences<br>Yu, Dan; The National Field Station of Lake Ecosystem of Liangzi Lake, College of Life Sciences<br>Liu, Chunhua; The National Field Station of Lake Ecosystem of Liangzi Lake, College of Life Sciences<br>Xiong, Wen; The National Field Station of Lake Ecosystem of Liangzi Lake, College of Life Sciences<br>Xie, Dong; The National Field Station of Lake Ecosystem of Liangzi Lake, College of Life Sciences |
| Keywords: | Carbon/nitrogen balance, Chlorophyll fluorescence, Invasion, Myriophyllum aquaticum, Water heterogeneity |
| | |

ORIGINAL ARTICLE

# Clonal integration facilitates invasiveness of a non-native plant under heterogeneous water availability in China

**Wenhua You · Dan Yu · Chuhua Liu · Wen Xiong · Dong Xie**

The National Field Station of Lake Ecosystem of Liangzi Lake,

College of Life Science, Wuhan University, Wuhan, 430072, P.R. China

Correspondence:

Dan Yu

College of Life Sciences, Wuhan University, Wuhan 430072, China

Tel: +86 27 68756834; Fax: +86 27 87882661

E-mail yudan01@public.wh.hb.cn

**Abstract**

Clonal plants benefit from the ability to translocate resources among interconnected ramets through clonal integration to colonise ubiquitous heterogeneous habitats, which may contribute to the invasiveness of exotic clonal plants. To test this hypothesis, a greenhouse experiment was conducted to investigate the effects of clonal integration on a non-native clonal plant, *Myriophyllum aquaticum*, subjected to spatial and temporal heterogeneity of water supply. The daughter ramets were grown with stolon connections either severed from or connected to the mother plant and subjected to different amounts or frequencies of water supply. Clonal integration significantly improved growth and photosynthetic performance of daughter ramets that were exposed to spatial and temporal heterogeneity of water supply. Biomass allocation to roots of offspring ramets changed with water supply to enhance the capacity for water uptake. The decrease of the maximum quantum yield of photosystem II ($F_v/F_m$) as a function of reduced water supply was greatly alleviated by stolon connection. Moreover, clonal integration facilitated stabilisation of foliar N concentration and C/N ratio to support healthy growth of the ramets. These results suggest that clonal integration may facilitate invasion of *M. aquaticum* when subjected to heterogeneity in resource supply under ever-changing environments in China.

**Key words** Carbon/nitrogen balance · Chlorophyll fluorescence · Invasion · *Myriophyllum aquaticum* · Water heterogeneity

## Introduction

According to IPCC (2007), extreme climatic events, such as seasonal droughts and floods, seem to have become more frequent. At a regional scale, spatial and temporal precipitation trends are expected, for instance, summer precipitation is increasing in Southern China (Piao et al. 2010), which would be expected to lower the mean and increase the variability in soil water content compared to normal precipitation patterns and hence to cause heterogeneity of water supply in the field. Therefore, water exhibits spatial and temporal variation in availability for plants, even among different microhabitats (Fay et al. 2003; James et al. 2003).

Clonality, a defining feature of clonal plants, may improve plants' exploitation of ubiquitous heterogeneous resources, help plants invade new environments and facilitate plants' spatial occupation of new habitats at a local scale (Maurer and Zedler 2002). Connected ramets of clonal plants possess the capacity to share water, carbohydrates and nutrients among individual subunits through clonal integration (Alpert and Mooney 1986; Marshall 1990; Stuefer *et al*. 1994, 1996; Alpert 1996, 1999). When exposed to different resource conditions, clonal ramets use reciprocal translocation to moderate their overall variation in resource availability and to facilitate efficient acquisition of resources in the face of heterogeneity (Hutchings and Wijesinghe 1997). For example, clonal integration may facilitate the colonisation and growth of the ramets in heterogeneous habitats with stressful conditions (Saitoh et al. 2002; Chidumayo 2006; Roiloa and Retuerto 2007; Otfinowski and Kenkel 2008), help genets to survive and to recover after severe environmental change (Yu et al. 2008; Moola and Vasseur 2009) and allow for occupation of new space (Brewer and Bertness 1996; Wang et al. 2008). These

43 positive effects of clonal integration may increase the performance of clonal plants over
44 non-clonal plants or other clonal plants with little integration (Herben 2004).

45 Plant invasion has become a great threat to biodiversity and global ecosystem stability
46 (Mack et al. 2000). A great fraction of the most notorious alien invasive plants have the ca-
47 pacity for vigorous clonal propagation (Kolar and lodge 2001; Liu et al. 2006, Xu et al. 2010).
48 For instance, two invasive aquatic clonal plants, *Myriophyllum aquaticum* (parrotfeather) and
49 *Eichhornia crassipes* (water hyacinth), can form highly stands that exclude almost all other
50 species in aquatic ecosystems (Timmons and Klingman 1958; Aston 1977). Some studies
51 have suggested that the invasiveness of alien clonal plants may be closely correlated to clonal
52 characteristics such as clonal integration (Reichard 1997; Maurer and Zedler 2002; Liu et al.
53 2006). Many studies have examined how individual plants respond either to spatially hetero-
54 geneous resources (e.g., Crick and Grime 1987; Gersani and Sachs 1992; Bilbrough and
55 Caldwell 1995; Day et al. 2003), or less often, to temporal heterogeneity of resources (e.g.,
56 Lodge et al. 1994; Maestre and Reynolds 2007; Hagiwara et al. 2008). To date, however, few
57 studies have addressed how clonal integration affects the growth of non-native species when
58 subjected to resources, such as water availability, that may be spatially and temporally heter-
59 ogeneous in non-native habitats.

60 Biomass allocation is a fundamental aspect of the competiveness of aquatic invasive plants,
61 which often allocate a large fraction of their biomass to form canopy and to support the rapid
62 spring growth that allows them to suppress other species (Madsen 1991; Sytsma and Ander-
63 son 1992). In heterogeneous habitats consisting of a mixture of rich and poor resource patches,

via clonal integration, clonal plants can alter biomass allocation and divert more biomass to shoots or roots for acquisition of more abundant resource, and exploration of more favourable space, a phenomenon called 'division of labour' (Stuefer et al. 1996; Alpert and Stuefer 1997; Hutchings and Wijesinghe 1997; Xiao et al. 2006). This pattern of biomass allocation is different from that used by non-clonal plants, or clonal plants grown in homogeneous conditions (Birch and Hutchings 1994; Stuefer et al. 1996; Alpert and Stuefer 1997; Hutchings and Wijesinghe 1997). The relationship between plant photosynthetic efficiency and clonal integration has not been widely studied (but see Hartnett and Bazzaz 1983; Roiloa and Retuerto 2005, 2006a, b; Wang et al. 2008). Photosynthetic efficiency can be estimated by measuring chlorophyll fluorescence (Schreiber et al. 1998). A sensitive indicator of plant photosynthetic performance derived from the parameters of chlorophyll fluorescence is the maximum quantum yield of photosystem II ($F_v/F_m$), which usually significantly decreases when plants are faced with environmental stress (Bj□rkman and Demmig 1987; Johnson et al. 1993; Roiloa and Retuerto 2005, 2006a, b). Environmental stress on ramets may be alleviated by clonal integration, which may markedly lower the negative effects of stress (such as water deficiency) on $F_v/F_m$. In addition, photosynthetic activity, measured in terms of the effective quantum yield of PSII (Yield), is closely related to plant performance. For healthy growth, plants must maintain sufficient nutrient concentrations and relatively stable nutrient ratios (stoichiometric balance) in their tissues (Marschner 1995; Han et al. 2011). However, the requirement for and the ability to acquire and to maintain specific ranges of nutrient concentrations and ratios (stoichiometric homeostasis) may differ among plant species subjected to variable environ-

mental conditions (Sterner and Elser 2002). Nutrient availability depends on water availability, which is influenced by heterogeneity of water supply (Lambers et al. 1998), that result in changes in nutrient concentration and ratio. Biomass allocation, photosynthetic efficiency, nutrient concentration (C, N) and nutrient ratio (C/N) can all contribute to the performance of clonal plants when exposed to variation in spatial and temporal water availability; however, their responses to clonal integration are still insufficiently understood, especially for non-native clonal plants.

*Myriophyllum aquaticum* is a native aquatic plant from South America and a common component of aquaria landscaping, which is regarded as the main vector of spread for this species (Sutton 1985). It is reported that this species has escaped from aquaria and naturalised in Taiwan (Xie et al. 2001). Although it is not considered to be a major noxious aquatic weed throughout most of its range, it is an increasingly troublesome species that can cause severe localised problems in shallow ditches, slow moving streams, ponds, shallow lakes and wetlands with heterogeneous habitats. For example, dense populations can block water movement in streams and ditches, resulting in increased flood duration and intensity and posing a severe threat to drinking water supplies in South Africa (Timmons and Klingman 1958; Sutton 1985). Moreover, this species provides mosquito larvae a refuge from predation, which creates a human health hazard (Orr and Resh 1989). Populations of *M. aquaticum* are usually found in habitats where water levels fluctuate across different seasons, they produce roots along riverbanks or shallow water along lake shorelines and extend creeping stolons over the surface of water. *M. aquaticum* can survive on moist soils with no overlying water column,

106 such as moist banks and sediments exposed by drawdown, even in relative dry environment.
107 Survival and spread of this species depend exclusively on vegetative reproduction by stem
108 fragments (Cook 1990; Xie et al. 2011).

109 A greenhouse growth experiment was conducted to investigate the effects of clonal inte-
110 gration on growth, biomass allocation to roots, photosynthetic efficiency, carbon/nitrogen
111 balance and thus potential invasiveness of the exotic clonal plant *M. aquaticum* as a function
112 of water availability (hereafter, 'water heterogeneity'). Ramets (the apical part of branches)
113 were either connected to or severed from an established mother plant under different combi-
114 nations of the amount or the frequency of water supply. This design mimics the expansion of
115 off-spring ramets from a well-established population into neighboring habitats subject to spa-
116 tial and temporal heterogeneity in water supply. Here, we use the non-native aquatic plant *M.*
117 *aquaticum* to test the following hypotheses. (1) Clonal integration will significantly improve
118 growth and photosynthetic performance of the ramets subjected to water heterogeneity to help
119 the genets colonise heterogeneous habitats. (2) Clonal integration will affect the changes of
120 biomass allocation to roots caused by water heterogeneity. For example, according to labour
121 division theory, we predict that biomass allocation to the roots of daughter ramets will de-
122 crease with decreasing water availability (relatively more biomass allocation to shoots for
123 light and space) and increase with increasing water supply (relatively more biomass allocation
124 to roots for water) by clonal integration, while biomass allocation to roots will increase with
125 decreasing water supply when stolons are severed (producing more roots to acquire sufficient
126 water to support growth). (3) The decrease in $F_v/F_m$ of the ramets subjected to water hetero-

127 geneity would be alleviated by clonal integration. (4) Changes of nutrient concentrations (C,
128 N) and ratios (C/N) with water heterogeneity will be modified to relatively stable nutrient
129 concentration and ratio to support healthy growth by clonal integration.
130
131 **Materials and methods**
132 Experimental design
133 The growth experiment was conducted in a glasshouse under natural sunlight and ambient
134 temperature at The National Field Station of the Lake Ecosystem of Liangzi Lake, Wuhan
135 University, from July to September 2010. Source material of *Myriophyllum aquaticum* was
136 collected from Liangzi Lake in the Hubei province of China and then propagated in the
137 greenhouse. Field-collected clonal fragments were planted in seedling pots in early May 2010
138 and then transplanted to plastic pots (25 cm in diameter, 15 cm tall). After the plants grew
139 roots at multiple nodes and developed into a dense patch within the pot, the plants were
140 trimmed to similar size and allowed to grow for an additional 5 weeks. These plants were
141 used as mother plants in the experiment to simulate well-established populations of this spe-
142 cies in the natural environment, which are composed of abundant groups of connected off-
143 spring ramets. The substrate in each pot was a 1:1 mixture of washed pure sand and lake clay
144 with 5 g of slow-release fertiliser (Osmocote®, N–P–K: 16–8–12). 12 mother plants with sim-
145 ilar size (approximately 60 cm in diameter, each containing at least 30 branches) were used
146 for the growth experiment. To mimic the vegetative growth from mother patches, 6
147 well-developed branches were selected from each mother plant and were left connected to the

parent as the daughter plants used in the experiment. For each branch, the top of approximately 4 cm of the apical part was placed on the surface of the same substrate as that of the mother plant in a culture pot (20 cm in diameter, 15 cm tall) with sufficient water to encourage root generation. After 5 days, all apical parts of the branches had developed roots (1–2 cm length), and the rooted part was potted in the culture pot and fixed in place with two pins.

The experiment was conducted with two main factors: water heterogeneity (three levels of spatial and temporal heterogeneity treatment, respectively, for two experimental set-ups), and clonal integration (stolon connection severed or kept intact). The water spatial heterogeneity treatment had three levels of water supply amount (0.5 L every 10 days, 1.0 L every 10 days and 2.0 L every 10 days). The water temporal heterogeneity treatment had three levels of water supply frequency (0.2 L every day, 1.0 L every 5 days and 2.0 L every 10 days) with the total amount of water provided kept constant throughout the experimental period. The water used in this study was lake water (TN 54.69 mmol $m^{-3}$, TP 1.41 mmol $m^{-3}$). Low amounts of water supply (0.5 L every 10 days) were applied only to maintain plant growth beyond wilting, moderate amounts of water supply (1.0 L every 10 days) were provided to keep soil saturated with water, and the maximum amount of water supply at each treatment was chosen to keep the soil with an overlying water column (no more than 2 cm) that would not exceed the capacity of culture pots (5-L volume). Mean soil moisture (volumetric water content) was recorded with soil moisture probes (TDR-3**,** Jinzhou Sunshine Technology, China) during the experimental period. The clonal integration treatment left three daughter ramets connected to and the other three ramets severed from the mother plant, so that each experimental unit con-

sisted of one mother plant, three connected ramets and three severed ramets. Connected ramets and severed ramets were subjected to water heterogeneity (spatial or temporal), while the mother plant (including all other branches and stolons) was kept in a constantly wet growth environment (Fig 1). The experiment used a two-way factorial randomised block design with each experimental unit was treated as a block, and the main factors were water heterogeneity and clonal integration. All of the 12 experimental units (6 for water spatial treatment and 6 for water temporal treatment) were randomly located in the greenhouse. The experimental units were randomly repositioned every two weeks to avoid the effects of possible environmental heterogeneity (such as light). The light intensity in the greenhouse was 1200-1500 μmol $m^{-2}$ $s^{-1}$ on a sunny day, and the mean air temperature was 15-28 °C during the experimental period.

Measurements

One week before harvesting the plants, the minimum ($F_0$) and the maximum ($F_m$) fluorescence yield were measured for a fully developed, healthy leaf on the second-youngest of the ramets in each daughter plant after a dark adaptation of at least 20 minutes sufficient for photosystemII (PSII) reaction centres to open by a portable chlorophyll fluorometer (DIVING-PAM, Walz, Effeltrich, Germany) with the saturation pulse method (Schreiber et al. 1998). The maximum quantum yield of PSII ($F_v/F_m$) was calculated as $(F_m - F_0)/F_m$. The effective quantum yield of PS II (Yield) was calculated as $(F'_m - F_t)/F'_m$, where $F'_m$ is defined as the maximal fluorescence yield reached in a pulse of saturating light after a actinic light pulse of 120 μmol $m^{-2}$ $s^{-1}$ for 10 seconds, and $F_t$ is the fluorescence yield of the leaf at that photo-

190 synthetic photon flux density (Bj□rkman and Demmig 1987; Johnson et al. 1993; Roiloa and
191 Retuerto 2006a, b).

192 At harvest, the number of branches in each daughter plant was counted, and the total
193 length of the stolons was measured for all treatments. The daughter plants of *M. aquaticum*
194 were then harvested and separated into leaves, stolons and roots, and their biomass was de-
195 termined after drying at 70 °C for 72 h.

196 Leaves of the same part (apical part of the third-youngest ramets) of the daughter plants
197 were selected and ground into powder; approximately 1.0 mg of the powder was used to de-
198 termine the concentration of leaf C and N for all the treatments with a CHNS/O Elemental
199 Analyzer (EA3000, EuROVECTOR, Italy).

200 Statistical Analyses

201 All data were log transformed to meet assumptions of normality and homoscedasticity before
202 analysis. The effect of water heterogeneity treatment on mean soil moisture was analyzed
203 with one-way ANOVA. Factorial ANOVA for a randomised block design was used to test the
204 effect of water heterogeneity and clonal integration on photosynthetic performance ($F_v/F_m$ and
205 Yield), element concentration (C, N) and element ratio (C/N). Two-way multivariate analysis
206 of variance (MANOVA) was employed to investigate the global effects of water heterogenei-
207 ty and clonal integration on growth parameters (including total biomass, stolon length and
208 number of branches) of *M. aquaticum*, and corresponding univariate analyses were also con-
209 ducted. If a significant treatment effect was detected, post-hoc pair-wise comparisons of
210 means were made to examine differences between treatments using Studentized Tukey's HSD

211 for multiple comparisons.

212 The effect of water heterogeneity and clonal integration on biomass allocation to roots
213 was tested using two-way ANCOVAs, with root biomass treated as dependent variable and
214 shoot biomass (leaves and stolons) as a covariate. Root: shoot ratios were not used because
215 the use of these ratios to test biological hypotheses has been criticised (Tremmel and Bazzaz
216 1995; Murphy and Dudley, 2007). Non-significant terms involving covariates were dropped
217 using backwards elimination. If a significant treatment effect was detected, estimated margin-
218 al means were computed to test (Bonferroni) for differences between treatments. Statistical
219 significance was assigned at $P < 0.05$. All data analyses were performed using SPSS 17.0
220 (SPSS, Chicago, IL, USA).

221

222 **Results**

223 Mean Soil Moisture

224 The mean soil moisture significantly differed across the water spatial heterogeneity treatment
225 ($F_{2,15} = 108.23$, $P < 0.001$) and markedly decreased in relation to the reduced amount of water
226 supply (Low-amount: 0.44 ± 0.03; middle-amount: 0.81 ± 0.05; high-amount: 0.95 ± 0.06,
227 means ± standard error). The mean value of soil moisture was not affected by water temporal
228 heterogeneity ($F_{2,15} = 1.02$, $P > 0.05$).

229 Growth

230 In summary, under spatial heterogeneity of water supply, water heterogeneity and clonal inte-
231 gration had significant effects on growth of daughter ramets (apical part of branches) of *M.*

*aquaticum*, but their interaction was not significant. The growth of ramets was only markedly affected by clonal integration in the water temporal heterogeneity treatment (Table 1a). Clonal integration greatly increased total biomass, stolon length and the number of branches of *M. aquaticum* in both experimental set-ups (Table 1b, Fig. 2). The growth parameters (including total biomass, stolon length and number of branches) were significantly reduced by decreasing amount of water supply but unaffected by water temporal heterogeneity (Fig. 2).

Biomass Allocation to Roots

Root biomass of daughter ramets was closely correlated with shoot biomass both in the water spatial and temporal heterogeneity treatments (Table 2). In the water spatial heterogeneity treatment, in relation to shoot biomass, water heterogeneity and the interaction between water heterogeneity and clonal integration significantly affected root biomass. Least square means comparisons showed that normalised root biomass was reduced by increased water availability without stolon connections, while normalised root biomass was greatest in the moderate treatment with clonal integration (Table 2, Fig. 3a). In the water temporal heterogeneity treatment, the relationship between root biomass and shoot biomass was only significantly affected by clonal integration; least square means comparisons showed that normalised root biomass was greatly reduced with clonal integration (Table 2, Fig. 3b).

Photosynthetic Performance

The value of $F_v/F_m$ of *M. aquaticum* in the apical sections of branches (daughter ramets) was significantly affected by water spatial heterogeneity, clonal integration and their interaction. The Yield of *M. aquaticum* was affected by the two factors, but not by their interaction (Table

253 3). The decreasing amount of water supply remarkably reduced the $F_v/F_m$ value of daughter
254 ramets without clonal integration, but the differences disappeared when the stolon was kept
255 intact. On the other hand, increased amount of water supply and clonal integration greatly in-
256 creased the Yield value (Table 3). Meanwhile, the value of $F_v/F_m$ of daughter ramets was not
257 affected by water temporal heterogeneity, clonal integration or their interaction, but clonal
258 integration significantly improved photosynthetic activity (Yield) in the water temporal het-
259 erogeneity treatment (Table 3).

260 Foliar Nutrients Concentration (C, N) and Ratio (C/N)

261 Water spatial heterogeneity, clonal integration and their interaction had significant effects on
262 foliar N concentration and C/N ratio, but not on C concentration in daughter ramets (Table 3).
263 Foliar N concentration tended to be higher, while C/N ratio tended to be smaller as the water
264 supply was increased when the stolon was severed. However, the differences between the
265 treatments disappeared when stolon connections were preserved (Table 3). N concentration
266 and C/N ratio were significantly affected by water temporal heterogeneity and clonal integra-
267 tion, but not by their interaction (Table 3). Water temporal heterogeneity and clonal integra-
268 tion had similar effects on N concentration and the C/N ratio as did the water spatial hetero-
269 geneity treatment with the stolon severed; furthermore, the differences among the treatments
270 tended to be smaller with the stolon kept intact (Table 3).

271

272 **Discussion**

273 Clonal integration markedly increased the growth of daughter ramets (apical sections of

branches) of *M. aquaticum* in both the water spatial and temporal heterogeneity treatments. This result most likely occurred because the well-established mother plant supported the growth of the interconnected young apical sections and facilitated the production of new tissue due to acropetal (from mother plant to daughter ramets) translocation of carbohydrates via clonal integration (Wang et al. 2008). The results agree with those obtained in previous studies on several other clonal plants (Hartnett and Bazzaz 1985; Hester et al. 1994; Brewer and Bertness 1996; Yu et al. 2002; Wang et al. 2008), which showed that clonal integration facilitates establishment of newly produced ramets, improves growth of adult ramets and helps genets to occupy open space in heterogeneous habitats. These observations indicate that clonal integration is critical in allowing *M. aquaticum* to explore new heterogeneous open space and thus enhances its potential invasiveness in natural habitats. In addition, the growth of daughter ramets was greatly reduced by decreasing the amount of water availability, either with the stolon severed or kept intact, and showed no interaction with clonal integration. These results are not in accordance with the studies (Saitoh et al. 2002; Chidumayo 2006, Wang et al., 2008) that have examined the growth of ramets in heterogeneous habitats with stressful conditions. This is most likely because clonal plant can preferentially facilitate establishment of newly produced ramets to more suitable environment and help genets occupy more favourable open space, a phenomenon that has been interpreted as foraging behaviour (Silvertown and Gordon 1989; Oborny and Cain 1997; Xiao et al. 2006). However, growth of ramets was not affected by water temporal heterogeneity either with the stolon severed or kept intact, which is not consistent with the results of previous studies (Novoplansky and Goldberg

295 2001; Maestre and Reynolds 2007). Differences in species used and/or frequencies and
296 amounts of water supply could have caused the difference between our results and those of
297 other studies. Overall, the effects of water heterogeneity and clonal integration on growth
298 were additive without interaction, suggesting that *M. aquaticum* benefits from clonal integra-
299 tion to colonise heterogeneous habitats, which may be related to its potential invasiveness to
300 new open space with water heterogeneity.

301 Biomass allocation of *M. aquaticum* was significantly influenced by clonal integration
302 which is consistent with previous findings for many other clonal plants (Salzman and Parker
303 1985; Friedman and Alpert 1991; Evans and Whitney, 1992; Birch and Hutchings 1994;
304 Stuefer et al. 1994, 1996; Wang et al. 2008). In the water spatial heterogeneity treatment, bi-
305 omass allocation to roots tended to increase with decreasing water supply, allowing plants to
306 obtain more water by allocating more biomass to roots when the stolon was severed
307 (Markesteijin and Poorter 2009). With the stolon connection intact, biomass allocation to
308 roots under moderate water supply was greatest among the water spatial heterogeneity treat-
309 ments (Table 2, Fig. 3a). This result indicates that the physiological integration effect on the
310 middle-amount of water supply was intermediate between the other two levels of water sup-
311 ply. More water was provided by the mother plant via clonal integration under low water sup-
312 ply, and less water was offered by the mother plant under high water supply because enough
313 water could acquire directly by roots. The results obtained so far suggest that the biomass al-
314 location to roots modified by clonal integration also exhibits selective capacity. Biomass al-
315 location to roots of the daughter ramets most likely depends on the subsidy of water resource

316 from the mother plant; that is, water resource from the mother plant always flows into the
317 connected ramets under water stress. The pattern of biomass allocation is not consistent with
318 the theory of labour division of clonal plants (Alpert and Stuefer 1997; Hutchings and Wi-
319 jestinghe 1997), perhaps because of sufficient resources (such as water) provided by the
320 mother plant with no resource restriction. In the water temporal heterogeneity treatment,
321 clonal integration significantly decreased the allocation of biomass to roots; mean water con-
322 tent in the soil exhibited no differences, and there was no stress of water deficiency for
323 daughter ramets (effects on $F_v/F_m$, no significance, Table 3). Via clonal integration, the moth-
324 er plant enhances the growth of its connected ramets and allows them to occupy more space
325 and then widely spread by means of increasing biomass allocation to shoots (leaves and sto-
326 lons). Thus, the effects of clonal integration on the biomass allocation pattern may improve
327 the capacity for resource uptake of *M. aquaticum* and enhance its potential invasiveness.

328 In favourable conditions, the value of $F_v/F_m$ for most plant species ranges from 0.8 to 0.84
329 (Butler and Kitajima 1975; Bj□rkman and Demmig 1987). In the water temporal heterogene-
330 ity treatment, the $F_v/F_m$ values of both severed and intact ramets were within the normal range
331 of healthy plants and exhibited no significant differences among all treatments. Under water
332 spatial heterogeneity, however, the $F_v/F_m$ values of severed ramets were remarkable lower
333 than those of intact ramets, indicating that water spatial heterogeneity imposed stress (water
334 deficiency) on daughter ramets (Table 3). However, the decrease of plants' $F_v/F_m$ values was
335 markedly alleviated by stolon connections, allowing the ramets to maintain $F_v/F_m$ values
336 within the normal range (Table 3). Therefore, the results support our third hypothesis and

337 suggest that clonal integration significantly buffered plants against water stress and signifi-
338 cantly increased plant photosynthetic performance. Previous studies (Roiloa and Retuerto
339 2006; Wang et al. 2008) also found that clonal integration significantly alleviated the decrease
340 in $F_v/F_m$ of ramets grown in soils with heavy metals or with severe competition by neigh-
341 bouring plants. Moreover, photosynthetic capacity, measured in terms of the effective quan-
342 tum yield of PS II (Yield), was significantly improved by clonal integration in both experi-
343 mental set-ups and by increased amount of water supply in the water heterogeneity treatment.
344 The responses of Yield values were closely correlated with the growth of daughter ramets in
345 response to clonal integration under different amounts or frequencies of water supply, thus
346 supporting our first hypothesis.

347 As we expected, clonal integration significantly buffered against changes in the foliar N
348 concentration and the C/N ratio of ramets caused by water heterogeneity, indicating that the
349 clonal plant *M. aquaticum* possesses the ability to maintain a relatively stable nutrient con-
350 centration and nutrient ratio by clonal integration (Marshner 1995). This result is most likely
351 due to nutrient translocation among interconnected ramets and changes of biomass allocation
352 pattern via clonal integration, because plants absorb nutrients in a manner mainly dependent
353 on root biomass and soil water content. That the C/N ratio maintains a relatively stable value
354 is important for plants because the C/N balance determines resource allocation to growth or
355 defense (Bryand 1983). When exposed to heterogeneous resource supply, ramets of *M.*
356 *aquaticum* may reach a nutrient balance by clonal integration to support growth and thus
357 avoid waste of resource for defense according to the carbon nitrogen balance hypothesis (eg.,

more chemical defensive substance induced by low C/N ratio, or more physical defensive substance caused by high C/N ratio). Overall, the results suggested that clonal integration might help the daughter ramets maintain sufficient element concentrations and stable element ratios to support healthy growth when subjected to heterogeneous conditions, which may contribute to the potential invasiveness of *M. aquaticum*. However, that foliar N concentration decreased with intact stolon connections was most likely due to biodilution: the biomass accumulation of daughter ramets was faster than N accumulation (Titus and Andorfer 1996).

In summary, when subjected to water heterogeneity, growth and photosynthetic performance of daughter ramets were significantly improved by clonal integration, indicating that clonal integration may help *M. aquaticum* colonise heterogeneous habitats and then widely spread. Moreover, clonal integration facilitated effective uptake of resources via changing biomass allocation patterns and maintenance of stable foliar N concentration and C/N ratio of offspring ramets to support their healthy growth. These results suggest that clonal integration may facilitate invasion of *M. aquaticum* when subjected to resource heterogeneity under ever-changing environments in China.

**Acknowledgements**

We thank Dr. KY Xiao for critical comments on an early version of the manuscript, Dr. LF Yu for help with the data analysis, and CM Han, DY Ma, J Chen and YQ Han for assistance with plant harvest. This study was supported by the National Science Foundation of China (30930011, 31170339).

379 **References**

380 Alpert P (1996) Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. J Ecol 84:
381 395–406
382 Alpert P (1999) Clonal integration in *Fragaria chiloensis* differs between populations: ramets
383 from grassland are selfish. Oecologia 120: 69–76
384 Alpert P, Mooney HA (1986) Resource sharing among ramets in the clonal herb, *Fragaria*
385 *chiloensis*. Oecologia 70: 227–233
386 Alpert P, Stuefer JF (1997) Division of labour in clonal plants. In: Kroon HD, Groenendal JV,
387 (eds) The ecology and evolution of clonal plants. Backhuys Publishing, Leiden, pp
388 137–154
389 Aston HI (1977) Appendix 1: the water hyainth (*Eichhornia crassipes*). Aquatic plants of
390 Australia. Melbourne University Press, Melbourne, pp 333—339
391 Bilbrough CJ, Caldwell MM (1995) The effect of shading and N status on root proliferation in
392 nutrient patches by the perennial grass *Agropyron desertorum* in the field. Oecologia 103:
393 10–16
394 Birch CPD, Hutchings MJ (1994) Exploitation of patchlly distributed soil resources by the
395 clonal herb *Glechoma hederacea*. J Ecol 82: 653–664
396 Bj□rkman O, Demmig B (1987) Photon yield of Oevolution evolution and chlorophyll fluo-
397 rescence characteristics at 77 K among vascular plants of diverse origins. Planta 170:
398 489–504
399 Brewer JS, Bertness MD (1996) Disturbance and intraspecific variation in the clonal mor-

400 phology of salt marsh perennials. Oikos 77: 107–116
401 Bryant JP, Chapin FS, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to
402 vertebrate herbivory. Oikos 40: 357–368
403 Butler WL, Kitajima M (1975) Fluorescence quenching in photosystem II of chloroplasts.
404 Biochimica et Biophysica Acta 376: 116–125
405 Chidumayo EN (2006) Fitness implications of clonal integration and leaf dynamics in a sto-
406 loniferous herb, *Nelsonia canescens* (Lam.) Spreng (Nelsoniaceae). Evol Ecol 20: 59–73
407 Cook CDK (1990) Aquatic Plant Book. SPB Academic Publishing, Amsterdam
408 Crick JC, Grime JP (1987) Morphological plasticity and mineral nutrient capture in two her-
409 baceous species of contrasted ecology. New Phytol 107: 403–414
410 Day KJ, John EA, Hutchings MJ (2003) The effects of spatially heterogeneous nutrient
411 supply on yield, intensity of competition and root placement patterns in *Briza media* and
412 *Festuca ovina*. Func Ecol 17: 454–463
413 Evans JP, Whitney S (1992) Clonal integration across a salt gradient by a nonhalophyte, *Hy-*
414 *drocotyle bonariensis* (Apiaceae). Am J Bot 79: 1344–1347
415 Farley RA, Fitter AH (1999) Temporal and spatial variation in soil resources in a deciduous
416 woodland. J Ecol 87: 688–696
417 Fay PA, Carlisle JD, Knapp AK, Blair JM, Collins SL (2003) Productivity responses to al-
418 tered rainfall patterns in a C4-dominated grassland. Oecologia 137: 245–251
419 Friedman D, Alpert P (1991) Reciprocal transport between ramets increases growth of
420 *Fragaria chiloensis* when light and nitrogen occurred in separate patches but only if

patches are rich. Oecologia 86: 76–80

Gersani M, Sachs T (1992) Developmental correlations between roots in heterogeneous environments. Plant Cell Environ 15: 463–469

Hagiwara Y, Kachi N, Suzuki JI (2008) Effects of temporal heterogeneity of watering on size of an annual forb, *Perilla frutescens* (Lamiaceae), depend on soil nutrient levels. Botany 86: 1111–1116

Han WX, Fang JY, Reich PB, Woodsward FI, Wang ZH (2011) Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. Ecol Lett 14: 788–796.

Hartnett DC, Bazzaz FA (1983) Physiological integration among intraclonal ramets in *Solidago canadensis*. Ecology 64: 779–788

Hartnett DC, Bazzaz FA (1985) The integration of neighbourhood effects by clonal genets of *Solidago canadensis*. J Ecol 73: 415–428

Herben T (2004) Physiological integration affects growth form and competitive ability in clonal plants. Evol Ecol 18: 493–520

Hester MW, Mckee KL, Burdick DM, Kock MS, Flynn KM, Patterson S, Mendelssohn IA (1994) Clonal integration in *Spartina patens* across a nitrogen and salinity gradient. Can J Bot 72: 767–770

Hutchings MJ, Wijesinghe DK (1997) Patchy habitats, division of labour and growth dividends in clonal plants. Trends Ecol Evol 12: 390–394

IPCC 2007 Climate change (2007): Impacts, Adaptation and Vulnerability Working Group II

442 Contribution to the Intergovernmental Panel on Climate Change Fourth Assessment Re-
443 port Summary for Policymakers. Cambridge University Press, Cambridge, United
444 Kingdom and NewYork, USA
445 Jackson RB, Caldwell MM (1993a) Geostatistical patterns of soil heterogeneity around indi-
446 vidual perennial plants. J Ecol 81: 683–692
447 Jackson RB, Caldwell MM (1993b) The scale of nutrient heterogeneity around individual
448 plants and its quantification with geostatistics. Ecology 74: 612–614
449 James SE, Pärtel M, Wilson SD, Peltzer DA (2003) Temporal heterogeneity of soil moisture
450 in grassland and forest. J Ecol 91:234–239.
451 Johnson GN, Scholes JD, Young AJ, Horton P (1993) The dissipation of excess excitation
452 energy in British plant species. Plant Cell Environ 16: 673–679
453 Kolar CS Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol
454 Evol 16: 199–204
455 Lambers H, Chapin FSIII, Pons TL (1998) Plant physiological ecology. Springer, New York.
456 Liu J, Dong M, Miao S, Li Z, Song M, Wang R (2006) Invasive alien plants in China: role of
457 clonality and geographical origin. Bio Invasions 8: 1461–1470
458 Lodge DJ, McDowell WH, McSwiney CP (1994) The importance of nutrient pulses in tropi-
459 cal forests. Trends Ecol Evol 9: 384–387
460 Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic inva-
461 sions: causes, epidemiology, global consequences, and control. Ecol Appl 10: 689–710
462 Madsen JD (1991) Resource allocation at the individual plant level. Aquat Bot 41: 67–86

463 Maestre FT, Reynolds JF (2007) Amount or pattern? Grassland responses to the heterogeneity
464 and availability of two key resources. Ecology 88: 501–511
465 Markesteijin L, Poorrter L (2009) Seedling root morphology and biomass allocation of 62
466 tropical tree species in relation to drought- and shade-tolerance. J Ecol 97: 311– 325
467 Marschner H (1995) Mineral Nutrition of Higher Plants. Aademic Press, New York
468 Marshall C. 1990. Source–sink relations of interconnected ramets. In: Kroon HD, Groenendal
469 VJ (eds) Clonal growth in plants: regulation and function. SPB Academic Press, The
470 Hague, pp 23–41
471 Maurer DA, Zedler JB (2002) Differential invasion of a wetland grass explained by tests of
472 nutrients and light availability on establishment and clonal growth. Oecologia 131:
473 279–288
474 Moola FM, Vasseur L (2009) The importance of clonal growth to the recovery of *Gaultheria*
475 *procumbens* L. (Ericaceae) after forest disturbance. Plant Ecol 201: 319–337
476 Murphy GP, Dudley SA (2007) Above- and below-ground competition cues elicit independ-
477 ent responses. J Ecol 95: 261–272
478 Novoplansky A, Goldberg DE (2001) Effects of water pulsing on individual performance and
479 competitive hierarchies in plants. J Veg Sci 12: 199–208
480 Oborny B, Cain ML. 1997. Models of spatial spread and foraging in clonal plants. In: Kroon
481 HD, Groenendael JV (eds) Ecology and Evolution of Clonal Plants.Backhuys Publishers,
482 Leiden, pp155–183
483 Orr BK, Resh VH (1989) Experimental test of the influence of aquatic macrophyte cover on

the survival of Anopheles larvae. J. Am. Mosq. Control Assoc 5: 579–585

Otfinowski R, Kenkel NC (2008) Clonal integration facilitates the proliferation of smooth brome clones invading northern fescue prairies. Plant Ecol 199: 235–242

Piao SL et al. (2010) The impacts of climate change on water resources and agriculture in China. Nature 407 43–51

Reichard S (1997) Invasive woody plant species in North America.University of Washington Press, Seattle

Roiloa SR, Retuerto R (2005_ Presence of developing ramets of *Fragaria vesca* L. increases photochemical efficiency in parent ramets. Int J Plant Sci 166: 795–803

Roiloa SR, Retuerto R (2006a) Physiological integration ameliorates effects of serpentine soils in the clonal herb *Fragaria vesca*. Physiol Plantarum 128: 662–676

Roiloa SR, Retuerto R (2006b) Small-scale heterogeneity in soil quality influences photosynthetic efficiency and habitat selection in a clonal plant. *Annals of Botany* 98: 1043–1052

Roiloa SR, Retuerto R (2007) Responses of the clonal *Fragaria vesca* to microtopographic heterogeneity under different water and light conditions. Enviro Exp Bot 61: 1–9

Saitoh T, Seiwa K, Nishiwaki A (2002) Importance of physiological integration of dwarf bamboo to persistence in forest understorey: a field experiment. J Ecol 90: 78–85

Salzman AG, Parker MA (1985) Neighbours ameliorate local salinity stress for a rhizomatous plant in a heterogeneous environment. Oecologia 65: 273–277

Schreiber U, Bilger W, Hormann H, Neubauer C (1998) Chlorophyll fluorescence as a diag-

505 nostic tool: basics and some aspects of practical relevance. In: Raghavendra AS (ed)
506 Photosynthesis: a comprehensive treatise. Cambridge University Press, Cambridge, pp
507 320–336
508 Silvertown J, Gordon DM (1989) A framework for plant behavior. Annu Rev Ecol Syst 20:
509 349–366
510 Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological re-
511 search, 2nd edn. Freeman, San Francisco
512 Sterner RW, Elser JJ (2002) Ecological Stoichiometry: The Biology of Elements from Mole-
513 cules to the Biosphere. Princeton University Press, Princeton, New York
514 Stuefer JF, Kroon HD, During HJ (1996) Exploitation of environmental heterogeneity by spa-
515 tial division of labor in a clonal plant. Funct Ecol 10: 328–334
516 Stuefer JF, During HJ, Kroon HD (1994) High benefits of clonal integration in two stolonif-
517 erous species, in response to heterogeneous light environments. J Ecol 82: 511–518
518 Sutton DL (1985) Biology and ecology of *Myriophyllum aquaticum*. Proceeding, 1st Interna-
519 tional Symposium on watermilfoil (*Myriophyllum spicatum*) and Related Haloragaceae
520 Species. 23–24 July 1985. Vancouver, B.C.: 59–71
521 Sytsma MD, Anderson LWJ (1992) Biomass, nitrogen, and phosphorus allocation in parrpt-
522 feather (*Myriophyllum aquaticum*). J Aquat Plant Manage 31: 244–248
523 Timmons FL, Klingman DL (1958) Control of aquatic and bank weeds. Soil Conserv 24:
524 102–107
525 Titus JE, Andorfer JH (1996) Effects of $CO_2$ enrichment on mineral accumulation and nitro-

526 gen relations in a submerged macrophyte. Freshwater Biol 36 (3): 661–671
527 Tremmel DC, Bazzaz FA (1995) Plant architecture and allocation in different neighborhoods:
528 implications for competitive success. Ecology 76: 262–271
529 Wang N, Yu FH, Li PX, He WH, Liu FH, Liu JM, Dong M (2008) Clonal integration affects
530 growth, photosynthetic efficiency and biomass allocation, but not the competitive ability,
531 of the alien invasive *Alternanthera philoxeroides* under severe stress. Ann Bot-London
532 101:671–678
533 Xiao KY, Yu D, Wang JW (2006) Habitat selection in spatially heterogeneous environments:
534 a test for forging behaviour in the clonal submerged macrophyte *Vallisneria spiralis*.
535 Freshwater biol 51: 1552–1559
536 Xie D, Yu D, Yu LF, Liu CH (2010) Asexual propagation of introduced exotic macrophytes
537 *Elodea nuttallii*, *Myriophyllum aquaticum*, and *M. propinquum* are improved by
538 nutrient-rich sediments in China. Hyrdrobiologia 655: 37–47
539 Xie Y, Li ZY, Gregg WP, Li DM (2001) Invasive species in China-an overview. Biodivers
540 Conserv 10: 1317-1341
541 Xu CY, Schooler SS, Klinken RDV (2010) Effects of clonal integration and light availability
542 on the growth and physiology of two invasive herbs. J Ecol 98: 833–844
543 Yu F, Chen Y, Dong M (2002) Clonal integration enhances survival and performance of
544 *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China. Evol
545 Ecol 15: 303–318
546 Yu FH, Wang N, He WM, Chu Y, Dong M (2008) Adaptation of rhizome connections in
547 drylands: increasing tolerance of clones to wind erosion. Ann bot-London 102: 571–577

548 **List of figure legends**

549

550 **Fig. 1** Schematic representation of the experimental design. Each experimental unit contains
551 one mother plant, three severed daughter ramets and three intact daughter ramets. Ramets
552 were subjected to three levels of water spatial heterogeneity or water temporal heterogeneity
553 (L: Low-amount or Low-frequency; M: Middle-amount or Middle-frequency; H:
554 High-amount or High-frequency)

555

556 **Fig. 2** Growth parameters (total biomass, stolon length and number of branches) of daughter
557 ramets of *Myriophyllum aquaticum*, grown with stolon connections either severed from or
558 connected to the mother plant and provided by three levels of amount or frequency of water
559 supply: (a, b, c) for water spatial heterogeneity; (d, e, f) for water temporal heterogeneity. Da-
560 ta indicate the means ± SE

561

562 **Fig. 3** Effects of water heterogeneity and clonal integration (severed or intact) on the rela-
563 tionship between shoot biomass and root biomass of the daughter ramets: (a) for water spatial
564 heterogeneity, (b) for water temporal heterogeneity. All data are log transformed

Table 1

(a) Multivariate ANOVA analyses on growth parameters of daughter ramets of *M. aquaticum*

| Source | Wilk's Lambda | *F* | d.f. | *P* |
|---|---|---|---|---|
| Spatial | | | | |
| Heterogeneity (H) | 0.142 | 12.65 | 6,46 | **< 0.001** |
| Integration (I) | 0.154 | 42.18 | 3,23 | **< 0.001** |
| H × I | 0.737 | 1.26 | 6,46 | 0.292 |
| Block | 0.244 | 2.84 | 15,64 | **0.002** |
| Temporal | | | | |
| Heterogeneity (H) | 0.728 | 1.32 | 6.46 | 0.268 |
| Integration (I) | 0.132 | 50.23 | 3,23 | **< 0.001** |
| H × I | 0.901 | 0.41 | 6,46 | 0.869 |
| Block | 0.294 | 2.38 | 15,64 | **0.009** |

Main factors: water heterogeneity (spatial heterogeneity: 0.5 L every 10 days for low-amount, 1.0 L every 10 days for middle-amount and 2.0 L every 10 days for high-amount; or temporal heterogeneity: 2 L every 10 days for low-frequency, 1 L every 5 days for middle-frequency and 0.2 L every day for high-frequency), clonal integration (stolon connections severed or kept intact). *F*-values are shown

Significant *P*-values are presented in bold

(b) Univariate test statistics on growth parameters

| Dependent variable | Heterogeneity (H) | Integration (I) | H× I | Block |
|---|---|---|---|---|
| Spatial | | | | |
| Total biomass | $F_{2, 25} = 9.34$** | $F_{1, 25} = 135.33$*** | $F_{2, 25} = 0.69$ | $F_{5, 25} = 2.82$* |
| Stolon length | $F_{2, 25} = 49.65$*** | $F_{1, 25} = 46.93$*** | $F_{2, 25} = 1.05$ | $F_{5, 25} = 3.36$* |
| Number of branches | $F_{2, 25} = 20.37$*** | $F_{1, 25} = 52.09$*** | $F_{2, 25} = 0.03$ | $F_{5, 25} = 5.12$** |
| Temporal | | | | |
| Total biomass | $F_{2, 25} = 0.01$ | $F_{1, 25} = 149.25$*** | $F_{2, 25} = 0.69$ | $F_{5, 25} = 4.92$** |
| Stolon length | $F_{2, 25} = 2.81$ | $F_{1, 25} = 5.44$* | $F_{2, 25} = 0.16$ | $F_{5, 25} = 3.32$* |
| Number of branches | $F_{2, 25} = 0.98$ | $F_{1, 25} = 15.47$** | $F_{2, 25} = 1.18$ | $F_{5, 25} = 1.96$ |

Main factor: water heterogeneity (spatial: low-amount, middle-amount, high-amount; or temporal: low-frequency, middle-frequency, high-frequency), clonal integration, *F*-values are shown, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2

Two-way ANCOVA analyses on root biomass of daughter ramets of *M. aquaticum*

| | Spatial | | | Temporal | | |
|---|---|---|---|---|---|---|
| Source | d.f. | *F* | *P* | d.f. | *F* | *P* |
| Shoot biomass (S) | 1 | 29.43 | **< 0.001** | 1 | 6.63 | **0.017** |
| Heterogeneity (H) | 2 | 5.13 | **0.014** | 2 | 0.03 | 0.971 |
| Integration (I) | 1 | 3.20 | 0.086 | 1 | 14.99 | **0.001** |
| H × I | 2 | 15.02 | **< 0.001** | 2 | 0.40 | 0.678 |
| Block | 5 | 3.60 | **0.014** | 5 | 0.38 | 0.858 |
| Error | 24 | | | 24 | | |

Main factor: water heterogeneity (spatial: low-amount, middle-amount, high-amount; or temporal: low-frequency, middle-frequency, high-frequency), clonal integration; with shoot biomass as covariate. *F*-values are shown

Significant *P*-values are presented in bold

Table 3

The results from two-way ANOVA of photosynthetic performance ($F_v/F_{m,}$ Yield), nutrient concentration (C, N) and ratio (C/N) of daughter ramets of *M. aquaticum*

| Dependent variable | Severed | | | Intact | | | ANOVAS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Low | Middle | High | Low | Middle | High | H | I | H × I | Block |
| **Spatial** | | | | | | | | | | |
| $F_v/F_m$ | 0.77 ± 0.014a | 0.79 ± 0.015b | 0.81 ± 0.003c | 0.82 ± 0.006c | 0.81 ± 0.008c | 0.81 ± 0.017c | 5.59** | 60.49*** | 15.42*** | 2.21 |
| Yield | 0.29 ± 0.011a | 0.30 ± 0.004b | 0.31 ± 0.006c | 0.37 ± 0.006d | 0.37 ± 0.009d | 0.39 ± 0.007e | 29.36*** | 96.12*** | 0.81 | 0.85 |
| C (%, g/g) | 45.30 ± 1.039a | 44.40 ± 1.251a | 43.89 ± 0.952a | 44.71 ± 1.011a | 45.12 ± 1.143a | 44.04 ± 2.199a | 2.08 | 0.04 | 0.72 | 1.24 |
| N (%, g/g) | 2.89 ± 0.241a | 3.45 ± 0.238ab | 3.68 ± 0.385b | 3.10 ± 0.320ab | 2.92 ± 0.379a | 3.19 ± 0.542ab | 5.51* | 6.80* | 5.64** | 3.77* |
| C/N | 15.14 ± 1.090c | 12.95 ± 1.281ab | 12.03 ± 1.157a | 14.54 ± 1.397bc | 15.65 ± 2.018c | 14.04 ± 1.785abc | 9.82** | 9.03** | 8.69** | 3.72* |
| **Temporal** | | | | | | | | | | |
| $F_v/F_m$ | 0.82 ± 0.045a | 0.81 ± 0.007a | 0.81 ± 0.008a | 0.82 ± 0.010a | 0.81 ± 0.005a | 0.82 ± 0.013a | 3.07 | 0.49 | 1.99 | 0.89 |
| Yield | 0.35 ± 0.015a | 0.35 ± 0.018a | 0.35 ± 0.016a | 0.41 ± 0.016b | 0.40 ± 0.018b | 0.41 ± 0.022b | 0.08 | 85.66*** | 0.22 | 0.67 |
| C (%, g/g) | 45.03 ± 0.940a | 44.32 ± 1.239a | 44.09 ± 1.150a | 44.32 ± 0.652a | 42.63 ± 4.013a | 44.16 ± 1.013a | 1.26 | 1.56 | 0.72 | 1.26 |
| N (%, g/g) | 3.38 ± 0.310c | 3.07 ± 0.370bc | 2.73 ± 0.310ab | 2.66 ± 0.378ab | 2.49 ± 0.145a | 2.41 ± 0.192a | 9.83** | 45.79*** | 1.54 | 4.32** |
| C/N | 13.43 ± 4.166a | 14.601 ± 1.716ab | 16.29 ± 1.712bc | 16.95 ± 2.413bc | 17.22 ± 2.175bc | 18.43 ± 1.232c | 6.20** | 26.92*** | 0.80 | 2.66* |

Main factor: water heterogeneity (spatial: low-amount, middle-amount, high-amount; or temporal: low-frequency, middle-frequency, high-frequency), clonal integration. *F*-values are shown

Data indicate means ± SE (n = 6), Significant differences of treatments are indicated by different letters, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

$F_v/F_{m,}$: the maximum quantum yield of photosystem II (PS II); Yield: the effective quantum yield of PS II

**Fig. 1**

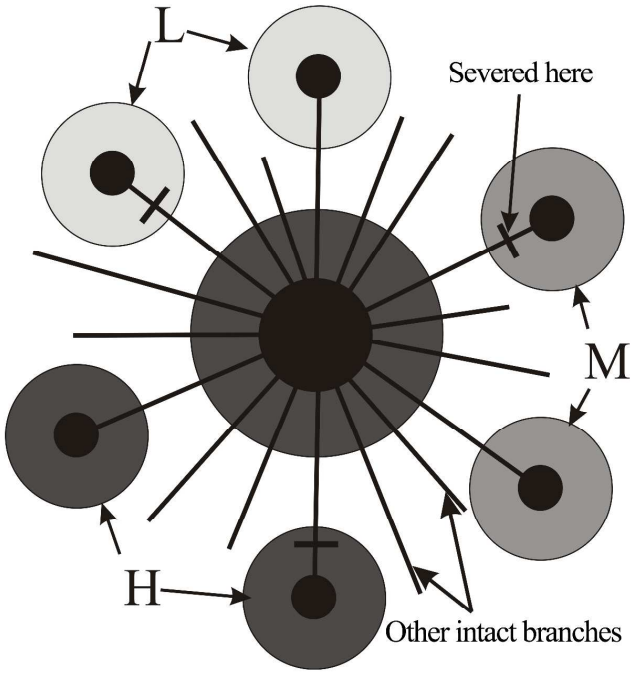

**Fig. 2**

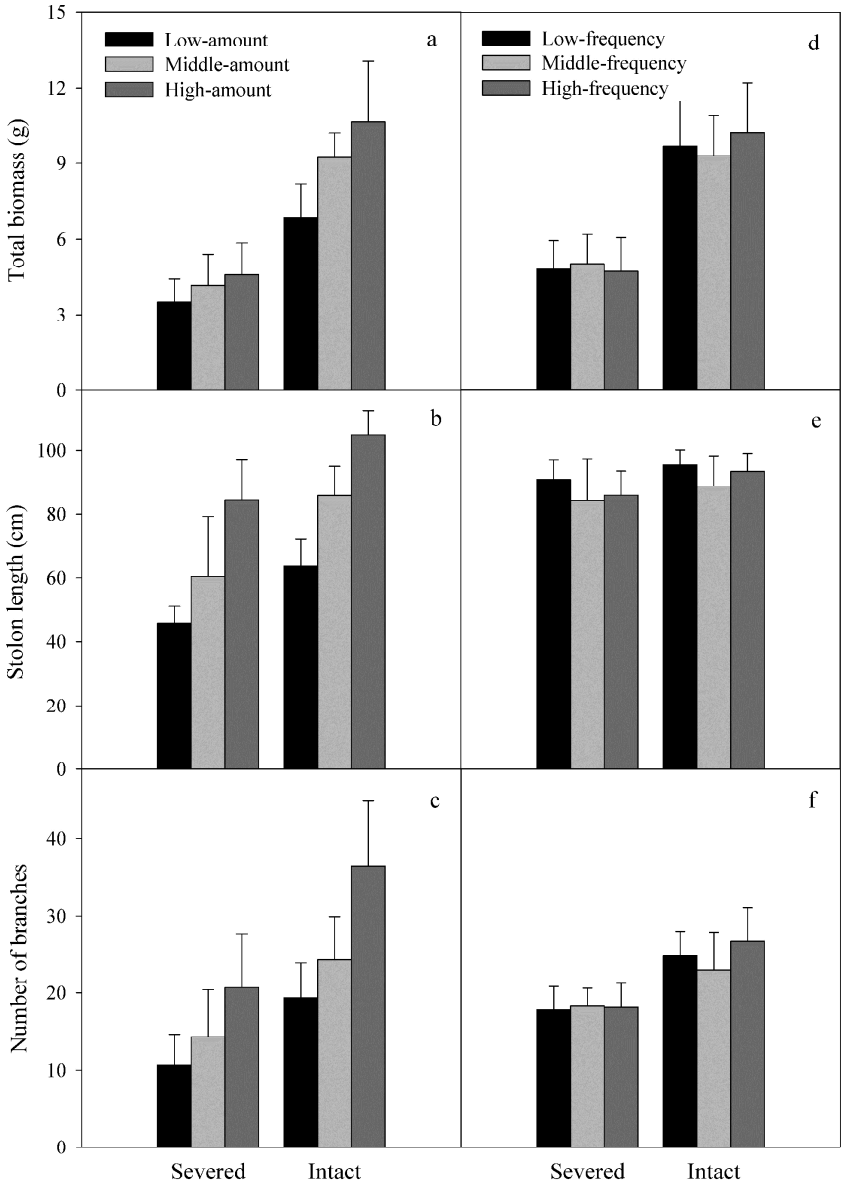
Low-amount
Middle-amount
High-amount
Low-frequency
Middle-frequency
High-frequency
a
b
c
d
e
f
Total biomass (g)
Stolon length (cm)
Number of branches
Severed
Intact
Severed
Intact

**Fig. 3**