**Running title:** Salinity and integration effects on *P. paspaloides*

**Effects of salinity and clonal integration on the amphibious plant *Paspalum paspaloides*: growth, photosynthesis and tissue ion regulation**

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**Abstract**

***Aims***

Clonal integration can increase performance of clonal plants suffering from environmental stress, and clonal plants in many wetlands commonly face stress of flooding accompanied by salinity. However, few studies have tested roles of clonal integration in amphibious plants expanding from terrestrial to aquatic saline habitats.

***Methods***

Basal (older) ramets of clonal fragments of *Paspalum paspaloides* were grown in soil to simulate terrestrial habitats, whereas their apical (younger) ramets were placed at the surface of saline water containing 0, 50, 150 and 250 mmol L-1 NaCl to mimic different salinity levels in aquatic habitats. Stolons connecting the apical and basal ramets were either intact (connected) to allow clonal integration or severed (disconnected) to prevent integration.

***Important Findings***

Increasing salinity level significantly decreased the growth of the apical ramets of *P. paspaloides*, and such effects on the leaf growth were much higher without than with stolon connection after 60-d treatment. Correspondingly, Fv/Fm and F/Fm′ of the apical ramets were higher with than without stolon connection in highly saline treatments. Due to clonal integration, Na+ could be translocated from the apical to the basal ramets to alleviate ion toxicity in apical ramets. Our results suggest that clonal integration benefits the expansion of *P. paspaloides* from terrestrial to aquatic saline habitats via maintained photosynthetic capacities and changed biomass allocation pattern.

**Keywords:** amphibious clonal plant, clonal integration, Na+, salt stress, wetland restoration

**Introduction**

Soil salinization is increasing over large areas of the world’s land (Bazihizina *et al.* 2012; Deinlein *et al.* 2014; Flowers and Colmer 2015; Munns and Tester 2008). It negatively impacts the growth and reproduction of plants due to reduction in water availability and over-accumulation of ionic Na+ and Cl- (Alamri *et al.* 2013; Deinlein *et al.* 2014; Flowers and Colmer 2015; Zhang *et al.* 2010). When exposed to salt stress, tolerant species often maximize water uptake to reduce water loss (Munns and Tester 2008; Teakle and Tyerman 2010; Wang *et al.* 2011). Some species such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) can also avoid ion toxicity by restricting the entry of Na+ and Cl- and maintaining high retention of K+ in tissues (Garthwaite *et al.* 2005; Gorham *et al.* 1986). Some species that cannot efficiently exclude Na+ can compartmentalize Na+ into vacuoles to minimize ion toxicity (Deinlein *et al.* 2014; Munns and Tester 2008; Tester and Davenport 2003).

Flooding accompanied by salinity is a common stress encountered by plants in many wetlands such as salt marshes (Costa *et al.* 2003; Emery *et al.* 2001; Pennings *et al.* 2005; Wang *et al.* 2011; Xiao *et al.* 2011). Partial O2 deficiency caused by flooding increases the rate of Na+ uptake, decreases K+ retention in roots and prevents K+ uptake into the shoots (Alamri *et al.* 2013; Barrett-Lennard and Shabala 2013; Colmer and Greenway 2011; Pang *et al.* 2006), which may exacerbate ion imbalance in tissues and cause severe leaf necrosis or even plant death (Bazihizina *et al.* 2012; Munns *et al.* 1995; Munns and Tester 2008). Therefore, the ability of salt tolerance is important for plants growing in flooded salt marshes (Costa *et al.* 2003; Emery *et al.* 2001; Pennings *et al.* 2005).

Many amphibious clonal plants spread quickly by clonal growth and span from terrestrial to aquatic habitats and *vice versa* (Costa *et al.* 2003; Glover *et al.* 2015; Klimes 2008; Wang *et al.* 2009; Xiao *et al.* 2011). These species can establish a connected ramet system via stolons or rhizomes, which allows the translocation of resources such as water, carbohydrates and nutrients between the connected ramets through clonal integration (Wang *et al.* 2009; Xiao *et al.* 2011). Clonal integration has been shown to facilitate the performances of the ramets suffering from various environmental stresses such as shading, drought, flooding, sand burial and contamination of heavy metals in heterogeneously stressful habitats (Glover *et al.* 2015; Gruntman *et al.* 2016; Luo *et al.* 2014; Roiloa *et al.* 2014; Wang *et al.* 2009; Xu and Zhou 2016; Yu *et al.* 2004). However, only a few studies have examined how amphibious clonal plants expand populations in terrestrial-aquatic ecotones with the help of clonal integration (Liu *et al.* 2016; Luo *et al.* 2014; Wang *et al.* 2009; Xu and Zhou 2016; Yan *et al.* 2013). Moreover, in most of these studies, neither terrestrial nor aquatic habitats are contaminated by pollution (but see Xu and Zhou 2016).

*Paspalum paspaloides* grows vigorously at terrestrial-aquatic transition zones of flooded salt marshes (McCarthy *et al.* 2006; Semple *et al.* 2001; Watt *et al.* 2007). To investigate the roles of clonal integration in the expansion of this species from unpolluted terrestrial to aquatic saline habitats, the basal ramets of clonal fragments of *P. paspaloides* were grown in soil to simulate unpolluted terrestrial habitats, whereas their apical ramets were placed on the surface of saline water containing 0, 50, 150 and 250 mmol L-1 NaCl to mimic different salinity levels in aquatic habitats. The connecting stolons between the apical and basal ramets were either intact (connected) to allow clonal integration or severed (disconnected) to prevent integration. Eutrophication is a common phenomenon in wetlands (Bergstrom and Jansson 2006; Mackay *et al.* 2014). To simulate moderate eutrophication, certain amounts of nitrogen (N) and phosphorous (P) were added to the saline water. We expected that (1) salinity will reduce the growth and photosynthetic capacity of *P. paspaloides*; (2) clonal integration will alleviate such negative effects; (3) the benefits of clonal integration will be amplified with increasing the salinity level.

**Material and methods**

**Plant species**

*Paspalum paspaloides* (Michx.) Scribn is an amphibious, perennial, stoloniferous clonal grass (Bhattacharya *et al.* 2010; Chen *et al.* 2015). It is widely distributed in China, as well as in tropical and subtropical regions of the world. This species produces creeping stolons that bear leaves, roots and axillary stolons at each node. Each node along the stolon with its leaves and roots can form a ramet. *Paspalum paspaloides* grows frequently in flooded salt marshes, and can spread quickly by asexual propagation of stolon fragments to occupy transition zones ranging from terrestrial to aquatic areas.

**Experimental material**

*Paspalum paspaloides* was collected from four riparian areas (geographical positions: 30°43'51"N, 104°32'19"E; 30°24'13"N, 103°58'59"E; 30°25'39"N, 103°49'39"E; 31°5'9"N, 103°50'54"E) in Chengdu, Sichuan Province, China. These areas are typical fluctuation zones along rivers with elevation ranging from 380 to 710 m and little disturbed by human activity. In these areas, *P. paspaloides* is a dominant species and its common, coexisting species include *Phalaris arundinacea*, *Saccharum spontaneum* and *Polygonum hydropiper*. In each area, plants were collected from four different locations spacing at least 10 m apart. These plants were mixed and vegetatively cultivated in a greenhouse of the Forest Science Company of Beijing Forestry University in Beijing. Before we started the experiment, the plant materials had been cultivated for > 2 years in the greenhouse to reduce potential maternal effects. Clonal fragments of *P. paspaloides* were propagated from stolon cuttings from these materials.

On August 2015, we selected clonal fragments of *P. paspaloides*, each having eight ramets and an apex. For each clonal fragment, the four older ramets were termed as 'basal part', and the other four younger ones with the apex as 'apical part'. Each clonal fragment was grown in a plastic box (50 cm × 36 cm × 17.5 cm, long × wide × high) that was physically separated by a plastic partition into two equal sections (25 cm × 36 cm × 17.5 cm) to simulate the transition zones of flooded salt marshes. The left section of the box was filled with a mixture of riverbank soil and sand (1:1, v:v, containing 0.33 ± 0.03 mg total N g-1 and 0.75 ± 0.04 mg total P g-1) to a depth of 10 cm to simulate terrestrial habitats, and was grown with the basal part of the clonal fragment. The right section was filled with a medium level of eutrophic water (1.76 mg total N L-1 and 0.16 mg total P L-1) to a depth of 10 cm to simulate aquatic habitats, and was grown with the apical part of the clonal fragment. The partition was sealed to the side walls and the bottom of the box by glue so that neither water nor nutrients in the two sections could interfere with each other. After two weeks for recovery (15 August 2015), 102 clonal fragments of *P. paspaloides* were selected and used in the experiment described below.

**Experimental design**

The experiment used a factorial design with four levels of salinity (0, 50, 150 and 250 mmol L-1NaCl) crossed with two levels of stolon connection (connected or disconnected), resulting in a total of eight treatments (Fig. 1). Six clonal fragments were randomly selected and harvested to evaluate the initial dry mass of apical and basal ramets on day 0 (0.22 ± 0.02 g for apical ramets; 0.16 ± 0.03 g for basal ramets). The remaining 96 fragments were randomly assigned to each of the eight treatments, with 12 replicates for each treatment.

For the treatments with salinity levels of 50, 150 and 250 mmol L-1, NaCl (Sigma-Aldrich, Shanghai, China) solutions of corresponding concentration were added to the right section of the boxes with the apical part, and for the treatment without salinity water (0 mmol L-1 NaCl), the same volume of deionised water was added. The salinity levels were set according to results of a pilot experiment which showed that *P. paspaloides* could 100% tolerate the saline level up to 250 mmol L-1. For the disconnected treatment, the stolon connecting the basal and apical part was severed. For the connected treatment, the stolon connecting the two parts remained untreated (intact) and passed over the top of the plastic partition (Fig. 1).

The experiment lasted for two months. The photosynthetically active radiation measured at plant level at noon was 800 - 1800 μmol photons m-2 s-1 (Li-250A photometer, Li-Cor Biosciences, Lincoln, NE, USA). The daily maximum air temperature ranged between 25 and 33°C during the experiment. No extra NaCl solution was added and only deionised water was added to the boxes to compensate for the loss due to evaporation. All plants survived at harvest.

**Growth measurements**

Six replicates were randomly selected and harvested on day 30 (14 September 2015), and the other six were harvested on day 60 (14 October 2015). Before harvest, we measured total number of ramets (the four original ramets plus their offspring ramets) of the apical and basal parts. Then, we harvested leaves, stolons and roots of the apical and basal parts separately, and dried them in oven at 80°C for at least 72 h. The dried samples were weighed, and then ground to fine powder for measuring Na+ and K+.

**Chlorophyll fluorescence measurements**

On days 30 and 60 and before harvest, chlorophyll fluorescence was measured on the youngest, fully expanded leaves of both apical and basal ramets by using a portable modulated fluorometer (PAM-2500, Heinz Walz, Germany) at 09:00 - 12:00 h. The maximal (Fm) and minimal (Fo) fluorescence intensity of dark-adapted leaves were measured after 30 minutes of dark adaptation using leaf clips. Thereafter, the maximal (Fm′) and steady-state (Fs) fluorescence intensity of light-adapted leaves were determined after illumination at 800 µmol m-2 s-1 for 4 min. The intensity and duration of the saturation pulse used to determine Fm and Fm′ were 3500 μmol m-2 s-1 and 1 s, respectively. The maximal quantum yield of PSII (Fv/Fm) was calculated as Fv/Fm = (Fm - Fo)/Fm. Dark-adapted values of Fv/Fm are used as sensitive indicators of plant photosynthetic performance, in particularly, the phenomenon of photoinhibition (Maxwell and Johnson 2000). The effective quantum yield of PSII is calculated as ΔF/Fm′ = (Fm′ - Fs)/ Fm′, which is an indication of overall photosynthesis. Non-photochemical energy quenching in PSII (NPQ), i.e. NPQ = (Fm - Fm′)/ Fm′, is linearly related to heat dissipation (Lysenko *et al.* 2015).

**Tissue Na+ and K+ measurements**

Dried powder of leaf, stolon and root samples (ca. 200 mg) was used for Na+ and K+ concentration measurements. The samples were digested with HNO3/HClO4 (4:1, v:v) for three hours in heat-resistant glass tubes on a heating block at 180 °C and the digests were diluted in deionised water. The concentrations of Na+ and K+ in dilutions of digests were determined using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto, Japan), according to the method described by Kotula *et al*. (2015). Data were calculated by taking standard curves of NaCl and KCl (Sigma-Aldrich, Shanghai, China) through the same procedures.

**Statistical analyses**

Data were checked for normality and homogeneity of variance before analyses. To increase normality and homogeneity of variance, data for root mass and ramet number of the apical ramets were transformed to logarithm, and data for root K+ concentration of the apical ramets were transformed to square root before analyses. Two-way ANOVA was used to examine the effects of stolon connection and salinity on growth parameters of the apical part, the basal part and the whole clonal fragment on days 30 and 60, and on chlorophyll fluorescence, Na+ and K+ concentration of the apical and basal parts on days 30 and 60. Planned contrasts following ANOVA were used to examine differences between the connected and disconnected treatments at each salinity level, using LMATRIX subcommands to control the family-wise error rate (Howell and Lacroix 2012; Sokal and Rohlf 1995). Leaf mass ratios and total mass ratios of the connected to the disconnected apical part were calculated as values of the connected treatment/mean values of the disconnected treatment. Differences in leaf mass ratios and total mass ratios of the apical part among salinity treatments were examined using one-way ANOVA followed by Duncan’s test. Some data of Na+ and K+ concentration in leaves or roots were not available because the sample amount was below the instrument detection. All analyses were done by using SPSS 16.0 (SPSS, Chicago, IL, USA). Effects were considered significant if *p* < 0.05.

**Results**

**Growth performance of apical and basal parts**

On day 30, salinity significantly decreased root mass, total mass and ramet number of the apical ramets of *P. paspaloides* (Table S1A; Fig. 2E, G and I). Leaf mass and ramet number of the apical ramets in saline water of 250 mmol L-1 NaCl were significantly higher with than without stolon connection to the basal ramets (Fig. 2A and I). Correspondingly, leaf mass ratio and total mass ratio of the connected to the disconnected apical part were significantly higher at the level of 250 mmol L-1 NaCl than at the other levels (Fig. 3A and C). However, root mass was higher without than with stolon connection in saline water of 50 mmol L-1 NaCl (Fig. 2E). For the basal ramets, biomass allocation to roots was higher with than without stolonconnection to the apical ramets (Table S2; Fig. S2).

On day 60, increasing salinity level significantly decreased all growth variables of the apical ramets (Table S1D; Fig. 2B, D, F, H and J), and such effects on leaf and total mass were much higher without than with stolon connection (Fig. 2B and H). Correspondingly, leaf mass ratio of the connected to the disconnected treatment was higher at the level of 250 mmol L-1 NaCl and total mass ratio was higher at the levels of 150 mmol L-1 NaCl than at the levels of 0 and 50 mmol L-1 NaCl (Fig. 3B and D). Again, root mass of the apical ramets was significantly higher without than with the connection (Fig. 2F). Correspondingly, biomass allocation to leaves in the apical ramets was significantly higher with than without stolon connection, whereas biomass allocation to roots was lower (Table S2; Fig. S2). Consequently, stolon connection did not significantly affect total mass (Fig. 2H), but changed biomass allocation pattern of the apical ramets (Fig. S2). For the basal ramets, neither salinity nor stolon connection significantly affected their growth (Table S1B and E; Fig. S1). However, stolon connection greatly increased biomass allocation to roots, but decreased biomass allocation to leaves, showing counteracting effects of the apical and the basal ramets (Fig. S2).

**Growth performance of the whole fragment**

On day 30, neither salinity nor stolon connection significantly affected the growth of the whole fragment (Table S1C). On day 60, salinity significantly decreased stolon mass and total mass of the whole fragment (Table S1F; Fig. 4D and H), with higher values at the saline levels of 0 and 50 mmol L-1 NaCl than at the levels of 150 and 250 mmol L-1 NaCl. However, stolon connection had no significant effects on any growth variables.

**Photosynthetic capacities of apical and basal parts**

On day 30, salinity significantly decreased Fv/Fm and ΔF/Fm′ of the apical ramets (Table S3A; Fig. 5A and C). ΔF/Fm′ was significantly higher with than without stolon connection (Fig. 5C). On day 60, Fv/Fm of the apical ramets significantly decreased with increasing salinity level, and was significantly higher with than without stolon connection (Table S3C; Fig. 5B). On the contrary, NPQ of the apical ramets was significantly smaller with than without stolon connection, and such effects increased with increasing salinity level (Table S3C; Fig. 5F). For the basal ramets, ΔF/Fm′ and NPQ were significantly smaller with than without stolon connection on day 60 (Table S3D; Fig. 5J and L).

**Na+ and K+ concentration in apical and basal parts**

On day 30, salinity significantly increased Na+ in leaves and stolons of the apical ramets, but decreased K+ in stolons (Table S4A; Figs. 6 and S3). Na+ in roots of the apical ramets was only detected at saline levels of 50 and 150 mmol L-1 NaCl with stolon connection(Fig. 6E). K+ in stolons and roots of the apical ramets was higher with than without stolon connection at saline level of 50 mmol L-1 NaCl (Fig. S3C and E). On day 60, salinity significantly increased Na+ in leaves, stolons and roots of the apical ramets, but decreased K+ in stolons and roots (Table S4C; Figs. 6 and S3). Na+ in roots of the apical ramets was significantly smaller with than without stolon connection at saline levels of 150 and 250 mmol L-1 NaCl (Fig. 6F).

For the basal ramets, salinity significantly increased Na+ in stolons (Table S4B; Fig. 6I), and such effects were much higher with than without stolon connection on day 30. K+ in leaves and stolons of the basal ramets was also higher with than without stolon connection at saline level of 50 mmol L-1 NaCl (Table S4B; Fig. S3G and I). On days 60, salinity significantly increased Na+ in leaves of the basal ramets, and such effects were much higher with than without stolon connection (Table S4D; Fig. 6H). Similarly, Na+ in stolons of the basal ramets was higher with than without stolon connection (Fig. 6J). At the saline level of 250 mmol L-1 NaCl, K+ in leaves and roots of the basal ramets was significantly higher with than without stolon connection (Fig. S3H and L).

**Discussion**

As expected, salinity significantly reduced the growth and thus imposed stress on the apical ramets of *P. paspaloides*, agreeing with previous findings (Bazihizina *et al.* 2012; Munns *et al.* 1995; Munns and Tester 2008; Zhang *et al.* 2010). With increasing salinity level, leaf mass of the apical ramets decreased much less with than without stolon connection to the basal ramets on day 60. Meanwhile, leaf and total mass ratios of the connected to the disconnected apical ramets were higher at the high than at the low saline treatments. These results suggest that clonal integration could benefit the spread of apical ramets from the terrestrial habitats into saline water, and that such a positive effect increased with increasing salinity level. Correspondingly, salinity significantly decreased Fv/Fm and ΔF/Fm′ of the apical ramets without clonal integration, but had no negative impact on them with integration. The maintained photosynthetic capacities could well support the resource demand for the initial establishment and the production of new ramets to extend the population (Liu *et al.* 2016; Yu *et al.* 2001). Benefits of clonal integration to recipient ramets importing resources have been also found in many other clonal species in response to environmental stresses such as nutrient deficiency, shading and flooding (Elgersma *et al.* 2015; Glover *et al.* 2015; Roiloa *et al.* 2014; Wang *et al.* 2009; Wang *et al.* 2017; Xiao *et al.* 2011). The results suggest that clonal integration can alleviate the negative effects of salinity on the growth and clonal reproduction of the apical ramets of *P. paspaloides* during its expansion from terrestrial to aquatic saline habitats.

Although clonal integration was found to reduce or increase the growth of donor ramets exporting resources in several studies (Glover *et al.* 2015; Pauliukonis and Gough 2004; Wang *et al.* 2017; Xiao *et al.* 2011; Xu and Zhou 2016), a meta-analysis has shown that clonal integration generally brings no significant costs to donor ramets (Song *et al*. 2013). We also found no cost of clonal integration to the basal ramets. As salinity did not significantly affect photosynthetic capacities of the apical ramets, the possible reasons for no costs to the basal ramets might be the independence of apical ramets or translocation of only surplus resources to the apical ramets (Liu *et al.* 2016; Song *et al.* 2013; Yu *et al.* 2001).

The leaf and root growth of the apical and basal ramets showed different responses to clonal integration, showing counteracting effects of the apical and basal ramets. Under saline stress, biomass allocation to leaves of the apical ramets and that to roots of the basal ramets were significantly greater with than without clonal integration on day 60, whereas biomass allocation to roots of the apical ramets and to leaves of the basal ramets were smaller. Salt-tolerant species can avoid ion toxicity by restricting the entry of Na+ and Cl- (Bazihizina *et al.* 2012; Garthwaite *et al.* 2005; Gorham *et al.* 1986; Munns and Tester 2008). In this study, the roots of the apical ramets were directly subjected to saline stress in aquatic conditions. Thus, the inhibited root growth could reduce ion accumulation and alleviated saline injuries in the apical ramets. The results also indicate that with stolon connection resource demand for the apical ramets may be mainly supplied from the connected basal ramets through clonal integration other than through the roots in saline water. Decreased biomass allocation to roots of stressed ramets has also been found with stolon connection to unstressed ramets when subjected to alkalinity, nutrient deficiency or Cu contamination (Xu and Zhou 2016; Zhang *et al.* 2006; Zhang *et al.* 2015). Without clonal integration, the apical ramets allocated more biomass to roots to uptake nutrients and water from saline water, which is a possible reason for high Na+ accumulation in the roots and severe necrosis in leaves of the apical ramets.

The integrative performance of apical and basal ramets determines the outcome of the whole fragment. Clonal integration greatly increased leaf growth of the apical ramets, but decreased their root growth. Such a contrasting effect on leaf and root growth led to no significant effect on the growth of the apical ramets. Meanwhile, the counteracting effects between apical and basal ramets discussed above resulted in no effect of the integration on the growth of the basal ramets. Furthermore, as the mass of the basal ramets accounted for about 60% of the total mass of the whole fragment during the experiment, the benefits to leaves and clonal reproduction of the apical ramets were not large enough to significantly increase the growth of the whole fragment. Therefore, clonal integration did not significantly increase the performance of the whole fragment. No benefits of integration at the level of the whole fragment was also observed in *Spartina alterniflora* under soil salt stress (Xiao *et al.* 2011), as well as *S. alterniflora* and *Alternanthera philoxeroides* when subjected to flooding (Wang *et al.* 2009; Xiao *et al.* 2010).

Na+ was significantly higher in roots of the apical ramets without than with clonal integration under highly saline stress, but lower in leaves and stolons of the basal ramets. The results suggest that clonal integration allows Na+ to be translocated from the apical to the basal ramets. Therefore, the saline stress suffered by the apical ramets could be shared with the connected basal ramets, which may greatly alleviate accumulated ions toxicity to the apical ramets in highly saline water. The horizontal translocation of ions such as Cu2+ via connected stolons between interconnected ramets has been found in some other clonal species (Roiloa and Retuerto 2012; Xu and Zhou 2016; Yan *et al.* 2013). For the basal ramets, there will be a potential risk of ion toxicity when Na+ accumulation reaches a toxic level, so that their growth would be greatly decreased by clonal integration (Garthwaite *et al.* 2005; Munns and Tester 2008). In our study, the Na+ concentration of the apical ramets in the saline water was appropriately 50 times higher than that of the basal ramets in the terrestrial soil at the end of the experiment, which was apparently not higher enough to become toxic as the growth of the basal ramets was not significantly affected by clonal integration.

*Paspalum paspaloides* may take a double-edged adaptive strategy during its expansion from terrestrial to aquatic saline habitats. With stolon connection to the basal ramets, the apical ramets maintained photosynthetic capacities, decreased growth moderately, changed biomass allocation, translocated Na+ to the basal ramets and maintained high K+ concentration during their expansion in aquatic saline habitats. On the other hand, once terrestrial and aquatic ramets were disconnected, a large fraction of absorbed light energy became excessive due to decreased ΔF/Fm′ of apical ramets in highly saline stress. The up-regulated NPQ of the apical ramets could greatly protect the photosynthetic apparatus and minimize photoinhibition. Meanwhile, the basal ramets greatly increased their photosynthetic capacities, resulting in higher shoot growth and biomass allocation to leaves than those with stolon connection in highly saline stress, which may facilitate the expansion the population in terrestrial habitats.

**Conclusions**

With the help of clonal integration, the apical ramets of *P. paspaloides* were able to establish and expand in highly saline water. However, the benefits of clonal integration to the apical ramets did not significantly increase the growth of the whole clonal fragments, which is probably due to counteracting effects of the apical and the basal ramets via changed biomass allocation pattern. As soil or water salinization happens at most (72 %) of the surface of the Earth (Flowers and Colmer 2015), resource translocation, risk sharing of ions (e.g. Na+, Cl-) and changed biomass allocation pattern between inter-connected ramets can benefitwetland clonal plants in aquatic-terrestrial ecotones of these regions.

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**Tables here** (if there is any)

**Figure legends**

**Figure 1:** Schematic representation of the experimental design.Each clonal fragment of *Paspalum paspaloides* consists of four basal ramets (closed circles) grown in soil and four apical ramets (open circles) with a stolon apex (an arrow) subjected to eutrophic water with four salinity levels (0, 50, 150 and 250 mmol L-1). The apical and basal ramets were either connected (on the left) or disconnected by severing (on the right).

**Figure 2:** Leaf mass (A, B), stolon mass (C, D), root mass (E, F), total mass (G, H) and ramet number (I, J) of the apical part of *Paspalum paspaloides* grown in eutrophic water with four salinity levels measured on days 30 and 60, connected or disconnected with the basal part grown in soil. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*\*** *p* < 0.01 and **\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 6).

**Figure 3:** Leaf mass ratio (A, B) and total mass ratio (C, D) of the connected to the disconnected apical part of *Paspalum paspaloides* on days 30 and 60. The apical part was subjected to eutrophic water with four salinity levels, connected or disconnected with the basal part grown in soil. Means sharing the same letter for different salinity treatments are not significantly different at *p* = 0.05. Bars are mean values (± s.e., *n* = 6).

**Figure 4:** Leaf mass (A, B), stolon mass (C, D), root mass (E, F), total mass (G, H) and ramet number (I, J) of the clonal fragment of *Paspalum paspaloides* measured on days 30 and 60, with its apical part subjected to eutrophic water with four salinity levels and connected or disconnected with its basal part grown in soil. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 6).

**Figure 5:** Maximal quantum yield of photosystem II (Fv/Fm, A, B, G, H), effective quantum yield of photosystem II (ΔF/Fm′, C, D, I, J) and non-photochemical energy quenching (NPQ, E, F, K, L) of the apical (A - F) and the basal parts (G - L) of *Paspalum paspaloides* measured on days 30 and 60. The apical part was subjected to eutrophic water with four salinity levels, connected or disconnected with the basal part grown in soil. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*\*** *p* < 0.01 and **\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 2 - 6).

**Figure 6:** Na+ concentration in leaves (A, B, G, H), stolons (C, D, I, J) and roots (E, F, K, L) of the apical (A - F) and the basal parts (G - L) of *Paspalum paspaloides* measured on days 30 and 60. The apical part was subjected to eutrophic water with four salinity levels, connected or disconnected with the basal part grown in soil. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*\*** *p* < 0.01 and **\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 2 - 6).

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**Supplementary Material**

**Figure S1:** Leaf mass (A, B), stolon mass (C, D), root mass (E, F), total mass (G, H) and node number (I, J) of the basal part of *Paspalum paspaloides* grown in soil measured on days 30 and 60, connected or disconnected with the apical part grown in eutrophic water with four salinity levels. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 6).

**Figure S2:** Biomass allocation proportion in leaves, stolons and roots of the apical (A, B) and the basal parts (C, D) of *Paspalum paspaloides* measured on days 30 and 60. The apical part was subjected to eutrophic water with four salinity levels, connected or disconnected with the basal part grown in soil. Bars are mean values (± s.e., *n* = 6).

**Figure S3:** K+ concentration in leaves (A, B, G, H), stolons (C, D, I, J) and roots (E, F, K, L) of the apical (A - F) and the basal parts (G - L) of *Paspalum paspaloides* measured on days 30 and 60. The apical part was subjected to eutrophic water with four salinity levels, connected or disconnected with the basal part grown in soil. Asterisks represent statistically significant differences between the connected and the disconnected treatments at each salinity level (**\*\*** *p* < 0.01 and **\*** *p* < 0.05). Bars are mean values (± s.e., *n* = 2 - 6).