Effects of clonal integration and light availability on the growth and physiology of two invasive herbs

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Summary

1. Clonal plants benefit from the ability to translocate resources among interconnected ramets to colonize heterogeneous habitats. Clonal integration affects the resource level and morphological traits of ramets, and thus may influence their physiology and general performance. Although leaf gas exchange and its associated physiological adjustments are key traits to assess plant fitness, the effect of clonal integration on these functional traits is insufficiently understood.

2. In a greenhouse experiment, we addressed how clonal integration affects gas exchange properties, leaf characters and growth of ramets in two invasive plants, *Alternanthera philoxeroides* and *Phyla canescens*, under full sun and 85% shade. We also used stable-isotope labelling to assess the maternal subsidy to daughter ramets.

3. Similar effects of connection were observed in both species for most gas exchange parameters and leaf characters. Clonal integration did not affect photosynthetic capacity and respiratory rates of ramets. When grown in shade, ramets connected with an unshaded mother plant displayed higher area-based leaf nitrogen and chlorophyll content than severed ramets, but the additional nitrogen and chlorophyll was not translated to increased photosynthetic capability. Overall, severed ramets displayed significant light response for leaf nitrogen (area-based), photosynthetic nitrogen use efficiency, chlorophyll to nitrogen ratio, and nitrogen stable isotope signature, but the light response was eliminated by clonal integration in connected ramets.

4. Both species displayed substantial maternal carbohydrate subsidy that benefited the growth of daughter ramets, but species-specific patterns were observed in the growth of daughter ramets and the amount of subsidy. The amount of subsidy was independent of ramet growth light levels for *P.canescens*, but shaded, connected ramets of *A. philoxeroides* received more subsidy than unshaded controls, facilitating a larger growth improvement relative to severed counterparts than *P. canescens*.

5. *Synthesis,* We observed increased leaf nitrogen and chlorophyll in shaded, connected ramets of two clonal invasive plants. Clonal integration may facilitate nitrogen assimilation and allow pre-acclimation to high-light conditions for shaded,

connected ramets, thus promoting the opportunistic expansion of these invaders on site scale. The species-specific maternal subsidy pattern demonstrated that clonal plants possess different strategies to subsidize ramets under light-limited conditions.

Introduction

Clonal plants possess the capacity to share resources, such as water, photosynthates and nutrients, among individual subunits (Alpert & Mooney 1986; Alpert 1996). The reciprocal translocation among ramets exposed to different resource conditions buffers the spatial variation in resource availability and facilitates efficient acquisition of heterogeneously distributed resources (Hutchings & Wijesinghe 1997). As a result, clonal integration may facilitate the colonization and growth of ramets in habitats with low resource availability or adverse conditions (Saitoh *et al.* 2002; Chidumayo 2006; Roiloa & Retuerto 2007; Otfinowski & Kenkel 2008), help ramets to survive and recover after abrupt environmental change (Yu *et al.* 2008; Moola & Vasseur 2009), and promote recovery after biomass loss from herbivores (Liu *et al.* 2007). These advantages may increase the performance of clonal plants over co-occurring non-clonal plants or clonal plants with little integration (Herben 2004). Physiological variables (e.g. rate of photosynthesis and respiration, nitrogen use efficiency) are key traits to assess plant fitness and general performance, so understanding physiological properties of clonal plants will provide insights into the functional mechanism of ecological advantage mediated by clonal integration. However, how clonal integration influences the physiology of ramets remains insufficiently understood.

In plants, photosynthesis and respiration are affected by leaf structural (e.g. leaf mass per area, LMA) and chemical properties (e.g. nitrogen content) (Evans 1989;

Niinemets & Tenhunen 1997; Oguchi *et al.* 2005), but the pattern could be altered in clonal plants due to the physiological integration among ramets. In non-clonal plants, numerous studies across diverse taxa and environments have confirmed strong, positive correlations between leaf nitrogen content and rates of photosynthesis (Field & Mooney 1986; Reich *et al.* 1998; Wright *et al.* 2004) and dark respiration (Ryan 1995; Griffin *et al.* 2002; Xu *et al.* 2007). The area-based relationships often result from the scaling of leaf mass per area (Poorter *et al.* 2009). Because clonal integration affects chemical and morphological properties of ramet leaves such as nitrogen content (Alpert 1991; Saarinen & Haansuu 2000; Saitoh *et al.* 2006), water potential (Alpert & Mooney 1986; Shumway 1995), carbohydrate concentration (Tissue and Nobel 1988; Saarinen & Haansuu 2000), chlorophyll content (Roiloa & Retuerto 2005, 2006; Roiloa *et al.* 2007), and LMA (Alpert 1999; Liao *et al.* 2003; Chu *et al.* 2006), modifications in these properties may alter the rate of photosynthesis and respiration, the acclimation of gas exchange rate to resource availability, and the pattern of gas exchange–leaf property relationships of clonal plants. Thus, it is expected that clonal integration may influence the carbon balance, growth rate and the overall competitive ability of ramets.

Plant invasion is a threat to biotic diversity and ecosystem services worldwide (Mooney & Hobbs 2000). Successful invaders often possess a high assimilation rate (Daehler 2003) and/or resource use efficiency (e.g. lower construction cost and maintenance respiration) (Nagel & Griffin 2001; Funk & Vitousek 2007; Xu *et al.* 2007), so that they may exploit and/or use resources more effectively than co-occurring native species. Many invasive plants also have the capacity for vigorous clonal propagation, and their invasiveness is related to clonality (Kolar & Lodge 2001;

Liu *et al.* 2006). To understand whether clonal integration would enhance or counteract the physiological advantage of plant invaders, the interaction of clonal integration and physiological traits needs to be addressed. However, studies on the influence of clonal integration on ramet physiology are rare, including cases investigating invasive species. The physiological parameters examined are very limited (e.g. chlorophyll florescence, Roiloa & Retuerto 2005, 2006, 2007), with only a few studies directly measuring photosynthetic rates in interconnected ramets of clonal invasive plants (Shumway 1995; Amsberry *et al.* 2000; Liu *et al.* 2008). Overall, previous studies found that clonal integration enhanced the photosynthetic efficiency, rate or capacity in resource-limited or adverse growth conditions. The effect of clonal integration on respiration and the physiological adjustment of gas exchange (e.g. nitrogen–photosynthetic rate relationship) has not been documented. In this study, we addressed the effect of clonal integration and light level on gas exchange properties, leaf characters and growth of two clonal plants that are invasive in Australia, *Alternanthera philoxeroides* (Martius) Grisebach (common name: Alligator weed, Amaranthaceae) and *Phyla canescens* (Kunth) Greene (common name: lippia, Verbenaceae). Comparison and contrast between two unrelated species allows a more solid extrapolation of the observed pattern.

Both *A. philoxeroides* and *P. canescens* are perennial herbs native to South America and both aggressively invade wetland ecosystems worldwide, including some communities where most native plant species are non-clonal (K. Reardon-Smith, unpublished data; Pan *et al.* 2006). *Alternanthera philoxeroides* and *P. canescens* both exhibit a creeping habit and a tendency to form dense mats. This facilitates rapid opportunistic recruitment and colonization, particularly in open habitats with low interspecies competition, rare extreme temperatures and high resource levels (Julien 1995). However, both species commonly exhibit a shorter stem height when compared to co-occurring plants (Lucy *et al.* 1995; C.-Y. Xu, personal observation), so juvenile ramets are vulnerable to light competition from dense wetland vegetation. For example, field studies observed that the establishment rate of *P. canescens* was reduced in the presence of existing vegetation (Macdonald 2008). In a related study, we found that clonal integration promotes the growth of juvenile ramets for both species during their early establishment in light-limited environments. We also found species-specific effects of clonal integration on morphological traits; clonal integration reduced the magnitude of light response in morphological traits for *A. philoxeroides*, but connected ramets of *P. canescens* consistently displayed larger organs than severed ramets (C.-Y. Xu unpublished data).

In this study, we investigated the physiological response of ramets under different light conditions that were either connected to or severed from an established mother plant (placed in full sun). This design simulates the expansion of daughter ramets from a mother plant established in an open habitat into neighbouring habitats with contrasting light conditions. We investigated the influence of clonal integration on the light responses of physiological traits, and compared and contrasted patterns between the two species. To gain a mechanistic understanding of the effect of clonal integration on physiological traits, we characterized photosynthetic – CO_2 concentration response, respiration rates and their relationships with leaf characters (chlorophyll, LMA and nitrogen). We also quantified the translocation of assimilates (using carbon stable-isotope labelling) and the concentration of non-structural carbohydrates in daughter ramets to assess the pattern of photosynthate subsidy from the mother plant. For each species, we tested five hypotheses. Because previous studies observed that clonal integration could enhance photosynthetic properties in an adverse environment and buffer the response of ramets, we expected that compared to severed ramets, connected ramets would possess (1) greater photosynthetic capacity, (2) higher chlorophyll content and leaf nitrogen concentration on a leaf area-based unit and (3) a lower magnitude of light response in the above-mentioned characters. In addition, because connected ramets may receive subsidy of assimilates from mother plants, we predicted that (4) connected ramets would grow faster than severed ramets, and (5) connected ramets in shade would obtain a larger maternal subsidy of photosynthates than connected ramets in full sun.

Material and methods

Species

Alternanthera philoxeroides has invaded many tropical and temperate areas around the world and become a problematic aquatic weed in more than 30 countries (Julien 1995; Holm *et al.* 1997). In introduced regions (e.g. USA, Australia and China), alligator weed rarely produces viable seeds in the field (Julien 1995) and reproduces mainly through vegetative structures such as roots and broken stems. In Australia, it mainly invades aquatic to mesophytic terrestrial habitats in coastal New South Wales (Burgin & Norris 2008). *Phyla canescens* was introduced worldwide as an ornamental plant, but became invasive in some countries; in the Murray–Darling Basin, Australia, it mainly invades riparian, wetland, and floodplain habitats (Lucy *et al.* 1995; Earl 2003). It reproduces both through seed (McCosker 1994) and vegetatively by rooting at nodes along either connected or broken stems (Lucy *et al.* 1995).

Plant materials and experimental design

Source material for *P. canescens* and *A. philoxeroides* was collected, respectively, from St Ruth’s Reserve near Dalby (27º 20’ 04” S, 151º 14’ 38” E, Queensland, Australia) and the town of Griffith (34º 17’ 21” S, 146º 2’ 37” E, New South Wales, Australia), and the material was propagated in the greenhouse of CSIRO Long Pocket Laboratory (Brisbane, Australia). Field-collected clonal fragments were planted in seedling pots in November 2006 and subsequently transplanted to 15 × 15 cm (diameter × height) plastic pots. These plants were allowed to grow roots at multiple nodes and developed into a dense patch within the pot. In April 2007, the plants were trimmed to similar size, transplanted to 25 × 25 cm pots and allowed one month additional growth. These plants were used as mother plants in the experiment to mimic well-established mother plants that are composed of groups of interconnected ramets and colonized on a favourable patch with abundant light. The potting mixture was 3:1 commercial potting mixer (H40, including composted pine bark and washed river sand, Centenary Landscape, Brisbane, Australia) to sand, and each litre contained 4 g Osmocote, 1 g Osmoform (Scott, Marysville OH, USA), 0.5 g urea formaldehyde, 0.5g IBDU and 1g $CaSO_4$:$MgSO_4$ mix (3:1 respectively) as fertilizer. The amount of total nitrogen (N), phosphoric pentoxide (P_2O_5) and potassium oxide (K_2O) supplied in 1 L of medium were 1.10g, 0.38g, and 0.55g, respectively. An additional 8 g of Osmocote was supplied to parent plants within two months during the experiment. Six mother plants with similar size (each had at least 30 branches) were used in the growth experiment for each species (Figure 1b). This medium and nutrient level is commonly used for rapidly growing herbal species in the nursery industry and is considered favourable for the growth of both *A. philoxeroides* and *P. canescens* (G. Fichera, personal communication).

To simulate the vegetative expansion of the two weeds from established patches, four well-extended branches were selected from each mother plant. These four branches were left connected to the parent and were the daughter plants measured in the experiments. For each branch, the top two nodes were cut and the third node was placed in a saucer with water to encourage root generation. After four days, all nodes had developed roots (1-2 cm length) and the rooted nodes were potted in a 15 cm plastic pot and fixed in place with two pins (Figure 1c). The soil medium contained the same amount of fertilizer as that used to grow the mother plants, but 1:3 commercial potting mixture:sand was used for more effective root harvesting. Compared with the added nutrient, the medium composition contained only trace amounts of nutrients, so difference in the medium composition was not likely to result in differential nutrient availability for mother and daughter plants.

All potted nodes were effectively rooted (examined by lightly shaking the rooted node) and had sprouted shoots after another six days. Two daughter ramets were severed from the mother (Figure 1c), so that each experimental unit consisted of one mother plant, two connected ramets and two severed ramets. Connected ramets and severed ramets were subjected to two light treatments (full sun and shaded by 85% shade cloth, Figure 1a) while the mother plant (including all other branches and stolons) was exposed to full sun. All six experimental units of each species were randomly located on benches in a greenhouse. Then, plants were grown for eight weeks. The photosynthetic photon flux density (PPFD) in the greenhouse was 1200–1400 μmol CO_2 m^{-2} s^{-1} on a sunny day, and the night and day temperatures were 10–17 ºC and 25 – 32 ºC, respectively, during the experiment period. All plants were watered to field capacity on a daily basis.

The experiment used a split-plot design; each experimental unit was treated as a ‘whole plot’; each whole plot was split into two subplots on which different light treatments were added; one connected and one severed ramet of the same mother plant were located in each light treatment. This design accounted for the effect of the mother plant and the location in the greenhouse before the effects of main factors (light and connection) were assessed. Because the mother plant was much larger than daughter ramets, we assumed that the existence of two ramets was not likely to alter the overall physiological status of the mother plant, and the physiology and growth of the two ramets would not affect each other directly. In addition, the mother plant appeared to have sufficient resources to support two ramets, as evidenced by its observed vigorous growth (C.-Y. Xu, personal observation). Thus, any disparity in the pattern of maternal subsidy between the full-sun and shaded ramet connected with the same mother, if observed, could be attributable to an allocation strategy, rather than the resource limitation, of the mother plant. In our study, the two ramets exposed to different light treatments were connected to the same mother. Because clonal plants often display labour division between ramets to capture locally abundant resources (Alpert & Stuefer 1997; Hutchings & Wijesinghe 1997), mother plants might allocate more subsidy to full-sun ramet to promote light harvest. This prediction can be an alternative hypothesis to our null hypothesis that shaded ramets would obtain more maternal subsidy full-sun ramets and was also examined in our experiment.

Measurements

Gas exchange

Leaf gas exchange characteristics were measured during the last week of growth with a portable photosynthesis system (Li-Cor 6400, Lincoln, NE, USA) equipped with CO_2 and temperature control modules. The 5th and the 6th leaves (on the 3rd node) from the tip of one branch were selected for gas exchange measurements (one for photosynthesis and one for respiration). A steady-state response of photosynthesis (A) to internal leaf CO_2 partial pressure (A-C_i Curve) was generated for each selected leaf at 25 ºC leaf temperature between 10 am and 4 pm. External CO_2 partial pressure (C_a) was set to 10 or 11 levels between 5 and 150 Pa (1500 ppm). At each C_a set point, photosynthetic parameters were recorded when gas exchange had equilibrated (taken to be when the coefficient of variation for C_a between the sample and reference analyser was below 0.3%), which typically took 1-2 min to achieve. A constant, saturating PPFD (1500 μmol m^{-2} s^{-1}) was supplied by blue–red light-emitting diodes mounted above the leaf cuvette. Dark respiration rates were measured at 15 ºC leaf temperature and 40 Pa CO_2 concentrations (400 ppm) between 8 pm and midnight. Following measurements, leaf portions sealed in the cuvette were cut and digital photos were taken to determine the measured leaf area by comparing leaf pixels with the pixels of a reference with known area (Photoshop, Adobe, San Jose, California, USA). The leaf temperature was controlled by a thermoelectric cooler.

The A-C_i curves were analysed to calculate the parameters potentially limiting to photosynthesis: maximum carboxylation rate of Rubisco (V_{cmax}) and RuBP regeneration capacity mediated by maximum electron transport rate (J_{max}) (Photosynthesis Assistant, Dundee Scientific, Scotland, UK). The calculations were made based on the biochemical model described by (Farquhar *et al.* 1980), and the model was parameterized according to (Bernacchi *et al.* 2001). The maximum photosynthetic rate (A_{max}) was calculated at PPFD of 1500 μmol m^{-2} s^{-1} and 30 Pa C_i and presented in area and mass based units (A_a, A_m). The photosynthetic nitrogen use efficiency (PNUE) at maximum photosynthetic rate was calculated as the photosynthetic rate per gram of leaf N. Respiration rates were presented in area, mass and nitrogen basis (R_a, R_m, R_N). The ratio of maximum photosynthetic rate to respiration (A/R) was calculated accordingly.

Stable isotope labelling

Mother plants were labelled by $^{13}CO_2$ 6 days before harvest to examine the carbon transportation between mother and daughter plants. The plant was enclosed inside a transparent plastic bag with vials containing 0.6 g $Ba^{13}CO_3$ (98% atom % ^{13}C), and

$^{13}CO_2$ was generated by injecting 2 mL of 1.4 M lactic acid. The mother plant was then allowed to assimilate $^{13}CO_2$ for two hours.

Harvest and leaf analysis

For all leaves used in gas exchange measurements, the leaf area-based relative chlorophyll content (RCC) was measured with a compact chlorophyll meter (Minolta SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan). Previous studies that calibrated SPAD in other C_3 species suggested the relationship between chlorophyll content and SPAD reading was linear (Neufeld *et al.* 2006), or approximately linear within a small range (<10 units, (Markwell *et al.* 1995; Uddling *et al.* 2007). Leaf mass per area (LMA) was calculated from the leaf area and dry weight (to 0.1 mg, Sartorius BA210S, Data Weighting Systems, Elk Grove, IL, USA). The leaf mass-based RCC was presented as RCC:LMA and the ratio of chlorophyll to nitrogen was calculated by RCC:nitrogen per unit leaf area (see below). All daughter ramets were harvested after 8 weeks of growth. The whole plant was dried in an oven at 60 ºC for at least 48 h and weighed to obtain total biomass. Leaf tissues were collected and ground into fine powder for chemical analysis.

For connected ramets, those in the light treatment receiving more maternal subsidy may display larger change in carbohydrate concentration relative to its severed counterpart. Thus, non-structural carbohydrates in ramet leaf tissues were quantified to examine whether they would provide any implication on the pattern of maternal subsidy. About 300 mg of dried and ground leaf material was used and the concentrations of soluble carbohydrates and starch were measured with a colorimetrical method in the Analysis Services Laboratory at the School of Land and

Food Sciences of the University of Queensland, Brisbane, Australia (see Karkalas (1985) for details of the method). In brief, about 100-200 mg of ground material was extracted in 80% ethanol at 80 ºC for soluble sugar; the remnant was treated with amylase to digest the starch. After reacting with a potassium ferricyanide reagent, the concentration of carbohydrate in the extract was determined by comparing the absorbance at 420 nm to a glucose standard on a UV-visible double beam spectrophotometer (Model 917, GBC Scientific Equipment, Dandenong, Victoria, Australia). Total non-structural carbohydrate (TNC) was calculated as the sum of soluble sugar and starch.

Leaf carbon and nitrogen concentrations and stable isotope ratios (carbon and nitrogen) were measured in the Isotope Core Laboratory of Washington State University (Pullman, USA) by a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen, Germany) coupled with an elemental analyser (ECS 4010, Costech Analytical, Valencia, CA, USA). For nitrogen, the concentration was presented in area and mass based units (N_a, N_m), and isotope ratios were expressed as δ^{15}N (‰) = $[R_\mathrm{sample} / R_\mathrm{standard} - 1] \times 1000$, where R is the molar ratio of ^{15}N:^{14}N. For carbon, molar ratio of ^{13}C:^{12}C was directly used to calculate the ^{13}C label incorporated in connected ramets in relevance to the severed ramet as: Concentration of ^{13}C label in a connected ramet (ppm) = $(R_\mathrm{con} - R_\mathrm{sev}) / (R_\mathrm{label} - R_\mathrm{sev}) \times 1000000$. R_sev is average ^{12}C:^{13}C ratio of all severed daughters of one light treatment. The total amount of label incorporated in the shoot of the connected ramet was calculated by multiplying the concentration of ^{13}C label by the biomass.

Statistical analysis

Factorial ANOVA for split-plot design was used to test the main effect and the interaction of connection and light on all parameters after the random effect of plant was fitted. The mean square values of connection × plant, light × plant and connection × light × plant were used as error terms to test the effect of connection, light and connection by light interaction, respectively. The effect of plant was not assessed in this design because there was no replicate for plant term. Multiple comparisons between treatments were made using Bonferroni tests. The relationships between gas exchange properties and leaf characters were addressed using a Pearson's correlation analysis. To examine whether the pattern of maternal subsidy to connected ramets was species-specific, the light effect on the amount of ^{13}C label incorporated in connected ramets was tested for each species. Because the concentration of assimilated ^{13}C of mother plants may affect the ^{13}C signal of daughter plants, ANCOVA was used with light as the mean effect and the $\delta^{13}C$ value of mother shoot tissue as a covariate. When species was used as a main factor in ANCOVA, the data did not fulfil the assumption of normality and homoscedasticity due to the large difference in the amount of ^{13}C integrated by *A. philoxeroides* and *P. canescens*, and no proper transformation was applicable. Thus, the pattern was separately analysed for the two species. Linear regression was run to explore the relationship between growth and A_a. Slopes of regression lines were compared between connected and severed ramets by running ANCOVA with connection as the main factor and A_{max} as the covariate. A significant effect of connection by covariate interaction suggests different slopes between connected and severed ramets. In addition, the non-structural carbohydrate concentrations of daughter ramets were compared using the ANOVA model above. Biomass and carbon isotope data of daughter ramets were square root-transformed to fulfil the assumption of normality and homoscedasticity. All analyses were conducted with Datadesk 6.0 (Data Description Inc., Ithaca, NY, USA).

Results

Gas exchange parameters

For *A. philoxeroides* and *P. canescens*, connection and connection × light interaction had no influence on most photosynthetic and respiratory parameters, but there was a significant connection × light effect on PNUE for both species (Table 1). The light effect was significant for all photosynthetic parameters except A_m for *A. philoxeroides* (Table 1). Ramets in full sun displayed a generally higher photosynthetic capacity and PNUE than shade treatments, regardless of the connection with the mother plant (Table 2a, Figure 2e, f). In addition, *A. philoxeroides* showed significant light effect for R_m while *P. canescens* displayed a light effect for A/R (Table 1). Overall, *P. canescens* displayed higher photosynthesis and respiration rates than *A. philoxeroides* on an area basis (Table 2a). These results do not support our hypothesis that connected ramets possess greater photosynthetic capacity and decreased photosynthetic light response.

Leaf mass per unit area, chlorophyll and nitrogen

The effect of connection on area-based chlorophyll content was significant in both species (Table 1), with connected ramets showing higher RCC values (Table 2c). The light effect on RCC was only significant for *A. philoxeroides* (Table 1). For both species, the shade treatment displayed higher mass-based chlorophyll content (RCC_m), and lower LMA than the full sun treatment, regardless of the connection with mother plant (Table 1, 2c). The connection × light interaction did not affect chlorophyll content and LMA for either species, but *P. canescens* displayed a significant connection effect (Table 1). However, connected ramets appeared to have a smaller difference in RCC between light treatments for both species (Table 2c).

Alternanthera philoxeroides and *P. canescens* had high N concentrations (> 50 mg N g^{-1}) in leaf tissue (Figure 2c, d), as regularly observed in these two species cultured under greenhouse condition with adequate nutrient supply (Xu *et al.* 2009, C.-Y. Xu, unpublished data), and both species displayed effects of light and the connection × light interaction on leaf N content (Table 1). The light response of N_a was significant only in severed ramets (Figure 2a, b), while the pattern was reversed for N_m (Figure 2c, d). Overall, connected ramets possessed higher nitrogen concentrations than severed ramets in the shade treatment (Figure 2a-d). The pattern of RCC and N support our hypothesis that connected ramets possess higher values, and reduced light responses of RCC and N_a.

The differential light response of leaf N between connected and severed ramets may explain the significant or marginally significant effect of connection × light interaction on PNUE and RCC/N_a (Table 1, Figure 2 e-h). Although *A. philoxeroides* and *P. canescens* showed different $\delta^{15}N$ values, the pattern of $\delta^{15}N$ response was consistent, i.e. severed ramets displayed lower $\delta^{15}N$ in shade than in full sun, whereas the light effect was eliminated in connected ramets (Figure 2i, j).

Relationships between gas exchange parameters and leaf properties

For both species, connected and severed ramets displayed a different relationship between photosynthetic capacity and leaf characters. In severed ramets, J_{max} and A_a displayed significant correlations with RCC; the photosynthetic capacity (V_{cmax}, J_{max}, and A_a) was also positively correlated to N_a and LMA, but not to N_m and RCC_m (Table 3). In contrast, connected ramets only exhibited a clear relationship between photosynthetic capacity and LMA (Table 3). The photosynthetic capacity–LMA relationships were similar among connected and severed ramets (ANCOVA with connection as main factor and LMA as covariate, $P > 0.07$ for connection × LMA interaction). The respiration rate of *A. philoxeroides* showed no covariance with leaf nitrogen, but R_a–N_a and R_a–LMA correlations were significant in severed ramets of *P. canescens* (Table 3). The correlations between photosynthetic parameters and $\mathrm{RCC_m}$ and N_m, if any, were all negative. In particular, for *P. canescens* the negative relationship was mainly observed in connected ramets, suggesting decreased efficiency of these resources associated with clonal integration.

Influence of clonal integration on ramet growth

Clonal integration and light intensity influenced the growth of both species. As expected in our hypothesis, daughter ramets accumulated greater biomass when connected with mother plants or when grown under full sun (Figure 3). However, the effect of clonal integration on the light response of biomass was species-specific. Only *A. philoxeroides* exhibited a significant interaction, with a reduced light response in connected ramets (Figure 3a).

The growth of daughter ramets was positively correlated to A_{max} (Figure 4), but the effect of connection on this relationship was different between species. The growth–photosynthesis regression lines were almost parallel between the connected and severed ramets of *P. canescens* (Figure 4b, P=0.61, ANCOVA). However, for *A. philoxeroides*, severed ramets showed a much steeper regression line than connected ramets (Figure 4a, P=0.01, ANCOVA). The difference in growth was large between connected and severed ramets with low photosynthetic rates (in shade), but negligible in ramets when photosynthetic rates were high (in full sun).

Non-structural carbohydrate concentration and ^{13}C label translocation

There was no significant effect of connection, light or their interaction on non-structural carbohydrate concentrations of *A. philoxeroides* (ANOVA, $P > 0.05$). However, the effect of the connection × light interaction was significant for soluble sugar (ANOVA, $P = 0.05$) starch (ANOVA, $P = 0.05$) and TNC (ANOVA, $P = 0.04$) for *P. canescens* (Figure 5b). For *P. canescens*, soluble sugar had a significant connection effect (ANOVA, $P = 0.05$), with connected ramets displaying a generally higher carbohydrate concentration, but the difference among treatments was not large enough to be detected in multiple comparisons (Figure 5b). Overall, both species mainly stored soluble sugar (>80% of TNC) in the leaf tissue, so starch concentration did not affect the light response pattern of TNC in ramets (Figure 5); this probably facilitates the transportation of assimilates among ramets.

In general, *P. canescens* incorporated more ^{13}C label in leaves of daughter ramets than *A. philoxeroides*. The concentration of ^{13}C label integrated in the biomass of connected ramets was higher in shaded treatments for both species (Figure 6a), suggesting maternal subsidy constituted a greater proportion of biomass when photosynthetic activity was limited. In *A. philoxeroides*, the total amount of ^{13}C label translocated to shaded ramets was higher than that translocated to ramets in full sun, with the light effect marginally significant ($P = 0.06$, Figure 6b). In contrast, the light effect on the total amount of translocated ^{13}C label was absent for *P. canescens*

(P=0.51, Figure 6b). Thus, our hypothesis that connected ramets in shade would obtain a larger maternal subsidy was therefore only supported for *A. philoxeroides*.

Discussion

The influence of clonal integration on photosynthetic and respiratory parameters, leaf characteristics and gas exchange–leaf property relationships were similar for *A. philoxeroides* and *P. canescens*. We initially hypothesized that connected ramets would have greater photosynthetic capacity, higher RCC and N_a, and reduced light response (of these parameters). However, the data only supported increased value and decreased light response for RCC and N_a. Overall, clonal integration did not affect the magnitude and the light response pattern of photosynthetic parameters, or the respiration rates of ramets under contrasting light conditions. In addition, the increase in chlorophyll and N in leaves of connected ramets was not translated into enhanced photosynthetic capacity. These results suggest a different pattern of N use and photosynthetic light acclimation between connected and severed ramets.

Clonal integration promoted the ramet growth of *A. philoxeroides* and *P. canescens*, but these two species displayed different modes of maternal subsidy. The results support our hypothesis that connected ramets would grow faster than severed ramets for both species, but our hypothesis that daughter ramets in shade would receive more photosynthates than those in full sun was only supported for *A. philoxeroides*, while the maternal subsidy to full sun and shaded daughter ramets was not significantly different for *P. canescens*. The difference in maternal subsidy affected the growth of ramets: the negative effect of shade on the growth of ramets was better ameliorated for *A. philoxeroides* than for *P. canescens*.

The influence of clonal integration on photosynthetic capacity

Clonal integration can improve the photosynthetic rate of ramets under unfavourable growth conditions. Previous studies of salt marsh species reported that clonal integration increases the photosynthetic rate of runners and ameliorates the negative effect of hypersalinity (Amsberry *et al.* 2000) by water translocation from connected runners located in a favourable habitat (Shumway 1995). It was also observed that the photosynthetic capacity of *A. philoxeroides* benefits from clonal integration across a N gradient (Liu *et al.* 2008). However, this was not the case across a light gradient in our study. The effect of clonal integration was absent for all photosynthetic parameters, probably because the photosynthetic acclimation pattern is physiologically different under light-limited conditions. The limited accessibility to light energy could not be compensated by translocation of other resources, so the leaf photosynthetic capacity would be mainly determined by the pattern of leaf photo-acclimation rather than clonal integration. In addition, light responses of photosynthetic capacity, either on an area (V_{cmax}, J_{max}, A_{a}) or mass basis (A_{m}), were not affected by clonal integration. Although translocation of photosynthates could alter the carbohydrate level of ramets and might further affect the photosynthetic capacity and efficiency of connected ramets (Hartnett & Bazzaz 1983; Roiloa & Retuerto 2005, 2006), it seems this regulation process did not affect photosynthetic parameters in our study.

Leaf properties and their association with photosynthetic capacity

Plants can adjust to grow under different light conditions by extensive modifications in leaf structure and physiology. Acclimation to high light often involves accumulation of rate-limiting photosynthetic proteins per unit leaf area, while enhancement of light-harvesting efficiency in low light occurs through increased chlorophyll per unit dry mass (Niinemets 2007). In our study, severed ramets of *A. philoxeroides* and *P. canescens* displayed light acclimation similar to what is observed in other species, with shaded leaves displaying lower LMA, N_a and RCC, and higher RCC_m than unshaded leaves. Both species also showed positive relationships on area-based quantities, which were mediated by the scaling of LMA (Table 3), between photosynthetic parameters (A_{max}, V_{cmax}, J_{max}) and nitrogen, and between light reaction-limited parameters (A_{max} and J_{max}) and chlorophyll content (Table 3). In contrast, leaf properties of connected ramets had substantially different light responses, with reduced response in N_a, RCC and RCC/N_a (Table 2, Figure 2). The positive associations among photosynthetic capacity and leaf N_a and RCC, as observed in severed ramets, were generally eliminated in connected ramets. These light response patterns of leaf nitrogen and chlorophyll may be explained by two possible mechanisms. Physiological integration may simply have dampened local plasticity of the ramets, as observed in some previous studies (Alpert 1999; Liao *et al.* 2003; Chu *et al.* 2006). Alternatively, the pattern is in line with the optimal biomass allocation theory and the division of labour theory for clonal plants. Non-clonal plants may allocate their biomass for acquiring resources that are limiting (Bloom *et al.* 1985), but clonal plants often display divisions of labour in resource-acquisition duties, with ramets specialized in capturing locally abundant resources (Alpert & Stuefer 1997; Hutchings & Wijesinghe 1997). In our study, severed ramets showed high RCC/N_a in shade to facilitate the light harvest, and low RCC/N_a to improve CO_2 fixation in full sun when CO_2 was the limiting factor of photosynthesis, but the response was buffered by clonal integration (Figure 2g, h). This different light response of leaf N and chlorophyll between connected and severed ramets observed in our study matches with the theoretical predictions, but has not been reported in empirical studies previously.

The additional leaf N and chlorophyll accumulation in connected ramets of *A. philoxeroides* and *P. canescens* were not translated into carbon gain capacity or direct promotion of photosynthesis under sub-optimal light conditions. Nonetheless, high leaf resource level in shaded, connected ramets may lead to other advantages. Clonal integration may stimulate the assimilation and storage of N. In our study, the alleviated light response of δ^{15}N values in connected ramets (Figure 2i, j) suggests that connected ramets received N from mother plants, so that the light-mediated, differential isotopic signature displayed in severed ramets was overridden. In addition, the uptake and allocation of N is also affected by photosynthates available in root material (Jordan *et al.* 1998; Delaire *et al.* 2005; Rogers *et al.* 2006), so mother plants probably supply energy and the carbon skeleton to promote the N assimilation of shaded ramets (grown in high-N medium). *Alternanthera philoxeroides* and *P. canescens* displayed very high N concentrations (5%-8%). Storage in the form of inorganic or small-molecular compounds (e.g. nitrate, ammonium, free amino acids, amides (Millard 1988)) is therefore not likely because of potential toxic and osmotic problems. Thus, we tentatively conclude that the additional N is accumulated, at least partially, as Rubisco in an inactive form. Over-investment in Rubisco has been reported in evergreen trees (Warren & Adams 2001; Warren *et al.* 2003) and interpreted as a mechanism of resource storage that is adaptive in nutrient-limited habitats (Millard 1988; Warren & Adams 2004). For these two invasive plants, the ability to accumulate N in shaded ramets may promote their N exploitation capacity, leading to an advantage over native non-clonal competitors. Further study examining the partitioning of N compounds is necessary to test this hypothesis in clonal herbaceous species.

The influence of clonal integration on respiration

The influence of clonal integration on the leaf respiration rate of ramets has not been previously addressed. In this study, we did not observe an effect of clonal integration on respiration rates in *A. philoxeroides* and *P. canescens* (Table 1). Area-based respiration rate was affected by LMA for severed ramets of both species, but not for connected ramets, probably due to the higher respiration rate of shaded, connected ramets (Table 2) that could be associated with the maintenance requirement of leaves with high N. Although respiration is often greater in leaves with higher concentrations of carbohydrates (e.g. Griffin *et al.* 2002; Turnbull *et al.* 2003; Xu & Griffin 2006), we did not observe a relationship between respiration rate and soluble sugar (data not shown). In *A. philoxeroides* and *P. canescens*, the majority of non-structural carbohydrate storage was soluble sugar rather than starch, suggesting that most soluble sugar may exist as forms for transport and storage (e.g. sucrose) rather than reducing monose, which is directly used as substrate for respiration. A previous study also showed that reducing monose is more closely related to respiration rate than sucrose (Xu & Griffin 2006). This may explain the absence of a correlation between respiration rate and soluble sugar.

Different pattern of maternal subsidy for ramet growth

The advantageous effect of clonal integration on the growth of ramets varies among species (Amsberry *et al.* 2000; Pennings & Callaway 2000). In this study, we observed a species-specific effect of clonal integration on the light response of ramet growth. When grown under contrasting light conditions, clonal integration mainly promoted the growth of shaded ramets of *A. philoxeroides*, whereas connection had similar effects on shaded and unshaded ramets of *P. canescens*. This phenomenon also indicates that ramets of *P. canescens* may generally be more dependent on clonal integration than *A. philoxeroides* ramets.

Currently, the mechanism that determines the extent of integration is not clear in clonal plants. The different subsidy pattern of *A. philoxeroides* and *P. canescens* probably is associated with root function of these two species. The thickened taproots of *A. philoxeroides* are the major resource pool to support its vegetative growth, and are an important factor determining its performance (Geng *et al.* 2007; Wilson *et al.* 2007). In contrast, taproots of *P. canescens* usually form woody structures with limited storage capacity (C.-Y. Xu, personal observation). Thus, the root of *A. philoxeroides* could form a sink for carbon assimilates, which may partially explain the differential effect of clonal integration on the growth and maternal subsidy in these two species. This hypothesis needs to be tested in future studies, and the ecological interpretation and the physiological mechanisms of the differential maternal subsidy pattern deserve further exploration.

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Table 1. ANOVA results of the photosynthetic parameters, respiration rates and leaf properties of *Alternanthera philoxeroides* and *Phyla canescens*. *P*-values of effects of connection, light and connection × light interaction are shown

Parameters / Effect	Photosynthesis				PNUE	Respiration			A/R	Chlorophyll		LMA	Nitrogen			RCC/N_a
	V_{cmax}	J_{max}	A_a	A_m		R_a	R_m	R_N		RCC	RCC_m		N_a	N_m	$\delta^{15}N$	
A. philoxeroides																
Connection	0.72	0.34	0.99	0.96	0.65	0.81	0.68	0.21	0.70	**0.01**	0.24	0.93	**0.01**	**0.005**	0.33	0.85
Light	**0.02**	**0.02**	**0.01**	0.24	**0.04**	0.80	**0.03**	0.19	0.12	**0.04**	**0.04**	**0.001**	**0.02**	**<0.001**	**0.02**	0.08
C×L	0.38	0.28	0.68	0.40	**0.05**	0.34	0.83	0.13	0.25	0.38	0.72	0.47	**0.003**	**<0.001**	**0.02**	**0.01**
P. canescens																
Connection	0.79	0.15	0.42	0.06	0.07	0.70	0.58	0.51	0.63	**0.01**	0.08	**0.01**	**0.01**	0.26	0.76	0.20
Light	**0.007**	**0.03**	**0.003**	**0.03**	**0.01**	0.93	0.33	0.76	**0.003**	0.20	**0.05**	0.06	0.14	**0.01**	**0.01**	0.30
C×L	0.46	0.64	0.75	0.92	**0.02**	0.47	0.62	0.44	0.16	0.30	0.67	0.83	**0.008**	**0.001**	**0.02**	0.06

Abbreviations: V_{cmax} Maximum carboxylation rate of Rubisco, J_{max} RuBP regeneration capacity mediated by maximum electron transport rate, A_a Maximum photosynthetic rate on an area basis, A_m Maximum photosynthetic rate on a mass basis, *PNUE* Photosynthetic nitrogen use efficiency at maximum photosynthetic rate, R_a Respiration rate on an area basis, R_m Respiration rate on a mass basis, R_N Respiration rate on a nitrogen basis, *RCC* Relative chlorophyll content on an area basis, RCC_m Relative chlorophyll content on a mass basis, *LMA* Leaf mass per unit area, N_a Leaf nitrogen on an area basis, N_m Leaf nitrogen on a mass basis, $\delta^{15}N$ Leaf nitrogen isotope ratio relative to ambient N_2

Table 2. Photosynthetic parameters, respiration rate, relative chlorophyll content and leaf mass per unit area of ramets of *Alternanthera philoxeroides* and *Phyla canescens*. Values shown are means (±SE), n=6. Means were compared with Bonferroni tests and values followed by the same letter are not significantly different at P = 0.05 level. Abbreviations follow those listed in Table 1

a. Photosynthetic parameters

Ramet	Light Level	V_{cmax} (μmol CO_2 m^{-2} s^{-1})	J_{max} (μmol CO_2 m^{-2} s^{-1})	A_a (μmol CO_2 m^{-2} s^{-1})	A_m (μmol CO_2 kg^{-1} s^{-1})
A. philoxeroides					
Connected	Full sun	108.2 (10.9)^{a}	237 (23)^{a}	31.5 (3.7)^{a}	775 (70)^{a}
	Shade	58.3 (6.1)^{b}	164 (24)^{ab}	17.6 (1.9)^{c}	610 (82)^{a}
Severed	Full sun	98.9 (13.5)^{ab}	234 (27)^{a}	30.5 (3.7)^{ab}	721 (43)^{a}
	Shade	62.2 (5.0)^{b}	129 (12)^{b}	18.5 (1.4)^{bc}	671 (67)^{a}
P. canescens					
Connected	Full sun	142.7 (14.1)^{ab}	280 (33)^{a}	36.3 (2.4)^{a}	722 (25)^{ab}
	Shade	79.6 (23.3)^{bc}	171 (26)^{a}	17.3 (4.3)^{b}	427 (111)^{c}
Severed	Full sun	155.2 (14.9)^{a}	319 (46)^{a}	38.9 (2.9)^{a}	879 (28)^{a}
	Shade	74.6 (5.6)^{c}	178 (36)^{a}	18.4 (1.3)^{b}	606 (90)^{bc}

b. Respiration rates and the ratio of maximum photosynthetic rate to respiration

Ramet	Light Level	R_a (μmol CO_2 m^{-2} s^{-1})	R_m (μmol CO_2 kg^{-1} s^{-1})	R_N (μmol CO_2 gN^{-1} s^{-1})	A/R
A. philoxeroides					
Connected	Full sun	0.28 (0.02)^{a}	7.5 (0.56)^{a}	0.14 (0.01)^{a}	112 (11)^{a}
	Shade	0.29 (0.05)^{a}	10.5 (1.6)^{a}	0.14 (0.02)^{a}	72 (16)^{b}
Severed	Full sun	0.30 (0.03)^{a}	8.1 (0.4)^{a}	0.13 (0.01)^{a}	101 (9)^{ab}
	Shade	0.28 (0.03)^{a}	10.7 (1.2)^{a}	0.17 (0.02)^{a}	76 (17)^{b}
P. canescens					
Connected	Full sun	0.57 (0.14)^{a}	12.9 (3.5)^{a}	0.28 (0.08)^{a}	85 (20)^{a}
	Shade	0.64 (0.17)^{a}	14.6 (1.9)^{a}	0.23 (0.03)^{a}	37 (11)^{b}
Severed	Full sun	0.60 (0.03)^{a}	15.2 (1.3)^{a}	0.27 (0.02)^{a}	66 (5)^{ab}
	Shade	0.52 (0.10)^{a}	16.5 (1.8)^{a}	0.32 (0.04)^{a}	44 (9)^{ab}

c. Relative chlorophyll content and leaf mass per unit area

Ramet	Light Level	RCC	RCC_m	LMA (g m^{-2})
A. philoxeroides				
Connected	Full sun	61.6 (3.0)^{a}	1.54(0.08)^{bc}	40.3 (2.0)^{ab}
	Shade	57.5 (1.0)^{a}	1.96(0.03)^{a}	29.4 (0.9)^{bc}
Severed	Full sun	59.2 (1.0)^{a}	1.45(0.10)^{c}	41.6 (2.8)^{a}
	Shade	50.9 (1.6)^{a}	1.83(0.05)^{ab}	27.8 (0.9)^{c}
P. canescens				
Connected	Full sun	51.0 (0.6)^{a}	1.03(0.07)^{a}	50.3 (2.9)^{a}
	Shade	49.2 (1.2)^{a}	1.28(0.04)^{a}	38.9 (2.0)^{a}
Severed	Full sun	48.4 (1.7)^{a}	1.11(0.07)^{a}	44.6 (3.7)^{a}
	Shade	42.2 (3.5)^{a}	1.29(0.11)^{a}	35.0 (6.4)^{a}

Table 3. Correlations between gas exchange parameters (V_{cmax}, J_{max}, A_{a} at 25 ºC, R_{a} at 20 ºC) and leaf properties (RCC, N_{a}, N_{m}, LMA) of *Alternanthera philoxeroides* and *Phyla canescens*. Values of correlation efficient (r) and P-values are shown. Abbreviations follow those listed in Table 1.

Species/ daughters	Gas exchange parameters	RCC		RCC$_{\mathrm{m}}$		N_{a} (g m^{-2})		N_{m} (%)		LMA (g biomass m^{-2})	
		r	P	r	P	r	P	r	P	r	P
A. philoxeroides											
Connected	V_{cmax} (μmol CO$_2$ m^{-2} s^{-1})	0.20	0.55	-0.77	**0.004**	0.24	0.82	-0.73	**0.006**	0.73	**0.007**
	J_{max} (μmol CO$_2$ m^{-2} s^{-1})	0.37	0.24	-0.45	0.14	0.35	0.28	-0.37	0.24	0.59	**0.04**
	A_{a} (μmol CO$_2$ m^{-2} s^{-1})	0.37	0.23	-0.68	**0.02**	0.28	0.37	-0.63	**0.03**	0.77	**0.004**
	R_{a} (μmol CO$_2$ m^{-2} s^{-1})	--	--	--	--	0.10	0.74	-0.00	0.91	0.00	0.86
Severed	V_{cmax} (μmol CO$_2$ m^{-2} s^{-1})	0.41	0.18	-0.85	**<0.001**	0.75	**0.005**	-0.66	**0.02**	0.86	**<0.001**
	J_{max} (μmol CO$_2$ m^{-2} s^{-1})	0.62	**0.03**	-0.85	**<0.001**	0.90	**<0.001**	-0.41	0.19	0.92	**<0.001**
	A_{a} (μmol CO$_2$ m^{-2} s^{-1})	0.57	**0.05**	-0.83	**<0.001**	0.84	**<0.001**	-0.54	0.07	0.90	**<0.001**
	R_{a} (μmol CO$_2$ m^{-2} s^{-1})	--	--	--	--	0.44	0.15	-0.50	0.10	0.52	0.08
P. canescens											
Connected	V_{cmax} (μmol CO$_2$ m^{-2} s^{-1})	0.55	0.06	-0.64	**0.02**	0.00	0.99	-0.69	**0.01**	0.71	**0.009**
	J_{max} (μmol CO$_2$ m^{-2} s^{-1})	0.46	0.13	-0.81	**0.001**	0.14	0.66	-0.68	**0.02**	0.85	**0.005**
	A_{a} (μmol CO$_2$ m^{-2} s^{-1})	0.53	0.08	-0.74	**0.006**	0.00	0.97	-0.75	**0.005**	0.78	**0.003**
	R_{a} (μmol CO$_2$ m^{-2} s^{-1})	--	--			0.35	0.27	0.10	0.72	0.14	0.64
Severed	V_{cmax} (μmol CO$_2$ m^{-2} s^{-1})	0.56	0.07	-0.50	0.10	0.84	**0.001**	0.17	0.60	0.83	**0.001**
	J_{max} (μmol CO$_2$ m^{-2} s^{-1})	0.77	**0.003**	-0.71	**0.01**	0.85	**<0.001**	-0.17	0.60	0.89	**<0.001**
	A_{a} (μmol CO$_2$ m^{-2} s^{-1})	0.76	**0.006**	-0.41	0.18	0.92	**<0.001**	0.30	0.35	0.89	**<0.001**
	R_{a} (μmol CO$_2$ m^{-2} s^{-1})	--	--	--	--	0.62	**0.03**	-0.36	0.25	0.71	**0.01**

Figure Legends

Figure 1. Schematic representation of the experimental design. Each experimental unit consists of one mother plant, two severed daughter ramets and two connected daughter ramets (a). Ramets were subjected to the light treatments of full sun or 85% shade. The mother plant (including all stolons in the pot and unused branches) was exposed to full sun. The mother plant (b), and the set up of daughter ramets (connected or severed, c) are shown for *Alternanthera philoxeroides*.

Figure 2. Light responses for leaf nitrogen content on area (N_a; a, b) and mass (N_m; c, d) basis, photosynthetic nitrogen use efficiency (PNUE; e, f), chlorophyll to nitrogen ratio (RCC/N_a; g, h) and nitrogen stable isotope ratio relative to ambient N_2 ($\delta^{15}N$; i, j) of *Alternanthera philoxeroides* (left panel) and *Phyla canescens* (right panel) daughter ramets (filled circle, connected; open circle, severed). Values are means (±SE), n=6. Means were compared with Bonferroni tests and values followed by the same letter are not significantly different at $P = 0.05$ level. Abbreviations follow those listed in Table 1.

Figure 3. Total biomass of daughter ramets (severed or connected with the mother plant) of a) *Alternanthera philoxeroides* and b) *Phyla canescens*. Values are means (+SE), n=6. *P*-values of ANOVA results, based on square root-transformed data, are shown for effects of plant, connection, light and connection × light interactions. Means were compared with Bonferroni tests and values followed by the same letter are not significantly different at $P = 0.05$ level.

Figure 4. Relationships between maximum photosynthetic rate (A_{max}) and total biomass (square root transformed) for daughter ramets of *Alternanthera philoxeroides* (a) and *Phyla canescens* (b). Regression lines (solid line, connected ramets; dashed line, severed ramets), slope (±SE), r^2 and *P*-values are shown.

Figure 5. Leaf non-structural carbohydrates of *Alternanthera philoxeroides* (left panel) and *Phyla canescens* (right panel) daughter ramets, including soluble sugar and starch. Values are means (+SE) for starch and total non-structural carbohydrate, and means (-SE) for soluble sugar, n=6. The error bars of total non-structural carbohydrates (TNC, the sum of soluble sugar and starch) are marked in bold lines. Means were compared with Bonferroni tests and values followed by the same letter (soluble sugar: normal, starch: *italic*, total non-structural carbohydrates: **bold**) are not significantly different at *P* =0.05.

Figure 6. The concentration (a) and total amount (b) of assimilated ^{13}C label in leaves of ramets connected with a mother plant. Values are means (+SE), n=6. *P*-values of ANOVA results, based on square root-transformed data, are shown for effects of covariant (δ^{13}C values of mother shoot tissue) and light.

Figure 1.

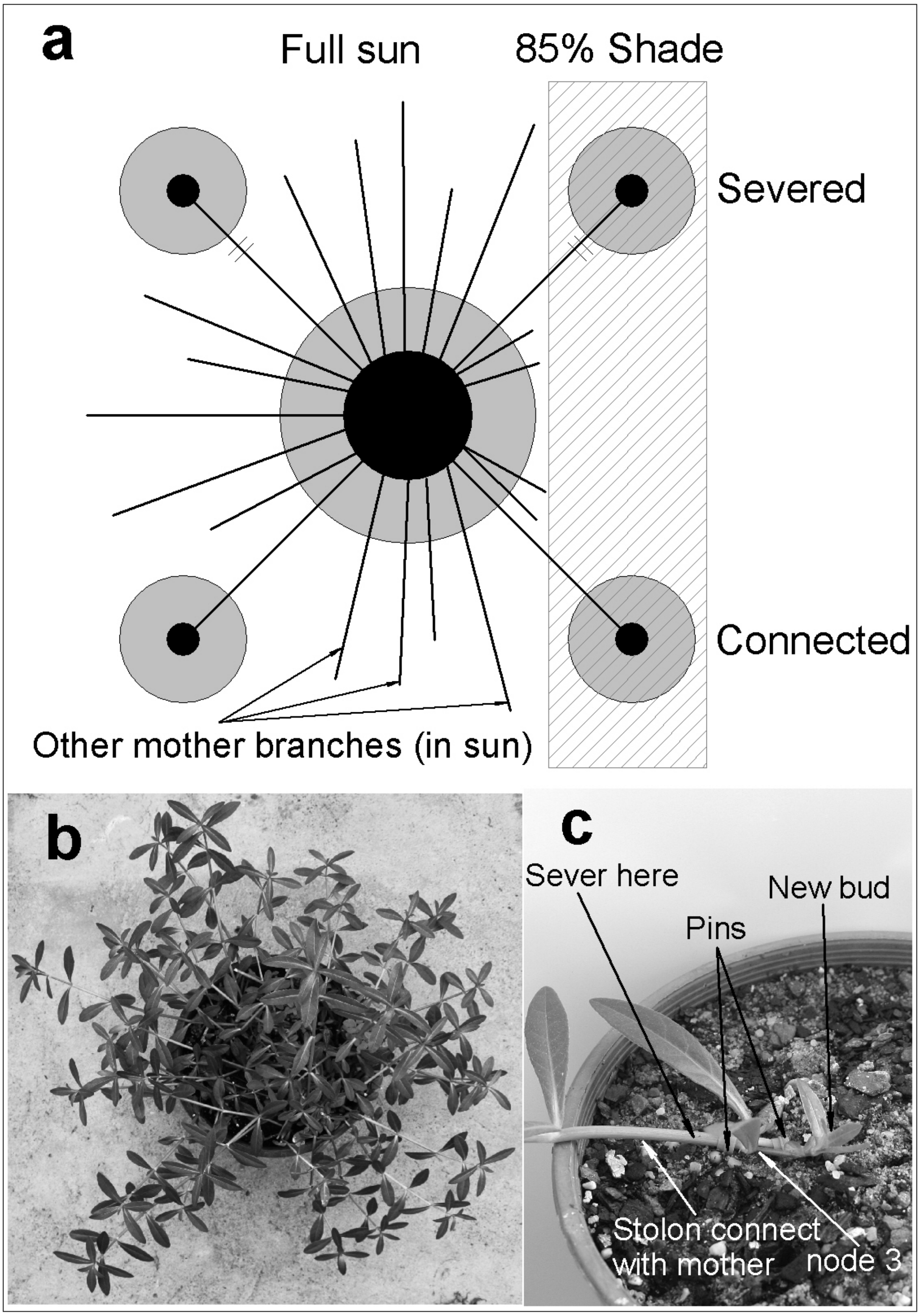


Figure 2.

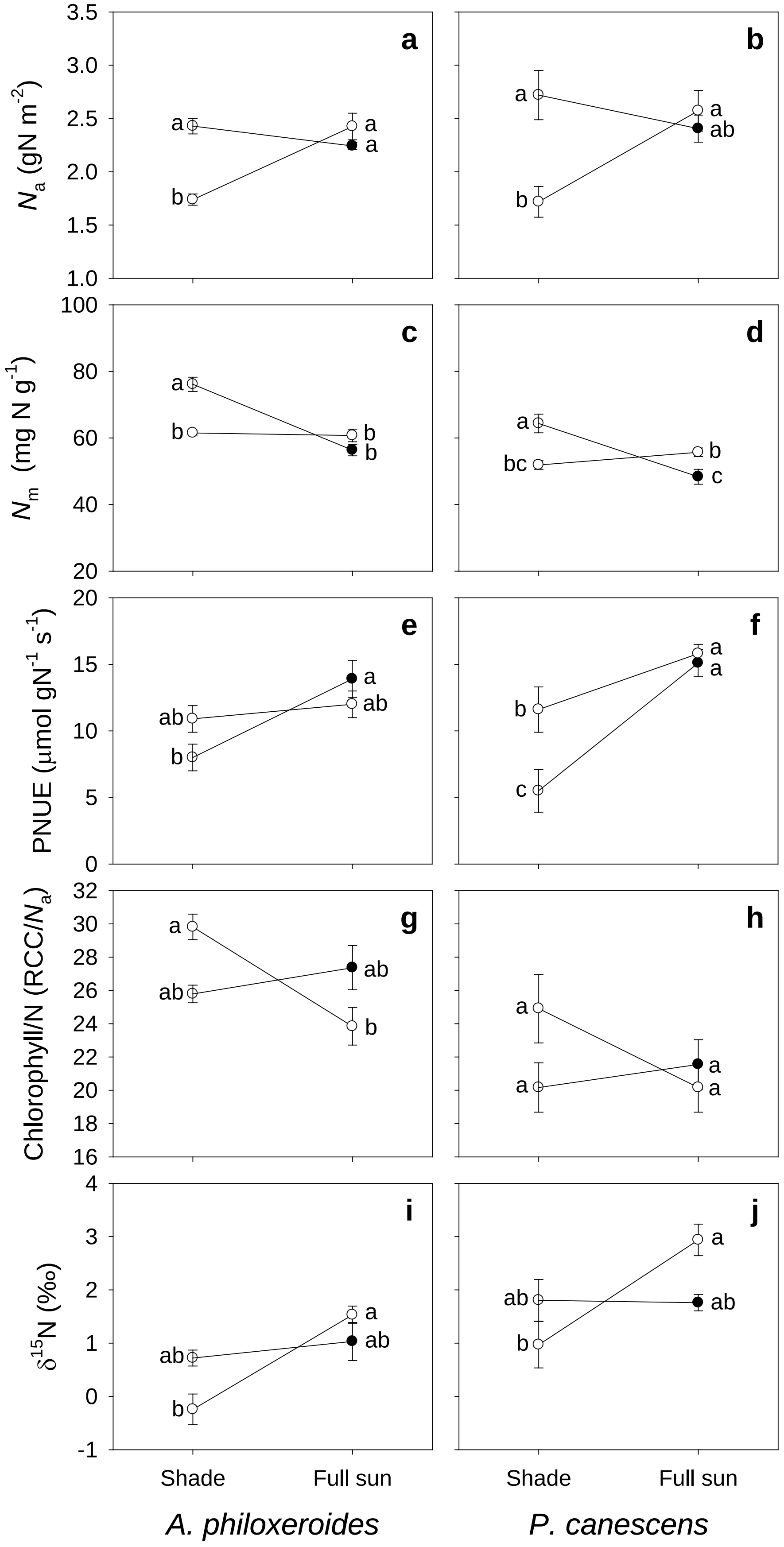

a
b
c
d
e
f
g
h
i
j
N_a (gN m^{-2})
N_m (mg N g^{-1})
PNUE (μmol gN^{-1} s^{-1})
Chlorophyll/N (RCC/N_a)
δ^{15}N (‰)
Shade
Full sun
Shade
Full sun
A. philoxeroides
P. canescens

Figure 3.

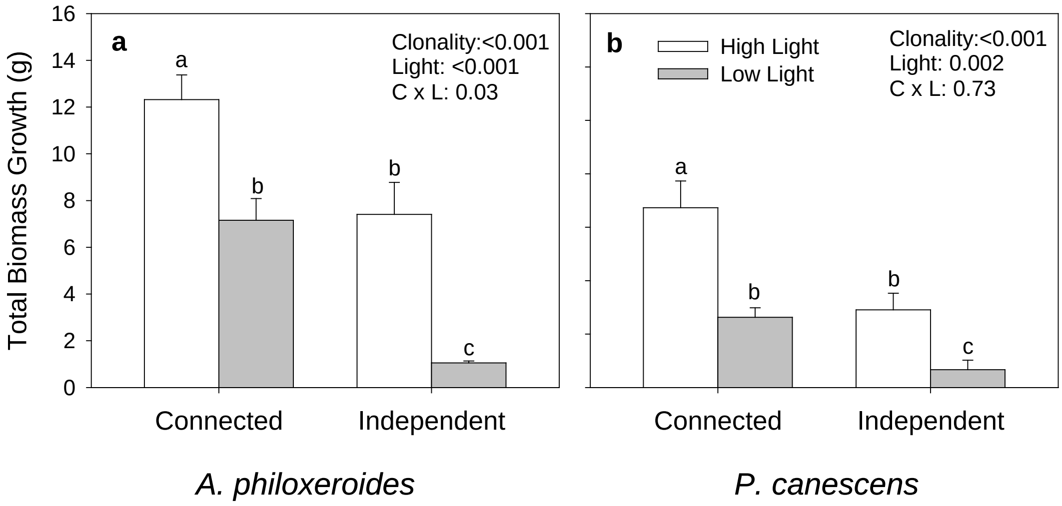

Total Biomass Growth (g)
0
2
4
6
8
10
12
14
16
a
Clonality:<0.001
Light: <0.001
C x L: 0.03
a
b
b
c
Connected
Independent
A. philoxeroides
b
High Light
Low Light
Clonality:<0.001
Light: 0.002
C x L: 0.73
a
b
b
c
Connected
Independent
P. canescens

Figure 4.

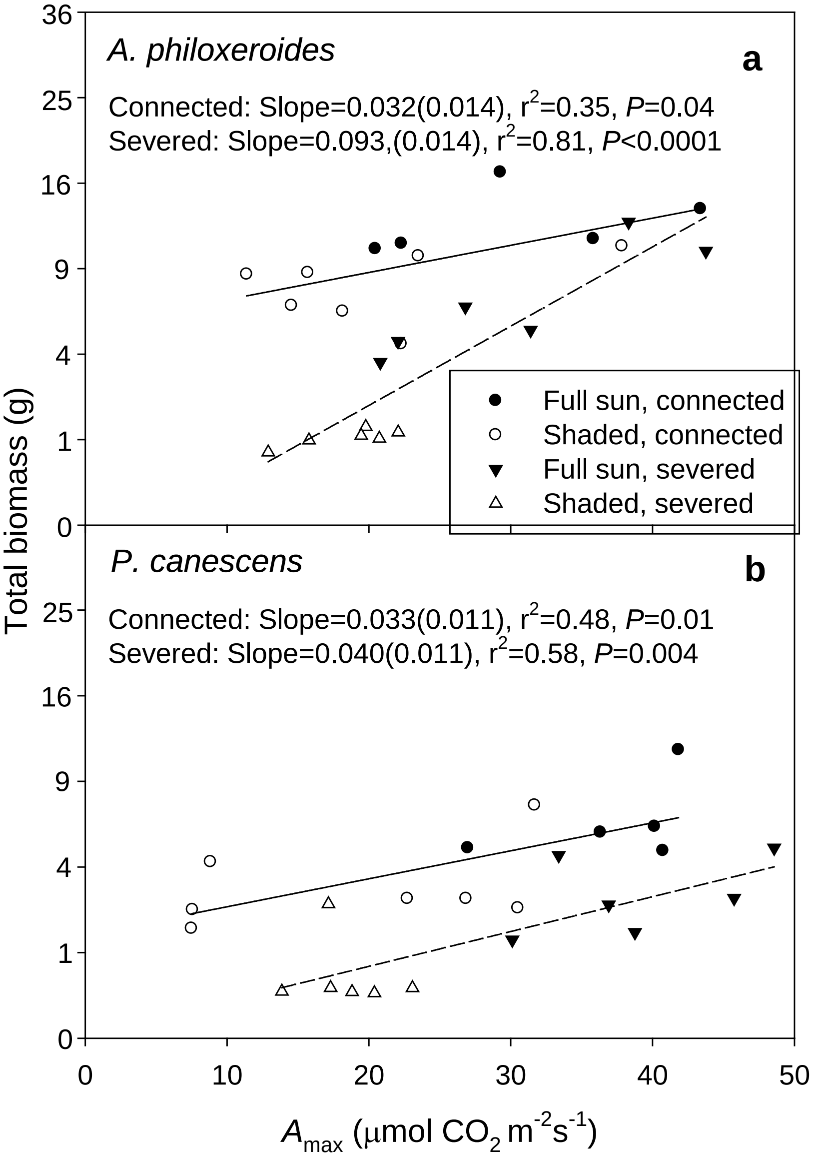

A. philoxeroides
a
Connected: Slope=0.032(0.014), r2=0.35, P=0.04
Severed: Slope=0.093,(0.014), r2=0.81, P<0.0001
● Full sun, connected
○ Shaded, connected
▼ Full sun, severed
△ Shaded, severed
P. canescens
b
Connected: Slope=0.033(0.011), r2=0.48, P=0.01
Severed: Slope=0.040(0.011), r2=0.58, P=0.004
Total biomass (g)
36
25
16
9
4
1
0
0
10
20
30
40
50
Amax (μmol CO2 m-2s-1)

Figure 5.

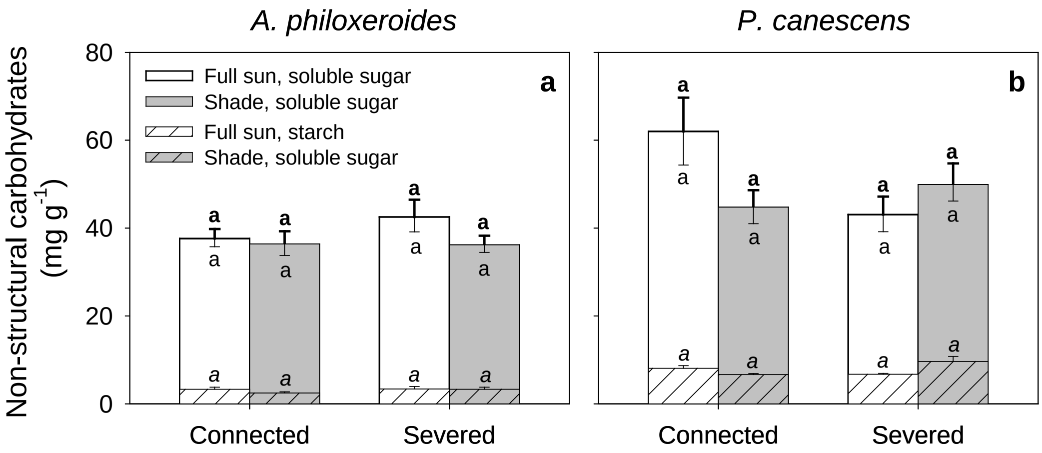
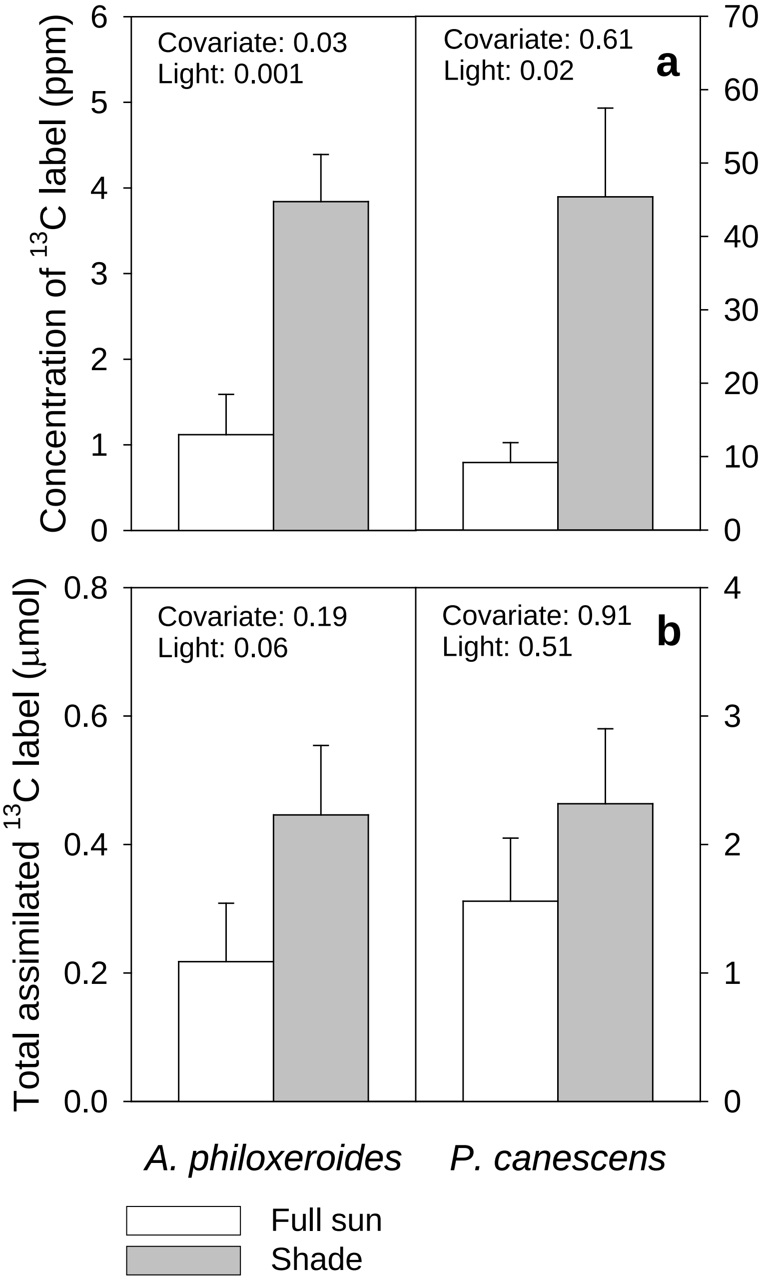


Figure 6.


Concentration of 13C label (ppm)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
Covariate: 0.03
Light: 0.001
Covariate: 0.61
Light: 0.02
a
Total assimilated 13C label (μmol)
0.0
0.2
0.4
0.6
0.8
0
1
2
3
4
Covariate: 0.19
Light: 0.06
Covariate: 0.91
Light: 0.51
b
A. philoxeroides
P. canescens
Full sun
Shade