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The effect of altered sink-source relations on photosynthetic traits and matter transport during the phase of reproductive growth in the annual herb *Chenopodium album*

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Abstract

Annual plants transport a large portion of carbohydrates and nitrogenous compounds from leaves to seeds during the phase of reproductive growth. This study aimed to clarify how reproductive growth affects photosynthetic traits in leaves and matter transport within the plant in the annual herb *Chenopodium album* L. Plants were grown in pots and either reproductive tissues or axillary leaves were removed at anthesis. Matter transport was evaluated as temporal changes in dry mass (as a substitute of carbohydrates) and nitrogen content of aboveground organs: leaves, axillary leaves, stems and reproductive tissues. Photosynthetic capacity (light-saturated photosynthetic rate under ambient CO_2 concentration), nitrogen, chlorophyll and soluble protein content were followed in the 20th leaf that was mature at the start of the experiment. Removal of reproductive tissues resulted in accumulation of dry mass in leaves and axillary leaves, and accumulation of nitrogen in stem as nitrogen resorption from leaves and axillary leaves proceeded with time. Removal of axillary leaves proportionally reduced dry mass and nitrogen allocation to reproductive tissues, thus affecting the quantity but not quality of seeds. Removal treatments did not alter the time course of photosynthetic capacity, nitrogen, chlorophyll or soluble protein content during senescence in the 20th leaf, but changed the photosynthetic capacity per unit of leaf nitrogen according to demand from reproductive tissues. Together, the results indicate that reproductive tissues affected carbon and nitrogen economy separately. The amount of carbon was adjusted in leaves through photosynthetic capacity and carbohydrate export from them, and the amount of nitrogen was adjusted by transport from stem to reproductive tissues. The plant's ability to independently regulate carbon and nitrogen economy should be important in natural habitats where the plant carbon-nitrogen balance can easily be disturbed by external factors.

Additional key words: leaf senescence, nitrogen resorption, photosynthetic nitrogen use efficiency, soluble protein.

Introduction

The life of annual plants consists of two main phases (Harper 1977). Firstly annual plants go through the phase of vegetative growth, in which they establish vegetative structures. Then they switch into the phase of reproductive growth, decelerating vegetative growth and developing reproductive organs. In this phase, reproductive tissues become the largest sink for resources such as carbohydrates and nitrogen (N). A large part of carbohydrate is provided by concurrent photosynthesis in a leaf, and N is mobilised from vegetative organs with the leaf being the major source (Millard 1988, Wardlaw 1990, Salon *et al.* 2001). Many species including *Chenopodium album* (Yasumura *et al.* 2007) become less dependent on N uptake from the soil than before (Tibodeau and Jaworski 1975, Malagoli *et al.* 2005).

During the phase of reproductive growth, leaves undergo senescing processes that gradually reduce their photosynthetic capacity, degrade proteins and other N compounds, and export N mainly in the form of amino acids (Stoddart and Thomas 1982, Smart 1994, Hörtensteiner and Feller 2002). Leaf senescence therefore determines the amount of carbohydrates and N made available in the leaf. Former studies have shown that reproductive growth can affect the rate of leaf senescence

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Abbreviations: Chl – chlorophyll; g_s – stomatal conductance to water vapour; LMA – leaf mass per leaf area; N_{area} – leaf nitrogen content per leaf area; P_{max} – light-saturated photosynthetic rate.

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in many annual species. For example, the rate of decline in photosynthetic capacity and N content (N_{area}) of the leaf was altered by removal of reproductive tissues in cowpea (Khanna-Chopra and Reddy 1988), sunflower (Sadras *et al.* 2000), rice (Nakano *et al.* 1995), and in maize (Christensen *et al.* 1981, Sadras *et al.* 2000) and by the partial defoliation that reduced the ratio of reproductive tissues to leaves in wheat (Guitman *et al.* 1991). As the majority of leaf N is associated with the photosynthetic apparatus (Evans and Seemann 1989), changes in photosynthetic capacity are likely to be associated with those in leaf N_{area}.

Carbohydrates and N are transported as solutes in the phloem from leaves to reproductive tissues (Winter *et al.* 1992, Lohaus *et al.* 1995). The transport of N, which is relatively small in amount, is accompanied by the bulk transport of carbohydrates. Reproductive growth can affect matter transport in the plant. For example, physical restriction of pod growth depressed the transport of starch to the reproductive tissues in soybean (Miceli *et al.* 1995).

Plants must regulate photosynthesis and the transport of carbohydrates to maximize reproductive yield on one hand, and N resorption and transport to ensure quality of seeds on the other one. However, due to their relatedness, plants may not be able to regulate carbon and N metabolism independently of each other. In this study, I examined how closely carbon and N economy is related with each other in the annual plant *Chenopodium album*, during the phase of reproductive growth. I removed either reproductive tissues or axillary leaves from plants at anthesis, and followed temporal changes in leaf properties and allocation of dry mass (as a substitute of carbohydrates) and N among different aboveground organs until the end of the reproductive phase. In particular, I focused on how excision treatments affect (*1*) light-saturated photosynthetic rate (photosynthetic capacity) and content of nitrogenous compounds in senescing leaves, (*2*) export of carbohydrates and N from leaves to stem, and (*3*) transport through stem to reproductive tissues.

Materials and methods

Plants and growth conditions: *Chenopodium album* L. is an annual herb commonly found in wasteland. This species develops small axillary leaves at the base of a "normal" leaf (hereafter simply referred to as leaf). I sowed seeds of *C. album* in 1.4 l pots filled with washed river sand on 1st July 2005, and grew the plants under 90 % of full sunlight in a greenhouse. I applied 50 ml of diluted Hyponex solution (20 mM N, mass of N:P:K= 6:10:5; *Hyponex Japan Corp., Ltd*) once a week from 11th July until anthesis on 30th August, and thereafter only tap water until the end of the season. Shortly before anthesis, I measured the shoot height (h) and stem diameter at the ground (d) and divided the plants into five different size groups according to their d^2h values. Within the same group, the plants were randomly allotted to one of the following treatments on 12th–14th September: control in which the plants were untouched; R-treatment in which reproductive buds were fully removed; A-treatment in which axillary leaves were fully removed. Thereafter, all the plants were grown with no additional excision treatments.

Measurements of leaf traits during senescence: I examined senescing processes in the 20th leaf from the ground, which reached full expansion around anthesis. Photosynthetic rates (P_{max}; $n = 5$) were measured weekly from 2nd September to 7th October with a portable photosynthesis system (*LI-6400; LI-COR Inc.*, Lincoln, Nebraska, USA) under saturating photon flux density (2000 μmol m^{-2} s^{-1}), ambient CO_2 concentration (370 ppm) and at average day temperatures of the preceding week. Stomatal conductance to water vapour (g_s) was recorded simultaneously. After the P_{max} measurements, the leaves were sampled and carried in damp plastic bags to the laboratory where leaf discs (0.8 cm in diameter) were punched out. Chlorophyll (Chl) was extracted from a fresh disc in dimethylformamide, and its concentration was determined spectrophotometrically (Porra *et al.* 1989). Leaf mass per area (LMA) was determined with several discs that had been dried at 70 °C for more than 72 hours. Leaf N concentration was determined on the same dry discs with an NC analyzer (*Sumigraph NC-80, Sumika Chemical Analysis Service Ltd.*, Tokyo, Japan) connected to a gas chromatograph (*GC-8A, Shimadzu Ltd.*, Kyoto, Japan). Fully-senescent 20th leaves were also collected when they were abscised from the plant, and their LMA, Chl and N concentration were determined as described above. Photosynthetic capacity of these fully-senescent leaves was not determined.

Soluble protein content was determined in frozen leaf discs stored at –80 °C. One or two leaf discs were powdered in liquid nitrogen in a mortar with a pestle, and homogenized in 100 mM Bicine-NaOH buffer (pH 8.0) containing 20 mM $MgCl_2$, 10 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 15000 × g for 20 min. Soluble protein in the supernatant was precipitated with trichloroacetic acid (10 % w/v) and recovered as the sediment after centrifugation at 15000 × g for 20 minutes. Soluble protein was re-suspended in 0.25 M NaOH, and its concentration was determined by Lowry's method (Lowry *et al.* 1951).

Destructive harvesting of the aboveground parts: The aboveground parts of the plants were harvested ($n = 5$) in the morning following the P_{max} measurements, and separated into four different organs: leaf, axillary leaf, stem, and reproductive tissues. Total leaf- and axillary-leaf area of each plant was determined with a leaf area meter (*LI-3000; LI-COR Inc.,* Lincoln, Nebraska, USA). Samples were dried at 70 °C for more than 72 h, weighed, ground, and N concentration was determined as described above.

Statistical analysis: Statistical tests were performed with *StatView software version 5.0* (*SAS Institute, Inc.*, Cary, NC, USA). Regression lines were obtained using the least-squares methods, and differences between the control and each treatment were analysed with *ANOVA*. The differences were regarded as being significant at $p<0.05$.

Results

Changes in dry mass of different organs: Total aboveground dry mass changed drastically with time (Fig. 1*A*). In the control plants, it increased during the early part of the reproductive phase, and decreased at the final stage of this phase. After the excision treatments, the dry mass of the R-plants increased to the level of that of the control plants, whereas the A-plants had consistently lower dry mass than the control plants.

Total leaf mass and area decreased steadily as leaves abscised one by one on the plant (Fig. 1*B*, 2*A*). The R-plants had apparently larger leaf dry mass than the control plants, but their leaf area was similar to that of the control plants. The A-plants had a similar leaf mass and area as the control plants. Axillary leaves remained on the plant longer than ordinary leaves, and their mass and area decreased mainly at the final stage (Fig. 1*C*, 2*B*). Again, total dry mass of axillary leaves increased markedly after the removal of reproductive tissues without a concomitant increase in area. In the A-plants, no new axillary leaves were produced after the excision treatment. Stem dry mass increased during the first few weeks and then became relatively stable (Fig. 1*D*). Excision treatments affected stem dry mass only slightly. Dry mass of reproductive tissues increased vigorously with time (Fig. 1*E*). In the R-plants, new reproductive buds were formed after the excision treatment, and they turned into flowers and seeds. Total mass of reproductive tissues was largest in the control plants, intermediate in the A-plants, and the lowest in the R-plants.

Changes in nitrogen content and concentration in different organs: Total aboveground N content was relatively stable during the reproductive phase (Fig. 3*A*), and was reduced by the excision treatments in both the R- and A-plants. Nitrogen concentration in the aboveground parts declined at first, and then became stabilized in the middle of the reproductive phase (Fig. 4*A*).

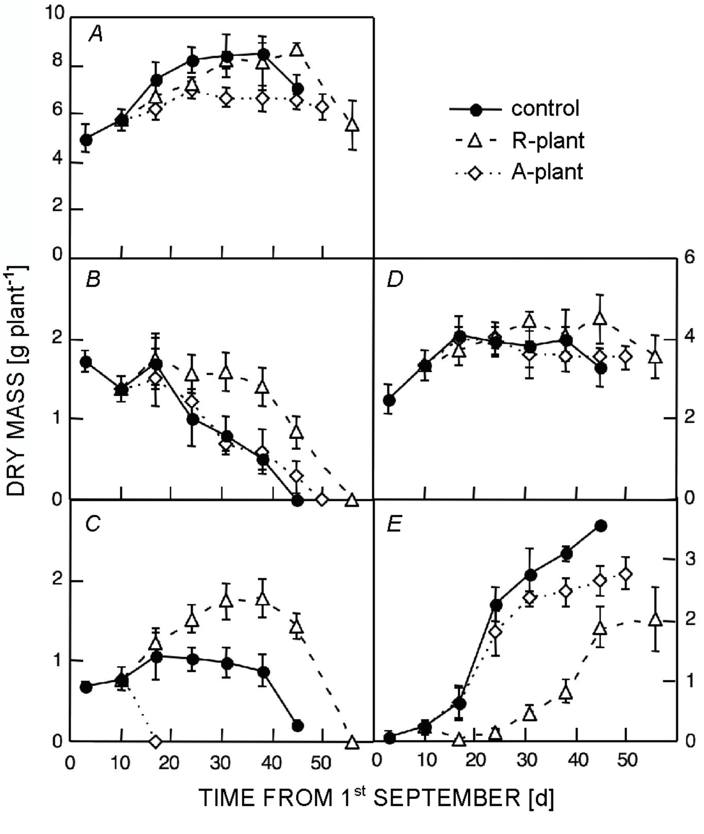

Fig. 1. Temporal changes in dry mass of *A*: total aboveground part, *B*: leaf, *C*: axillary leaf, *D*: stem, and *E*: reproductive tissues. Means±SD (n = 5). C-plant: control plant, R-plant: plant of which reproductive tissues were removed on 12–14 September, A-plant: plant of which axillary leaves were removed on 12–14 September.

The total N content in the leaves, axillary leaves, and stems continued to decrease with time, with a slower rate of decline in the R-plants than in the control plants (Fig. 3*B-D*). The total N content in reproductive tissues, on the other hand, increased continuously until the end of the reproductive phase (Fig. 3*E*). Both the R- and A-plants had a lower N content in reproductive tissues than the control plants. Nitrogen concentration in all aboveground organs declined with time (Fig. 4*B-E*). Nitrogen concentration in the leaves tended to be higher in the A-plants and lower in the R-plants than in the control plants, while N concentrations in the stems and reproductive parts were higher in the R-plants, and similar between the control and A-plants.

The rate of leaf senescence: Light-saturated photosynthetic rates, stomatal conductance (g_s), N_{area}, Chl and soluble protein content of the 20th leaf continued to decrease throughout the phase of reproductive growth, and LMA declined moderately after an initial increase (Fig. 5). The excision treatments did not notably affect the decline rate of P_{max}, N_{area}, Chl or soluble protein content, but increased the LMA in the R-plants. When fully senescent, the leaves had only a small amount of N and soluble protein left, and Chl had been degraded almost completely. Final N, Chl and soluble protein contents in dead leaves were similar between the excised and the control plants.

During leaf senescence, Chl and soluble protein contents were closely correlated with N_{area} (Fig. 6*A,B*). The slope and y-intercept of the Chl-N_{area} and soluble protein-N_{area} relationships were not significantly affected by removal of reproductive tissues (ANOVA, $p>0.05$), and only the slope of the Chl-N_{area} relationship differed significantly between the A- and control plants ($p=0.037$). Light-saturated photosynthetic rate was correlated with N_{area} (Fig. 6*C*), with a significantly larger and smaller y-intercept in the R- and A-plants, respectively, than in the control plants ($p<0.0001$ for R-plants, $p=0.005$ for A-plants). The slope of the P_{max}-N_{area} relationship did not differ between the excised and control plants ($p>0.05$).

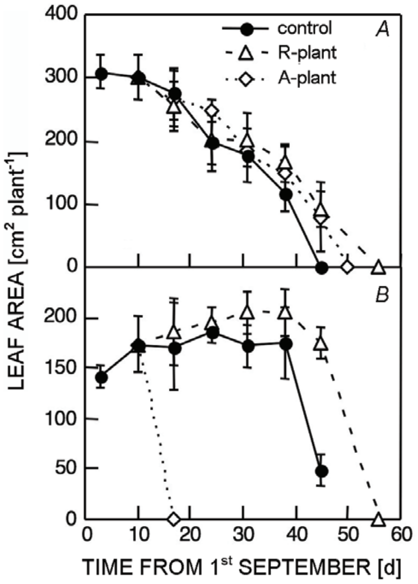

Fig. 2. Temporal changes in *A*: total leaf area and *B*: total axillary-leaf area. Means±SD (n = 5). Abbreviations are as in Fig. 1.

Discussion

Removal of reproductive tissues affected dry matter allocation in the plants. Total mass of the leaves and axillary leaves increased (Fig. 1*B,C*) without a marked increase in their area (Fig. 2). This indicates that the dry mass increase was caused by accumulation of carbohydrates rather than by development of new tissues. Therefore, it was likely that the removal of reproductive tissues suppressed the transport of carbohydrates out from the leaves or axillary leaves. There was a slight increase in stem dry mass in the R-plants (Fig. 1*D*). The stem can probably also accommodate a part of excess carbohydrates (Miceli *et al.* 1995). The removal of axillary leaves did not affect total leaf or stem dry mass (Fig. 1*B,D*), and only reduced the reproductive yield (Fig. 1*E*). Similarly, the amount of reproductive tissues decreased after the removal of leaves in wheat (Barneix and Guitman 1993, Guitman *et al.* 1991) and in barley (Dreccer *et al.* 1997). Thus, reproductive yield seems to be determined by the amount of photosynthetic tissues operating at the time of reproductive growth.

Annual plants remobilize N from various vegetative tissues for the development of reproductive tissues (Millard 1988). The decline in N content and concentration indicates that N was remobilized from the leaves, axillary leaves and stems in *C. album* (Fig. 3*B-D*, 4*B-D*). The total aboveground N content which was relatively stable, suggests that there was also N remobilization from the belowground parts that offset N loss through the abscission of leaves and axillary leaves. In previous studies, taproots were shown to be an important N source for reproductive growth in oilseed rape (Rossato *et al.* 2001) and in *Rumex acetosa* (Bausenwein *et al.* 2001).

Removal of reproductive tissues affected N content in the leaves and axillary leaves less strongly than their dry mass (Fig. 3*B,C*) and therefore moderately reduced their N concentration (Fig. 4*B,C*), indicating that N resorption from these organs proceeded in the absence of a strong sink, in contrast to carbohydrate export. In the R-plants, N accumulated in their stems (Fig. 3*D,* 4*D*), which probably act as a buffer when there is more internal N than is required for reproductive growth. Removal of

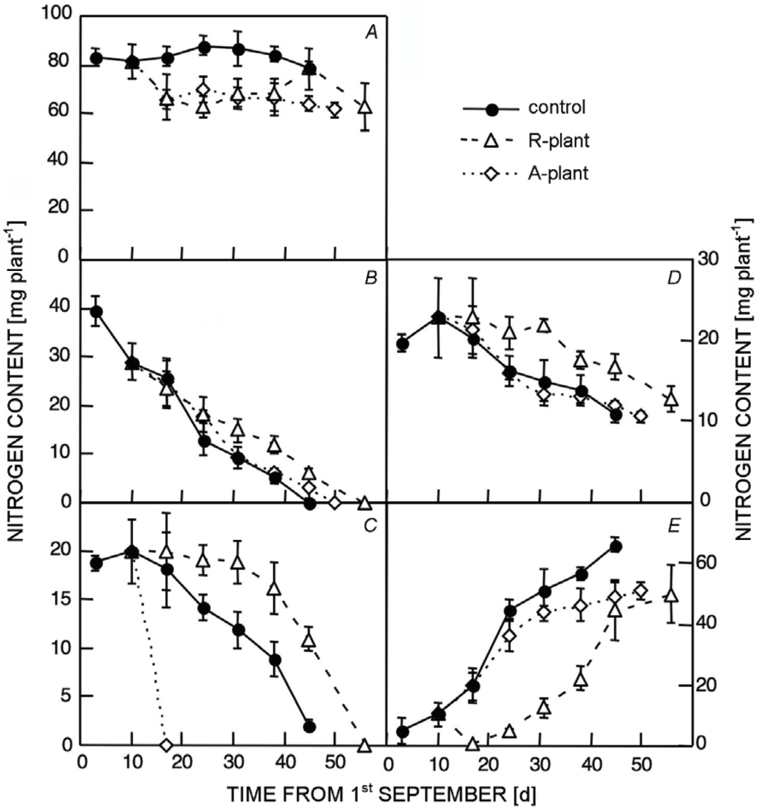

Fig. 3. Temporal changes in nitrogen content in *A*: total aboveground part, *B*: leaf, *C*: axillary leaf, *D*: stem, and *E*: reproductive tissues. Means±SD ($n = 5$). Abbreviations are as in Fig. 1.

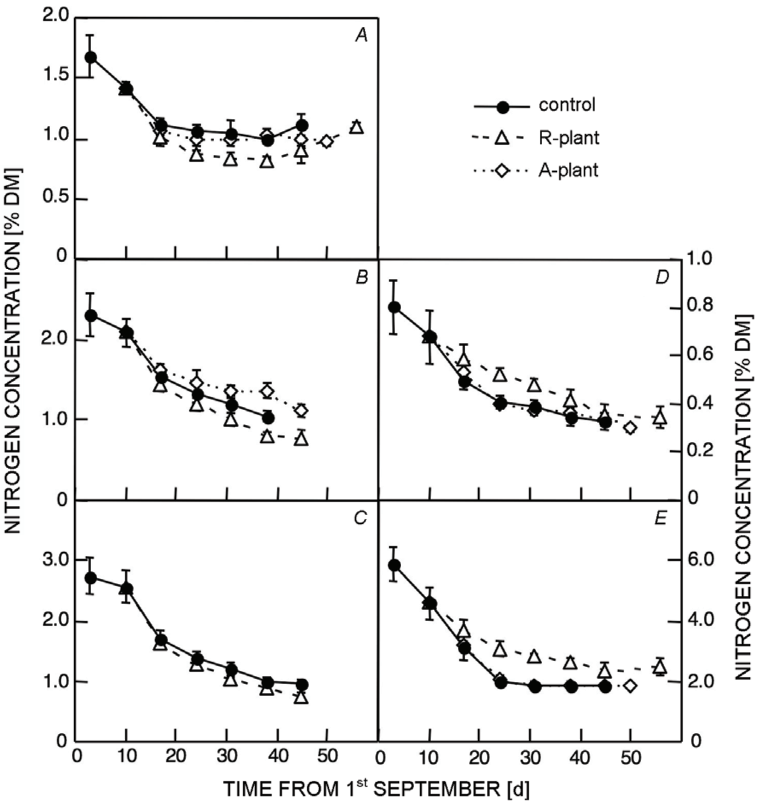

Fig. 4. Temporal changes in nitrogen concentration of *A*: aboveground parts, *B*: leaf, *C*: axillary leaf, *D*: stem and *E*: reproductive tissues. Means±SD ($n = 5$). Abbreviations are as in Fig. 1.

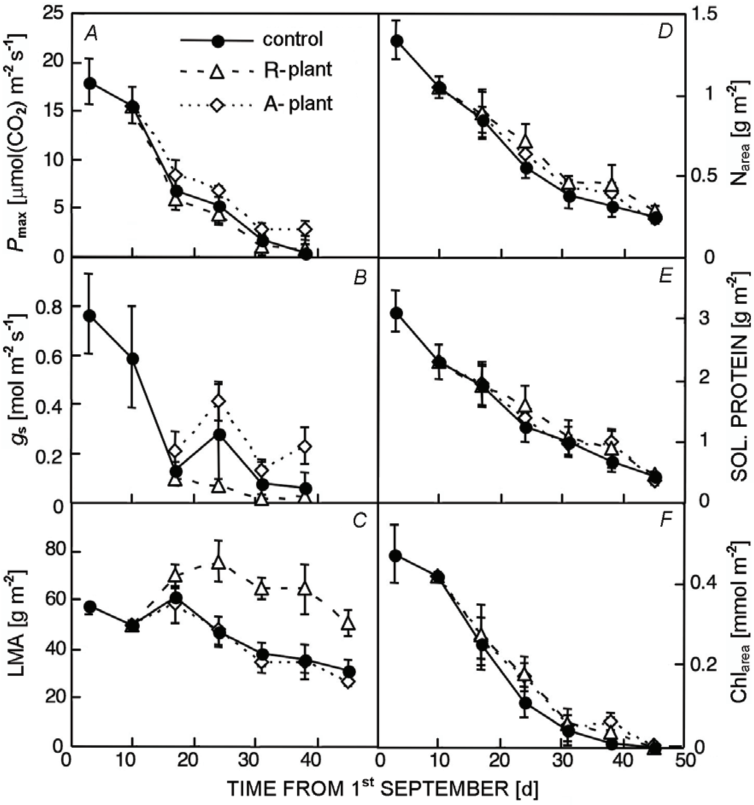

Fig. 5. Temporal changes in *A*: light-saturated photosynthetic rate (P_{max}), *B*: stomatal conductance (g_s), *C*: leaf mass per area (LMA), *D*: nitrogen content (N_{area}), *E*: soluble protein content, *F*: chlorophyll (Chl) content of the 20th leaf. Data on day 45 denote dead leaves. Means±SD (n = 5). Abbreviations are as in Fig. 1.

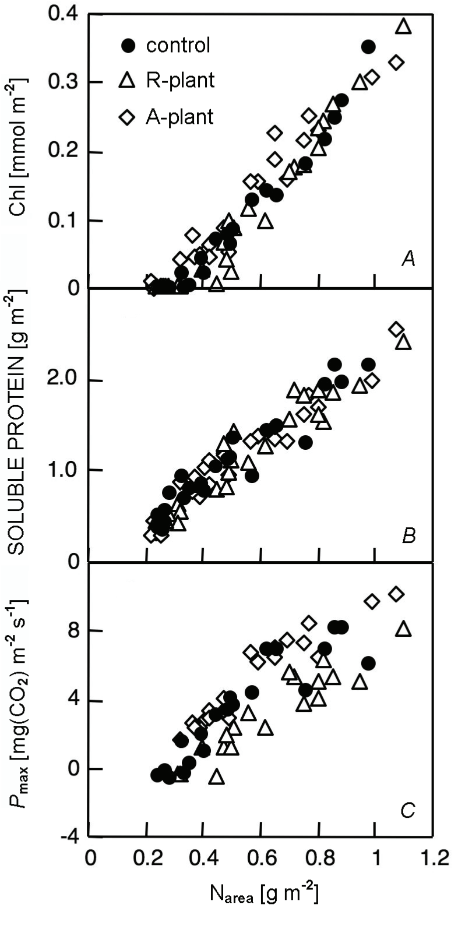

Fig. 6. *A*: Chlorophyll (Chl) content, *B*: soluble protein content, and *C*: light-saturated photosynthetic rate (P_{max}) as functions of leaf nitrogen content (N_{area}) in the 20th leaf. Abbreviations are as in Fig. 1. *See* text for *ANOVA* results for differences in regression lines between treated and control plants.

axillary leaves did not affect N partitioning between the leaf and stem (Fig. 3*B,D*), but reduced N content in reproductive tissues (Fig. 3*E*). N concentration in reproductive tissues did not change after this treatment, implying that the decline in dry mass was proportional to that in N. Annual plants may ensure a certain N concentration in their seeds by regulating the amount of carbohydrate import into reproductive tissues, especially when N is deficient. In *Xanthium canadense*, elevated CO_2 concentration did not reduce the seed N concentration even though it increased photosynthesis in the leaves (Kinugasa *et al.* 2003).

Removal of reproductive tissues or axillary leaves caused only small changes in the decline rate of P_{max} and N_{area} in the 20th leaf (Fig. 5*A,D*). While many annual species either accelerate or delay leaf senescence (Christensen *et al.* 1981, Khanna-Chopra and Reddy 1988, Nakano *et al.* 1995, Sadras *et al.* 2000), some species seem to be relatively insensitive to alternation of sink-source relations (*e.g.* barley, Dreccer *et al.* 1997). Leaf age may also have affected the fate of the 20th leaf in this study, because the reversibility of leaf senescence disappears with leaf age (Smart 1994). It has been reported that the rate of leaf senescence was either delayed or accelerated in response to N deficiency depending on leaf age in oilseed rape (Etienne *et al.* 2007).

Chl and soluble protein represent the light-harvesting and light-utilizing components of the photosynthetic apparatus in the leaf, respectively (Evans and Seemann 1989). The decline rate of Chl or soluble protein content in the 20th leaf was unaffected by removal of reproductive tissues or axillary leaves (Fig. 5*E,F*), similarly to N_{area} (Fig. 5*D*). The final N_{area}, Chl and soluble protein contents were also similar among different treatments. Therefore, degradation of N compounds and N resorption from the 20th leaf proceeded irrespective of the extent of reproductive growth. It has been suggested that N resorption from senescing leaves can proceed independently of the sink-source relations (Dreccer *et al.* 1997). Also, the extent of N resorption seems to be insensitive to changes in the sink-source relations except at an extreme condition (Yasumura *et al.* 2007).

Although P_{max} or N_{area} alone was affected only minutely, the P_{max}-N_{area} relationships (photosynthetic nitrogen-use efficiency) were changed significantly by the excision treatments (Fig. 6*C*). Removal of reproductive tissues reduced P_{max} per N_{area}, while the removal of axillary leaves increased P_{max} per N_{area} compared with the control plants. Therefore, photosynthesis could be either depressed or enhanced with the same N_{area}, in response to carbon demand. In a former study, changes in photosynthetic capacity were associated with changes in the amount of photosynthetic components such as cyt *f* in rice (Nakano *et al.* 1995). In this study, however, Chl or soluble protein contents per N_{area} were similar among the treatments (Fig. 6*A,B*) and thus at least these two components cannot account for changes in the P_{max}-N_{area} relationships. Possibly, photosynthesis was affected by feedback inhibition (Paul and Foyer 2001) that occurs as leaf sugar content or leaf C:N ratio increases (Paul and Driscoll 1997, Pourtau *et al.* 2006, Wingler *et al.* 2006). The R-plants had a remarkably high LMA, which suggests massive accumulation of carbohydrates.

In conclusion, the amount of carbohydrate and N import into reproductive tissues could be regulated quite independently from each other; carbon economy was adjusted mainly in the photosynthetic organs (photosynthesis and export of carbohydrates from the leaf and axillary leaf), and N economy was adjusted mainly in the stem (N transport therein). Alternation of the sink-source relations did not conspicuously affect the photosynthetic capacity or the rate of degradation and resorption of nitrogenous compounds in senescing leaves, but modified the photosynthesis-leaf N relationships according to carbon demand for reproductive growth.

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