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The contribution of drought-related decreases in foliar nitrogen concentration to decreases in photosynthetic capacity during and after drought in prairie grasses

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While stomatal closure usually limits photosynthesis during drought, our previous results suggest that drought-related decreases in foliar nitrogen concentration (N_L) limit photosynthesis during recovery from drought in prairie grasses. Here we estimate the importance of decreases in N_L to decreased photosynthetic capacity (PS_{cap}) during drought and a subsequent recovery period in three perennial C_4 prairie grasses. PS_{cap} (O_2 evolution at light and CO_2 saturation) decreased 69 to 78% during drought in these grasses, and full recovery of PS_{cap} required 8 to 12 days, until younger leaves were expanded or older leaves were repaired, depending on species. Decreases in N_L explained 38 to 51% of the loss of PS_{cap} during drought and accounted for 51 to 69% of the total loss of PS_{cap} integrated over the post-drought recovery period. N-related loss of PS_{cap} appeared to result more from decreases in ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39), phosphoenolpyruvate carboxylase (4.1.1.31), and other soluble photosynthetic enzymes, than from decreases in thylakoid N-containing compounds. Decreases in quantum yield of O_2 evolution and F_v/F_m (variable-to-maximum fluorescence of dark-adapted leaves) during drought were small, so we assumed that little damage to photsystem II (PSII) and thylakoid membrane function occurred. Further, F_o (minimum F) decreased or remained unchanged, dark F_o was greater than light F_o, and decreases in photochemical quenching (the fraction of oxidized PSII) were reversed within 1–3 days after drought. Therefore, prolonged increases in non-photochemical quenching (q_n; thermal dissipation of excess light energy) during and after drought were indicative of protective downregulation and were likely associated with disproportionate loss of soluble photosynthetic proteins during drought. In support of this, post-drought recovery of q_n paralleled recovery of N_L and PS_{cap}. Thus, in C_4 prairie grasses, loss of PS_{cap} during drought is largely the result of decreases in shoot N_L and of associated protective downregulation, decreasing carbon assimilation for 1–2 weeks after drought.

Key words – Drought, nitrogen, photosynthesis, prairie grasses, water stress.

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Introduction

Perennial C_4 grasses of tallgrass prairie exhibit decreases in maximum rates of photosynthesis with drought that may persist for a week or more following the end of drought, despite recovery of leaf water status and stomatal conductance (Heckathorn and DeLucia 1994, Heckathorn 1995). Prolonged recovery of photosynthesis from water deficits may be costly in terms of whole-plant carbon assimilation, particularly if droughts are recurrent as is common in tallgrass prairie (Weaver and Fitzpatrick 1932, Borchert 1950, Heckathorn 1995).

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The decrease in photosynthesis with drought in these prairie grasses is accompanied by a decrease (25–40%) in leaf nitrogen concentration (N_L) that also persists for a week or more upon rewatering (Heckathorn and DeLucia 1994, 1995), suggesting that changes in leaf N status may influence photosynthesis during and after drought.

The majority of leaf N (e.g. 75% or more) is associated with photosynthesis and photosynthetic rates are strongly correlated with leaf N content (Field and Mooney 1986). Degradation of both thylakoid and stromal N-containing compounds can occur in response to water stress, recovery from which may require more than a week (Kulshreshtha et al. 1987, Castrillo and Calcagno 1989, Chaves 1991). Such decreases in photosynthetic proteins would provide a potential explanation for correlations between decreased photosynthesis and N_L during and after drought in prairie grasses.

In our previous greenhouse studies (Heckathorn and DeLucia 1994, 1995), decreases in foliar N concentration during drought in prairie grasses were attributable to three processes: (1) drought-induced retranslocation of shoot N to roots and rhizomes; (2) volatilization of foliar N; and (3) drought-related dilution of shoot N resulting from a greater impact of drought on soil N uptake than on growth (Chapin 1991 and references therein). The relative importance of each of these processes was species dependent. Decreases in leaf N status from these processes may not fully explain the loss of photosynthesis with drought in these species; however, as damage to and protective downregulation of photosynthetic metabolism may also occur (Kaiser 1987, Chaves 1991, Schulze and Caldwell 1994). Damage to photosynthetic machinery is usually minimal and downregulation rapidly reversible (< 24 h) with moderate water stress and low light, but damage or downregulation may be greater under high-light and high-temperature conditions (Kaiser 1987, Chaves 1991), as typical of tallgrass prairie.

While the contribution of damage and downregulation to decreased photosynthesis with drought has been appreciated for some time, the potential impact of drought-related decreases in leaf N status on photosynthesis has been largely ignored (Kaiser 1987, Chaves 1991). The primary purpose of the present study was to estimate the importance of decreases in foliar N concentration to the loss of photosynthesis during and especially after drought in perennial prairie grasses. During mild-to-moderate drought, photosynthesis is mostly limited by stomatal closure (Kaiser 1987, Chaves 1991). However, because limitations to photosynthesis associated with stomatal conductance and leaf water status do not appear to increase following drought in prairie grasses (Heckathorn and DeLucia 1994, Heckathorn 1995), post-drought recovery of net CO_2 assimilation in these species may be limited by foliar N status until leaf N is restored to pre-drought levels.

In the present study, after estimating the photosynthetic consequences of decreases in bulk leaf N status, we then examined the importance of degradation of thylakoid vs stromal N-containing compounds to N-related decreases in photosynthesis. We also assessed whether photodamage or protective downregulation contributed to decreases in photosynthesis with drought in prairie grasses. Lastly, we partitioned N-related effects on photosynthesis among shoot N retranslocation, volatilization and growth dilution, using our previous estimates of the contribution of each of these processes to decreased foliar N concentration during drought (discussed above).

Abbreviations – N_L, leaf nitrogen concentration; PEPC, phosphoenolpyruvate carboxylase; PNUE, photosynthetic N-use efficiency; PPDK, pyruvate P_i dikinase; PS_{cap}, photosynthetic capacity; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

Materials and methods

Plant material and growth conditions

We examined the loss of photosynthetic capacity (PS_{cap}) during drought in three rhizomatous perennial C_4 grasses native to tallgrass prairie. These species exhibit a gradient of drought tolerance from mesic to xeric as follows: *Spartina pectinata* Link (prairie cordgrass), *Andropogon gerardii* Vitman (big bluestem), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem) (Weaver and Fitzpatrick 1932, Heckathorn and DeLucia 1991, Heckathorn 1995). Plants were grown in a greenhouse at the University of Illinois-Urbana from seed collected at the Konza Prairie Research Natural Area (Manhattan, KS, USA). Individual plants were grown in 18-l pots (to prevent pot binding) containing loam, calcite clay, and sand (1:1:1, v:v:v) under 15 h, 30°C days and 20°C nights. Daytime photosynthetic photon flux density (PPFD) (natural plus supplemental light) at pot height was 700–2 000 μmol m^{-2} s^{-1}. Plants were watered daily at the soil surface and a commercial N:P:K (20:20:20) fertilizer (Peters, Milpitas, CA, USA) was applied weekly (300 ml of a 10 g l^{-1} solution). Plants were not fertilized during the drydown period.

A controlled drought similar in duration (17–24 days) and severity to what might be experienced in the field (Heckathorn 1995) was imposed after 8 weeks of growth as described previously (Heckathorn and DeLucia 1994); i.e. each species was subjected to minimum leaf water potentials similar to those occurring naturally in the same species. Data were collected from four to six previously unused plants for each species-treatment combination. Measurements were made on the most recently fully expanded leaves prior to drought, at mid-drought when leaf rolling was nearly complete, late in the drought when stomatal closure was approximately complete, and throughout a subsequent post-drought recovery period following resumption of daily watering. Midday water potentials were measured with a pressure chamber (PMS Instr. Co., Corvallis, OR, USA).

PS_{cap}, N_L, and estimating N effects on PS_{cap}

Photosynthetic capacity, defined here as net O_2 evolution at light and CO_2 saturation, was measured throughout

drought and recovery with a gas-phase oxygen electrode (Hansatech Ltd, Norfolk, UK), as described in Day et al. (1991) and Heckathorn (1995). Measurements were made on flat unrolled leaf segments (adaxial surface up). Maximum potential photosynthesis under saturating CO_2 levels (i.e. PS_{cap}) was monitored, rather than net CO_2 uptake under ambient CO_2, in order to study N-related effects on photosynthesis during and after drought independent of stomatal limitations associated with plant water status.

Decreases in PS_{cap} associated with changes in N_L concentration were estimated from pre-drought PS_{cap} vs N_L relationships determined separately for each species; i.e. PS_{cap}-N_L curves were used to predict photosynthesis from N_L during drought and recovery. Decreases in PS_{cap} below levels predicted from N_L were then attributed to photodamage, protective downregulation, etc. We judged recovery from damage and downregulation to have been complete when the observed rate of photosynthesis could again be predicted from the pre-drought relationship between PS_{cap} and N_L.

As we have operationally defined it, damage and downregulation collectively include disproportionate degradation of some photosynthetic proteins such that the amount of these proteins then limits photosynthesis and lowers the slope of the PS_{cap}-N_L relationship. We did not expect apriori that drought-stressed and unstressed plants would have similar photosynthetic N-use efficiencies (PNUE; i.e. similar PS_{cap}-N_L slopes); to the contrary, if anything, we expected drought-stressed plants to have lower PNUE than unstressed plants. If we did, in fact, observe lower PNUE in stressed plants, then we asked whether lower PNUE in these plants resulted from drought-related photodamage, changes in photosynthetic-N allocation, etc.

To generate pre-drought PS_{cap}-N_L relationships, photosynthetic capacity and N_L were measured in well-watered plants receiving various levels of N fertilizer (0×, 1×, and 2× levels of the fertilization regime described above). Leaf nitrogen content was determined colorimetrically (Traacs 800, Bran-Luebbe, Buffalo Grove, IL, USA) following acid (Kjeldahl) digestion. Nitrogen concentrations are expressed on a dry mass basis (g N g^{-1} dry mass), although results would be similar on a leaf area basis since leaf mass per area did not change significantly during the study (see legend, Fig. 2).

Partitioning N effects on PS_{cap}

Based on our previous examinations of whole-plant N allocation patterns (Heckathorn and DeLucia 1994) and direct measurements of N volatilization (Heckathorn and DeLucia 1995), we partitioned N-related decreases in PS_{cap} among retranslocation, volatilization, and drought-related growth dilution. The contribution of retranslocation, volatilization, and dilution are based on data from two experimental droughts of either 2 or 3 weeks duration; hence the use of ranges to express changes in PS_{cap} in Tab. 1. Decreases in PS_{cap} associated with changes in N_L were calculated from pre-drought values and end-of-drought rates predicted from the PS_{cap}-N_L relationships described above. Differences between predicted values and observed rates of photosynthesis were used to estimate the contribution of damage and downregulation.

Thylakoid membrane N-content and function

To determine whether drought-related decreases in shoot N_L and PS_{cap} resulted from damage and degradation of thylakoid membrane proteins, we monitored leaf chlorophyll (Chl) content, the ratio of variable-to-maximum chlorophyll fluorescence in dark-adapted leaves (F_v/F_m), and the quantum efficiency or yield (Φ) of oxygen evolution (i.e. the initial slope of the photosynthesis-irradiance relationship). Changes in total chlorophyll content indirectly reflect changes in total thylakoid N, provided growth irradiance remains constant (Evans 1989a,b), as it did in this experiment. Changes in Chl *a*:*b* provide information about degradation of light-harvesting complexes relative to photosystem reaction centers (Barber 1987). For example, increases in Chl *a*:*b* indicate disproportional degradation of light-harvesting complexes. Quantum yield and F_v/F_m are both measures of the efficiency of photosystem II (PSII) function (Björkman and Demmig 1987, Schulze and Caldwell 1994), and decreases in these parameters reflect damage to PSII reaction centers.

Chlorophyll content (Chl *a*, *b*, *a*:*b*, and total) was determined spectrophotometrically following dimethyl sulfoxide extraction (Barnes et al. 1992). F_v/F_m was monitored following 30 min of dark adaptation using a modulated fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Quantum yield, on an absorbed-light basis, was determined from rates of oxygen evolution at four PPFDs between 30 and 120 μmol m^{-2} s^{-1}. Leaf absorptance was determined for both well-watered and drought-stressed plants using a spectroradiometer (OL-752, Optronics Lab, Inc., Orlando, FL, USA) and integrating sphere (Li-Cor, Lincoln, NE, USA).

Chlorophyll fluorescence quenching analysis of light-adapted leaves was also employed to help determine if photodamage or protective downregulation contributes to drought-related decreases in PS_{cap}. Quenching analysis included determination of photochemical (q_p) and nonphotochemical (q_n) quenching parameters (Krause and Weis 1991, Schulze and Caldwell 1994), and quenching of minimum fluorescence (F_o quenching, denoted q_o; Bilger and Schreiber 1986, Demmig-Adams 1990). The coefficient q_p represents the proportion of PSII reaction centers that are open or oxidized, and increases in q_p indicate that photochemical reactions on the donor side of PSII are limiting electron transport relative to reactions on the acceptor side of PSII. Nonphotochemical quenching is a measure of the thermal dissipation of excess absorbed light energy, and increases in q_n indicate an increase in photoprotective downregulation of PSII.

Increases in dark-adapted F_o, and in light-adapted F_o (F_o') relative to dark F_o, have been interpreted as an indication of photodamage to either PSII or the light-harvesting complexes, while decreases in F_o and F_o':F_o have been interpreted as an indication of increased protective down-regulation. The change in F_o' relative to F_o is expressed by q_o, which equals $(F_o - F_o')/F_o$. Steady-state quenching coefficients were determined at a moderate PPFD of 700 rather than 2000 μmol m^{-2} s^{-1}, to avoid photoinhibitory damage during the measurement.

Soluble photosynthetic enzyme content

Degradation of soluble stromal enzymes was monitored by measuring total leaf soluble protein content and changes in the content of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39), and phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) plus pyruvate P_i dikinase (PPDK; 2.7.9.1), as in Heckathorn et al. (1996). Leaf tissue that had been stored at –70°C was ground with a mortar and pestle in liquid N_2 and then in extraction solution consisting of 50 m*M* Tris-HCl (pH 8.0), 5 m*M* Na-EDTA, 1 m*M* DTT, 1 m*M* phenylmethylsufonyl fluoride, 10 μ*M* leupeptin, 1 m*M* benzamidine-HCl, 5 m*M* ε-aminocaproic acid, 1 m*M* benzidine (Gegenheimer 1990). Samples were then boiled and centrifuged at 14000 *g* to remove insoluble material. Aliquots from equal leaf area from each plant (2 leaves per plant; 5–7 plants) were pooled and then analyzed in duplicate for content of soluble protein and photosynthetic enzymes.

Soluble protein content of crude leaf extract was determined by the Bradford assay (Bradford 1976, reagent from Bio-Rad, Richmond, CA, USA). Relative (to pre-drought) changes in Rubisco and PEPC plus PPDK content were quantified by laser densitometry (LKB Biochrom Ltd, Cambridge, UK) following gel electrophoresis and staining. Crude leaf extract and molecular mass standards were subjected to SDS-PAGE (Laemmli 1970) and stained with Coomassie Blue. Optical density of bands at ca 55 kDa (Rubisco large subunit; Andrews and Lorimer 1987) and 96 kDa (PEPC and PPDK; Podestá et al. 1990) was then determined. The identity of the 55-kDa band as the Rubisco large subunit was confirmed by western blotting and antibody probe. Because PEPC and PPDK are of similar molecular mass and were not resolvable as separate bands, we refer to the 96-kDa band as PEPC+PPDK throughout this paper.

Statistical analysis

Results were analyzed statistically using two-way (species × H_2O status) analysis of variance (ANOVA). Analyses were limited to data collected before and during drought (i.e. pre-, mid-, and late-drought) and from well-watered age controls. Soluble protein and enzyme data were not analyzed because of the pooled sampling protocol. Tukey's multiple-comparison test was used to identify significant differences among treatments and species. Variances were tested for homogeneity using Bartlett's test. Percentage data were arcsine transformed prior to analysis, following Zar (1974); however, means and error bars in figures are untransformed values. Photosynthetic capacity vs N_L data were subjected to least-squares linear-regression analysis.

Results

In well-watered plants, the PS_{cap}-N_L relationships in these species were linear, with steep slopes, and they ex-

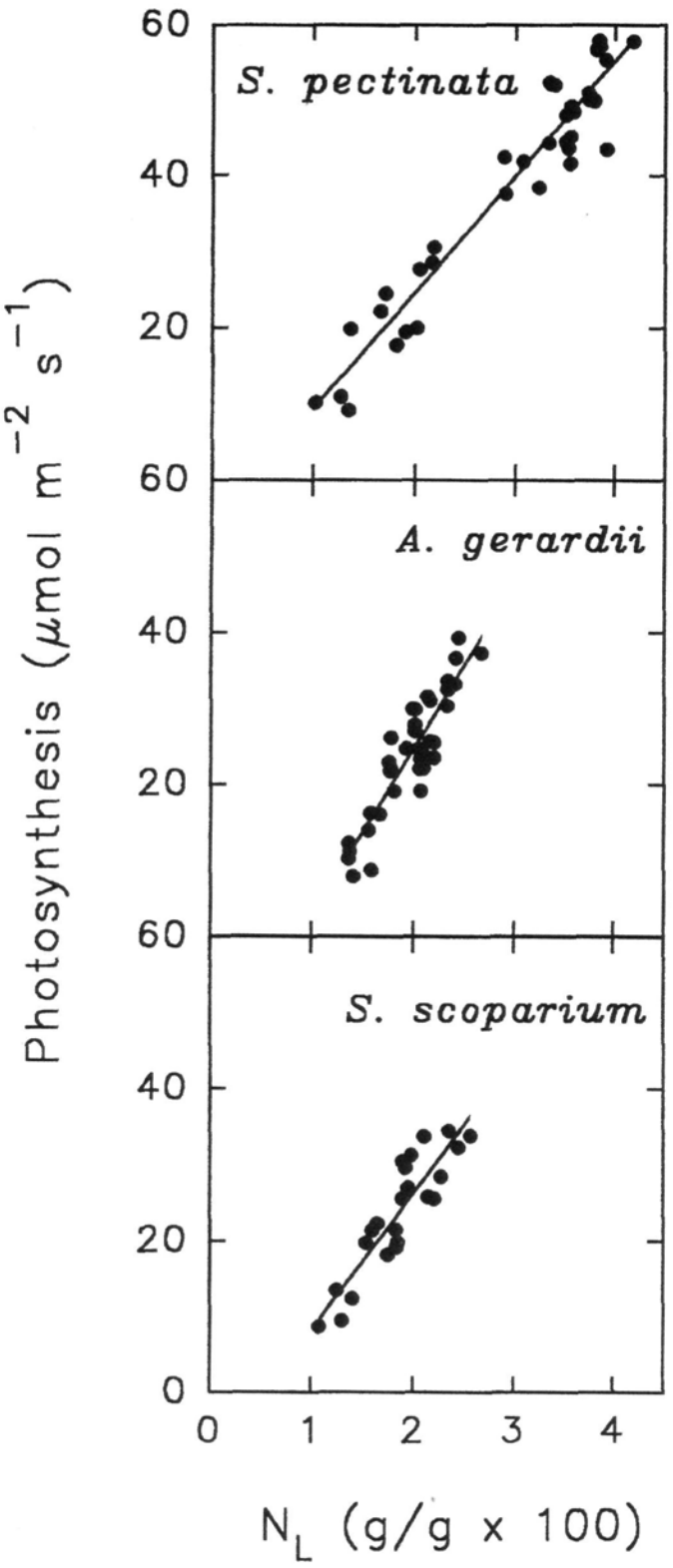

Fig. 1. Relationship between PS_{cap} and N concentration in the most recently fully expanded leaves of well-watered *Spartina pectinata*, *Andropogon gerardii* and *Schizachyrium scoparium* plants grown at different fertilization levels. Best-fit linear equations and coefficients of determination are as follows: *S. pectinata*, $y = 15.3x - 6.0$, $r^2 = 0.92$; *A. gerardii*, $y = 22.0x - 19.3$, $r^2 = 0.82$; *S. scoparium*, $y = 18.0x - 10.0$, $r^2 = 0.80$.

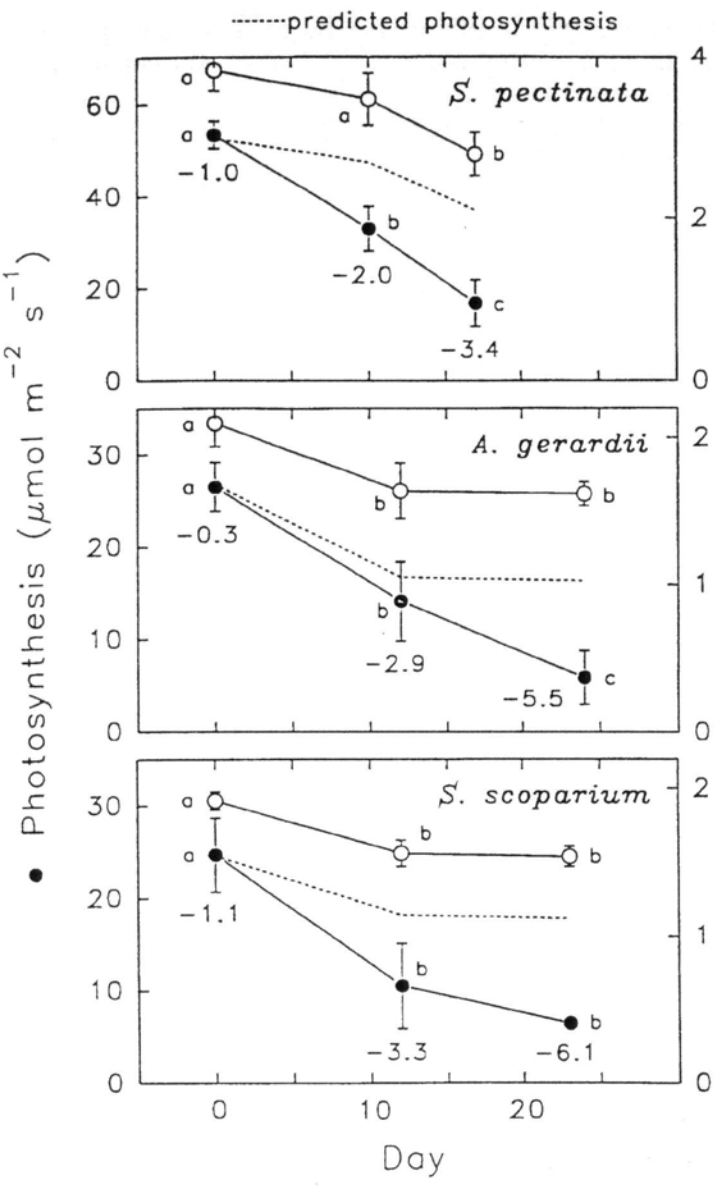

Fig. 2. Photosynthetic capacity and N concentration of leaves of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium* during drought. Data are from the most recently fully expanded leaves present at the onset of drought. Photosynthetic capacity predicted from leaf N, using the relationships in Fig. 1, is indicated with a dashed line. Midday leaf water potentials (MPa) are also indicated for each species by numbers inside of panels. Significant differences ($P < 0.05$) in photosynthesis and N among drought stages are indicated by different letters. Error bars = 1 SD; n = 4–6. Leaf mass per area (mg cm^{-2} ± 1 SD) for each species for pre-, mid-, and end-of-drought, respectively, was as follows: *S. pectinata* = 7.0 ± 0.6, 7.4 ± 0.5, 7.5 ± 0.8; *A. gerardii* = 6.6 ± 0.7, 6.4 ± 0.4, 6.7 ± 0.9; *S. scoparium* = 6.5 ± 0.1, 6.7 ± 0.5, 6.3 ± 0.2.

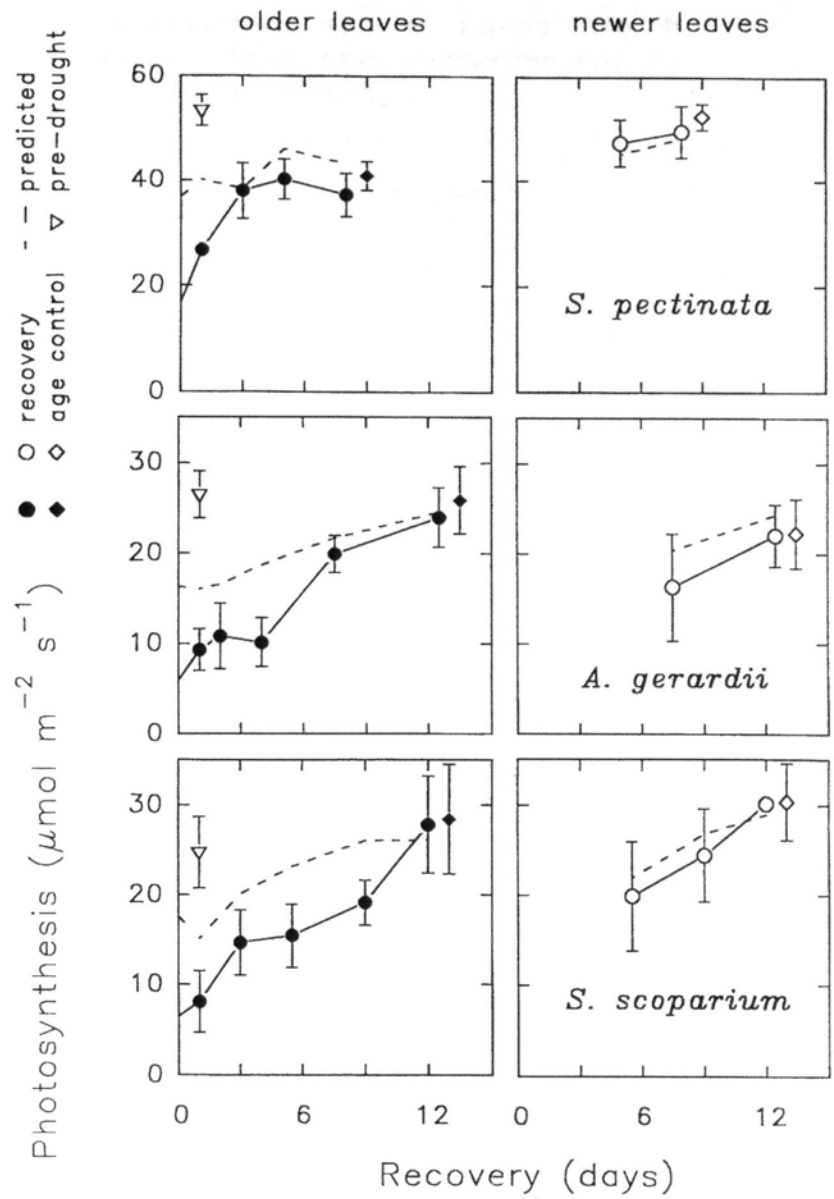

Fig. 3. Post-drought recovery of PS_{cap} of recently expanded leaves of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium*. Data are from either the most recently fully expanded leaves present at the onset of the drought as in Fig. 2 (i.e. older leaves, closed symbols) or the next youngest leaves expanded after the drought (i.e. newer leaves). The dashed lines are predicted photosynthesis based on Fig. 1. Age controls (diamonds) were well-watered throughout the experiment. Error bars = 1 SD; n = 4–6.

hibited high determination coefficients (r^2, Fig. 1). Leaf nitrogen concentration thus provides a reliable estimate of PS_{cap} in these species prior to drought and, relative to many other species, small changes in N_L result in large changes in photosynthesis (Field and Mooney 1986).

As expected, PS_{cap} decreased during drought in all species (69–78%), as did leaf N_L, exhibiting relative decreases of 27, 23, and 20% in *S. pectinata*, *A. gerardii*, and *S. scoparium*, respectively, compared to pre-drought values (Fig. 2). Nitrogen-related decreases in photosynthesis, estimated from the relationship between PS_{cap} and N_L status (Fig. 1), accounted for only 38 to 51% of the total decrease in maximum photosynthesis during drought (Fig. 2, the difference between the filled symbols and the dashed line), indicating substantial damage to or downregulation of photosynthesis.

Recovery of PS_{cap} and N_L to pre-drought levels following resumption of daily watering required at least 6 days in each species (Figs 3 and 4). In *S. pectinata*, PS_{cap} and N_L never recovered to pre-drought levels in the most recently fully expanded leaves present at the end of the drydown (i.e. "older" leaves). It was not until younger leaves were expanded after the drought that PS_{cap} and N_L were restored to within 90% of pre-drought levels (by day 8). Photosynthesis could again be predicted solely from leaf N status by day 3 in older leaves, indicating that drought-related damage or downregulation was repaired within that time.

Leaf N status did not recover to within 90% of pre-drought levels until day 12 in both older and younger leaves of *A. gerardii*. Recovery of PS_{cap} to predicted levels required 8 days for older leaves, while re-

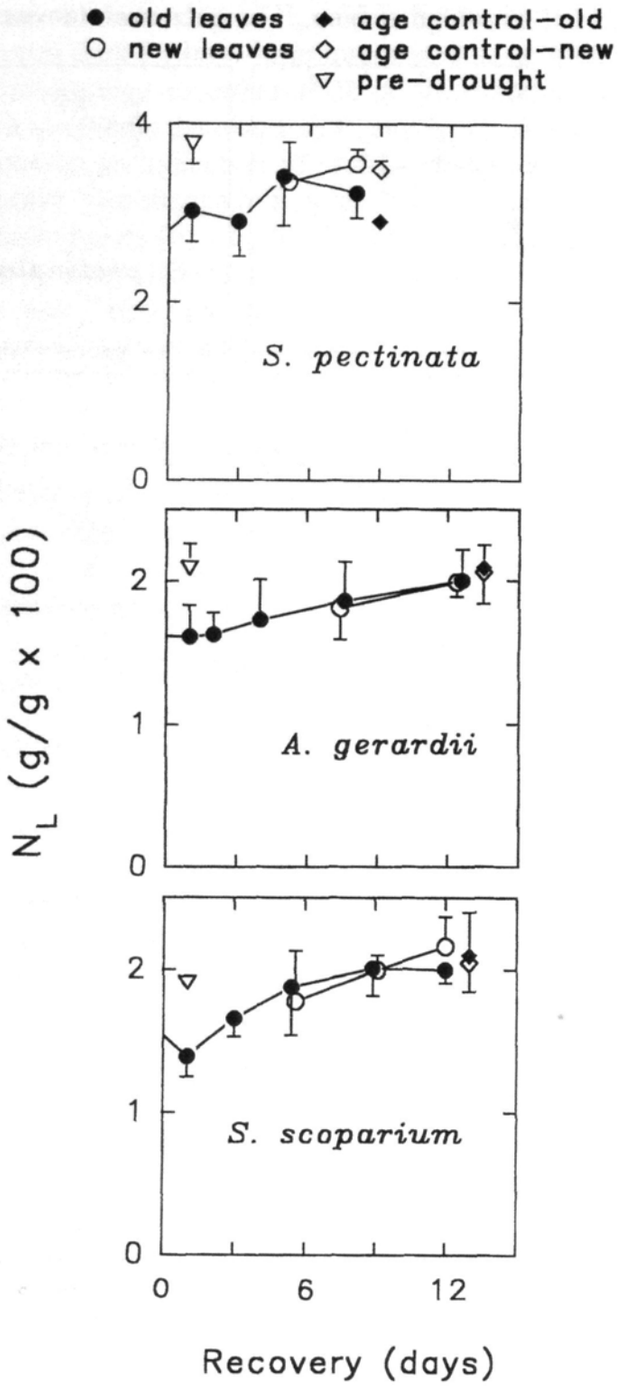

Fig. 4. Post-drought recovery of N concentration of recently expanded leaves of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium*. Data are from the most recently fully expanded leaves present at the onset of the drought (old leaves) and the next youngest leaves expanded after the drought (new leaves). Age controls (diamonds) were well-watered throughout the experiment. Error bars = 1 SD; n = 4–6.

covery to pre-drought levels required 12 days. Photosynthetic capacity of older leaves was greater than that of younger leaves in this species. Recovery of leaf N status to 90% of pre-drought levels was more rapid in *S. scoparium* than in the two more mesic species, occurring within 6 days; full recovery occurred within 9 days. Restoration of PS_{cap} to pre-drought levels in *S. scoparium* occurred earliest in younger leaves, which exhibited predicted levels of photosynthesis at day 6 and pre-drought levels at day 9. Recovery of older leaves to predicted and pre-drought levels required 10 to 12 days.

Tab. 1. Decreases (%) in photosynthetic capacity during a 2–3-week drought explained by decreases in leaf N concentration and damage or downregulation. All decreases are relative to pre-drought values. Nitrogen-related changes are partitioned into decreases associated with retranslocation, volatilization, and growth dilution.

	Spartina pectinata	*Andropogon gerardii*	*Schizachyrium scoparium*
Decrease related to:			
Retranslocation	17–21	9–14	2–2.5
Volatilization	0.5–1	2–4	1–2
Growth dilution	8–12.5	22–29	23.5–25
Total decrease predicted from N_L	30	40	28
Decrease due to damage/down-regulation	39	38	46
Total decrease	69	78	74

Nitrogen-related decreases in PS_{cap} were associated mostly with retranslocation in *S. pectinata*, but with drought-related growth dilution in *A. gerardii* and *S. scoparium* (Tab. 1). The contribution of volatilization to reductions in leaf N was small in all species, but may increase with larger decreases in foliar N status during drought (Heckathorn and DeLucia 1995) or in older leaves (Weiland and Stutte 1979). Importantly, PS_{cap} recovered to values predicted from leaf N (i.e. downregulation and damage were reversed) in either younger or older leaves of each species before recovery to pre-drought values. Therefore, the relative importance of decreases in leaf N during drought to whole-plant post-drought PS_{cap} is greater than indicated in Tab. 1. We estimate that, for recently fully expanded leaves, the proportion of total potential fixed carbon lost during post-drought recovery due to decreased N_L and photodamage or downregulation is 23, 38, and 32%, for *S. pectinata*, *A. gerardii*, and *S. scoparium*, respectively. Integrated over the post-drought recovery period, decreases in foliar N status are responsible for 69, 59 and 51% of this lost carbon (for *S. pectinata*, *A. gerardii*, and *S. scoparium*, respectively).

The changes in Φ, F_v/F_m, and total chlorophyll content during drought in these species were smaller than for PS_{cap} or N_L (Fig. 5). In *S. pectinata* and *S. scoparium* only F_v/F_m changed with drought, decreasing 4 and 8%, respectively, and recovered to pre-drought levels within 1 to 3 days. Somewhat greater effects of drought were seen in *A. gerardii*. Chlorophyll content decreased 19% and F_v/F_m decreased 14% during the drydown (compared to pre-drought values) and both were fully recovered within 8 days after rewatering.

Changes in total leaf soluble protein during drought exceeded those for total nitrogen and chlorophyll in *S. pectinata* and *S. scoparium*, decreasing 33 and 32% from pre-drought levels respectively, but were similar in *A. gerardii*, decreasing 24% (Fig. 6). Relative decreases

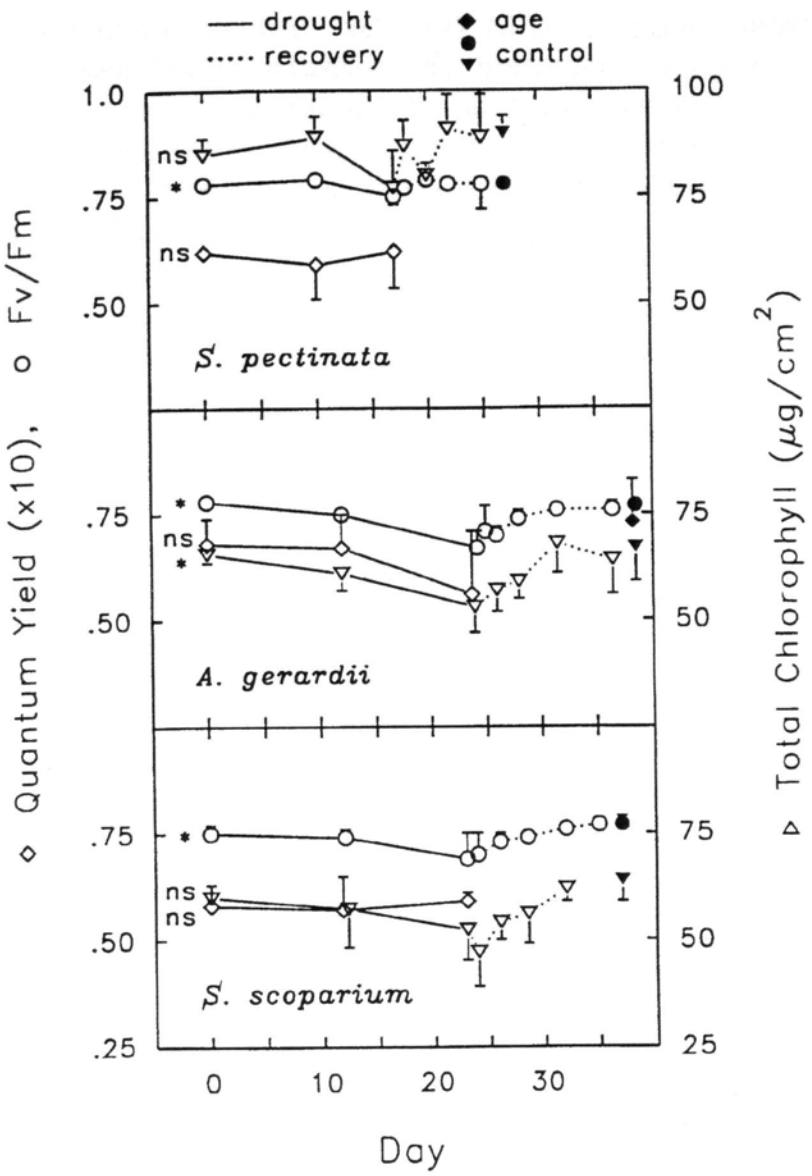

Fig. 5. Quantum yield of O_2 evolution, dark-adapted F_v/F_m, and total chlorophyll concentration ($a + b$) of leaves of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium* during drought (solid line) and recovery (dashed line). Data are from the most recently fully expanded leaves present at the onset of the drought. Age controls (solid symbols) were well-watered throughout the experiment. Significant ($P < 0.05$) effects of drought within each species are indicated with an asterisk; ns indicates nonsignificance. Error bars = 1 SD; n = 4–6.

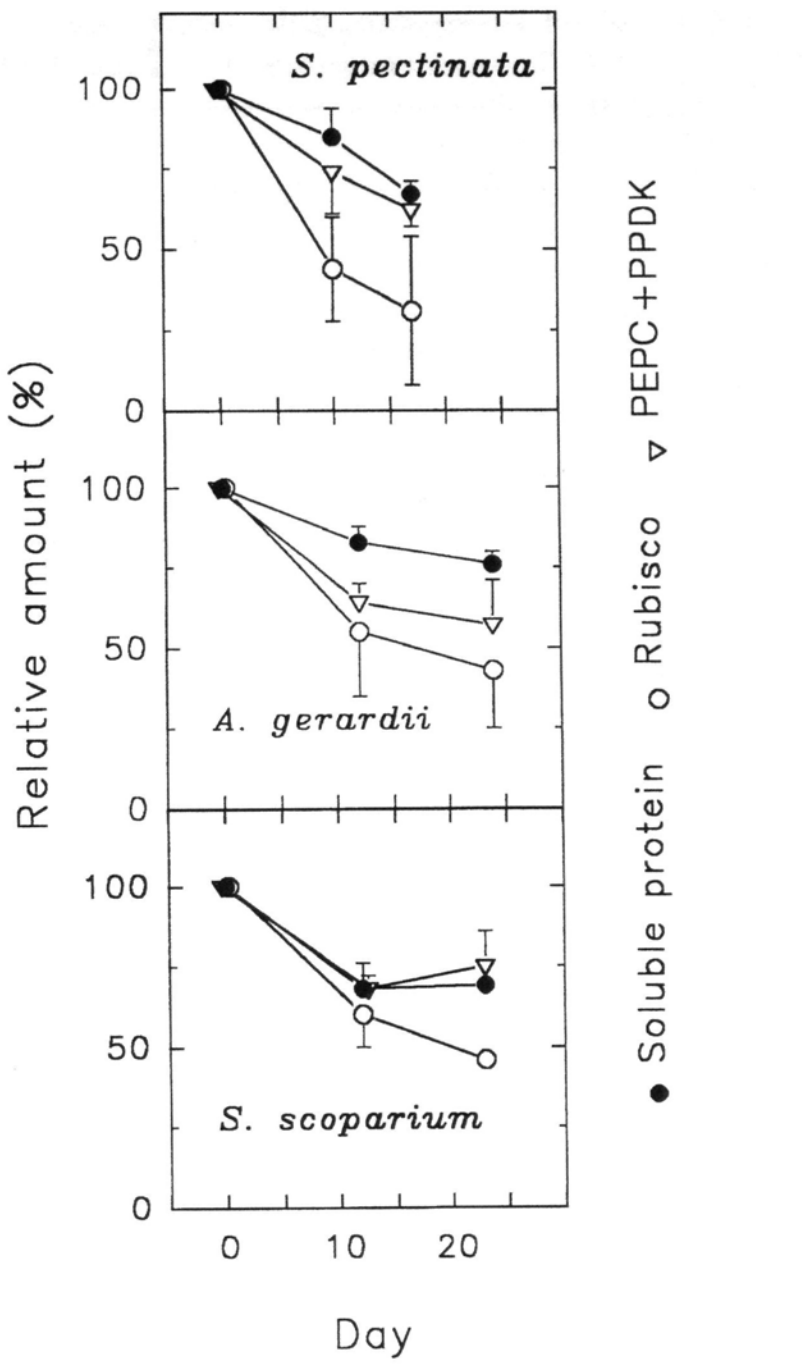

Fig. 6. Relative decreases during drought in concentrations (leaf area basis) of total leaf soluble protein, Rubisco, and PEPC+PPDK of the most recently fully expanded leaves present at the onset of drought of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium*. Data are normalized to pre-drought values. Errors bars = 1 SD; n = 2 replicates of pooled samples.

in Rubisco content were greater than for soluble protein in all three species, while this was true for PEPC+PPDK only in *A. gerardii* (Fig. 6).

Nonphotochemical quenching increased during drought in all three species, although this increase appeared to be age-related in *S. pectinata* (Fig. 7). Concomitant increases in F_o quenching (q_o) and decreases in q_p occurred in *A. gerardii* and *S. scoparium*, but not in *S. pectinata*. Recovery of q_p was rapid, occurring within 1 to 3 days in all species; however, complete recovery of q_n and q_o required 8 to 12 days in the two more xeric species.

Discussion

Photosynthetic capacity decreased 69 to 78% during drought in recently expanded leaves of *S. pectinata*, *A. gerardii* and *S. scoparium*. Approximately one-third to one-half of this decrease could be explained by decreases in leaf N concentration, while the remainder was attributed to protective downregulation of or damage to photosynthetic metabolism. Recovery of PS_{cap} occurred first in fully expanded leaves present at the end of the drought in *A. gerardii*, but in younger leaves expanded after the drought in *S. pectinata* and *S. scoparium*. Older leaves were also fully repaired in *S. scoparium*, as in *A. gerardii*, but not in the most mesic species, *S. pectinata*, which exhibited more-rapid leaf turnover than the two more xeric species (S. A. Heckathorn, personal observation).

Post-drought recovery of PS_{cap} was prolonged and required 8 to 12 days following resumption of daily watering, decreasing potential post-drought carbon gain by 23 to 38%. Photodamage and downregulation recovered more quickly after drought than N_L; therefore, when integrated over the recovery period, decreases in N_L ac-

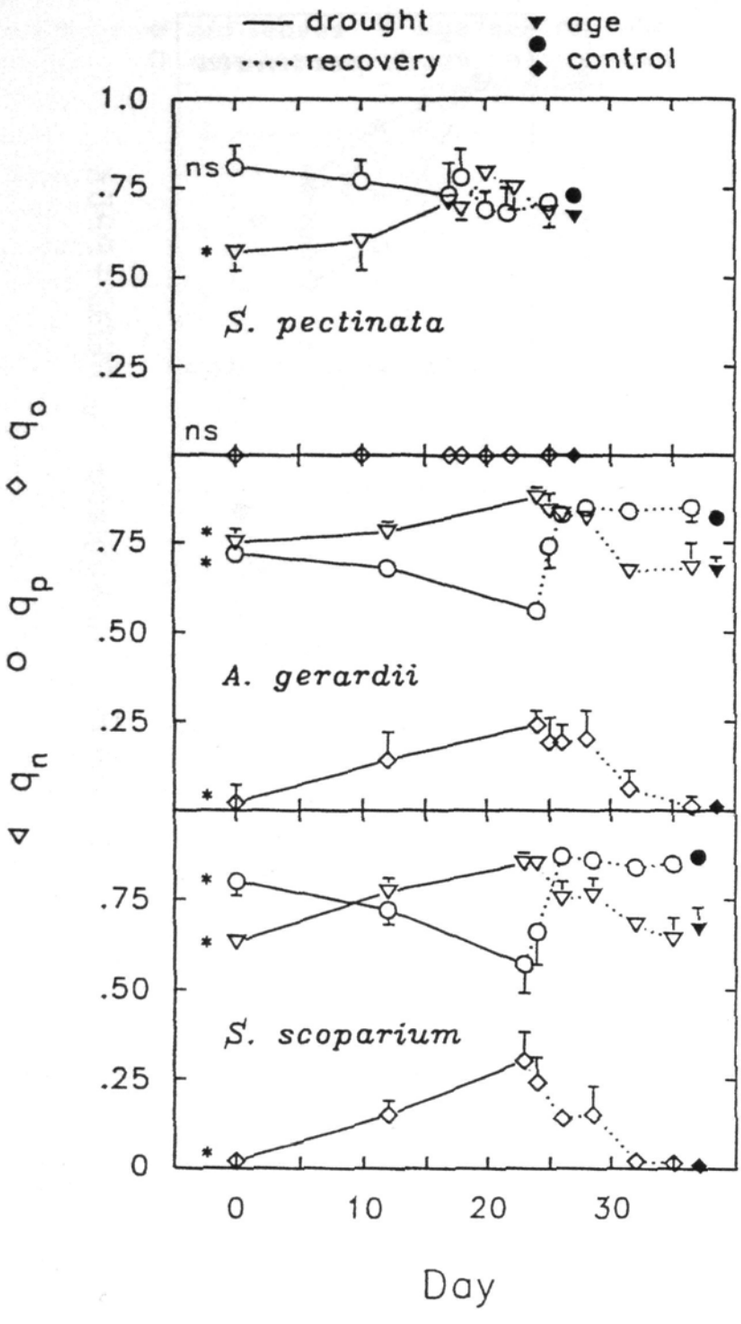

Fig. 7. Steady-state nonphotochemical (q_n), photochemical (q_p), and minimum-fluorescence (q_o) quenching coefficients determined at a PPFD of 700 μmol m^{-2} s^{-1} for recently expanded leaves of *Spartina pectinata*, *Andropogon gerardii*, and *Schizachyrium scoparium* during drought (solid line) and recovery (dashed line). Data are from the most recently fully expanded leaves present at the onset of the drought. Age controls (solid symbols) were well-watered throughout the experiment. Significant ($P < 0.05$) effects of drought within each species are indicated with an asterisk; ns indicates nonsignificance. Error bars = 1 SD; n = 4–6.

counted for 51–69% of the decrease in post-drought carbon gain. Because decreases in N_L during drought in recently expanded leaves, on which these calculations are based, are of smaller magnitude than for the entire shoot (Heckathorn and DeLucia 1995), these estimates of the importance of leaf N decreases may be conservative. Furthermore, these estimates are for loss of PS_{cap} per unit leaf area, and so ignore increases in leaf area per plant following drought. Since the rate of leaf area production usually increases with foliar N concentration, and retranslocation, volatilization and growth dilution decrease N concentration, this further renders these estimates conservative at the whole-plant level.

Nitrogen-related decreases in PS_{cap} were associated mostly with retranslocation in *S. pectinata*, but with drought-related growth dilution in *A. gerardii* and *S. scoparium*. Further, the decrease in PS_{cap} associated with decreased leaf N concentration during drought resulted primarily from degradation of soluble stromal enzymes rather than thylakoid N-containing compounds (inferred from changes in chlorophyll content). In *S. pectinata* and *S. scoparium*, relative decreases in total chlorophyll concentration (0 and 13%, respectively) were smaller than decreases in N_L (27 and 20%), while decreases in soluble protein concentration, Rubisco, and PEPC+PPDK exceeded those for N_L. In *A. gerardii*, decreases in chlorophyll, soluble protein and total N were of similar magnitude (19, 24, and 23%, respectively). Nevertheless, decreases in Rubisco and PEPC+PPDK in *A. gerardii* were greater than for chlorophyll, soluble protein, and total N. These results are consistent with other studies that show little or no effect of drought on chlorophyll content or thylakoid membrane N content, particularly in drought-tolerant species, but larger decreases in Rubisco and PEPC activity or amount (Kulshreshtha et al. 1987, Castrillo and Calcagno 1989, Stuhlfauth et al. 1990, Chaves 1991, Majumdar et al. 1991).

In all three species, relative decreases in Rubisco concentration were greater than for total soluble protein, supporting the idea that Rubisco serves as a labile nitrogen storage protein (Millard 1988). Decreases in PEPC+PPDK were smaller than for Rubisco but at least as large as decreases in soluble protein. Rubisco, PEPC, and PPDK activity have all been found to be strongly associated with rate of photosynthesis in C_4 species (Usuda et al. 1984, Crafts-Brandner and Poneleit 1987), and activity of these enzymes is related to concentration (Sugiyama et al. 1984), thereby providing a plausible mechanistic explanation for N-related decreases in PS_{cap} in the present study.

Decreases in PS_{cap} with drought, beyond those caused by decreases in bulk leaf N status, are thought to result from damage or downregulation (Kaiser 1987, Chaves 1991). However, with the exception of *A. gerardii*, we saw little evidence of photodamage during drought, despite the high irradiances that plants experienced in the greenhouse during the drydown (see Materials and methods). Quantum yield was unaffected by drought in *S. pectinata* and *S. scoparium*, and F_v/F_m decreased only 4–8%, indicating little damage to PSII. In *A. gerardii*, Φ decreased 18% and F_v/F_m 14%, but F_v/F_m recovered within 8 days, at least 4 days before PS_{cap} recovered to pre-drought levels (Φ was not measured during recovery). Although small decreases in Chl *a*:*b* in all species during drought (data not shown) indicated disproportionate degradation of reaction center proteins rather than light-harvesting complexes (Barber 1987), the small decreases in F_v/F_m and Φ indicate that remaining

reaction centers were functional. These results agree with most studies wherein little damage to PSII function is observed unless drought stress is especially severe or additional stresses are imposed along with drought stress (Kaiser 1987, Stuhlfauth et al. 1990, Chaves 1991).

Chlorophyll fluorescence quenching analysis also indicated little photosynthetic damage during drought in these species, but suggested substantial downregulation. Increases in q_n and decreases in q_p during drought, as in these species, are consistent with either damage or downregulation (Krause and Weis 1991). However, q_p, the fraction of open PSII centers during steady-state illumination, recovered within 1 to 3 days in old leaves of all three species, indicating that damage downstream from PSII was repaired by that time. Furthermore, as discussed above, decreases in F_v/F_m and Φ were relatively small. Together, these results indicate that changes in q_n and q_p likely resulted primarily from protective downregulation and were perhaps related to a disproportionate loss of soluble photosynthetic enzymes. In support of this, decreases in Rubisco were similar or greater in magnitude to decreases in N_L (this was not the case for chlorophyll), and recovery of PS_{cap}, N_L, and q_n were well correlated, particularly in *A. gerardii* and *S. scoparium*.

Additionally, F_o quenching (q_o) remained unchanged with drought in *S. pectinata*, as did dark-adapted F_o (data not shown). This suggests that no damage on the donor side of PSII occurred, and interestingly, that this species may exhibit little photoprotective xanthophyll-cycle activity (Demmig-Adams 1990). As there was no evidence of photoinhibitory damage directly to PSII (i.e. no decrease in F_v/F_m or Φ), increases in q_n in this species, which were mostly age-, rather than drought-related, were presumably related entirely to ΔpH-dependent fluorescence quenching (Krause and Weis 1991, Schulze and Caldwell 1994). Taken together, these observations suggest that the lack of photoinhibitory damage in *S. pectinata*, the least drought-tolerant species in the present study, is primarily the result of changes in leaf angle and displayed area following leaf rolling (Heckathorn and DeLucia 1991), rather than protective metabolic changes.

Increases in q_n in *A. gerardii* and *S. scoparium* were accompanied by increases in q_o and by decreases in dark-adapted F_o (not shown). Post-drought recovery of q_n in old leaves was protracted, requiring 8 to 12 days, and appeared to be correlated with recovery of q_o. These data are indicative of photosynthetic downregulation and implicate protective activity of the xanthophyll cycle (Demmig-Adams 1990) or protective changes in light-harvesting complexes (Ruban et al. 1993). Recovery of PS_{cap} to predicted and pre-drought levels was more closely correlated with recovery of q_n in old leaves of *S. scoparium*, than with leaf N concentration. Thus, reversal of downregulation and recovery of leaf N status are both necessary for restoration of PS_{cap}.

In summary, the present study indicates that nitrogen, protective downregulation, and, to a lesser extent, damage-related decreases in PS_{cap} in response to water stress decrease potential carbon gain during and after drought in perennial prairie grasses. While the importance of photodamage and protective downregulation has been appreciated for some time, the contribution of changes in leaf N status to drought-related decreases in photosynthesis has been largely unstudied. Most of the carbon cost of drought-related decreases in photosynthesis will be incurred during recovery from drought, since water availability and stomatal closure will limit photosynthesis during drought. It is important to note that not all of the decrease in PS_{cap} during drought and recovery represents a net cost to the plant as a whole in terms of potential fixed carbon. While decreases in leaf N status resulting from shoot N retranslocation and volatilization decrease carbon fixation at the whole-plant level, growth dilution of existing shoot N during drought does not. Increases in shoot mass and leaf area during drought in these species more than offset decreases in maximum photosynthesis per unit leaf area associated with dilution, resulting in an increase in whole-plant photosynthetic rate after the drought has ended (Heckathorn and DeLucia 1994). Likewise, while photosynthetic downregulation may contribute to decreased PS_{cap}, protective downregulation is presumably less costly than the alternative, photoinhibitory damage.

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