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Plasticity of nitrogen allocation in the leaves of the invasive wetland grass, *Phalaris arundinacea* and co-occurring *Carex* species determines the photosynthetic sensitivity to nitrogen availability

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ABSTRACT

Phalaris arundinacea displaces the slower-growing, native sedge, *Carex stricta*, where nitrogen availability is high. Our aim was to address whether morphological and physiological traits associated with carbon gain for *P. arundinacea* and *C. stricta* responded to nitrogen supply differently and if the species exhibited different degrees of plasticity in these traits. The plants were grown in gravel and provided modified Hoagland's solution containing four nitrogen concentrations from 0.15 to 15 mM for 6 to 7 weeks. Supplied nitrogen affected the leaf nitrogen content to the same degree for both species. Increasing supplied nitrogen strongly increased CO_2 assimilation (A), photosynthetic nitrogen use efficiency (PNUE), and respiration for *P. arundinacea* but had only a small effect on these parameters for *C. stricta*. Relative to growth at 15 mM nitrogen, growth at 0.15 mM for young leaves decreased carboxylation capacity and efficiency and the capacity for electron transport for *P. arundinacea* and a larger, stouter *Carex* species, *Carex lacustris*, by 53 to 70% but only 20 to 24% for *C. stricta*. Leaf nitrogen decreased approximately 50% for all species, but vacuolar nitrate did not decrease for *P. arundinacea* and *C. stricta*, suggesting that it does not serve as a nitrogen reserve for use during nitrogen deprivation in these species. After 4 months of nitrogen deprivation, *P. arundinacea* doubled A in 12 days after being supplied 15 mM nitrogen, whereas A for *C. stricta* increased only 22%. We propose that one factor linking *P. arundinacea* abundance to nitrogen availability involves this species' plastic response of carbon gain to nitrogen supply. *C. stricta* appears to be adapted to tolerate low nitrogen availability but cannot respond as rapidly and extensively as *P. arundinacea* when nitrogen supply is high.

Introduction

Plant species fall into a consistent axis of leaf trait variation called the leaf economics spectrum (Westoby et al., 2002; Reich et al., 2003; Diaz et al., 2004; Wright et al., 2004). Position on this spectrum is, in part, associated with strategies of resource use (Reich et al., 2003; Diaz et al., 2004; Wright et al., 2004; Sorrell et al., 2012). At one end of this spectrum are the "fast" species (Reich, 2014) that are rapidly-growing, highly productive, often invasive species with high rates of CO_2 assimilation (A) and a small investment in leaf development, as indicated by their high specific leaf area (SLA). Generally, such species exhibit high phenotypic plasticity (both morphological and physiological) with changes in resource availability (Davidson et al., 2011). At the other end of the spectrum are the "conservative" or "slow" species (Reich, 2014) that are slowly-growing plants of less productivity with a low A and low SLA, which are often adapted to infertile soils (Berendse and Aerts, 1987; Aerts and de Caluwe, 1995; Aerts and Chapin, 2000; Adamidis et al., 2014). Interestingly, many of these species maintain "greater fitness homeostasis when comparing growth between low and average resource availability" (Davidson et al., 2011).

Of the resources that are linked to this spectrum, the supply of nitrogen (N) and leaf-level N are of great importance, if for no

Abbreviations: A, rate of CO_2 assimilation; A_{amb}, rate of CO_2 assimilation at ambient pCO_2; A_{max}, rate of CO_2 assimilation at saturating pCO_2; CE, carboxylation efficiency; J_{max}, light-saturated maximum rate of electron transport; PNUE, photosynthetic nitrogen use efficiency; PrNUE, soluble protein nitrogen use efficiency; R, rate of dark respiration; SLA, specific leaf area; V_{cmax}, maximum carboxylation capacity.

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other reason than the close relationship between N availability and carbon gain *via* photosynthesis (Evans, 1989; Poorter and Evans, 1998). Indeed, the success of many rapidly-growing, often invasive, species has been attributed to high photosynthetic nitrogen use efficiency (PNUE) relative to that for slower-growing species (Poorter and Evans, 1998; Onoda et al., 2004; Feng, 2008a,b; Feng et al., 2008, 2009, 2011; Funk et al., 2013). The high PNUE indicates that these invasive species allocate proportionately more of their leaf N to photosynthetic proteins than to other N uses, such as cell wall proteins or storage pools. In contrast, the species that the invaders displace generally have lower PNUE. Thus, it is possible that N availability could affect the ability of rapidly-growing, invasive species to displace other species and become abundant through its effects on photosynthetic capacity. A high rate of net carbon gain is important to high productivity and is a trait of many invasive species (Leishman et al., 2007; Feng et al., 2011).

We sought to identify the physiological and morphological responses for these disparate strategies of N allocation in co-occurring species from opposite ends of the leaf economics spectrum. By identifying the physiological basis for any leaf-level plasticity in response to N availability, we strived to improve our understanding of why N influences the distribution of certain species and the competitive success of some invasive species over more conservative species. Also, given that considerable agricultural plant research is focused on the improvement in yield relative to the amount of N required (Kant et al., 2011), such a study could also provide some insights into what mechanisms might be of value to improve the performance of crop species under low N availability.

Nowhere is it more evident that N availability affects species growth rates and success than in wetlands. Wetlands are landscape sinks, accumulating large amounts of nutrients, sediments, and water that originate at agricultural and industrialized, urban sites (Kercher and Zedler, 2004; Zedler and Kercher, 2004; Zedler, 2009). The periodic input of excessive N can have a large impact on the plant communities that originally became established during earlier periods of much lower, more evenly dispersed N inputs. These communities become increasingly susceptible to invasion by more rapidly-growing, invasive species that take advantage of the disturbances to the communities and eutrophication of the wetland, converting the wetlands from species-rich, native vegetation to a near monotype of the invasive species (Lindig-Cisneros and Zedler, 2002). The success of two common invasive species in North America, *Phalaris arundinacea* and *Phragmites australis*, over more conservative species, such as members of the genus, *Carex*, is related to increases in N availability (Green and Galatowitsch, 2002; Rickey and Anderson, 2004; Kercher et al., 2006; Martina and von Ende, 2008; Bartodziej, 2011). There appears to be a direct relationship between the availability of N, especially nitrate, and the extent of invasion (Rickey and Anderson, 2004; Martina and von Ende, 2008; Bartodziej, 2011) for these species.

For our study, we chose the wetland grass species, *P. arundinacea*, as the representative of an invasive species and the common sedge that *P. arundinacea* displaces, *Carex stricta* as a representative of a conservative species. We hypothesized that photosynthesis and associated leaf traits for *P. arundinacea* and *C. stricta* would respond differently to growth at different levels of N supply, with the responses for *P. arundinacea* being the most plastic. A major focus of our experiments was to identify any differences in the plasticity of specific photosynthetic components. In particular, given that wetlands can experience pulses of nutrients, we hypothesized that *P. arundinacea* would have a more rapid physiological response, especially with respect to *A*, to a rapid increase in N supply than would *C. stricta*.

Materials and methods

Plant material, experimental growth conditions, and leaf sampling for the first set of experiments

Seeds of *Phalaris arundinacea* L., reed canary grass, were collected in the field over an area of approximately 0.4 ha in Marshall County, Indiana, U.S.A., and bulked before germinating as described in He et al. (2011). Seedlings of *Carex stricta* Lam., tussock sedge, were supplied by Dr. Joy Zedler (University of Wisconsin, U.S.A.). For *Carex lacustris* Willd., common lake sedge, tillers were collected in the field with a minimum of 10 m and a maximum of 12 km between sample points in Marshall County, Indiana, U.S.A. Initially, the seedlings were planted into pots containing a coarse, non-soil medium that provided no nutrients (Turface Athletics Soil, Profile Products LLC, Buffalo Grove, IL, U.S.A.) and fertilized with a nutrient solution based on that of Hoagland and Arnon (1950) (Hoagland's solution) containing 15 mM N and adjusted to a pH of ~6.5. After several tillers had been produced, the tillers were separated to establish clones from the original seedlings or tillers.

In the first set of experiments, four separate clones (based on four original, sexually-reproduced seedlings per species) of *P. arundinacea* and *C. stricta* were grown in a greenhouse at 25–27/18 °C (day/night) with a 15-h photoperiod maintained by 400 W metal halide lamps. The plants were grown in 16-L pots containing the non-soil medium described above, and the pots stood in plastic rectangular pans (two pots per pan) that were 6.5 cm deep. Root growth into the solution of the pans occurred through holes in the pots. To minimize the growth of cyanobacteria and algae, aluminum foil covered the sides of the pots and the pans to restrict light reaching the solution in the pans. Every 4 d, the pots and the pans were flushed with de-ionized water to remove the old nutrient solution. After removing the excess water from the pans, the new solution was applied to the top of the gravel in each pot. Further watering with de-ionized water between nutrient additions was controlled to maintain the water content of the gravel while not causing a loss of water from the pans.

These experiments were conducted twice, once in the late winter through the spring, and once in the spring through summer. However, in the latter experiment, leaf N content was not determined. Therefore, the PNUE and soluble protein NUE (PrNUE) were calculated from the data obtained from the experiment performed in the late winter–spring. To establish the plants in a phase of maximum growth rates and to develop enough initial leaf mass for the analyses to be performed, the plants were fertilized with 400 mL of full strength Hoagland's solution (Hoagland and Arnon, 1950), containing 15 mM N as NH_4^+ and NO_3^- (NH_4^+:NO_3^- was 2:13) every 4 d for 4 weeks before the N treatments were imposed. This exposure to high N supply allowed the plants to develop any storage pools of N before their exposure to any reduced N supply. For the N treatments, the plants were supplied with 500 mL of a modified Hoagland's solution containing the following concentrations of N: 0.15, 0.75, 3.0, and 15 mM. these concentrations were chosen, not only because they are typical of concentrations used in other controlled experiments addressing responses to N (Martin et al., 2002; Kant et al., 2008), but also because the middle concentrations were similar to concentrations measured in wetlands where *P. arundinacea* and sedge species occur (Bartodziej, 2011). Because the ammonium phosphate, potassium nitrate, and calcium nitrate concentrations were lowered in creating the different N concentrations in the modified nutrient solution, sufficient potassium phosphate, calcium carbonate, and potassium chloride were added to provide the amount of potassium, phosphate, and calcium as in normal Hoagland's solution. The gas-exchange measurements and leaf sampling on the plants supplied with 15 mM N were performed 3 weeks after the imposition of the lower N treatments.

The remaining plants were sampled and measured 6 to 7 weeks after their lower N treatments were imposed. Therefore, all plants received a particular N treatment for 6 to 7 weeks prior to any measurements or sampling being performed. An additional set of plants (four clones per species) were fertilized every 4 d with a modified Hoagland's solution containing 0 mM N for 5 months to determine the leaf N content of young and old leaves after an extended period of N deprivation.

The young, but mature middle portions of leaves of comparable development from both species were used for all analyses. Generally, the leaves that were measured or sampled were developing as the N treatments were commenced. For determining protein and N contents and specific leaf area (SLA), one leaf per plant was removed during the middle of the photoperiod, and the middle portion sectioned to determine values for each parameter. Each section was kept moist until its area could be measured with a Li-Cor, Li-3100 Leaf Area Meter (Li-Cor, Lincoln, NE, U.S.A.). Then the sections for C, N, and SLA analyses were dried in an oven at 65 °C for 48 h to determine their dry mass. The leaf sections to be used for soluble protein contents were frozen in liquid N_2 and stored at −80 °C until they could be processed.

The response to a rapid increase in the nitrogen supplied following N deprivation

Four plants (genets) per species from the first experiment were allowed to grow with 0.15 mM N supplied for 4 months. They were repotted in larger pots (23 L) and allowed to grow for an additional 10 d at 0.15 mM N to improve root growth. At the end of this period, roots were protruding out of the holes in the pots and into the nutrient solution of the trays by approximately 2 cm, indicative of rapid new root growth. Gas-exchange measurements were performed on the young, fully-expanded leaves at that time, and these leaves were tagged so that their gas exchange could be measured periodically for the next 12 d while the plants were receiving the normal Hoagland's solution containing 15 mM N every 3 d. The 15 mM N concentration was chosen to minimize any differences in N absorption at the root level, allowing a focus on N allocation and metabolism in the leaves. At the end of the experiment, sections of the leaves were removed and dried for the measurement of total N content.

Plant material, experimental growth conditions, and leaf sampling for the second experiment

The second experiment, which was performed in the late winter through the spring, included plants of a second *Carex* species, *C. lacustris*, which grow approximately 50% taller than *C. stricta* plants and have leaves that are approximately twice the size of the leaves of *C. stricta* (our unpublished observations). Five genets each of *C. stricta*, *C. lacustris*, and *P. arundinacea* were grown under the same greenhouse conditions established for the first set of experiments, except that the plants were fertilized with 500 mL of Hoagland's solution containing 15 mM N for 9 weeks in an attempt to maximize the leaf N content. Each circular pan holding three, 16-L pots was 7 cm deep. After this period, the plants were provided 500 mL of a modified Hoagland's solution containing 0.15 mM N every 4 d for 6 weeks. At the end of each N-treatment period, leaf sections were removed to determine leaf nitrate, total leaf N and chlorophyll content, and SLA. For *P. arundinacea*, the first fully-expanded leaf was designated a young leaf, and for measurements taken at the end of the high N treatment, the fourth leaf below it (fifth or sixth leaf from the shoot tip) was designated an old leaf. The old leaves sampled at the end of the low N treatment were 6 leaves below the first fully-expanded leaves (seventh or eighth leaves from the tip). For the two *Carex* species, a leaf about to reach full elongation was designated a new leaf and sampling was performed from the mid-portion. The old *Carex* leaves were leaves that were approximately 10 weeks old at the time of measurement. Similar leaves were used for the analyses of the response of *A* to the leaf internal CO_2 concentration (*Ci*), and all of the leaves were sampled during full-sun illumination from 09:00 to 12:00 local time.

Determination of leaf nitrogen and carbon content

Dried leaf samples were ground to a fine powder by hand using a mortar and pestle with a small amount of liquid nitrogen or ground using a mechanical grinder (Cianflone Scientific Instruments Corporation, Pittsburgh, PA, U.S.A.). To determine leaf C and N contents, either a CE Elantech CN 2500 analyzer (Carlo Erba, Milan, Italy) at Texas Tech University was used or the samples were sent to Raymond Lee, Washington State University, U.S.A. for analysis using a Costech elemental analyzer (Costech Anatalytical Technologies Inc.).

Gas-exchange measurements

Gas-exchange analyses were performed in the greenhouse from 09:00 to 16:00 local time using a Li-Cor, Li-6400 XT portable photosynthesis system (Li-Cor Inc, Lincoln, NE, U.S.A.). In the first set of experiments, the measurements were conducted at the ambient CO_2 partial pressure (38.5 Pa). The blue and red light emitting diodes in the leaf chamber of the Li-6400A supplied a photon flux density of 1800 $\mu mol\ m^{-2}\ s^{-1}$ (growth irradiance), and the relative humidity in the leaf cuvette was 45–50%. The temperature within the leaf cuvette was 25 °C (determined to be optimum). The measurements were made on the first fully expanded leaf from the apex of *P. arundinacea* plants or the mid-section of the young leaves of *C. stricta* plants. In the second experiment, the environmental conditions were the same, except that the leaves were sequentially exposed to CO_2 concentrations of 0, 4, 8, 10, 20, 30, 40, 60, 80, 100, 120, 160, and 200 Pa to provide data on the carboxylation efficiency (CE) of Rubisco, the maximum carboxylation capacity (V_{cmax}), and the light-saturated maximum rate of electron transport (J_{max}). The values of V_{cmax} and J_{max} were calculated using the "plantecophys" package (Duursma, 2013) for the R statistical program (R Development Core Team, 2014).

The values of respiration (*R*) were measured as the rate of CO_2 released with the light source switched off and the entire leaf and chamber covered by a black cloth for 30 min prior to measurement during the same portion of the photoperiod in which measurements of *A* had been performed. The leaves measured were similar to the leaves used for measurements of *A* but on adjacent tillers of similar age.

Chlorophyll analysis

For chlorophyll determinations, frozen leaf sections of known fresh mass were ground at liquid N_2 temperature, and the chlorophyll was extracted in a known volume of 80% acetone. After centrifugation at 10,000 × *g*, the total chlorophyll (*a* and *b*) content was measured following the procedure of Lichtenthaler (1987).

Determination of the total leaf soluble protein

After grinding frozen leaf sections in liquid N_2 in a mortar, the frozen leaf powder was transferred to an ice-cold glass tissue grinder containing a 100 mM borate-buffered solution with 0.1% Triton-X 100 detergent at pH 7.8. After centrifugation at 16,000 × *g* for 20 min, the protein content of the extract was determined by the method of Bradford (1976). The amount of protein in the extract was determined from a standard protein curve developed using bovine serum albumin.

Measuring the leaf nitrate content

In the second experiment, leaf sections in full sun were sampled and their fresh mass and area rapidly measured before freezing in liquid N_2 for nitrate measurements. To determine the total leaf nitrate content, the frozen samples were ground to a powder at liquid N_2 temperature. The extraction and assay of the extract for nitrate followed the procedures of Catalado et al. (1975).

Determination of SLA

To determine the SLA, the areas of fresh leaf sections were measured using a Li-Cor, Li-3100 Area Meter (Li-Cor, Lincoln, NE, U.S.A.) prior to drying them in an oven at 65 °C for 48 h. Using the masses of the dried leaf sections, the SLA was calculated as:

$$\text{SLA} = \frac{\text{fresh leaf area } (\text{cm}^2)}{\text{leaf dry mass (g)}}$$

Statistical analyses

All analyses were conducted using the R statistical software (R Development Core Team, 2014). For the first set of experiments, analysis of covariance was used to test for significant species differences in the responses to supplied N concentration (log transformed). For analyses that used data from both of the experiments (*A*, soluble protein, and SLA), "experiment" was included as a fixed effect to account for growing condition and season differences (plants grown winter–spring *vs.* plants grown spring–summer). Of greatest interest was relative plasticity: that is, the relative slopes of measured variables in response to supplied nitrogen concentration. Significant interactions between supplied nitrogen concentration and species ($p \leq 0.05$) were interpreted as significant differences in plasticity. In the second experiment, the effects of supplied nitrogen and of species were tested using a mixed-effects ANCOVA in R using the lme function of the nlme package (Pinheiro et al., 2014) to account for repeated measures on individual plants.

Results

The response of A, R, and leaf soluble protein to supplied nitrogen concentration for P. arundinacea and C. stricta

In the first set of experiments, there was a significant positive effect of supplied N concentration on *A* for young, fully-expanded leaves of *P. arundinacea* and *C. stricta* ($p < 0.0001$, Fig. 1A). *P. arundinacea* exhibited a steeper slope of the response of *A* to supplied N than did *C. stricta* (greater plasticity) (species by supplied N interaction, $p < 0.0001$). There was no effect of experiment (winter–spring *vs.* spring–summer). We noted no consistent effect of supplied N concentration on stomatal conductance (not shown). Therefore, any effect of supplied N on *A* was realized largely at the biochemical level. Supplied N had a significant positive effect on *R* for *P. arundinacea* ($p = 0.023$), but there was no significant effect on *R* for *C. stricta* (Table 1).

The N supplied affected the total leaf soluble protein content, an estimation of the metabolic protein content, to the same degree for both species ($p = 0.862$, Fig. 1B). However, over the range of N concentrations supplied, the leaf soluble protein content was significantly ($p < 0.0001$) greater for *P. arundinacea* than for *C. stricta*. There was no effect of experiment (winter–spring *vs.* spring–summer).

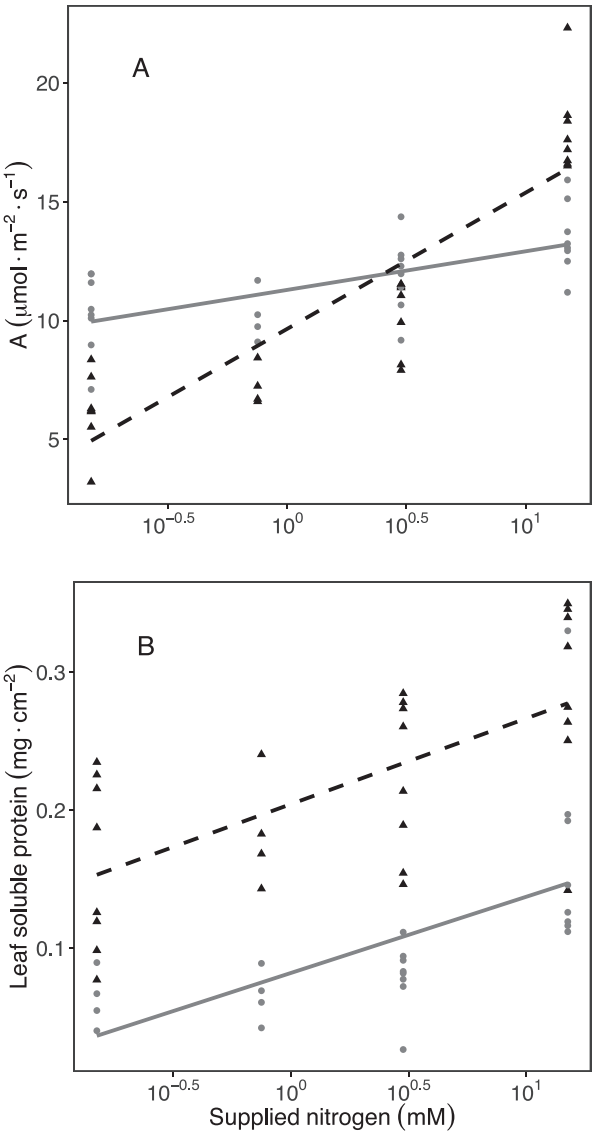

Fig. 1. The response of the rate of CO_2 assimilation (*A*, panel A) and the leaf soluble protein content (panel B) to growth at different concentrations of supplied nitrogen ($\log_{10}$) for *P. arundinacea* (triangles and dashed black line) and *C. stricta* (circles and solid gray line). There was a species by supplied nitrogen interaction ($p < 0.0001$) for *A* but not for soluble protein. Species soluble protein content was significantly different ($p < 0.0001$), but there was no effect of experiment (winter–spring *vs.* spring–summer).

The response of leaf nitrogen content to supplied nitrogen concentration for P. arundinacea and C. stricta

Leaf N on an area basis exhibited a significant and positive response to the concentration of supplied N ($p = 0.0005$) for both species (Fig. 2A and B). There was no difference in slope

Table 1
Rates of dark respiration (*R*) at 25 °C measured during the middle of the photoperiod 6 to 7 weeks after each nitrogen treatment was commenced for *P. arundinacea* and *C. stricta*. The values are means ± standard deviation, $N = 4$. Different letters indicate that the means are significantly different ($p < 0.05$).

Species	Nitrogen treatment (mM)	Respiration (*R*) (μmol m^{-2} s^{-1})
P. arundinacea	0.15	0.60 ± 0.12b
P. arundinacea	15.0	1.22 ± 0.30a
C. stricta	0.15	1.04 ± 0.41a
C. stricta	15.0	0.88 ± 0.25a

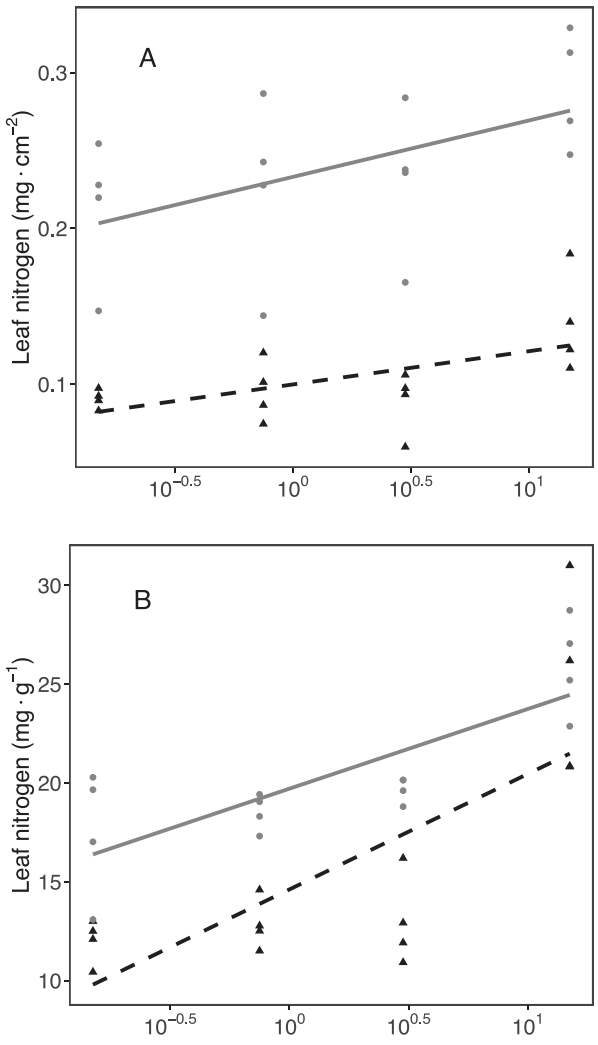

Fig. 2. The response of leaf nitrogen content on a leaf area (panel A) and on a leaf dried mass (panel B) basis to growth at different concentrations of supplied nitrogen ($\log_{10}$) for *P. arundinacea* (triangles and dashed black line) and *C. stricta* (circles and solid gray line). There was no species by supplied nitrogen interaction on an area basis but a significant interaction on a mass basis ($p = 0.0304$). Species differed with respect to the leaf nitrogen content on both bases ($p < 0.0001$).

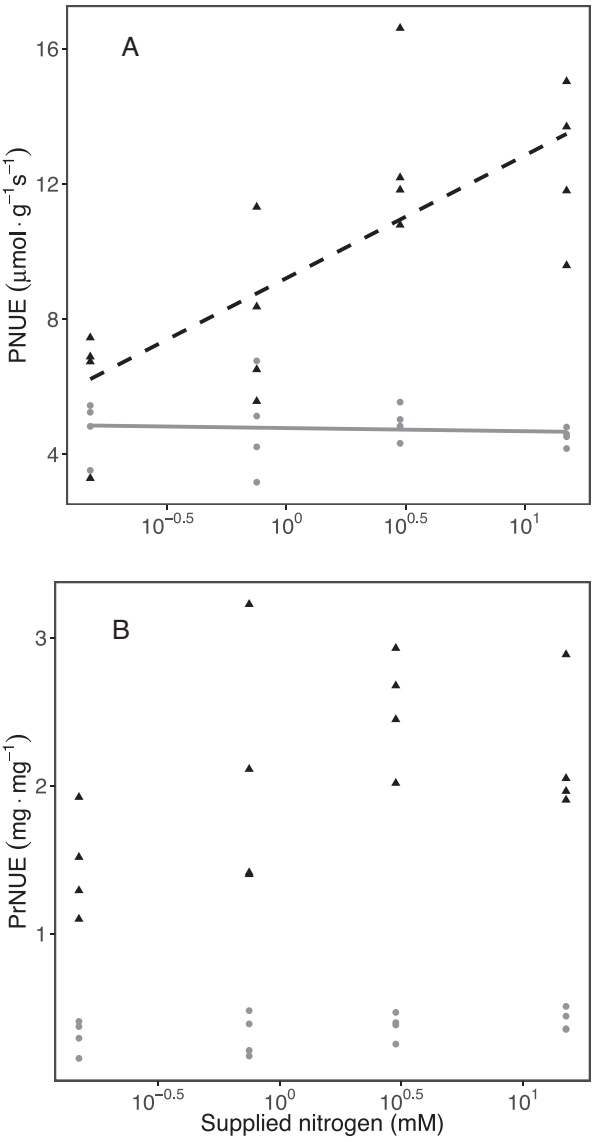

Fig. 3. The response of photosynthetic nitrogen use efficiency (PNUE, panel A) and the soluble protein nitrogen use efficiency (PrNUE, panel B) to growth at different concentrations of supplied nitrogen ($\log_{10}$) for *P. arundinacea* (triangles and dashed black line) and *C. stricta* (circles and solid gray line). There was a significant species difference in PNUE and PrNUE ($p < 0.0001$). There was a species by supplied nitrogen interaction for PNUE ($p = 0.0017$) but not for PrNUE.

between the species' responses (no significant interactions). On a mass basis, leaf N content also exhibited a significant and positive response to the concentration of supplied N ($p < 0.0001$), and *P. arundinacea* exhibited a steeper response (species by supplied N interaction, $p = 0.0304$). *C. stricta* had significantly higher leaf N than did *P. arundinacea* across all levels of supplied N in these experiments ($p < 0.0001$) on both an area and a mass basis. Although the concentration of supplied N had a significant negative effect on C:N ($p < 0.0001$, Fig. S1), neither the magnitude nor the slope of the response differed significantly between species. When plants were grown for 5 months without any N after an initial 4 weeks at 15 mM N, the leaf N for young, fully-expanded leaves of growing tillers was similar for both species, being 0.086 ± 0.025 and 0.165 ± 0.039 mg N cm^{-2} (18.47 ± 3.18 and 17.66 ± 4.54 mg N g^{-1}) for *P. arundinacea* and *C. stricta*, respectively.

The response of PNUE and PrNUE to supplied nitrogen concentration for P. arundinacea and C. stricta

The NUE was calculated to explore the relationships of *A* and soluble protein to leaf N content (*i.e.,* *A*/leaf N content (g) per leaf area and mg soluble protein/mg leaf N), allowing an estimation of species differences in N allocation to leaf processes at the different levels of supplied N (Fig. 3). A high NUE with respect to *A* (PNUE) or soluble protein (PrNUE) reflects a greater proportion of N allocated to photosynthesis or the soluble protein pool, respectively, than to other processes or N pools. The PNUE was greater for *P. arundinacea* than for *C. stricta* ($p < 0.0001$, Fig. 3A), and the supplied N concentration had a greater positive effect on PNUE for *P. arundinacea* than for *C. stricta* (species by supplied N interaction, $p = 0.0017$). At all concentrations of N supplied, PrNUE was greater for *P. arundinacea* than for *C. stricta* ($p < 0.0001$) (Fig. 3B). However, supplied N did not have a significant effect on PrNUE, indicating that the proportion of N in soluble proteins relative to the total leaf N content, does not change significantly when these plants are grown at different concentrations of supplied N.

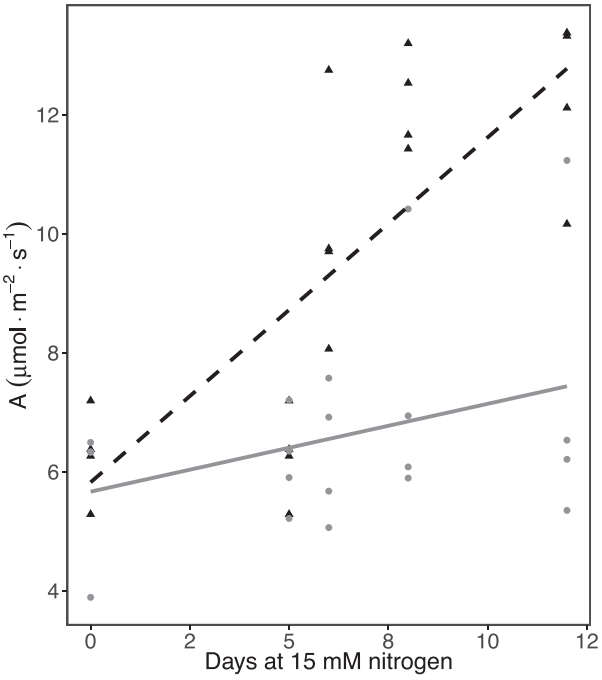

Fig. 4. The change in the rate of CO_2 assimilation (A) for *P. arundinacea* (triangles and dashed black line) and *C. stricta* (circles and solid gray line) over a 12-d period when the plants were supplied 15 mM nitrogen following 4 months when the plants were supplied 0.15 mM nitrogen. There was a significant species difference in the change in A with time ($p = 0.004$).

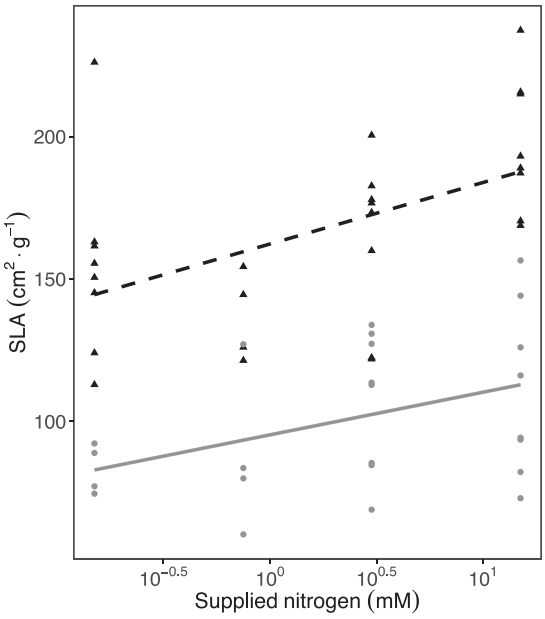

Fig. 5. The response of the specific leaf area (SLA) to growth at different concentrations of supplied nitrogen ($\log_{10}$) for *P. arundinacea* (triangles and dashed black line) and *C. stricta* (circles and solid gray line). There was a significant species difference ($p < 0.0001$), and there was a significant species by supplied nitrogen interaction ($p = 0.0090$).

The response of A to an increase in nitrogen supply following growth at 0.15 mM nitrogen for P. arundinacea and C. stricta

After growing plants of both species for 4 months with only 0.15 mM N, followed by 10 d in larger pots to grow new roots, values of A were similar for both species (Fig. 4), indicating that *C. stricta* cannot maintain A under N deprivation indefinitely. The values of A for both species responded positively to the addition of 15 mM N every 3 d over the next 12 d ($p < 0.0001$, Fig. 4), but the response of A for *P. arundinacea* was significantly greater than the response of A for *C. stricta* ($p = 0.004$). Over the 12 d of the experiment, the total N content in the young leaves of *P. arundinacea* increased from 16.0 ± 0.9 to 26.9 ± 2.6 mg g^{-1} dry mass (0.08 ± 0.01 to 0.10 ± 0.01 mg cm^{-2} leaf area), which was 109% of the value for leaves of plants grown for 7 weeks with 15 mM N supplied (Fig. 2A). During this period, the total N content of the young leaves of *C. stricta* increased from 12.2 ± 2.4 to 16.3 ± 1.4 mg g^{-1} dry mass (0.14 ± 0.01 to 0.2 ± 0.03 mg cm^{-2} leaf area), which was 63% of the value for leaves of plants grown for 7 weeks with 15 mM N supplied. More importantly, the PNUE for *P. arundinacea* increased from 7.28 ± 0.45 to 12.10 ± 0.28 μmol CO_2 (g N)$^{-1}$ s^{-1}, whereas the PNUE for *C. stricta* declined slightly from 4.44 ± 0.27 to 3.10 ± 0.73 μmol CO_2 (g N)$^{-1}$ s^{-1}.

The response of leaf morphological parameters to supplied nitrogen concentration for P. arundinacea and C. stricta

The supplied N concentrations had no significant effect on the development of the total area of individual leaves for *P. arundinacea* and *C. stricta* over the 6 to 7 week period (not shown). Over all of the supplied N concentrations, *P. arundinacea* had maintained a significantly greater SLA than *C. stricta* ($p < 0.0001$) (Fig. 5). The SLA for *P. arundinacea* was more sensitive to supplied N concentration than was the SLA for *C. stricta* (species by supplied N interaction, $p = 0.0090$). For SLA, there was a significant effect of the timing of the experiments (winter–spring *vs.* spring–summer) ($p < 0.0001$).

The response of photosynthetic parameters, chlorophyll, and leaf nitrogen to low nitrogen supply in the second experiment

In the second experiment, providing 15 mM N for 9 weeks did increase the N content of the young leaves relative to the N content measured in the first set of experiments for both species but primarily for *P. arundinacea* (Table 2 and Fig. 2B). There was also an increase in SLA and a decrease in the C:N values (Table S1). However, the proportional changes in the values of all of these parameters after 6 weeks of growth with 0.15 mM N supplied were similar to the changes observed in the first experiments.

To further assess the responses of specific components of photosynthesis for *C. stricta*, *C. lacustris*, and *P. arundinacea* to low N availability, *A vs. Ci* analyses were performed on young and old leaves of plants of all three species before and after 6 weeks of growth at 0.15 mM supplied N. Growth for 6 weeks at 0.15 mM supplied N significantly reduced the V_{cmax} and CE ($p < 0.0001$) for young and old leaves for all species, indicating that both sets of leaves had reduced *in vivo* Rubisco activity (Fig. 6A and B). The largest decreases of these two parameters occurred for the young leaves of *P. arundinacea* and *C. lacustris*, being approximately 60 to 70%, whereas for *C. stricta* young leaves, the decrease was only approximately 24%. The values of J_{max} were also negatively affected significantly for all species ($p = 0.0001$) in young and old leaves, indicating a reduction in electron transport capacity (Fig. 6C). For the young leaves of *P. arundinacea* and *C. lacustris*, the reduction in J_{max} was 53 and 58%, respectively, whereas it was only 20% for the young leaves of *C. stricta*. In addition, growth at 0.15 mM supplied N negatively affected the chlorophyll content of *P. arundinacea* leaves ($p < 0.0001$) but not for the *Carex* species, with no effect of leaf age for all species ($p = 0.1424$) (Fig. 6D).

There was no main effect of the age of a leaf on A measured at near ambient pCO_2 (A_{amb}, 40 Pa) or at CO_2 saturation (A_{max}, 200 Pa) under either N treatment for any species, but there was an

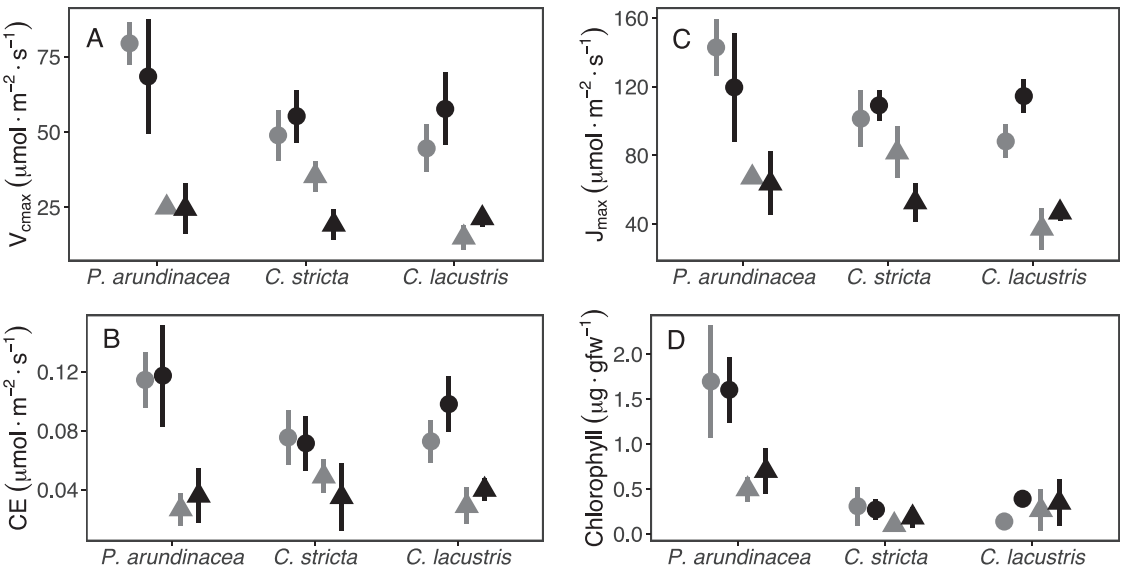

Fig. 6. The maximum carboxylation capacity (V_{cmax}, panel A), carboxylation efficiency (CE, panel B), maximum electron transport capacity (J_{max}, panel C), and chlorophyll content (panel D) for young (lightly shaded) and old (darkly shaded) leaves of *P. arundinacea*, *C. stricta*, and *C. lacustris* when grown with 15 mM supplied nitrogen for 9 weeks (circles) followed by growth with 0.15 mM supplied nitrogen for 6 weeks (triangles). The values are means ± standard deviation ($N = 5$). There was a significant negative effect of growth at low supplied nitrogen on V_{cmax}, CE, and J_{max} ($p \leq 0.0001$). There was a significant negative effect of low supplied nitrogen on chlorophyll content for *P. arundinacea* ($p < 0.0001$) but no effect for *C. stricta*.

interaction of leaf age and species as well as leaf age and N treatment for all species ($p = 0.0002$ and $p = 0.0001$ for A_{amb} and $p < 0.0001$ and $p = 0.0272$ for A_{max}, respectively) (Table 3). Therefore, consistent with the response of *A* to low N supply in the first set of experiments (Fig. 1A), growth for 6 weeks with 0.15 mM N supplied reduced *A* measured near ambient pCO_2 (40 Pa) by only 14% for young *C. stricta* leaves, whereas it reduced *A* by 69 and 62% for young leaves of *P. arundinacea* and *C. lacustris*, respectively, and by 53% for the old leaves of *C. stricta* (Table 2). Similarly, the values of *A* at CO_2 saturation for young leaves of *C. stricta* were reduced less than they were for the other two species (Table 2). Comparable to the results of the first set of experiments, the young leaves and even the old leaves of *C. stricta* maintained slightly increased PNUE when grown at 0.15 mM N supplied, whereas the PNUE decreased 50 to 60% for both sets of leaves of *P. arundinacea* and *C. lacustris* (Table 2).

The response of leaf nitrate accumulation to low nitrogen supply

When grown for 8 weeks at 15 mM supplied N, the young and old leaves of *P. arundinacea* and *C. stricta* and the young leaves of *C. lacustris* accumulated comparable amounts of nitrate (Table 2). However, the nitrate accumulation for the old leaves of *C. lacustris* was considerably less. Unexpectedly, over the 6 weeks of growth at 0.15 mM supplied N, the nitrate content in the young and old leaves increased for plants of *P. arundinacea* or did not change for plants of *C. stricta*. Only for the young leaves of *C. lacustris* was there a large decrease in the nitrate content with growth at low N.

Discussion

Phalaris arundinacea and C. stricta exhibit essentially opposite responses of leaf primary metabolism to nitrogen supply over 6 to 7 weeks

A high abundance of the invasive grass, *P. arundinacea*, is associated with moderate to high soil N (Martina and von Ende, 2008; Bartodziej, 2011), and its competitive success in stands of *Carex* species is dependent, to a large extent, on a high N supply (Green and Galatowitsch, 2002; Perry et al., 2004). These findings strongly suggest that this species is an opportunist, taking advantage of increased N to outcompete sedges, such as *C. stricta*. Our finding that *P. arundinacea* and *C. stricta* respond differently to high N supply with respect to *A* and PNUE is consistent with the results of these earlier studies. At high N availability, the strategy of N use for *P. arundinacea* is to maximize carbon gain, as indicated by its high PNUE, which is typical for many invasive species (Feng, 2008a,b; Feng et al., 2008, 2009, 2011). The high PNUE is associated with a high carboxylation capacity and efficiency (V_{cmax} and CE), a high capacity for electron transport (J_{max}), and a high capacity for CO_2-saturated photosynthesis. It is likely that the high leaf soluble protein content is due to a high allocation of N to Rubisco and other enzymes of photosynthesis and respiration (Kant et al., 2011). Thus, it is not surprising that *P. arundinacea* thrives in N-rich soils, as do most crop species. On the other hand, *C. stricta* allocates proportionately less N to photosynthesis than *P. arundinacea* (a lower PNUE) resulting in a lower *A* measured at ambient or at saturating CO_2 due to a lower carboxylation capacity and efficiency and a lower electron transport capacity. Studies of some other species with these traits indicate that, compared to species with a high PNUE, proportionately more N is being allocated to proteins of leaf cell walls or possibly vegetative storage proteins than to metabolic proteins (Onoda et al., 2004; Funk et al., 2013). Given the low SLA for *C. stricta* leaves, such N allocation may reflect an emphasis on protection from herbivores or the existence of many, diverse N sinks, but a thorough investigation of cellular allocation of N in *C. stricta* leaves is required to verify this idea. We propose that it is the emphasis on maximizing carbon gain by *P. arundinacea* to a greater degree than *C. stricta* in N-rich conditions that supports the rapid growth needed to out-compete *C. stricta*.

P. arundinacea demonstrates considerable leaf physiological plasticity when switched to a low N supply for a 6- to 7-week period. Associated with a decline in leaf N content and protein is a decline in leaf primary metabolism, whether the leaves are newly formed (the young leaves) or were formed prior to the switch to a low N supply (the old leaves). Carboxylation and electron transport capacity, chlorophyll content, and CO_2-saturated *A* are all strongly negatively affected, indicating diminished support of all components

Table 2
Leaf nitrogen (N) content, photosynthetic nitrogen use efficiency (PNUE), and leaf nitrate (NO_3^-) content after 9 weeks of growth with 15 mM supplied nitrogen and after an additional 6 weeks of growth with 0.15 mM supplied nitrogen for young and old leaves of *P. arundinacea*, *C. stricta*, and *C. lacustris* ($N = 5$). For all parameters, there was a significant difference between species ($p < 0.005$). For leaf N and PNUE there were significant differences between treatments ($p < 0.001$) and an interaction between species and treatment ($p < 0.0001$). There was no effect of age for any of the parameters or a treatment affect in leaf nitrate.

N treatment	*Phalaris arundinacea*				*Carex stricta*				*Carex lacustris*			
	High N (15 mM)		Low N (0.15 mM)		High N (15 mM)		Low N (0.15 mM)		High N (15 mM)		Low N (0.15 mM)	
Leaf age	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old
Leaf N ($mg\,g^{-1}$)	45.6 ± 6.56	44.9 ± 5.60	19.5 ± 3.02	19.0 ± 3.22	29.6 ± 5.06	30.3 ± 3.80	15.1 ± 1.39	15.1 ± 3.65	23.0 ± 6.27	33.6 ± 2.74	16.2 ± 5.29	16.2 ± 1.43
PNUE ($\mu mol\ s^{-1}\ g\,N^{-1}$)	11.3 ± 2.60	10.7 ± 2.01	4.83 ± 1.63	5.31 ± 2.10	5.29 ± 2.24	4.07 ± 2.05	8.40 ± 4.37	5.29 ± 3.77	5.66 ± 3.08	5.80 ± 0.83	2.27 ± 0.60	3.77 ± 0.54
Leaf NO_3^- ($mMol\,cm^{-2}$)	0.648 ± 0.26	0.971 ± 0.31	1.51 ± 0.67	1.46 ± 0.54	2.37 ± 1.69	1.46 ± 0.50	2.05 ± 0.70	2.04 ± 0.65	2.56 ± 1.66	0.600 ± 0.21	0.844 ± 0.41	0.928 ± 0.36

Table 3
The rate of CO_2 assimilation (A, $\mu mol\,CO_2\,m^{-2}\,s^{-1}$) at near ambient pCO_2 (40 Pa, A_{amb}) and the CO_2-saturated A (A_{max}, 200 Pa) after 9 weeks of growth with 15 mM supplied nitrogen and after an additional 6 weeks of growth with 0.15 mM supplied nitrogen for young and old leaves of *P. arundinacea*, *C. stricta*, and *C. lacustris* ($N = 5$). For both parameters, there were significant differences between species ($p < 0.0001$) and treatment ($p < 0.0001$), as well as interaction between species and treatment ($p < 0.0002$) and between treatment and age ($p = 0.0001$). There was no significant effect of leaf age for either parameter.

N treatment	*Phalaris arundinacea*				*Carex stricta*				*Carex lacustris*			
	High N (15 mM)		Low N (0.15 mM)		High N (15 mM)		Low N (0.15 mM)		High N (15 mM)		Low N (0.15 mM)	
Leaf age	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old
A_{amb}	19.6 ± 1.32	18.2 ± 1.82	5.63 ± 1.13	6.13 ± 2.23	10.7 ± 1.74	11.3 ± 2.07	9.21 ± 1.61	5.37 ± 1.82	8.90 ± 2.51	14.0 ± 1.36	3.49 ± 0.74	5.31 ± 0.27
A_{max}	32.5 ± 3.58	28.9 ± 4.42	14.7 ± 2.82	14.6 ± 4.28	22.7 ± 2.75	24.6 ± 2.27	18.9 ± 3.04	12.7 ± 3.26	20.2 ± 1.46	28.6 ± 1.74	8.81 ± 2.68	11.8 ± 1.19

of photosynthesis. In this regard, *P. arundinacea* responded much as major crop species would to low N supply (Kant et al., 2011). The plastic response of photosynthesis to changes in N supply for *P. arundinacea* is consistent with mesocosm experiments showing large changes in total biomass, shoot:root ratio, plant height, tiller number, and total leaf area with changes in nutrient supply (Miller and Zedler, 2003; Herr-Turoff and Zedler, 2007). The decline in *R* that we measured for *P. arundinacea* indicates a shift of N allocation away from proteins of primary metabolism, in general, not just from photosynthesis. Thus, *P. arundinacea* sacrifices considerable carbon gain and leaf respiratory activity in favor of other processes, possibly in other parts of the plant, such as roots. *P. arundinacea* has been observed to develop a larger root system along with a slowing of shoot growth under nutrient deficiency, presumably to improve nutrient absorption (Mauer and Zedler, 2002).

In contrast to the situation with *P. arundinacea*, primary metabolism in young *C. stricta* leaves is less plastic (less responsive) to growth at low N supply. *C. stricta* supports carboxylation and electron transport processes of photosynthesis, as well as respiratory metabolism in young leaves in the first 6 to 7 weeks of low N supply, but not for an extended period of 4 months. What is important to keep in mind is that this slower decline in primary metabolism occurs despite a decline in total leaf N content and a decline in leaf soluble protein similar to the declines in *P. arundinacea* leaves. Given that PNUE does not decrease and even increases in one of our experiments, *C. stricta* is clearly changing its N allocation strategy when N availability is low so that carbon gain and leaf respiration are supported. Thus, leaf primary metabolism is an important focus of N allocation in young *C. stricta* leaves during N deprivation, at least in the short term.

As we hypothesized, when *P. arundinacea* experiences a high N supply following prolonged N deprivation, leaf N content increases rapidly to equal the N content of plants grown for 6 weeks with a high N supply. More importantly, *P. arundinacea* allocates a high proportion of the new N absorbed to photosynthesis, as indicated by a rise in PNUE, further attesting to the plasticity of these traits for this invasive species. In contrast, the N content of young leaves of *C. stricta* rises more slowly. In addition, increasing *A* is not the main focus of N allocation for *C. stricta*. Thus, *C. stricta* allocates a considerable portion of the newly assimilated N to processes other than carbon gain. This slow response of *A* to an increase in N supply may explain, at least in part, the small effect (only 10 to 15%) that a high application of N and phosphorous fertilizer had on plant biomass for *C. stricta* (Lawrence and Zedler, 2011) in mesocosms.

C. lacustris is interesting in that its N allocation to photosynthesis (PNUE) is similar to that for *C. stricta* at high N supply but similar to that for *P. arundinacea* at low N supply. This discovery indicates that the disparate strategies of N allocation typified by *C. stricta* and *P. arundinacea* can overlap and are not mutually exclusive. The poor response of photosynthesis for *C. lacustris* when N supply is low suggests that this large, stout *Carex* species may be abundant only where N supply is sufficient to sustain substantial photosynthetic capacity. Therefore, in wetlands with high N availability, it is reasonable to speculate that *C. lacustris* may possess mechanisms to allow it to flourish in sites not favorable to *P. arundinacea*, such as water-saturated soils, as we have observed in wetlands of the central U.S.A. (A.S. Holaday and E.F. Waring, unpublished observations).

Possible mobilization of nitrogen reserves

Plants have the ability to mobilize N from organic N sources (Rubisco, non-metabolic storage proteins, other organic N compounds, such as polyamines) and possibly accumulated nitrate (Staswick, 1994; Aerts and Chapin, 2000; Diaz et al., 2008; Kant et al., 2011). Compared to young leaves, the greater decline in photosynthetic parameters for the old leaves of *C. stricta*, suggests that their metabolic proteins, such as Rubisco, are serving as a source of N for the young leaves. However, PNUE is maintained after 6 to 7 weeks of N deprivation in the old leaves to nearly the same degree as in the young leaves. Thus, the photosynthetic proteins are not being degraded to a proportionally greater extent than other sources of N. Contrary to our expectation, accumulated leaf nitrate does not serve as a net source of N for the leaves of *P. arundinacea* and *C. stricta*, as it may serve in the young leaves of *C. lacustris*, during N deprivation.

Conclusions

We propose that the link between N availability and the abundance of *P. arundinacea* is due, at least partially, to this species' physiological plasticity of N allocation, emphasizing allocation to photosynthesis when N supply is high and shifting N allocation to other processes when N supply is low. For this invasive species, a high N supply favors more carbon gain to support the increase in above-ground biomass needed to shade competitors, such as *C. stricta*. At low N supply, *P. arundinacea* sacrifices high carbon gain for the maintenance of other processes, diminishing its ability to out-compete native sedges, but possibly allowing for persistence. *Carex stricta* lacks plasticity with respect to photosynthetic and respiratory metabolism during short-term N deprivation by emphasizing N allocation to these processes despite a decline in total leaf N in young leaves. Such a response to N supply appears to be an adaptation to wetland conditions in which brief periods of moderate or high N availability are separated by longer periods of low N availability. We hypothesize that, in N-enriched wetlands, *C. stricta*'s inability to attain a high level of N allocation to carbon gain reduces its competitive potential relative to opportunistic, rapidly-growing species. This problem may also exist for *C. lacustris*. Our results support the contention of Perry et al. (2004) that a realistic management practice to control *P. arundinacea* invasion into *C. stricta* wet meadows is to reduce N availability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2015.01.008.

References

Adamidis GC, Kazakou E, Fyllas NM, Dimitrakopoulos PG. Species adaptive strategies and leaf economic relationships across serpentine and non-serpentine habitats on Lesbos, eastern Mediterranean. PLoS ONE 2014;9:e96034.

Aerts R, de Caluwe H. Inter-specific and intra-specific differences in shoot and leaf life span of four *Carex* species which differ in maximum dry matter production. Oecologia 1995;102:467–77.

Aerts R, Chapin FS III. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 2000;30:1–67.

Bartodziej WM. Assessing urban wetland soils for reed canary grass management (Minnesota). Ecol Restor 2011;29:329–31.

Berendse F, Aerts R. Nitrogen-use-efficiency: a biological meaningful definition. Funct Ecol 1987;1:293–6.

Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

Catalado DA, Harron M, Schrader LE, Youngs VL. Rapid colormetric determination of nitrate in plant tissue by nitrification of salicyclic acid. Commun Soil Sci Plant Anal 1975;6:71–80.
Davidson AM, Jennions M, Nicotra AB. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol Lett 2011;14:419–31.
Diaz C, Lemaitre T, Christ A, Azzopardi M, Kato Y, Sato F, et al. Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in Arabidopsis under low nitrogen nutrition. Plant Physiol 2008;147:1437–49.
Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jalili A, et al. The plant traits that drive ecosystems: evidence from three continents. J Veg Sci 2004;15:295–304.
Duursma R. Plantecophys modelling and analysis of leaf gas exchange data. In: Version 0.4. R Package; 2013.
Evans JR. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 1989;78:9–19.
Feng Y-L. Nitrogen allocation and partitioning in invasive and native *Eupatorium* species. Physiol Plant 2008a;132:350–8.
Feng Y-L. Photosynthesis, nitrogen allocation and specific leaf area in invasive *Eupatorium adenopphorum* and native *Eupatorium japonicum* grown at different irradiances. Physiol Plant 2008b;133:318–26.
Feng Y-L, Fu G-L, Zheng Y-L. Specific leaf area relates to the differences in leaf construction cost, photosynthesis, nitrogen allocation, and use efficiencies between invasive and noninvasive alien congeners. Planta 2008;228:383–90.
Feng Y-L, Lei Y-B, Wang R-F, Callaway RM, Valiente-Banuet A, et al. Inderjit Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. Proc Nat Acad Sci USA 2009;106:1853–6.
Feng Y-L, Li Y-P, Wang R-F, Callaway RM, Valiente-Banuet A, Inderjit. A quicker return energy-use strategy by populations of a subtropical invader in the non-native range: a potential mechanism for the evolution of increased competitive ability. J Ecol 2011;99:1116–23.
Funk JL, Glenwinkel LA, Sack L. Differential allocation to photosynthetic and non-photosynthetic nitrogen fractions among native and invasive species. PLoS ONE 2013;8:e64502.
Green EK, Galatowitsch SM. Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities. J Appl Ecol 2002;39:134–44.
He Z, Bentley LP, Holaday AS. Greater seasonal carbon gain across a broad temperature range contributes to the invasive potential of *Phalaris arundinacea* (Poaceae; reed canary grass) over the native sedge *Carex stricta* (Cyperaceae). Am J Bot 2011;98:20–30.
Herr-Turoff A, Zedler JB. Does morphological plasticity of the *Phalaris arundinacea* canopy increase invasiveness. Plant Ecol 2007;193:265–77.
Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. Calif Agric Exp Stn Circ 1950;347:4–9.
Kant S, Bi Y-M, Weretilnyk E, Barak S, Rothstein SJ. The Arabidopsis halophytic relative *Thellungiella halophila* tolerates nitrogen-limiting conditions by maintaining growth, nitrogen uptake, and assimilation. Plant Physiol 2008;147:1168–80.
Kant S, Bi Y-M, Rothstein SJ. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J Exp Bot 2011;62:1499–509.
Kercher SM, Herr-Turoff A, Zedler JB. Understanding invasion as a process: the case of *Phalaris arundinacea* in wet prairies. Biol Invasions 2006;9:657–65.
Kercher SM, Zedler JB. Multiple disturbances accelerate invasion of reed canary grass (*Phalaris arundinacea* L.) in a mesocosm study. Oecologia 2004;138:455–64.
Lawrence BA, Zedler JB. Formation of tussocks by sedges: effects of hydroperiod and nutrients. Ecol Appl 2011;21:1745–59.
Leishman MR, Haslehurst T, Ares A, Baruch Z. Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. New Phytol 2007;176:635–43.
Lichtenthaler HK. Chlorophylls and carotenoids. Pigments of photosynthetic biomembranes. Meth Enzymol 1987;148:350–82.
Lindig-Cisneros RA, Zedler JB. Relationships between canopy complexity and germination microsites for *Phalaris arundinacea* L. Oecologia 2002;133:159–67.
Martin T, Oswald O, Graham IA. Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. Plant Physiol 2002;128:472–81.
Martina JP, von Ende CN. Correlation of soil nutrient characteristics and reed canarygrass (*Phalaris arundinacea*: Poaceae) abundance in northern Illinois (USA). Am Midl Nat 2008;160:430–7.
Mauer DA, Zedler JB. Differential invasive of a wetland grass explained by test of nutrients and light availability on establishment and clonal growth. Oecologia 2002;131:279–88.
Miller RC, Zedler JB. Responses of native and invasive wetland plants to hydroperiod and water depth. Plant Ecol 2003;167:57–69.
Onoda Y, Hikosaka K, Hirose T. Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. Funct Ecol 2004;18:419–25.
Perry LG, Galatowitsch SM, Rosen CJ. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. J Appl Ecol 2004;41:151–62.
Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Development Core Team. nlme: linear and nonlinear mixed effects models. In: Version 3.1-1184. R Package; 2014.
Poorter H, Evans JR. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 1998;116:26–37.
R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014, ⟨http://www.R-project.org/⟩.
Reich PB. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. J Ecol 2014;102:275–301.
Reich PB, Wright IJ, Craines JM, Oleksyn J, Westoby M, Walters MB. The evolution of plant functional variation: traits, spectra and strategies. Int J Plant Sci 2003;164:S143–64.
Rickey MA, Anderson RC. Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. J Appl Ecol 2004;41:888–96.
Sorrell BK, Brix H, Fitridge I, Konnerup D, Lambertini C. Gas exchange and growth responses to nutrient enrichment in invasive *Glyceria maxima* and native New Zealand *Carex* species. Aquat Bot 2012;103:37–47.
Staswick PE. Storage proteins of vegetative plant tissue. Annu Rev Plant Physiol Plant Mol Biol 1994;45:303–22.
Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. Plant ecological strategies: some leading dimensions of variation between species. Annu Rev Ecol Syst 2002;33:125–59.
Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, et al. The worldwide leaf economics spectrum. Nature 2004;428:821–7.
Zedler JB. Feedbacks that might sustain natural, invaded and restored states in herbaceous wetlands. In: Hobbs R, Suding KN, editors. New models for ecosystem dynamics and restoration. Washington, DC: Island Press; 2009. p. 236–58.
Zedler JB, Kercher S. Causes and Consequences of Invasive plants in wetlands: opportunities, opportunists, and outcomes. Crit Rev Plant Sci 2004;23:7–22.