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Nutrient limitation and morphological plasticity of the carnivorous pitcher plant *Sarracenia purpurea* in contrasting wetland environments

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Summary

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- Plasticity of leaf nutrient content and morphology, and macronutrient limitation were examined in the northern pitcher plant, *Sarracenia purpurea* subsp. *purpurea*, in relation to soil nutrient availability in an open, neutral pH fen and a shady, acidic ombrotrophic bog, over 2 yr following reciprocal transplantation of *S. purpurea* between the wetlands.

- In both wetlands, plants were limited by nitrogen (N) but not phosphorus (P) (N content < 2% DW⁻¹, N : P < 14) but photosynthetic quantum yields were high ($F_V/F_M > 0.79$). Despite carnivory, leaf N content correlated with dissolved N availability to plant roots (leaf N vs NO₃⁻, $r^2 = 0.344$, $P < 0.0001$); carnivorous N acquisition did not apparently overcome N limitation.

- Following transplantation, N content and leaf morphological traits changed in new leaves to become more similar to plants in the new environment, reflecting wetland nutrient availability. Changes in leaf morphology were faster when plants were transplanted from fen to bog than from bog to fen, possibly reflecting a more stressful environment in the bog.

- Morphological plasticity observed in response to changes in nutrient supply to the roots in natural habitats complements previous observations of morphological changes with experimental nutrient addition to pitchers.

Key words: bog, fen, morphological plasticity, nitrogen, nutrient stoichiometry, phosphorus, *Sarracenia purpurea* (pitcher plant), wetland.

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Introduction

The carnivorous northern pitcher plant, *Sarracenia purpurea* L. subsp. *purpurea* Wherry (hereafter *S. purpurea*), is a long-lived herb that is widespread in North American wetlands (Ellison *et al.*, 2004). *S. purpurea* produces ‘pitchers’, modified leaves which have dual roles in photosynthesis and in the capture of insects to supplement mineral nutrition (Newell & Nastase, 1998; Kneitel & Miller, 2002; Anderson & Midgley, 2003; Ellison *et al.*, 2003), but the quantitative contributions of carnivory vs acquisition through roots to pitcher plant nutrition are unclear (Adamec, 1997; Miller & Kneitel, 2005).

Sarracenia purpurea shows marked plasticity in leaf form across latitudinal and environmental gradients (Ellison *et al.*, 2004). Morphology changes can reflect a trade-off between optimization for light capture and carnivory (Mandossian, 1966; Givnish *et al.*, 1984; Ellison & Gotelli, 2001, 2002). Ellison & Gotelli (2002) showed that when mineral nutrition limitation is alleviated by inorganic nutrient supply directly to pitchers, wider pitcher wings and flattened leaves result, which decrease prey capture efficiency but increase the capacity for photosynthesis. However, it is unknown whether leaf morphology can be similarly regulated by changes in nutrient uptake by the roots resulting from disturbance, changing hydrological regimes and atmospheric deposition (Mitsch & Gosselink,

2000; Hogan & Walbridge, 2007). In aquatic bladderworts, where investment in carnivory can be easily quantified, variable investment in carnivorous structures was shown to be more responsive to range of water nutrient concentrations than to prey availability (Knight & Frost, 1991). It is also unclear how closely *S. purpurea* plant nutrient content reflects nutrient availability to the roots, or whether carnivorous nutrient acquisition can significantly buffer variable wetland nutrient availability.

Sarracenia purpurea subsp. *purpurea* (Godt & Hamrick, 1999) thrives in adjacent acidic bog and neutral pH fen environments in south-east Wisconsin. In these contrasting wetlands, *S. purpurea* shows distinct morphology with short, upright and open pitchers in the fen but elongated pitchers and 'phyllode' leaves (*sensu* Schnell, 2002) in the bog. The presence of these morphologically distinct populations of *S. purpurea* plants in contrasting environments provides an opportunity for studying the role of soil nutrient availability and wetland conditions in plant morphological plasticity. Genotypic and phenotypic differences have been classically differentiated between populations using reciprocal transplant experiments (Clausen *et al.*, 1940). We followed nutrient content and leaf morphology of *S. purpurea* plants for 2 yr after reciprocal transplantation between the two contrasting wetlands. This study aimed to examine how the differences in macronutrient availability and physical environment between the fen and the bog influence leaf nutrient content, nutrient limitation and morphology of *S. purpurea*. We addressed these aims by focusing on three key questions:

- Is nutrient content of *S. purpurea* leaves limited by nutrient availability to the roots, or can carnivory overcome differences in surface wetland macronutrient availability?
- Is *S. purpurea* leaf morphology correlated with, and responsive to, changes in soil nutrient availability between the two wetlands?
- Does *S. purpurea* leaf morphology respond to current nutrient availability and wetland environment, or is it constrained by genetic identity of the population from which the plants originated?

Materials and Methods

Site descriptions

Cedarburg fen (43°23.2'N, 88°0.63'W) is the largest peatland (c. 1000 ha) in southern Wisconsin and the southernmost string bog in North America (Grittinger, 1970), characterized by low open marshy areas alternating with slightly elevated areas supporting tamaracks (*Larix laricina*) and cedars (*Thuja occidentalis*). The fen has groundwater inputs flowing through limestone glacial till (Reinartz, 1985). *S. purpurea* grows among sedges and grasses in the open marshy and slightly elevated areas. Site access was gained via a boardwalk.

Approximately 2 km from the Cedarburg fen site, but isolated by agricultural landscape, the smaller (3.3 ha) Sapa

Bog (43°23.64'N, 88°1.4'W) is the southernmost black spruce bog in Wisconsin (Kline, 1991) and has no groundwater input. *S. purpurea* grows among *Sphagnum* moss with some shrubs and is shaded by a dense tamarack and black spruce (*Picea mariana*) canopy.

Site characteristics – dissolved nutrients and irradiance

To characterize surface nutrient availability of the wetlands, water samples were collected from near the base of the 60 plants used for the reciprocal transplant experiment in October 2004, and over the following two growing seasons (field collection timetable summarized in the Supporting Information, Table S1). The pH of samples was measured before gravity filtration (coarse P5 filter; Fisher Scientific, Chicago, IL, USA) and then vacuum filtration (GF/A, Whatman Inc., Florham Park, NJ, USA). Filtrates were frozen at -20°C for later dissolved nutrient analysis and later thawed overnight at 4°C. NO₃⁻ plus NO₂⁻ was assayed by spectrophotometric determination of NO₂⁻ following Cd-column reduction (Parsons *et al.*, 1984). Dissolved PO₄³⁻ was assayed by spectrophotometric determination using the ammonium molybdate reaction (Parsons *et al.*, 1984) and measured in a microplate reader (Versa_{max}, Molecular Devices, Sunnyvale, CA, USA). NH₄⁺ was assayed in a fluorescence microplate reader (Spectra_{max} Gemini XS, Molecular Devices) using the method of Holmes *et al.* (1999).

Diel and seasonal changes in irradiance (photosynthetically active radiation (PAR) over the waveband 400–700 nm) were recorded with *in situ* light loggers with 2 pi sensors (Odyssey Data Recorders, Dataflow Systems, Christchurch, New Zealand), at three locations in each wetland to represent low, medium and high irradiance exposure for each wetland. Loggers were on permanent mounts 200–300 mm above the wetland surface. Loggers recorded PAR every 10 min during April–November 2006, and values were integrated over the whole 2006 growing season. The loggers were calibrated against a handheld light meter with 2 pi sensor (Li-250A, Li-Cor Biosciences, Lincoln, NE, USA) at the time of deployment and logger downloading. The irradiance at each of the 60 plants was measured over 2–3 min on a sunny July day and, together with a visual assessment of each plant exposure, the plants were allocated to light-exposure categories characterized as very low (deep shade), low (partial shade), medium low (dappled sun), medium (light shade), high (open canopy) and very high (no shade).

Reciprocal transplant experiment

To investigate the influence of wetland environment on plant morphology, we carried out a reciprocal transplant of *S. purpurea* between the two wetlands on October 25, 2004. Thirty *S. purpurea* plants were selected from each wetland. All 60 plants had leaves emerging from a single apex; plants with

multiple growth apices were avoided. Plants were photographed and flagged with numbers. Plants were randomly assigned to treatment groups: 10 plants from each wetland were assigned as controls; 10 plants were transplanted between wetlands; and a third group of 10 from each wetland was transplanted within the original wetland to control for the effects of transplantation. In each wetland, 20 plants were dug up, conserving the soil around the roots, and placed in clean plastic bags inside plastic pots for protection during transport. Any flower stalks or fruits were removed. To prevent transfer of distinct pitcher inquiline communities between the wetlands, pitchers of all 40 transplants were emptied and sterilized with 10% sodium hypochlorite solution for 5 min, then drained and rinsed thoroughly with clear well water. The between-wetland transplants were planted into holes left by plant removal, and within-wetland transplants were moved to the next available hole within the same wetland. Over the 2 yr experiment, a few plants were lost as a result of natural senescence or herbivory. When a control plant was lost, sample size was restored at the start of the following season by selecting another nearby plant, but when transplants were lost, they were not replaced.

Leaf nutrient stoichiometry

Leaf samples were collected for nutrient content analysis at the time of transplantation, and during the following 2 yr (Table S1). Preliminary analyses of all leaf parts showed the highest nutrient content of mature leaves was in the leaf tip (Bott, 2007) and this part was used for sampling. Leaf tip samples (0.5 cm × 3 cm) were trimmed from a healthy, mature pitcher from each of the 60 plants and air-dried in microcentrifuge tubes in a box with desiccant. Dried samples were crushed and weighed into tin foil cups for CN elemental analysis (Flash EA1112 CE Elantech, Inc., Lakewood NJ, USA). Total P content was determined by ashing dried samples (500°C muffle-furnace for 2 h) and digesting in 0.17 M HCl at 120°C in an autoclave for 30 min (after Stainton *et al.*, 1974), before assay of dissolved phosphate as already described.

Photosynthesis – chl *a* fluorescence

The maximum quantum yield of photosystem II photochemistry (F_V/F_M) was measured *in situ* on 15–24 *S. purpurea* plants growing in the bog and fen in August 2005, using a pulse amplitude modulated fluorometer (Walz GmbH, Effeltrich, Germany) with a fibreoptic held in a leaf clip, 2–3 mm from the leaf surface (White & Critchley, 1999). After 10–15 min dark acclimation, achieved by placing a black plastic bag over the plant, F_V/F_M was measured on mature leaves produced in that season, in conjunction with chl *a* fluorescence-based photosynthesis vs irradiance curves (rapid light curves; White & Critchley, 1999). The curves

were modeled to derive the irradiance required for saturation of photosynthesis using least-squares nonlinear regression to an exponential model in Sigmaplot (Systat Software Inc. San Jose, CA, USA) described by Young & Beardall (2005).

Leaf morphology measurements

Leaf morphological traits (Ellison & Gotelli, 2002) were measured before transplantation in October 2004, and over the next 2 yr (Table S1). For each plant, flowering status was recorded and leaves counted. A tape measure or electronic calipers were used to measure the diameter of the pitcher mouth or aperture, the wing width at the widest point, the combined widest width of pitcher and wing, and the length of the pitcher from the rhizome to the tip, on all green and nonsenesced pitchers (Fig. S1). Plant diameter was recorded for all plants in October each year. If a plant developed a secondary apex over the course of the experiment, morphological measurements and leaf counts were only made on the original apex. For leaf morphology traits, all the leaves on each plant were measured and the median value for each plant was used in analyses. Two ratios were calculated for each leaf on each plant: relative pitcher aperture (= pitcher aperture/pitcher length) and relative wing width (= wing width/(total width of pitcher + wing)), and the median ratio for each plant was used in analyses.

Statistical analysis

Dissolved nutrients Differences in dissolved nutrient over the two growing seasons were analyzed using one-way repeated-measures ANOVA with wetland treatment as the main effect and all sample dates as the time effect. Dates were examined individually when the time effect or wetland × time interaction was significant ($P < 0.05$). Sample size ranged from 24 to 30 per wetland, as during very dry periods some combined samples were collected for plants which were < 1 m apart.

Leaf nutrient stoichiometry and photosynthesis Differences in mean leaf N content, N : P ratios from October 2006, and F_V/F_M and irradiance values required to saturate photosynthesis were compared between bog and fen *S. purpurea* plants using a *t*-test or Mann–Whitney rank-sum test when transformation failed to resolve unequal variances between the groups.

Reciprocal transplant experiment Pretreatment data (before transplantation in October 2004) were not included in the analyses of plant traits (Bott, 2007 reports analysis of pretreatment data). Plant nutrient content and trait data were analyzed by repeated-measures ANOVA, including all sampling dates over both years. If a significant time effect or treatment × time interaction ($P < 0.05$) was observed, one-way ANOVA was run for each sampling date. If the one-way ANOVA indicated a significant treatment effect for a sampling date, treatment

differences were analyzed using *a priori* contrasts (see later). The experimental design included six treatments: control plants, within-wetland transplants, and between-wetland transplants for each of the two wetlands. Initial analysis showed that there were no significant differences in leaf nutrients between controls and within-wetland transplants for either wetland (Bott, 2007), so these two treatments within each wetland were combined for analysis. Initial analysis of the leaf morphology data also indicated that there were no significant differences between control plants and within-wetland transplants for either wetland in year 1, and also for the fen in year 2 (Bott, 2007). However, in October of year 2, within-bog transplants had longer leaves with wider wings than control plants (control: length = 146.9 ± 5.97 , wing width = 15.3 ± 0.96 ; within-bog transplant: length = 182.9 ± 6.95 , wing width = 19.2 ± 0.76 , $P = 0.02$ and 0.01 for length and wing width, respectively, contrast following one-way ANOVA). Within-wetland transplants were included to detect any deleterious effects of the transplant procedure, which might be expected to show up soon after transplantation. The effects did not indicate harm to the plant and appeared only 2 yr after transplantation, so do not suggest plant damage during transplantation. Control plants and within-wetland transplants within each wetland were therefore combined to simplify the data presentation, although analyses of all six original treatments are reported by Bott (2007).

Contrast analysis For leaf N and P content, and for leaf morphology traits, three orthogonal contrasts (Sokal & Rohlf, 1981) were used. The contrasts compared plants of fen vs bog origin, plants transplanted from fen to bog vs fen plants, and plants transplanted from bog to fen vs bog plants. A fourth contrast was added to compare plants that originated and remained in the bog with those that originated and remained in the fen. Sample size was 14–20 for controls plus within-wetland transplants and eight to 10 for between-wetland transplants.

All statistical analyses were performed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) or Sigmapstat V. 3.11 (Systat Software, Inc.).

Results

Water nutrient chemistry

The fen surface water pH was neutral and the bog was *c.* 2 pH units more acidic over the entire experiment (Fig. 1a, $P < 0.0001$, wetland effect in repeated-measures ANOVA, Table S2). The nitrate concentration was not always higher in the bog than the fen over the whole experiment (Fig. 1b, $P > 0.05$, Table S2), but during the second year (2006), nitrate was consistently higher in the bog than in the fen ($P < 0.05$, Table S2). Ammonium concentrations were not significantly different between wetlands over the

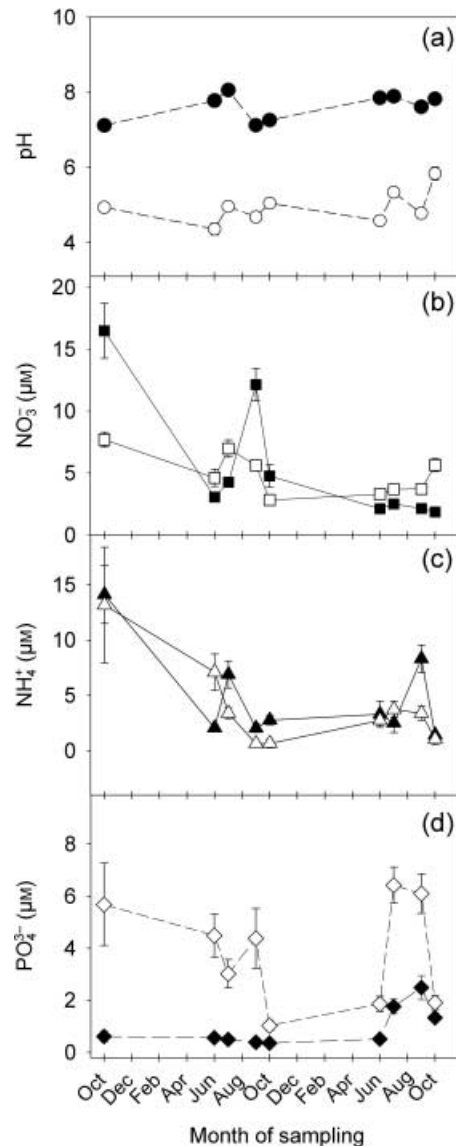


Fig. 1 Changes in surface water pH and dissolved nutrient concentrations in fen (closed symbols) and bog (open symbols) over 2 yr. (a) pH, (b) nitrate, (c) ammonium, (d) phosphate. Points are means of values from 24 to 30 water samples. Error bars (where visible) are standard error.

whole experiment (Fig. 1c, $P > 0.05$, Table S2). Throughout the study, the bog had significantly higher phosphate concentrations than the fen (Fig. 1d, $P < 0.001$, Table S2); the magnitude of this difference varied over time ($P < 0.0001$, Table S2).

Photosynthesis and nutrient limitation in bog and fen populations

The plants in the bog had significantly higher N content and N : P ratio in leaf tips than the plants growing in the fen

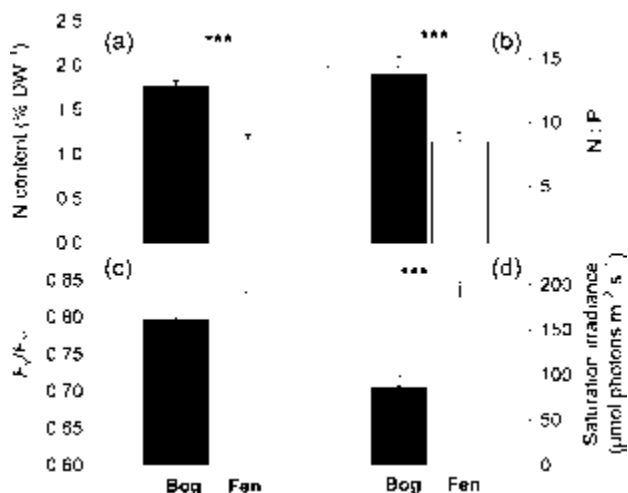


Fig. 2 Indices of nutrient limitation and photosynthesis in bog and fen populations of *Sarracenia purpurea* subsp. *purpurea*. (a) leaf N content, (b) leaf N : P ratio. The dashed horizontal lines represent threshold levels below which N is considered limiting. (c) Maximum quantum yield of photosystem II photosynthesis; and (d) irradiance required to saturate photosynthesis. Bars represent means + SE; $n = 26\text{--}28$ (a, b), $n = 15\text{--}24$ (c, d). ***, significant differences between fen and bog populations ($P < 0.001$).

(Fig. 2a,b, $P < 0.001$, Mann–Whitney rank-sum test) and the N content and N : P ratio for both populations were below thresholds indicating N limitation (Ellison, 2006). However, maximum quantum yields of photosystem II photochemistry were not significantly different between wetlands ($P > 0.05$, Mann–Whitney rank-sum test) and were all high ($F_v/F_m > 0.7$) (Fig. 2c). The bog population showed a saturation of photosynthesis at a lower irradiance than for the fen population (Fig. 2d, $P < 0.001$, t -test).

Transplant experiment – leaf nutrient content

Nitrogen Over the two growing seasons, plants that remained in the bog had consistently higher leaf N content than plants remaining in the fen (Fig. 3a, Table 1, fen vs bog contrast) and between-wetland transplants had leaf N content closer to that

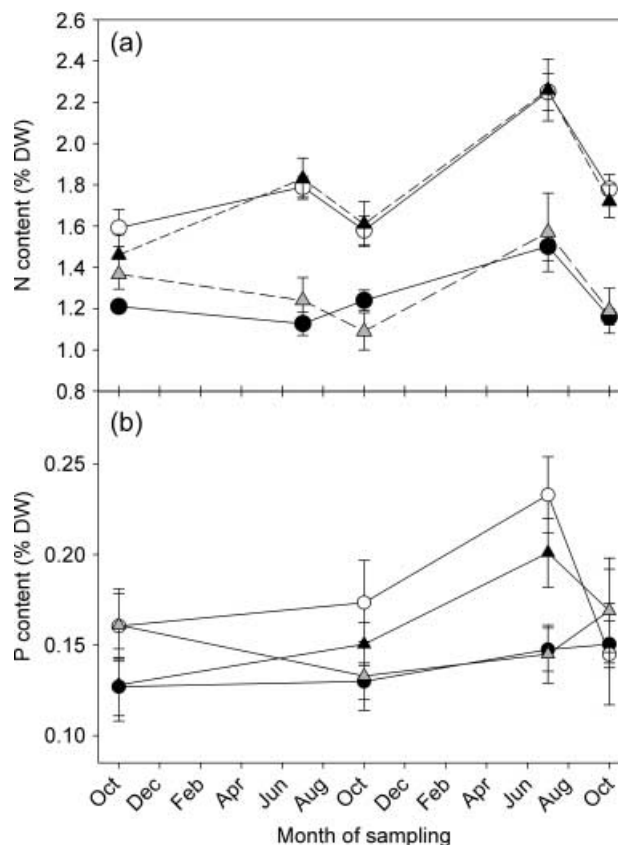


Fig. 3 Changes in *Sarracenia purpurea* subsp. *purpurea* leaf macronutrient content within bog, fen, or reciprocal transplant treatments over 2 yr following transplantation in October 2004. Treatments are fen controls (closed circles), bog controls (open circles), fen to bog transplants (black triangles) and bog to fen transplants (gray triangles). (a) Leaf N content; (b) leaf P content. All points are means of 14–20 (fen and bog) or 8–10 (fen to bog and bog to fen) plants \pm SE.

of plants in their new environment than plants from their wetland of origin (Fig. 3a, Table 1, fen to bog vs fen, bog to fen vs bog contrasts).

Phosphorus Leaf P content was not consistently different in bog and fen plants over the two seasons (Fig. 3b, Table S3).

Table 1 Results (P values) of contrast analysis for *Sarracenia purpurea* subsp. *purpurea* plant nutrient content (shown in Fig. 3)

Contrast	N content				P content
	July 2005	October 2005	July 2006	October 2006	July 2006
Fen vs Bog	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001
Fen to Bog vs Fen	< 0.0001	0.004	< 0.0001	< 0.0001	0.02
Bog to Fen vs Bog	< 0.0001	0.0002	0.0004	< 0.0001	0.0002
Fen origin vs Bog origin	0.56	0.32	0.82	0.60	0.36

Contrast analyses were only run for dates where there was a significant treatment effect in one-way ANOVA ($P < 0.05$, Table S3). Significant P values are indicated in bold.

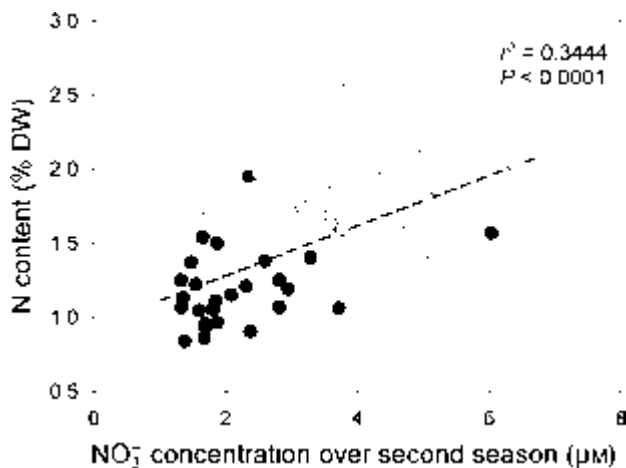


Fig. 4 Relationship between surface water NO_3^- concentration and leaf N content in *Sarracenia purpurea* subsp. *purpurea* plants in fen (closed symbols) and bog (open symbols). NO_3^- concentrations are means of four sampling dates during the second growth season, and leaf N is for samples collected in October of the second season. Points are mean values for each plant. The dashed line was fitted by linear regression to all points in the plot.

Only in July 2006 did bog plants have higher P content than fen plants, and between-wetland transplants had P content more similar to their new environment than to their wetland of origin (Fig. 3b, Table 1).

Carbon Plant C content did not vary significantly between wetland, treatment, or over time (data not shown, $P > 0.05$).

Plant nutrient content in relation to water nutrient availability

There was a strong correlation between average nitrate concentration at each site during year 2 (over four sampling dates) and N content of mature leaves from all bog and fen *S. purpurea* plants sampled at the end of year 2 (Fig. 4, $r^2 = 0.344$; $P < 0.0001$). All other correlations between leaf N and P content and dissolved ammonium or phosphate were not significant ($P > 0.05$) and showed low r^2 values (not shown).

Plant morphology measurements

Over the 2 yr, plants growing in the fen were more likely to flower than plants in the bog (16/30 fen plants vs 8/29 bog plants, chi-square = 4.05, $df = 1$, $P < 0.05$). More of the transplants flowered, but there were too few observations for statistical testing (3/10 fen controls, 8/10 within fen, and 5/10 bog to fen transplants, and 2/10 bog controls, 4/9 within bog and 2/10 fen to bog transplants). More fen plants (10/30) than bog plants (4/30) developed secondary apices during the experiment, but this difference was not significant (chi-square = 3.35, $df = 1$, $P > 0.06$). Transplants also seemed

more likely to develop secondary apices, but there were too few data for a statistical test (4/10 fen controls, 0/10 within fen, 6/10 bog to fen transplants, and 0/10 bog controls, 2/10 within bog, and 2/10 fen to bog transplants).

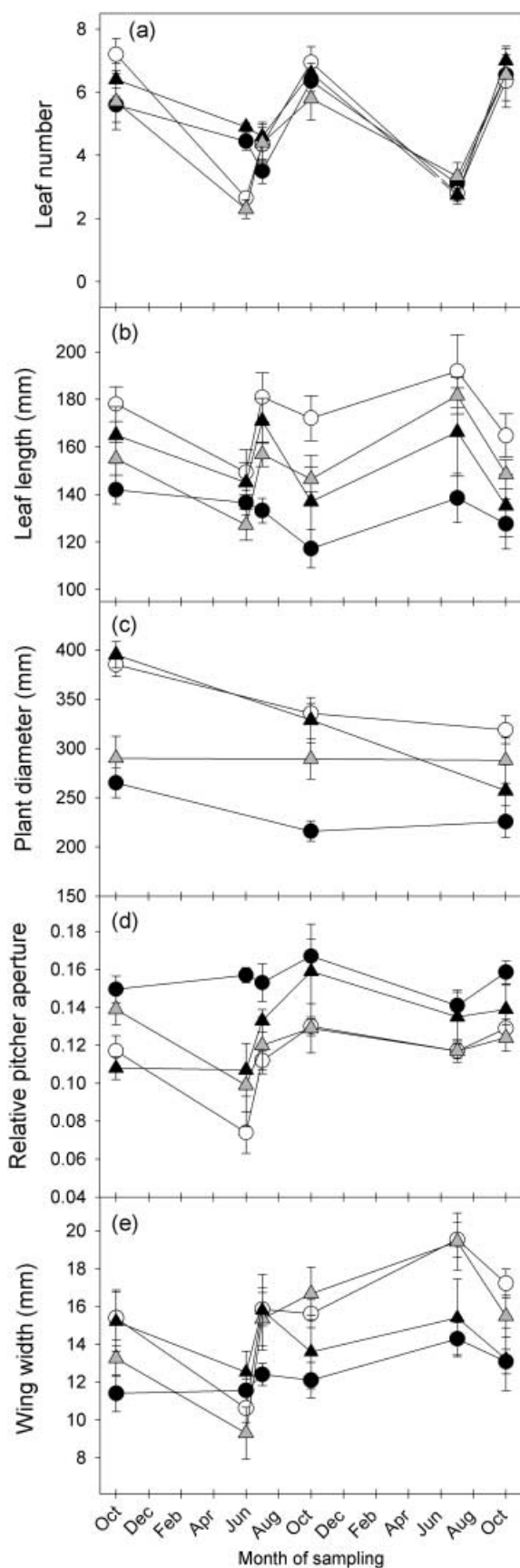
Leaf number Plants in the bog lost more leaves over winter than plants in the fen, and early in the growing season (June 2005) had significantly fewer leaves (Fig. 5a, Table 2, fen vs bog contrast). However, by July, there were no significant differences in leaf number between bog and fen plants (Fig. 5a, Table 2).

Leaf length and plant diameter Bog plants had longer leaves than fen plants (Fig. 5b, Table 2, fen vs bog contrast). Plants transplanted between wetlands changed leaf length to reflect these differences between bog and fen (Table 2, fen to bog vs fen, bog to fen vs bog contrasts). Plant diameter was measured at the end of each season to reflect a full season's growth. Bog plants had larger diameters than fen plants (Fig. 5c, Table 2, fen vs bog contrast) and fen to bog transplants showed larger diameters than the plants remaining in the fen (Table 2, fen to bog vs fen contrast). Changes in leaf length and diameter were faster in fen to bog than in bog to fen transplants (Table 2, bog to fen vs bog contrast).

Relative pitcher aperture Bog plants had narrower pitcher apertures relative to leaf length than fen plants (Fig. 5d, Table 2, fen vs bog contrast). By June following transplantation and for the rest of the experiment, relative pitcher apertures of fen to bog transplants had become similar to bog plants (Table 2, fen to bog vs fen contrast). By contrast, the bog to fen transplants maintained relative aperture ratios not statistically different from bog plants for the duration of the experiment (Table 2, bog to fen vs bog contrast).

Wing width Bog plants had wider wings than fen plants on all sampling dates except June 2005 (Fig. 5e, Table 2, fen vs bog contrast). By October 2005, fen to bog transplants had significantly wider wings than plants remaining in the fen (Table 2, fen to bog vs fen contrast), while bog to fen transplants retained wider wings typical of bog plants (Fig. 5e, Table 2, bog to fen vs bog contrast). However by 2006, wing widths of between-wetland transplants resembled plants in their new environment more than those of their original wetland (Fig. 5e, Table 2). Total leaf width showed similar changes to wing width (data not shown; Bott, 2007).

Univariate correlations between wetland variables pH, dissolved nitrate, phosphate and leaf morphological traits showed significant relationships ($P < 0.02$), but with poor predicative ability (r^2 values 0.103–0.28) (data not shown). Leaf morphology traits were significantly correlated with plant N content ($P < 0.005$) but r^2 values were low (0.1–0.3). The wetland water chemistry and plant nutrient content variables were correlated and distinct between the two wetlands (Figs 1–4),



making it difficult to isolate the effect of a single factor on plant traits.

Effect of irradiance

The integrated total photon dose over the 2006 growing season, representing a range of irradiances (as mol photons m^{-2} for April–November) for the plant locations in the two wetlands, were as follows: bog, very low, 0.704; low, 0.955; medium, 2.496; and fen, low–medium, 1.302; high, 6.755; and very high, 7.324. Despite the differences in photon dose, nutrient stoichiometry and plant morphology (leaf N content, wing width, and relative pitcher aperture), differences seemed more strongly grouped by wetland than by photon dose (Fig. 6), although diameter of fen plants declined with increasing light availability (Fig. 6b).

Discussion

Despite carnivory, is *S. purpurea* nutrient content limited by supply to the roots?

Pitcher plants growing in both bog and fen showed N content and N : P stoichiometry indicative of N limitation, as $< 2\%$ of dry mass as N and N : P ratios of < 14 indicate N limitation in *S. purpurea* (Wakefield *et al.*, 2005; Ellison, 2006). Except for bog plants in July of year 2 (Fig. 3a), plants were N-limited, as can commonly occur in wetlands (Olde Venterink *et al.*, 2003). The higher surface water-dissolved nitrate and phosphate observed in the bog, particularly in year 2, seemed to be driving N content of *S. purpurea* leaves; N and P leaf content was higher in bog than fen plants, and leaf N content of between-wetland transplants changed to reflect the new environment. The correlation between nitrate and N content (Fig. 4) also suggests that N uptake via the roots is a major determinant of *S. purpurea* leaf N content. This effect was less significant for P, although P content of transplants did change to reflect P availability of the new environment (Fig. 3). These data also suggest that carnivory was not sufficiently supplementing N acquisition to overcome N limitation. Although leaf nutrient content also responds to variable prey additions to *S. purpurea* pitchers (Chapin & Pastor, 1995; Wakefield *et al.*, 2005; Farnsworth & Ellison, 2008), naturally *Sarracenia* has been shown to have low prey capture efficiency (Cresswell, 1991; Newell & Nastase, 1998). Comprehensive data are

Fig. 5 Changes in plant morphology in *Sarracenia purpurea* subsp. *purpurea* in bog and fen following transplantation in October 2004. (a) leaf number, (b) leaf length, (c) plant diameter, (d) relative pitcher aperture, and (e) wing width. Treatments are fen controls (closed circles), bog controls (open circles), fen to bog transplants (black triangles) and bog to fen transplants (grey triangles). Points are means \pm SE, $n = 14$ –20 for bog and fen, and $n = 8$ –10 for bog to fen and fen to bog treatments.

Table 2 Results (*P* values) of contrast analysis for *Sarracenia pupurea* subsp. *pupurea* leaf morphological traits (shown in Fig. 5)

Parameter	Leaf number			Leaf length			Plant diameter			Relative pitcher aperture			Wing width			Relative wing width		
	June 2005	July 2005	October 2005	July 2005	October 2005	October 2006	October 2005	June 2005	October 2005	October 2006	October 2005	July 2005	October 2005	October 2006	July 2005	October 2005	October 2006	June 2005
Fen vs bog	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0006	0.0009	0.0004	0.0005	0.0005	0.01	0.66
Fen to bog vs fen	< 0.0001	0.02	0.05	0.02	0.16	0.002	0.001	0.03	0.002	0.002	0.03	0.02	0.03	0.009	0.08	0.009	0.52	0.47
Bog to fen vs bog	< 0.0001	0.33	0.02	0.17	0.05	0.75	0.06	0.03	0.33	0.33	0.09	0.13	0.03	0.03	0.03	0.006	0.85	0.003
Fen origin vs bog origin	0.20	< 0.0001	0.03	0.14	0.25	< 0.0001	0.003	0.12	< 0.0001	0.37	0.76	0.15	0.37	0.63	0.35	0.35	0.002	0.24

Contrast analyses were only run for dates where there was a significant treatment effect in one-way ANOVA ($P < 0.05$, Table S4). Plant diameter was only measured in October. Significant *P* values are indicated in bold.

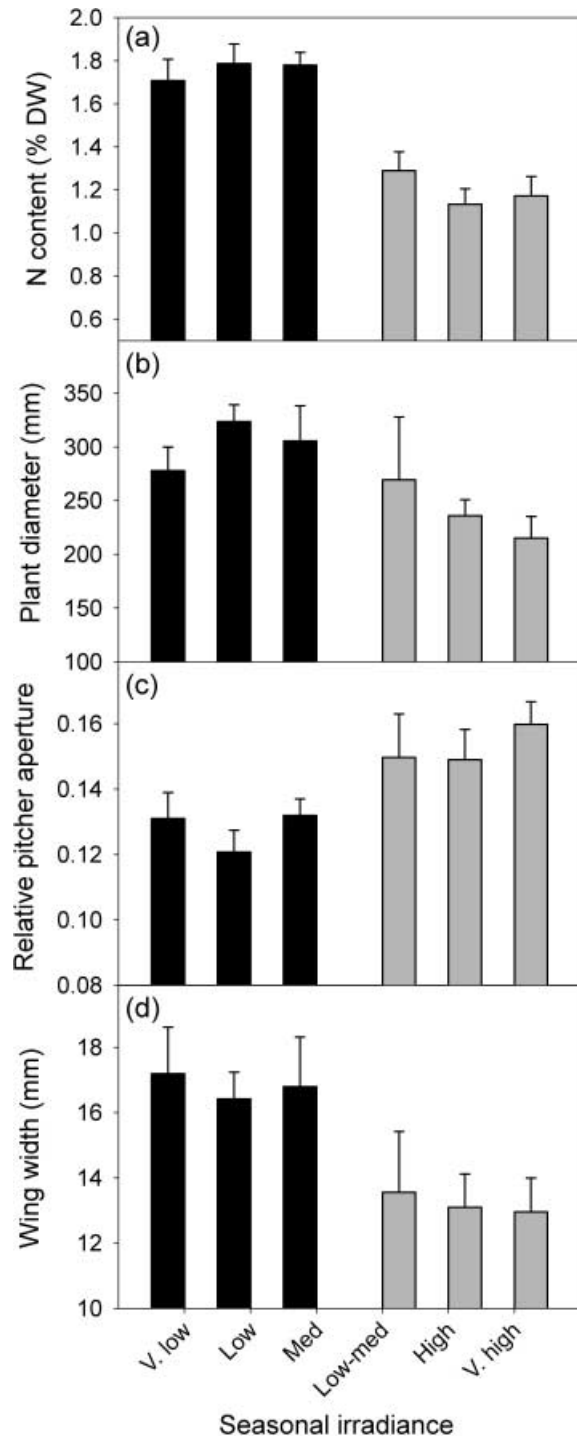


Fig. 6 Relationship between N content and leaf traits measured in October 2006 and estimated total seasonal photon dose over 2006 in *Sarracenia pupurea* subsp. *pupurea* plants in bog (black bars) and fen (gray bars). (a) N content, (b) plant diameter, (c) relative pitcher aperture, (d) leaf wing width. All bars are means + SE, $n = 6-16$. Total seasonal irradiance doses (in mol photons m^{-2}), along with the number of plants in each category, correspond to the following: v. low, 0.704 ($n = 8$); low, 0.955 ($n = 16$); med, 2.496 ($n = 6$); low-med, 1.302 ($n = 6$); high, 6.755 ($n = 15$); v. high, 7.324 ($n = 9$).

lacking, but *S. purpurea* may derive as little as 10% of N from carnivory (Chapin & Pastor, 1995; reviewed by Ellison, 2006). From the strong correlation between surface water N availability and leaf N content in *S. purpurea*, we conclude that despite the capability for carnivory, N content of leaf tissue of *S. purpurea* was constrained by N availability to the roots in the two natural wetland environments.

By contrast, there was no evidence for nutrient limitation of photosynthesis. High maximum quantum yield of photosystem II photochemistry (F_V/F_M) was indicative of healthy, nonlimited plants (White & Critchley, 1999). Farnsworth & Ellison (2008) recently showed higher photosynthesis rates and F_V/F_M in *Sarracenia* pitchers fed small amounts of prey compared with unfed plants, but also reported no physiologically significant suppression of F_V/F_M with lower leaf nutrient content. Previous studies suggested that photosynthesis in *S. purpurea* does not respond as readily to relative N limitation as does leaf morphology (Ellison & Gotelli, 2002; Butler & Ellison, 2007). Therefore, despite a lack of effect on photosynthesis, differences in leaf N content between fen and bog populations of *S. purpurea* may have been driving expression of *S. purpurea* leaf morphology traits.

Carnivory can also be important for P, S, K, Ca, Mg and micronutrient acquisition (Adamec, 1997; Ellison, 2006). By contrast with N, there was no evidence for P limitation in *S. purpurea* plants. Bott (2007) showed that only tissue from older leaves had leaf P contents below the threshold of 0.1% DW⁻¹ (Olde Venterink *et al.*, 2003; Ellison, 2006). The lack of a relationship between leaf P content and dissolved phosphate concentration in the wetlands may be the result of differential 'luxury' uptake and storage under excess P supply (Chapin & Pastor, 1995), or of variable P acquisition from carnivory.

Is the leaf morphology of *S. purpurea* correlated with natural wetland environment?

The significant differences in *S. purpurea* N content were associated with differences in leaf morphology traits between bog and fen plants. Leaves of bog plants were longer and more decumbent with wider wings, resulting in larger-diameter plants with narrower relative pitcher apertures than fen plants (Fig. 5). Wider pitcher apertures in fen plants may promote greater prey capture in the more N-limited fen plants (Ellison *et al.*, 2004), while longer leaves and wider wings in bog plants may promote photosynthetic performance under higher nutrient supply in the bog (Ellison & Gotelli, 2002; Butler & Ellison, 2007). This trade-off between optimizing carnivorous nutrient acquisition and photosynthesis in the same leaf structure is consistent with the cost-benefit model prediction that an adequate supply of nutrients would reduce the investment in carnivorous structures (Givnish *et al.*, 1984; Knight & Frost, 1991). We observed that this prediction can be met even under unmanipulated conditions within natural variations of

macronutrient availability in different wetland types, and could apply within wetlands undergoing changing nutrient regimes associated with hydrological disturbance.

Light is an important stimulus in plant phenotypic plasticity (Valladares *et al.*, 2007). Seasonal irradiance was higher in most fen sites than in the bog (Fig. 6), and plants had acclimated to the irradiance in each wetland (Fig. 2d). Greater investment in carnivory among carnivorous plants has been related to increasing irradiance (Mandossian, 1966; Givnish *et al.*, 1984; Zamora *et al.*, 1998; Brewer, 1999, 2003; but see Ellison *et al.*, 2004). Higher irradiance will increase demand for nutrients, but also provide energy for growth. Higher light and lower N availability in the fen may have worked synergistically to increase N demand to support vegetative growth and the higher reproductive output observed in fen plants. Conversely, lower irradiance and higher macronutrient availability in the bog may have stimulated growth of longer leaves more adaptive to photosynthetic light harvesting than carnivory. However, although irradiance may have contributed to differences in morphology between wetlands, light was not the primary driving force in morphological plasticity of *S. purpurea* (Fig. 6), except possibly for plant diameter (Fig. 6b). *Sarracenia alata* showed plasticity in above-to-belowground investment in response to irradiance (Brewer, 1999), and may also be a factor in *S. purpurea*.

Is leaf morphology determined by current environment or wetland of origin?

Transplantation of *S. purpurea* plants between wetlands showed that morphological traits were strongly determined by the current growing environment rather than genetic identity of the population of origin. Distinct patterns between wetlands emerged for phenology, leaf number, leaf length, plant diameter, wing width and relative pitcher aperture, with new leaves becoming more similar to plants in the new environment. This rapid morphological response was observed despite the first few new leaves of the season being formed from dormant leaf buds initiated the previous season, prior to transplantation. By contrast, the carnivorous *Pinguicula vulgaris* showed that preformed leaf primordia could not re-differentiate in response to changing resource availability (Worley & Harder, 1999). This suggests that leaf development in *S. purpurea* is unusually plastic in response to nutrient availability and is not genetically predetermined during formation of leaf primordia.

The bog environment appeared to exert a faster influence on *S. purpurea* than the fen; fen to bog transplants more closely resembled bog plants by the next season, but bog to fen transplants continued to resemble bog plants until the second season. This asymmetry in acclimation rate may relate to the acidic growing environment and lower light conditions of the bog, which may have imposed greater stress. Fen plants produced more secondary apices and flowers, both of which require significant resource investment (N and photosynthate)

(Ne'eman *et al.*, 2006), suggesting that fen plants may have been less physiologically stressed than bog plants.

Nitrogen content of *S. purpurea* may have been driving observed morphological changes in between-wetland transplants. Experimental addition of inorganic nutrients to *S. purpurea* pitchers both increased leaf nutrient content and changed morphology (Chapin & Pastor, 1995; Ellison & Gotelli, 2002). This reciprocal transplant study demonstrates that, under natural conditions, plasticity of leaf nutrient content and morphology in *S. purpurea* can also respond rapidly to nutrients acquired through the roots. This complements previous reports of morphological plasticity in leaf traits of widely dispersed *S. purpurea* populations, across latitudinal and environmental gradients, and in response to experimental inorganic nutrient fertilization (Mandossian, 1966; Ellison & Gotelli, 2002; Ellison *et al.*, 2004). Functional plant macronutrient limitation is often defined as N or P content thresholds (Olde Venterink *et al.*, 2003; Wakefield *et al.*, 2005), but leaf morphology traits of *S. purpurea* were more responsive than photosynthetic performance to relative N limitation in the two wetlands. Phenotypic plasticity in leaf form in response to environmental variability is uniquely important in the long-lived carnivorous plant *S. purpurea*, which balances investment in leaves adapted for the dual roles of photosynthesis and carnivorous nutrient acquisition and may provide a model for investigation of morphological plasticity, across more extreme trait gradients than might typically be found in noncarnivorous plants.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Diagram of leaf morphological traits measured.

Table S1 Timetable of water and plant sample collection

Table S2 Statistical analysis (repeated-measures ANOVA) of wetland surface dissolved nutrients

Table S3 Statistical analysis (repeated-measures ANOVA) of leaf macronutrient content

Table S4 Statistical analysis (repeated-measures ANOVA) of leaf morphology traits

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