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Prey availability directly affects physiology, growth, nutrient allocation and scaling relationships among leaf traits in 10 carnivorous plant species

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Summary

1. Scaling relationships among photosynthetic rates, leaf mass per unit area (LMA), and foliar nitrogen (N) and phosphorus (P) content hold across a diverse spectrum of plant species. Carnivorous plants depart from this spectrum because they dedicate substantial leaf area to capturing prey, from which they derive N and P. We conducted a manipulative feeding experiment to test whether scaling relationships of carnivorous plant leaf traits become more similar to those of non-carnivorous taxa when nutrients are not limiting.

2. We examined the effects of prey availability on mass-based maximum photosynthetic rate (A_{mass}), chlorophyll fluorescence, foliar nutrient and chlorophyll content, and relative growth rate of 10 *Sarracenia* species. We hypothesized that increased prey intake would stimulate A_{mass}, reduce stress-related chlorophyll fluorescence, increase photosynthetic nutrient-use efficiencies ($PNUE_N$, $PNUE_P$), and increase relative biomass allocation to photosynthetically efficient, non-carnivorous phyllodes.

3. Two plants per species were assigned in a regression design to one of six weekly feedings of finely ground wasps: 0–0.25 g for small plant species; 0–0.5 g for intermediate-sized species; and 0–1.0 g for large species. The first two leaves emerging on each plant were fed.

4. Increased prey availability increased photosystem efficiency (F_v/F_m ratio) in the first two leaves, and chlorophyll content and A_{mass} in younger leaves as older leaves rapidly translocated nutrients to growing tissues. Higher prey inputs also led to lower N : P ratios and a shift from P- to N-limitation in younger leaves. $PNUE_P$ was significantly enhanced whilst $PNUE_N$ was not. Better-fed plants grew faster and produced a significantly higher proportion of phyllodes than controls.

5. Feeding shifted scaling relationships of P relative to A_{mass}, N and LMA from outside the third bivariate quartile to within the 50th bivariate percentile of the universal spectrum of leaf traits; other scaling relationships were unaffected. Carnivorous plants can rapidly reallocate P when nutrients are plentiful, but appear to be less flexible in terms of N allocation.

6. *Synthesis*. Our results support the general hypothesis put forward by Shipley *et al.* (2006) that observed scaling relationships amongst leaf traits derive from trade-offs in allocation to structural tissues vs. liquid-phase (e.g. photosynthetic) processes. These trade-offs appear to be especially constraining for plants growing in extremely nutrient-poor habitats such as bogs and other wetlands.

Key-words: carnivorous plants, fluorescence, leaf production, N : P ratio, nutrients, photosynthesis, *Sarracenia*, stoichiometry, universal spectrum of leaf traits

Introduction

A central aim of plant ecology is to understand the mechanisms by which plants assimilate nutrients and allocate essential resources to physiological processes, growth and leaf production. Levels of foliar nitrogen (N) and phosphorus (P) are broadly correlated with maximal net photosynthetic rates and leaf mass per unit area (LMA) across thousands of plant species (Reich *et al.* 1999; Castro-Diéz *et al.* 2000; Shipley & Lechowicz 2000; Wright *et al.* 2004; Westoby & Wright 2006). Indeed, the predictable scaling relationships among these core leaf traits collectively have been termed the 'world-wide leaf economics spectrum', derived from the Global Plant Trait Network (GLOPNET) data base (Wright *et al.* 2004).

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However, plants are highly sensitive to the ratios of nutrients available to them, and limitation of one or more nutrients constrains the ability of plants to use other nutrients, water and light in growth and competition with other plants. Thus, recent research has illustrated that site- and species-specific differences in nutrient availability and allometric constraints, respectively, can contribute to significant variability in leaf-trait relationships and significant departures from the overall leaf economics spectrum (Santiago & Wright 2007).

Carnivorous plants, which derive the bulk of their nutrients by trapping and digesting prey, exhibit scaling relationships among leaf traits that diverge from the leaf economics spectrum (Ellison & Farnsworth 2005; Ellison 2006). For example, carnivorous plants have extremely low photosynthetic rates, and their photosynthetic nitrogen use efficiency ($PNUE_N$) and photosynthetic phosphorus use efficiency ($PNUE_P$) are less than 50% of those predicted by scaling relationships of the leaf economics spectrum (Ellison 2006). Carnivory is thought to have evolved in a diversity of plant lineages in high-light, low-nutrient environments in which the marginal photosynthetic benefit gained from prey capture (i.e. through allocation of supplemental nutrients to increased production of chlorophyll and RuBisCO) has exceeded the marginal costs of modifying leaves into photosynthetically inefficient traps (the cost-benefit model of Givnish *et al.* 1984). Existing data are inadequate to evaluate this model, however, as the few existing experimental studies, involving only a handful of carnivorous plant species, test only a subset of the links between prey capture, photosynthetic physiology and plant growth (Weiss 1980; Knight 1992; Chapin & Pastor 1995; Méndez & Karlsson 1999; Ellison & Gotelli 2002; Wakefield *et al.* 2005; Ellison 2006).

Here, we describe the first study to examine the effects of prey availability simultaneously on the net photosynthetic rate, chlorophyll fluorescence, growth, morphology, and foliar nutrient and chlorophyll content in 10 species of carnivorous pitcher plants. We explore four questions related to the position of these plants within the world-wide leaf economics spectrum. (i) How does supplemental prey alter leaf-level physiology, including maximal mass-based photosynthetic rate (A_{mass}), photosynthetic nutrient use efficiency ($PNUE_N$ and $PNUE_P$) and overall photosystem efficiency? (ii) How does supplemental prey affect plant relative growth rate, biomass allocation and tissue nutrient content of the leaves produced by pitcher plants over the course of the growing season? (iii) Does supplemental feeding result in consistent changes in nutrient stoichiometry or in the scaling relationships among foliar N, P, A_{mass} and LMA of carnivorous species? (iv) How do the scaling relationships for fed and unfed plants carnivorous species compare with scaling relationships of non-carnivorous plants in the GLOPNET data set? The answers to these questions allow us to elucidate the constraints and trade-offs that limit the adaptability of carnivorous plants to variation in nutrient availability – namely, the 'costs of carnivory' (Givnish *et al.* 1984; Knight 1992; Ellison & Farnsworth 2005). They also create a framework for understanding physiological and evolutionary constraints in other highly specialized plant species.

Methods

THE PITCHER PLANTS

Ten congeneric species of pitcher plant that have disparate geographical ranges, contrasting morphologies and different digestive physiologies were used in this study: *Sarracenia rosea* Naczi, Case & Case, *S. purpurea* L., *S. psittacina* Michx., *S. alabamensis* Case & Case, *S. jonesii* Wherry, *S. rubra* Walt., *S. minor* Walt., *S. alata* (Wood) Wood, *S. flava* L., and *S. leucophylla* Raf. (we did not work with the federally endangered species, *Sarracenia oreophila* (Kearney) Wherry). These species occur throughout the eastern seaboard of North America and overlap in range (expressed as the northernmost and southernmost latitudes from which populations are reported; Schnell 2002); *S. purpurea* extends the farthest north, whilst *S. minor*, *S. alata* and *S. psittacina* range furthest south (Table 1). The 10 species vary in size (e.g. height of the mature pitcher) by an order of magnitude (Table 1). All these species produce specialized leaves modified into pitchers which act as pitfall traps to capture insect and occasionally vertebrate prey (Butler *et al.* 2005). The prey is digested by enzymes secreted by the plant and/or a food web of bacteria and small invertebrates (Ellison *et al.* 2003). Four species produce rhizomes, whilst the other six produce roots only (Table 1).

Table 1. General traits of the 10 species used in this study. Plant size refers to height of fully expanded pitcher of mature plant measured at harvest, averaged across plants within species grown in this study (cm ± 1 SE); rhizome production (1 if yes, 0 if no); enzyme production (1 if yes, 0 if no); latitude to nearest degree of northernmost occurrences (Northernlat); latitude to nearest degree of southernmost occurrences (Southernlat); phyllode production (1 if yes, 0 if no)

Species	Plant Size	Rhizomes	Enzymes	Northernlat	Southernlat	Phyllodes
S. alabamensis	19.2 ± 1.8	0	1	32	31	1
S. alata	29.4 ± 2.4	1	1	33	29	0
S. flava	35.1 ± 2.2	1	1	35	32	1
S. jonesii	21.8 ± 1.4	0	1	33	31	1
S. leucophylla	46.8 ± 2.3	1	1	35	32	1
S. minor	14.9 ± 0.7	1	1	33	29	1
S. psittacina	4.7 ± 0.3	0	1	33	29	1
S. purpurea	8.7 ± 0.8	0	0	50	38	1
S. rosea	4.3 ± 0.2	0	0	30	30	1
S. rubra	20.7 ± 0.9	0	1	35	31	1

Plants were grown from wild-collected seed for 6 years beginning in 1999 (Ellison 2001) in a milled *Sphagnum* medium in a climate-controlled greenhouse at Harvard Forest (Petersham, Massachusetts, USA) and were watered at the base daily during the growing season. Plants entered dormancy at first frost (mid-October) and recommenced growth in March–April of each year. In year two of growth (2000), plants were transplanted into 10-cm diameter pots. In April 2005, 12 6-year-old plants of each species ($N = 120$ plants) were chosen randomly for the feeding experiment. We used 6-year-old plants in these experiments because, under optimal conditions in a greenhouse, pitcher plants reach reproductive maturity after 4–6 years and thus we could be certain that our experimental plants had the characteristics of mature plants. This is important because juvenile plants (seedlings 1–3 years old) often have non-functional pitchers (i.e. they are too small to capture prey) and display distinct heterophylly relative to adult plants (Franck 1976).

By 6 June 2005, all plants had emerged from winter dormancy and had produced at least one new pitcher. The first pitchers to emerge were labelled at the base with a plastic red bird band (National Band & Tag Co., Newport, Kentucky, USA); the second pitchers to emerge were labelled with a yellow bird band. This early pair of fully expanded pitchers constituted almost all of the above-ground biomass of the plant at the outset of the experiment. Because the two pitchers did not grow appreciably during the experiment, all subsequent mass-based above-ground growth coincided with the production of additional pitchers and phyllodes during the season, a fact that we exploited in calculating relative growth rates (see below).

SUPPLEMENTAL FEEDINGS

The first and second pitchers produced on each plant were fed once each week with a fixed dose of dried and finely ground hymenoptera (species collected locally: *Polistes fuscatus* Fabricius and *Dolichovespula maculata* L.) moistened in 5 mL of distilled water. Wasps are common prey of the majority of *Sarracenia* species in the field (Gibson 1983) and in our greenhouse (A. M. Ellison, personal observation) and have nutrient contents similar to ants, which are the common prey of the smaller pitcher plants, *Sarracenia purpurea*, *S. rosea* and *S. psittacina* ($N_{wasp} = 10.7\%$; $N_{ant} = 12.1\%$; $P_{wasp} = 1.75\%$; $P_{ant} = 1.52\%$; $K_{wasp} = 1.01\%$; $K_{ant} = 0.93\%$; values are means of three samples of dried and ground wasps (*Dolichovespula maculata* and *Polistes fuscatus*) or ants (*Tapinoma sessile* (Say) and *Formica subaenescens* Emery)). Doses were scaled to the size of each plant species to avoid overfeeding and injuring leaves and to reflect the ranges of prey capture observed in the wild. Feeding levels ranged from 0 g (control plants) to 0.25 g (0–0.0268 g N and 0–0.0044 g P per week) for the small species, *S. rosea*, *S. purpurea*, *S. psittacina*, *S. alabamensis*, *S. jonesii* and *S. rubra*; 0 g to 0.5 g (0–0.0535 g N and 0–0.0088 g P per week) for the intermediate-sized species, *S. minor* and *S. alata*; and 0 g to 1.0 g (0–0.1070 g N and 0–0.0176 g P per week) for the largest species, *S. flava* and *S. leucophylla*. We used a regression treatment design, with six feeding levels ranging from 0 g (no feeding) to the maximum dose. Two plants per species were assigned at random to each feeding level; initial plant size distributions did not differ significantly among treatments.

To enable natural digestion of prey, the two plant species that naturally host a food web of bacteria and small invertebrates (*S. purpurea* and *S. rosea*) were inoculated in June with a standardized volume and mixture of naturally occurring bacteria and protozoa from pitcher water collected at nearby Tom Swamp in Petersham, Massachusetts. The other species, which enzymatically digest prey directly, were not inoculated.

We fed plants for 7 weeks. The apparent brevity of this experiment, relative to the age of the plants, is justified by the brief phenology of leaf production. By 7 weeks into the growing season, most of the pitchers and phyllodes that would be produced in a given season had expanded and hardened up. The three smaller species (*S. purpurea*, *S. rosea*, and *S. psittacina*) can continue producing pitchers for another 4–5 weeks, but these end-of-season pitchers are very small (often < 4 cm long) and contribute little to the overall nutrient budget of the plant (Butler & Ellison 2007). In other experiments, we have found that *S. purpurea* assimilates 55–69% of N supplied to pitchers and translocates only *c.* 8% to root storage for overwintering (Butler & Ellison 2007). Two of the larger, rhizomatous *Sarracenia* species, *S. flava* and *S. alata*, also take up 50–60% of N supplied as prey, but in contrast to *S. purpurea*, these larger species translocate 25–35% of the captured N into below-ground structures (Butler & Ellison 2006). However, responses to feeding did not differ among rhizomatous and non-rhizomatous species (see Results below). More than 90% of the N previously stored in overwintering pitchers or roots is translocated into the first pitcher produced in the growing season, which then rapidly translocates nutrients from captured prey to younger leaves (Butler & Ellison 2007). Comparable data for P uptake and translocation do not exist. By focusing our measurements of photosynthesis and foliar traits of pitchers and phyllodes produced later in the season, we are confident that the vast majority of their standing stock of nutrients (and their responses to treatments) was due to the prey that we supplied.

MEASUREMENTS OF PITCHER PLANT PHYSIOLOGY

We sought to evaluate the effects of supplemental feeding on the physiology of the first and second pitchers produced (the ones that were fed), as well as on younger leaves and phyllodes produced later in the growing season that had received translocated nutrients from the fed pitchers (Butler & Ellison 2007). To assess whether feeding enhanced photochemical efficiency within the fed leaves, chlorophyll fluorescence was measured on healthy tissues halfway up the tube of the first and second pitchers of half of the plants at the conclusion of the experiment on 31 July 2005 (the other six plants per species were left intact in case the fluorescence measurements damaged pitchers, which, we later determined, they did not). Following overnight acclimation, dark-adapted F_o (minimum chlorophyll fluorescence yield in the dark-adapted state), F_v (maximum variable fluorescence yield in the dark-adapted state) and F_m (maximum chlorophyll fluorescence yield in the dark-adapted state) were measured using a LI-6400 system (Li-Cor BioSciences, Lincoln, Nebraska, USA). Twenty-four hours later, after plants had reacclimated to light, post-treatment A_{mass} was measured on the first and second leaves produced (the same pitchers on plants on which we had measured chlorophyll fluorescence), as well as on a fully expanded younger pitcher and on a phyllode (if present). Total foliar chlorophyll (*a* and *b*) contents were measured on all these same leaves using a CCM-200 Chlorophyll Content Meter (Opti-Sciences, Tyngsboro, Massachusetts, USA). Relative units (ranging 1–100) reported by the meter were converted to actual contents (μg m^{-2}) after calibrating the device directly using the acetone extraction procedure of Porra *et al.* (1989); the conversion equation was $391.8 + 16.78 \times$ CCM units; $r^2 = 0.89$.

MEASUREMENTS OF PITCHER-PLANT GROWTH AND NUTRIENT CONTENT

Pitchers and phyllodes produced over the course of the experiment were counted on 1 August 2005. Each plant was then harvested, above-ground and below-ground biomass separated, and the areas of the first, second and younger leaves measured using a Li-Cor LI-3100 leaf area meter. Areas and biomass of leaves that had been measured for net photosynthetic rate inside the Li-Cor cuvette were measured separately from the portion of leaves outside the cuvette so that accurate area- and mass-based photosynthetic rates could be calculated. All tissues were dried to constant mass for *c.* 1 week at 70 °C and dry biomass determined. Above-ground relative growth rates were calculated for all species as $[\ln(W_2) - \ln(W_1)]/60$ days, where W_1 was the initial biomass calculated as the sum of the biomass of the first and second-emerging leaves, W_2 was the total above-ground biomass of the harvested plants following the feedings, and 60 is the total duration in days of the experiment from initial leaf-labelling to harvest.

Because we observed significant differences among treatments in A_{mass} of younger leaves and phyllodes (see Results, below), and because nutrients are readily translocated from fed pitchers into younger tissues (Butler & Ellison 2006), we focused nutrient analysis and chlorophyll measurements on the same young, fully expanded leaf (and one phyllode per plant, if available) for which we had measured A_{mass}. Tissue samples from the younger pitchers and one fully expanded phyllode (as available) were ground in a Wig-L-Bug ball mill (International Crystal Laboratories, Garfield, New Jersey, USA) prior to sending them to the University of Vermont Agricultural and Environmental Testing Laboratory for determination of C, N and mineral content (P, Ca, Mg, K, Na and S). Carbon and N content of the finely ground, dried samples were determined using a Leeman Laboratory model 440 CHN elemental analyser (Teledyne Technologies, Los Angeles, California, USA). Mineral content was determined on microwave-digested samples using a PerkinElmer Optima 3000 DV inductively coupled plasma (ICP) atomic emission spectrometer (PerkinElmer, Wellesley, Massachusetts, USA). Photosynthetic nitrogen use efficiency ($PNUE_N$) and photosynthetic phosphorus use efficiency ($PNUE_P$) were calculated using the methods of Aerts & Chapin (2000).

STATISTICAL ANALYSES

The experimental design was a mixed response-surface/ANOVA design (Cottingham *et al.* 2005). All response variables that were normally distributed were analysed using a fixed-factor ANCOVA in which feeding level was treated as a continuous variable (one degree of freedom) and species as a factor (nine degrees of freedom). Any reductions in expected residual degrees of freedom reported (maximum possible = 100) would result from, for example, using only a subset of plants for a given measurement (e.g. fluorescence was measured on only one-half of the plants) or absence of phyllode production. Root : shoot ratios and F_v/F_m ratios were log-transformed prior to analysis. Feeding level was expressed as ln(absolute feeding level + 1). The Kruskal–Wallis rank sum test was used to test whether the proportion of phyllodes produced by plants differed among feeding levels. Data were analysed using S-Plus 2000 Professional Release 3.0 (Insightful Corp., Seattle, Washington, USA). Linear and non-linear regression lines were fitted using SigmaPlot 2001 for Windows, version 7.101 (SPSS Inc., Chicago, Illinois, USA).

Data on core leaf traits (LMA, foliar N, foliar P and A_{mass} of the younger leaves of each plant) from this experiment were compared with the large GLOPNET data set (kindly supplied by Ian Wright and the GLOPNET collaborative) from which existing data on carnivorous plants ($N = 2$ species) had been excluded. We examined relationships between the four leaf traits using reduced major axis regression (coded in S-Plus) on logarithmically transformed data (Wright *et al.* 2004). We determined the empirical 50th, 75th and 90th bivariate percentiles (as ellipses) of the GLOPNET data using the data.ellipse function in the car package (Fox 2002) of the R statistical software package (http://www.r-project.org/). Distributions of points from the 10 species examined in this experiment, generated by the seven scaling relationships among the variables A_{mass}, LMA, foliar N and foliar P, were graphed against the GLOPNET data. Proportions of unfed and fed carnivorous species falling within the ellipse describing the median (0–50th percentile), upper quartile (51–75th percentile), upper fence (76–90th percentile), or outer decile (91–100th percentile) around the centroid of the distribution of points of the GLOPNET data were counted and compared using chi-square tests. Previous analyses of differences in leaf traits of carnivorous vs. non-carnivorous plants have focused on single leaf traits (Ellison 2006) or on the difference between observed A_{mass} of a single species of carnivorous plant from the A_{mass} predicted by the GLOPNET scaling relationships (Ellison & Farnsworth 2005). The bivariate analysis conducted here compares the distribution in space of 10 carnivorous plant species relative to empirical bivariate quantiles of the GLOPNET data. It thus permits us to characterize more fully the position of carnivorous plant species relative to the scaling relationships observed for the global set of species.

All raw data collected during this experiment are available on-line from the Harvard Forest Data Archive (http://harvardforest.fas.harvard.edu/data/p10/hf109/hf109.html).

Results

EFFECTS OF SUPPLEMENTAL PREY ON LEAF-LEVEL PHYSIOLOGY

The mass-based net photosynthetic rate (A_{mass}) of the young (unfed) pitcher significantly increased with small increases in supplemental prey, and then levelled off asymptotically at higher levels of feeding (Fig. 1a, Table 2). The first and second pitchers did not show differences in photosynthetic rate attributable to feeding (data not shown). Photosynthetic nitrogen-use efficiency of the younger leaf was not significantly related to feeding level (Table 2), but photosynthetic phosphorus-use efficiency increased linearly with amount of prey supplement (Fig. 1b, Table 2).

Variable to maximal fluorescence ratios of both the first and second leaves (F_v/F_m; reflecting the efficiency of Photosystem II; Roháček 2002) were positively and significantly correlated with feeding level (Table 2). The mean ratio for unfed plants, across species, was 0.766 ± 0.008 (SE; maximum = 0.815), whilst that for maximally fed plants was 0.822 ± 0.007 (SE; maximum = 0.854). These effects were asymptotic (Fig. 1c), as F_v/F_m ratios seldom exceed 0.9 in nature, but even minimally fed plants exhibited a strong positive response to feeding, indicating a reduction in photosystem stress in fed leaves.

EFFECTS OF SUPPLEMENTAL PREY ON PLANT GROWTH AND TISSUE NUTRIENT CONTENT

The majority of species showed enhancements in above-ground relative growth rate at higher feeding levels relative to unfed controls (Fig. 1d, Table 2); exceptions, giving rise to the significant feeding × species interaction term in Table 2, were

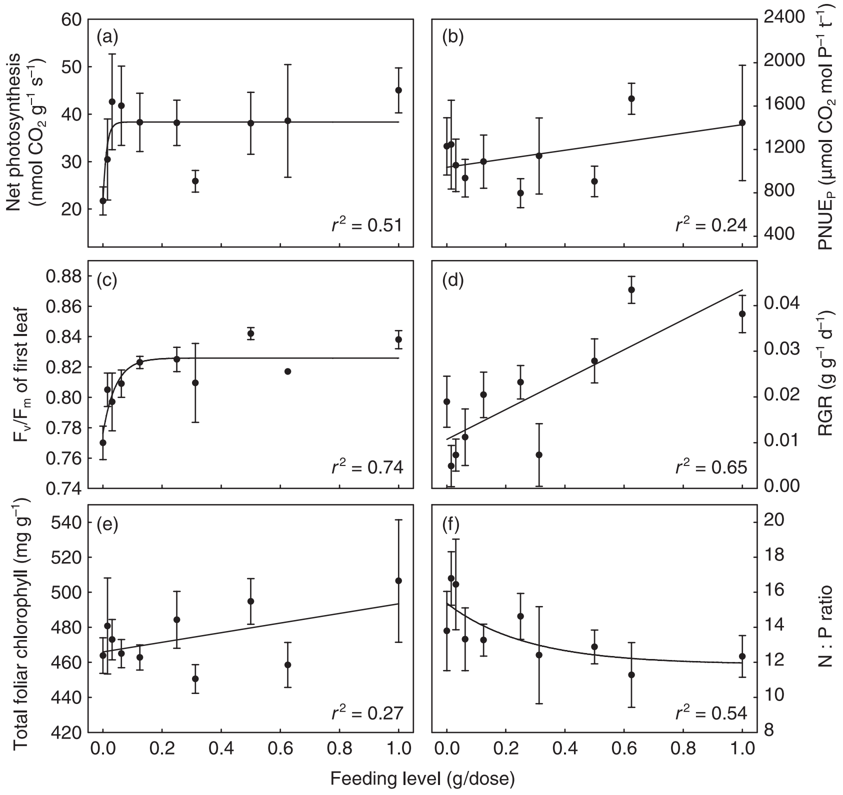

Fig. 1. Response of six variables to levels of feeding in 10 *Sarracenia* species. The variables that showed a feeding effect ($P < 0.1$) in the fixed-factor ANOVA are shown (statistics in Table 2); data are from young, mature leaves unless otherwise indicated. Points are means of all plants at each feeding level; error bars correspond to 1 SE of the mean. Lines for panels (a) and (c) were fitted with a non-linear regression describing a three-variable exponential rise to maximum ($y = y_0 + a(1 - e^{-bx})$), where a and b are constants). Data in panel (f) were fitted using a three-variable exponential decay function ($y = y_0 + ae^{-bx}$). Data in panels (b), (d) and (e) were fitted with linear regression.

Table 2. F-statistics (subscripted with degrees of freedom) and probabilities testing the effect of ln(feeding treatment), species and their interaction on foliar chemistry, physiology and growth rates of plant compartments measured on young, mature pitchers (unless otherwise indicated) at harvest. Units are given in column 1. P-values significant below the 0.05 level are shown in bold

Variable	Feeding level ($F_{\text{d.f.}}$, P)	Species ($F_{\text{d.f.}}$, P)	Feeding × species ($F_{\text{d.f.}}$, P)
Mass-based A_{max} (nmol CO$_2$ g^{-1} s^{-1})	**$F_{1,89}$ = 8.94, 0.004**	**F9,89 = 4.06, 0.0002**	$F_{9,89}$ = 1.49, 0.16
F_v/F_m ratio (first pitcher produced during season)	**$F_{1,25}$ = 7.97, 0.009**	$F_{9,25}$ = 1.76, 0.13	$F_{8,25}$ = 0.79, 0.62
F_v/F_m ratio (second pitcher produced during season)	**$F_{1,27}$ = 10.67, 0.003**	**$F_{9,27}$ = 2.98, 0.01**	$F_{9,27}$ = 1.37, 0.25
Total foliar chlorophyll concentration (mg g^{-1})	**$F_{1,87}$ = 2.18, 0.03**	**$F_{9,87}$ = 10.46, 0.001**	$F_{9,87}$ = 1.08, 0.39
Foliar N content (%)	$F_{1,85}$ = 1.71, 0.19	**$F_{9,85}$ = 5.44, < 0.0001**	$F_{9,85}$ = 0.54, 0.84
Foliar P content (%)	$F_{1,85}$ = 1.41, 0.2	**$F_{9,85}$ = 2.817, 0.006**	$F_{9,85}$ = 1.15, 0.34
Tissue K concentration (%)	$F_{1,85}$ = 0.94, 0.3	**$F_{9,85}$ = 3.23, 0.002**	$F_{9,85}$ = 1.27, 0.26
N : P ratio	$F_{1,85}$ = 3.11, 0.08	**$F_{9,85}$ = 10.65, < 0.0001**	$F_{9,85}$ = 0.73, 0.68
PNUE$_N$ (µmol CO$_2$ mol N^{-1} t^{-1})	$F_{1.85}$ = 0.38, 0.54	**$F_{9,85}$ = 17.64, < 0.0001**	$F_{9,85}$ = 1.55, 0.15
PNUE$_P$ (µmol CO$_2$ mol P^{-1} t^{-1})	**$F_{1,82}$ = 5.76, 0.02**	**$F_{9,82}$ = 9.27, < 0.0001**	$F_{9,82}$ = 0.60, 0.79
Estimated above-ground relative growth rate (g day^{-1})	**$F_{1,96}$ = 61.65, < 0.0001**	**$F_{9,96}$ = 30.39, < 0.0001**	**$F_{9,96}$ = 2.49, 0.01**
Leaf Mass Area (g m^{-2})	$F_{1,85}$ = 0.77, 0.38	**$F_{9,85}$ = 23.89, < 0.0001**	**$F_{9,85}$ = 3.35, 0.001**
Specific Leaf Area (cm^2 g^{-1})	$F_{1,90}$ = 0.34, 0.56	**$F_{9,90}$ = 7.549, < 0.0001**	$F_{9,90}$ = 1.32, 0.24
Root : shoot ratio	$F_{1,100}$ = 0.63, 0.43	**$F_{9,100}$ = 7.49, < 0.0001**	$F_{9,100}$ = 1.89, 0.06

S. rosea and *S. alata*. The total number of leaves produced per plant was slightly but not significantly enhanced at increased feeding levels ($P = 0.06$). However, for the nine species that produce phyllodes (Table 1), the relative proportion of phyllodes was significantly higher among better-fed plants than among plants receiving smaller supplements or no additional prey (Kruskal–Wallis rank sum test, $\chi^2 = 17.15$; $P < 0.05$).

Both LMA and specific leaf area (SLA) of pitchers differed significantly among species, but neither outcome was significantly affected by the feeding treatments (Table 2). Supplemental feeding had no effect on the areas of the first, second or younger pitchers produced by the plant (data not shown). We also did not observe an effect of supplemental feeding on root : shoot ratios (Table 2); well-fed plants of half of the species exhibited small increases in root : shoot ratios, whilst fed plants of the other five species showed small declines. The four rhizomatous species did not differ from the non-rhizomatous species in any aspect of their response to feeding.

Although well-fed plants had slightly higher foliar nitrogen, phosphorus and potassium contents than controls (Figs 2 and 3), absolute foliar nutrient concentrations were not significantly related to feeding levels (Table 2). Overall,

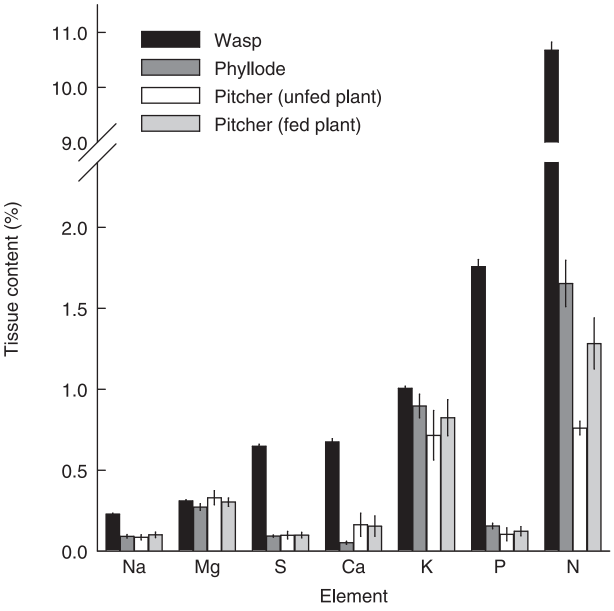

Fig. 2. Elemental contents in tissues of the ground wasps, phyllodes (all treatments pooled), pitchers of fed plants, and pitchers of unfed plants. Means are shown; error bars are ± 1 SE of the mean.

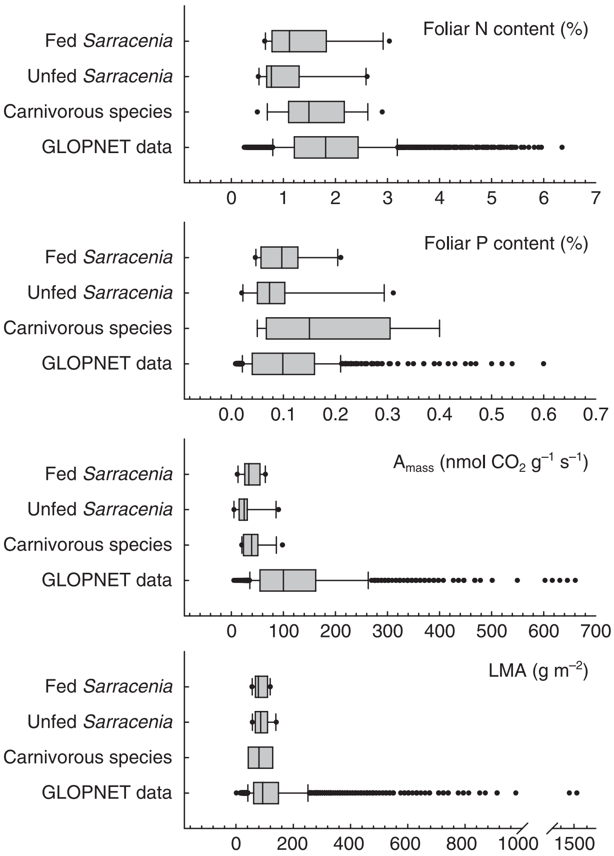

Fig. 3. Box plots showing foliar concentrations of N and P, mass-based net photosynthetic rate (A_{mass}), and leaf mass per unit area (LMA) for young, fully expanded leaves of unfed *Sarracenia* (this experiment), young leaves of maximally fed *Sarracenia* (this experiment), carnivorous plants generally (data compiled by Ellison 2006), and GLOPNET species pooled (Wright *et al.* 2004).

supplemental prey did not significantly alter the absolute concentrations of metals or trace nutrients in pitcher plants (Fig. 2).

Phyllodes differed from pitchers in their mineral composition (Fig. 2). Phyllodes had higher foliar nitrogen (Wilcoxon signed-rank test comparing pitchers and phyllodes across species: $Z = –2.00$, $P < 0.05$); higher phosphorus ($Z = –2.62$, $P < 0.01$); and marginally higher potassium contents ($Z = –1.91$, $P = 0.06$) than pitchers (Fig. 2). However, feeding levels did not appear to significantly affect the absolute levels of nutrients in phyllodes. Thus, although phyllodes and pitchers differed somewhat in nutrient content, they responded similarly to supplemental feeding.

Total chlorophyll content of the younger pitchers was positively and significantly correlated with feeding level, and this relationship was consistent among species (Table 2). Well-fed plants exhibited higher chlorophyll contents than unfed plants (Fig. 1e). Chlorophyll content was not correlated with either foliar nitrogen ($r^2 = 0.021$, $P = 0.15$) or phosphorus ($r^2 = 0.001$, $P = 0.77$).

EFFECTS OF SUPPLEMENTAL PREY ON NUTRIENT STOICHIOMETRY AND LEAF-TRAIT RELATIONSHIPS

Prey additions resulted in a non-linear decrease in N : P ratio (Fig. 1f; Table 2). Small amounts of additional prey (0.0156 g–0.03125 g dose^{-1}) were associated with a modest increase in N : P ratio, but doses of 0.0625 g and higher resulted in decreased N : P ratios (Fig. 1f). N : P ratios decreased in younger pitchers of fed plants (Fig. 1f, Table 2); this trend was significant when both phyllodes and pitchers were pooled for analysis ($F_{1,107} = 4.37$, $P = 0.04$). N : P ratios of phyllodes were significantly lower than those of pitchers ($Z = 2.17$, $P = 0.03$). Foliar N : P ratio was not correlated with A_{mass} ($r^2 = 0.013$; $P = 0.24$). N : K and K : P ratios (means = 1.7 and 8.9, respectively) were unaffected by feedings.

Fed and unfed *Sarracenia* species were similar to other carnivorous species in terms of values of the four key leaf traits: LMA, foliar N, foliar P, and A_{mass} (Fig. 3). The number of species falling in each of the four percentile categories (median, upper quartile, upper fence and outer decile) shifted slightly in all cases under the feeding regime (Fig. 4). Unfed species tended to lie within the bivariate upper quartile, upper fence or outer decile of the GLOPNET data, whilst fed species tended to lie within the bivariate median or upper quartile of the GLOPNET distribution (Fig. 5). However, this shift in frequency was statistically significant only for the relationship between P and N ($\chi^2_3 = 9.7714$, $P = 0.021$).

Discussion

This study, examining 10 of the 11 species recognized in the genus *Sarracenia*, more than doubles the number of carnivorous plants for which comprehensive ecophysiological data are available. *Sarracenia* species are similar in many ecophysiological attributes to other species of carnivorous plants: foliar nitrogen, phosphorus and chlorophyll contents all were low in unfed plants and increased with supplemental feeding

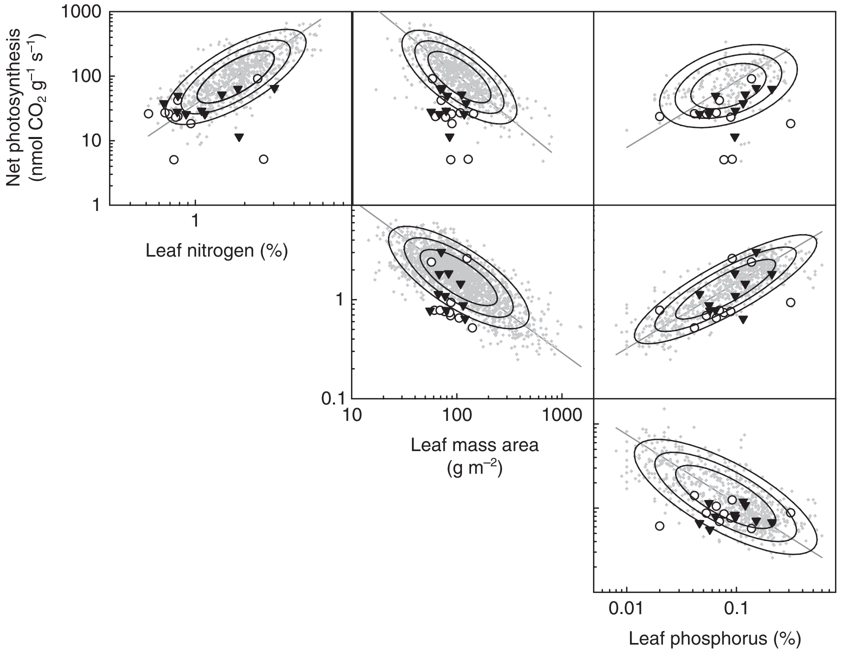

Fig. 4. Scaling relationships between mass-based net photosynthetic rate (A_{mass}), leaf mass area (LMA), leaf N (%) and leaf P (%). The light grey points are the GLOPNET data (Wright *et al.* 2004) used to generate the world-wide leaf economics spectrum; the light grey line corresponds to the reduced major axis regression line for these data. The ellipses (from smallest to largest) enclose the median (0–50th percentile), upper quartile (51–75th percentile) and the upper fence (76–90th percentile) of the GLOPNET data. Data beyond the outer ellipse are in the outer decile (90–100th percentile) of the GLOPNET data set. Mean values per species for unfed *Sarracenia* are depicted with open circles. Mean values for *Sarracenia* species receiving the maximum dosage of feeding per species are designated with filled triangles.

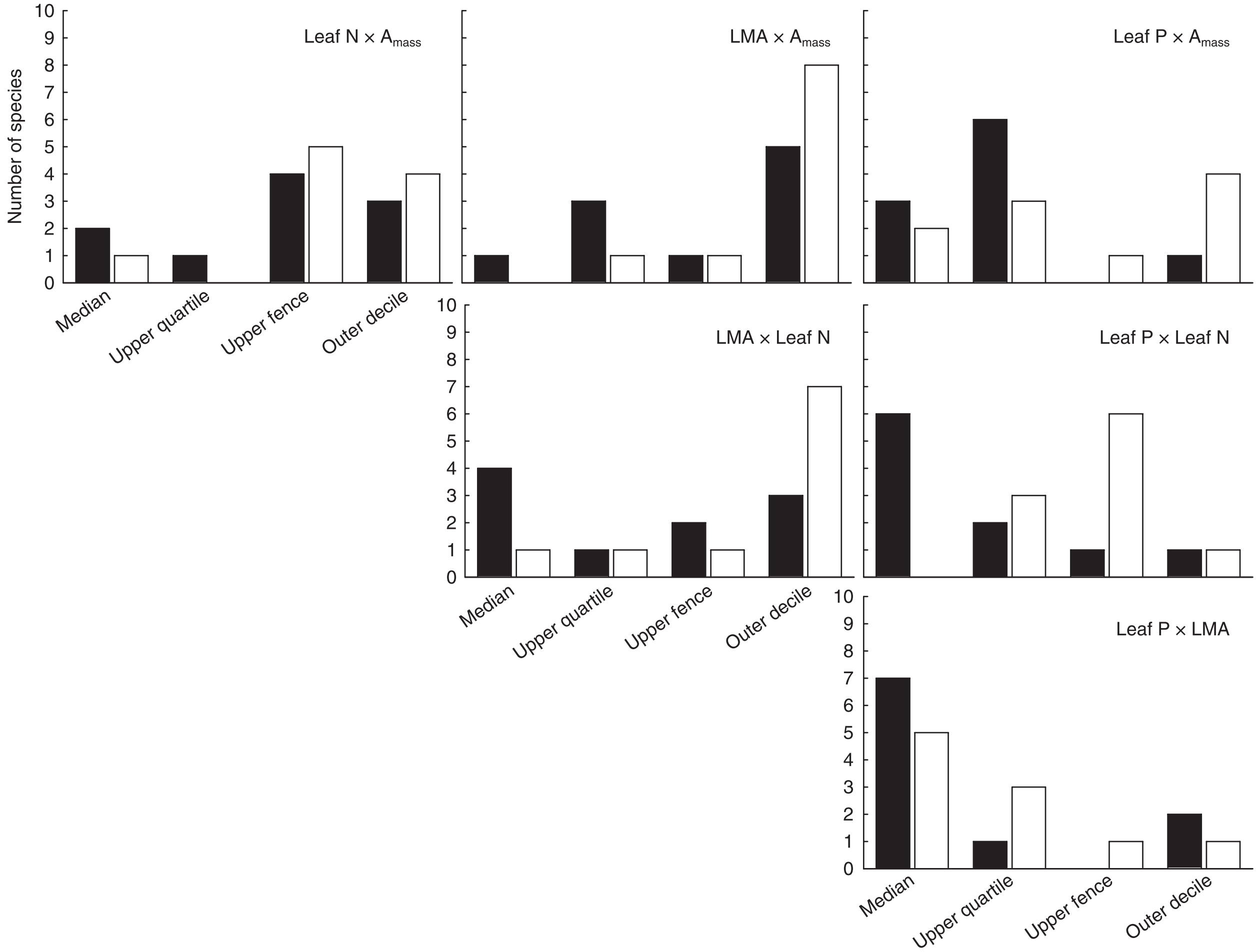

Fig. 5. Numbers of *Sarracenia* species falling within the median ellipse (0–50th percentile), upper quartile (51–75th percentile), upper fence (76–90th percentile) and outer decile (91–100th percentile) of the GLOPNET data. Open bars correspond to unfed plants; filled bars correspond to maximally fed plants of each species.

(Figs 1–3). Slow mass-based photosynthetic rates (Fig. 1a) may reflect limitations in the nutrients available to carry out basic physiological functions. A_{mass} was exceptionally low in unfed *Sarracenia* species and typical of those measured in other carnivorous species (Fig. 3). *Sarracenia* plants receiving increased inputs of insect prey showed significantly enhanced photosynthetic rates (Table 2, Fig. 1a), although these, too, were quite low relative to non-carnivorous species (Fig. 3).

Biologists who study carnivorous plants assume that these species are chronically stressed, especially when prey capture rates are low. The only study to date that has directly quantified photosystem stress in a carnivorous plant, which examined foliar reflectance patterns in starved *Nepenthes rafflesiana* plants (Moran & Moran 1998), did indicate that chlorophyll content is depressed and anthocyanin content is increased in stressed pitchers. In our experiments with *Sarracenia*, increased prey availability alleviated photosystem stress in the fed pitchers (Table 2, Fig. 1c). Feeding also significantly enhanced foliar chlorophyll content of the younger pitchers produced later in the growing season.

Young pitchers of fed plants exhibited higher photosynthetic nutrient-use efficiencies in terms of phosphorus ($PNUE_P$; Fig. 1b), but not of nitrogen ($PNUE_N$; Table 2). Increasing availability of prey also reduced the N : P ratio (Fig. 1f). Using values for the relationship between stoichiometric ratios and nutrient limitation (Olde Venterink *et al.* 2003), our data suggest that unfed plants were P-limited or N + P co-limited (N : P > 14.5; Fig. 1f), but fed plants were more strongly N-limited (N : P < 14.5; Fig. 1f). Stable isotope data have illustrated that *Sarracenia* species take up only about 40% of the N available from prey (Butler & Ellison 2006); the remainder appears to be sequestered by bacteria in the pitchers (Butler *et al.* in press). Taken together, these results suggest strongly that plants fed more insects exhibited increasing N-limitation (resulting in a lower N : P ratio) during the growing season, and that they used prey-based P rather than surplus N to enhance photosynthetic efficiency (resulting in higher $PNUE_P$). The few existing studies of other carnivorous species (Méndez & Karlsson 1999; Wakefield *et al.* 2005; Ellison 2006) also indicate that this P-sensitive response to supplemental prey may be a universal characteristic of carnivorous plants. However, direct studies of P uptake and translocation are lacking and experiments using labelled P are needed (cf. Plummer & Kethley 1964). Because excess nutrients can be allocated directly to storage (Schulze *et al.* 1997; Butler & Ellison 2007), current growth and construction costs (Shipley *et al.* 2006), or physiological processes, it may not be advisable to use photosynthetic rate alone as an index of the cost or benefit of carnivory (Givnish *et al.* 1984). It would be valuable to construct a full nutrient budget and to examine the interplay of nutrient absorption and root uptake for a greater range of carnivorous species (Adamec 2002).

The reduction of photosystem stress and the enhancement of photosynthetic rates in *Sarracenia* receiving supplemental prey translated directly into more rapid relative growth rates (Fig. 1d). Well-fed plants also increased production of non-carnivorous phyllodes, which themselves are more photosynthetically efficient. Similar enhancements of phyllode production have been found for *S. purpurea* growing in bogs that receive high levels of inorganic N from atmospheric deposition (Ellison & Gotelli 2002), and the bladderwort *Utricularia macrorhiza* produces fewer traps when nutrient concentrations in its aquatic habitat are high (Knight & Frost 1991).

The 10 *Sarracenia* species studied here, like other carnivorous plants (Ellison & Farnsworth 2005; Ellison 2006), depart significantly from the scaling relationships described by the universal spectrum of leaf traits found for non-carnivorous plant species, in terms of their positions in bivariate space (Figs 4 and 5). Variability among plant species and individual differences among them are certainly to be expected when only a small set of species (relative to the thousands used in GLOPNET) is studied in detail. It is notable, however, that most *Sarracenia* species, whether or not supplied with supplemental prey, tend to fall outside the 75th bivariate percentile of scaling relationships of leaf N, A_{mass} and LMA (Figs 4 and 5). This finding is consistent with the apparent constraints on N uptake discussed above. In contrast, in fed plants, the scaling relationships between P and other variables (N and A_{mass}) fall more in line with those of non-carnivorous plants (Figs 4 and 5), consistent with a greater capacity to take up and allocate P to physiological and growth processes.

Recent studies have shown that scaling relationships of A_{mass} with respect to LMA, N and K differ among life forms of plants exposed to different light environments (Santiago & Wright 2007). Carnivorous plants typically grow in high-light conditions, but are extremely constrained in terms of both nutrient availability and uptake. Although leaf traits vary even among closely related species within a single genus, differential sensitivity to nutrient availability imposes limits on plastic responses of which these carnivorous taxa are capable. We observed, for example, that leaf mass per unit area (LMA) of pitchers was relatively insensitive to feeding levels (Fig. 4; Table 2). In addition to nutrient stoichiometry, another key to understanding why these species depart so strongly from universal scaling relationships may lie in examining differences among carnivorous leaves (e.g. pitchers) and photosynthetic leaves (e.g. phyllodes; see also Pavlovič *et al.* 2007).

Shipley *et al.* (2006) posited that within-leaf trade-offs between photosynthetic rates, leaf density, and leaf longevity may in part result from trade-offs in allocation to structural tissues vs. liquid-phase (e.g. photosynthetic) processes. This may be particularly true for carnivorous plants, which allocate a substantial portion of biomass to essentially structural leaves optimized for prey capture rather than light absorption. The apparently aberrant scaling relationships of carnivorous plants observed here points to fundamental constraints inherent to botanical carnivory, and suggests a need for similar, comparative studies of other specialized adaptations of plant species to habitats such as 'skeletal soils' (*sensu* Thompson *et al.* 1997) and wetlands (Shipley & Lechowicz 2000). Such an approach should be applied more generally to other plant species that produce dimorphic leaves, such as those that inhabit aquatic environments and are also outliers with respect to GLOPNET (Shipley & Lechowicz 2000; Ellison 2002).

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