

Nitrogen cycling dynamics in the carnivorous northern pitcher plant, *Sarracenia purpurea*

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Summary

1. In nutrient-poor environments, plants employ many strategies to acquire and recycle scarce nutrients. Predictable relationships among a variety of leaf traits, including leaf nitrogen (N) suggest that how plants obtain, use, store and re-use N is a major component of plant fitness. The northern pitcher plant, *Sarracenia purpurea*, receives nitrogen from multiple sources: NH_4 and NO_3 dissolved in precipitation; N mineralized from captured prey; the scant N in saturated peat; and N remobilized from storage. In two greenhouse experiments, we examined N cycling in *S. purpurea* and consider how these dynamics relate to prior observations that this carnivorous plant has an unusually low photosynthetic rate for its tissue N content.

2. In the first experiment we assessed assimilation, translocation, storage and remobilization of ^{15}N supplied to pitchers and roots. In the second experiment, we examined how ^{15}N assimilated by the first pitcher produced at the start of the growing season contributed to the production and maintenance of subsequent pitchers, roots and rhizomes.

3. Patterns of N cycling were similar at the individual-leaf and whole-plant level. Pitchers assimilated 55%–69% of available ^{15}N and served as both the largest sink for newly assimilated N (> 90% of the ^{15}N assimilated during 2004) and the largest source of N remobilization the following spring. In contrast, N assimilated by roots was low and accounted for < 2.5% of the overall *S. purpurea* N budget. *Sarracenia purpurea* used both stored N and newly-acquired N throughout the growing season. The importance of stored N decreased throughout the growing season as newly assimilated N contributed more to later pitcher production.

4. Our detailed mechanistic analysis of nitrogen cycling dynamics of *S. purpurea* suggests why this plant has a low photosynthetic rate for its tissue N content. Excess nitrogen is stored for future use, and production of new pitchers is primarily aimed at enhancing prey capture rather than increasing photosynthetic tissue.

Key-words: ^{15}N , carnivorous plants, nitrogen cycling, *Sarracenia*, universal spectrum of leaf traits

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Introduction

In many ecosystems, nitrogen (N) is the nutrient that most limits plant growth (Berendse & Aerts 1987) and plants have generally adapted to this limitation by employing a variety of strategies to acquire and conserve nutrients (Killingbeck 1996). Predictable relationships among leaf N content, photosynthetic rate, leaf life span and specific leaf mass across a wide range of plant species and life-forms – the ‘universal spectrum of leaf traits’ (Wright *et al.* 2004) – suggest that how plants obtain, use, store and re-use N is a major component of plant fitness. Shipley *et al.* (2006)

suggested a mechanism underlying this universal spectrum: a trade-off in allocation of limiting nutrients to structural tissues vs liquid-phase processes such as photosynthesis. Assessing the validity of this mechanism requires a detailed understanding of within-plant nutrient use and allocation, which can be followed using isotopically labelled N (reviewed by Dawson *et al.* 2002). Here, we describe the results of two experiments in which we used ^{15}N to examine within-plant N cycling in a carnivorous plant whose leaf traits and physiological characteristics place it at the extreme lower end of Wright *et al.*'s (2004) spectrum (Ellison 2006).

Most plants assimilate nutrients primarily through their roots and translocate them above-ground. In contrast, the ± 600 species of carnivorous plants, which generally inhabit nutrient-poor habitats (Givnish *et al.*

1984) acquire some of their nutrients from captured animal prey (Ellison & Gotelli 2001). Results of the few studies examining nutrient uptake by roots of carnivorous plants suggest that prey capture rate may influence soil nutrient acquisition (Hanslin & Karlsson 1996; Adamec 2002; Adlassnig *et al.* 2005). But in general, N cycling dynamics within carnivorous plants is poorly understood. Many carnivorous plants, including the pitcher plant studied here, depart from the universal spectrum of leaf traits (E.J. Farnsworth & A.M. Ellison, unpublished; Ellison 2006). In particular, the photosynthetic rate of carnivorous plants is *c.* 50% lower for a given tissue N content than is predicted by the universal spectrum (E.J. Farnsworth & A.M. Ellison unpublished; Ellison 2006). By developing a more precise understanding of how carnivorous plants obtain, use, store and re-use N, we can begin to assess the generality of the mechanism that Shipley *et al.* (2006) proposed to explain the universal spectrum of leaf traits.

Sarracenia purpurea L. is one of the most widespread carnivorous plants in North America (Schnell 2002). We examined N cycling dynamics both within (assimilation and translocation) and across (storage and remobilization) growing seasons. Further, we explored the dynamics of N cycling at both the whole-plant and the individual-leaf levels. In two experiments we addressed the following questions: (i) What is the fate of N assimilated by pitchers and roots in terms of assimilation, translocation, storage and remobilization? (ii) To what extent does N assimilated by the first pitcher produced in a growing season contribute to plant growth later in the growing season? In the first experiment, we hypothesized that pitcher uptake of N would far exceed root uptake and that N stored in the previous year's above-ground biomass would contribute most to early season pitcher production; newly acquired N becomes more important as the season progresses and N stores are depleted. In the second experiment we predicted that pitchers produced later in the growing season would rely more heavily on N assimilated by pitchers produced early in the current growing season, as N stored in the previous season's pitchers was already depleted.

Materials and methods

STUDY SPECIES

Sarracenia purpurea is a long-lived (*c.* 50 years) carnivorous, perennial herb found in bogs, fens and seepage swamps across the coastal plain of eastern North America and in Canada westward to the Rocky Mountains (Schnell 2002). In northern bogs, pitchers are generally produced every 2 weeks, with 5–10 pitchers produced each growing season (Fish & Hall 1978). In northeastern North America, pitchers live 1–2 years; the previous year's pitchers senesce as the current year's pitchers develop. Unlike many carnivorous plants that produce digestive enzymes to break down

captured prey, *S. purpurea* relies on a food web of bacteria, protists, rotifers, mites and mosquito, midge, and fly larvae that inhabits its water-filled pitchers to decompose prey and mineralize nutrients (Juniper, Robins & Joel 1989). As captured prey moves through the food web, plants use excreted or mineralized nutrients that become available (Bradshaw & Creelman 1984). The root system of *S. purpurea* is weakly developed (the ratio of above- to below-ground biomass was *c.* 6 in this study); because of its small root system and its nutrient-poor habitat, it is thought that *S. purpurea* relies predominantly on decomposition of captured prey for its nutrients (Chapin & Pastor 1995). In addition, where N deposition is relatively high, *S. purpurea* likely uses NH_4 and NO_3 dissolved in rainfall (Bledzki & Ellison 1998).

EXPERIMENTAL DESIGN

We conducted two ^{15}N labelling experiments using five atom percentage excess $^{15}\text{NH}_4^{15}\text{NO}_3$ (Isotec, Miamisburg OH). NH_4^+ is present in pitcher water, whilst NO_3^- is generally found there in low concentrations (Bradshaw & Creelman 1984; J.L. Butler & A.M. Ellison, unpublished data). However, we chose to add N as NH_4NO_3 to represent what plants receive from atmospheric N deposition (Ellison & Gotelli 2002). In addition to assimilating NH_4^+ (Bradshaw & Creelman 1984), *S. purpurea* can assimilate NO_3^- ; both roots and pitchers contain nitrate reductase (J. Karagatzides, J.L. Butler & A.M. Ellison, unpublished data).

These experiments were carried out in the greenhouse to avoid over-harvesting of natural populations, and to limit food web development and prey capture. Because we were interested in characterizing N dynamics controlled by the plant itself, we used an inorganic N source (NH_4NO_3) and removed the food web from the experimental plants. All *S. purpurea* plants used in these experiments were 5 years old and had been grown in the greenhouse from wild-collected seed. Plants were grown in milled Sphagnum (*Sphagnum magellanicum* Brid.) as the growth medium (10 × 10 cm pots), and had not been fertilized prior to this experiment. Milled Sphagnum is a virtually nutrient-free potting medium commonly used for germinating seeds. It is used in horticultural conditions when careful control of nutrient levels by external nutrient additions is required. The high water-holding capacity of milled Sphagnum limits losses of added nutrients before the plant can take them up.

Experiment One: Dynamics of root and pitcher-assimilated N

One hundred *S. purpurea* plants were randomly assigned to one of four treatments: Control (no N added), Root-fed ('R-fed,' N added to growing medium), Pitcher-fed ('P-fed,' N added to individual pitchers), and Root + pitcher-fed ('RP-fed,' N added both to growth medium

and pitchers). Each R-fed plant received 3.51 mg of N throughout the growing season, which represented an average of 54% (range: 26.2%–92.9%) of those plants' N pools. The total N fed to pitchers at the end of labelling averaged 6.15 mg N, which represented 46% (range: 42.4%–49.4%) of those plants' N pools. The mass of N fed to plants in these studies is comparable to the mass of prey-N captured by *S. purpurea* (Heard 1998). To trace the fate of N within *S. purpurea* in the four treatments, nutrient solution containing five atom percentage excess ^{15}N was supplied throughout the 2004 growing season ('pulse' period), whilst unlabelled nutrient solution was supplied in 2005 ('chase period'). Production of new pitchers by all plants in each treatment group was monitored throughout the experiment. Once a new pitcher had fully opened and hardened (about 2 weeks after opening) it received a modified Hoagland's nutrient solution containing: 457.19 mmol m⁻³ NH₄NO₃ (provided as $^{15}\text{NH}_4^{15}\text{NO}_3$ in 2004 and unlabelled NH₄NO₃ in 2005), 138.59 mmol m⁻³ MgSO₄, 69.29 mmol m⁻³ KH₂PO₄, 352.97 mmol m⁻³ CaCl₂, 353.08 mmol m⁻³ KCl, 32.41 mmol m⁻³ H₂BO₃, 6.40 mmol m⁻³ MnCl₂, 0.56 mmol m⁻³ ZnCl₂, 0.21 mmol m⁻³ CuCl₂, 0.07 mmol m⁻³ Na₂MoO₄, 14.56 mmol m⁻³ Na₂HPO₄ and 14.02 mmol m⁻³ FeSO₄. Root-fed plants received a dilute Hoagland's solution (15 : 1 dilution) applied directly to the peat once the plant's first pitcher had fully established. Because our focus was on N cycling, we added a complete nutrient solution in order to more closely mimic the suite of nutrients available to pitchers if insect prey were captured, thereby avoiding potential deficiencies in other plant essential nutrients.

Nutrient addition took place twice weekly. Individual pitchers received a volume of nutrient solution equal to two-thirds the volume of that particular pitcher; volumes ranged from 0.5 to 7 mL. At each feeding, the solution added the previous time was withdrawn from pitchers and replaced with fresh nutrient solution. It should be noted that we measured NH₄⁺ and NO₃⁻ concentrations of pitcher solution withdrawn from P-fed and RP-fed pitchers once during the 2005 season. Whilst NH₄⁺ was not detected, NO₃⁻-N averaged 1.6 mg N L⁻¹ (about 8% of a biweekly N dose). R-fed plants were fed twice each week with 75 mL of nutrient solution; efforts were made to avoid splashing nutrient solution on pitchers. Distilled-deionized water (dd-H₂O) was added to control pitchers and pots not receiving nutrients. The milled Sphagnum was kept moist by watering with dd-H₂O as necessary. All pitchers were plugged with a fine nylon mesh screen to reduce prey capture.

Five randomly chosen plants from each treatment were harvested at each of five destructive harvests: (i) Mid-growing season 2004 (June), when fourth pitchers had formed but not been treated/fed; (ii) End of growing season 2004 (early October); (iii) Dormancy (mid-January 2005); (iv) Mid-growing season 2005 (June), when third pitchers had formed but not yet

been fed; and (v) End of growing season 2005 (late September). Fewer plants were harvested in some cases due to damage caused by scale in the greenhouse.

Pitcher heights were measured (± 1 mm) before each plant was harvested and at the end of each growing season. During harvests, pitchers, roots and rhizomes were separated and thoroughly washed in tap water, followed by several rinses in dd-H₂O. Plant parts were dried for 48 h at 65 °C, weighed (± 0.001 g), and ground to a fine powder using a Wig-L-Bug grinder (Bratt Technologies, LCC, East Orange, NJ). Subsamples (c. 2 mg) were then weighed into 8 × 5 mm tin capsules (Elemental Microanalysis, Mason, OH) and analyzed for total N and ^{15}N abundance at Yale University on a Finnigan DELTAplus Advantage continuous flow isotope ratio mass spectrometer and element analyzer.

Experiment Two: Fate of N assimilated by first pitcher

To examine in more detail the contribution of N assimilated by the first pitcher formed in a growing season to the remainder of the plant, 15 *S. purpurea* plants received a similar treatment to the P-fed treatment as described in Experiment One. However, in this experiment, only the first pitcher formed on each plant received ^{15}N -enriched nutrient solution, whilst the remaining pitchers received nutrient solutions containing natural abundance ^{15}N . Three destructive harvests were used to quantify the fate of ^{15}N assimilated by first pitchers (five replicate plants at each harvest): (i) when third pitchers had formed and opened (Harvest 1); (ii) when fourth pitchers had formed and opened (Harvest 2); and (iii) when fifth pitchers had formed and opened (Harvest 3). This allowed us to examine translocation of N obtained by the first pitcher into subsequent pitchers, roots and rhizomes. Pitchers that had opened just before each harvest period are referred to as 'target pitchers' (e.g. the third pitcher is the target pitcher in Harvest 1). Harvest procedures were similar to those described in Experiment One. Three unlabelled control plants were harvested at the end of the experiment to assess natural ^{15}N abundance.

DATA ANALYSIS

Recovery of ^{15}N in plants was calculated relative to unlabelled control plants. Uptake efficiency (UE) was calculated as:

$$\text{UE} = 100 \times (\text{g}^{15}\text{N}_{\text{plant}} / \text{g}^{15}\text{N}_{\text{fed}})$$

where g $^{15}\text{N}_{\text{plant}}$ and g $^{15}\text{N}_{\text{fed}}$ refer to the total g of ^{15}N recovered within the plant and the total g of ^{15}N supplied to the plant, respectively. Because uptake of N by root systems was so low in Experiment One (see Results below), the amount of N added to RP-fed plants was adjusted in our calculation of uptake efficiency. We subtracted the average percentage of ^{15}N assimilated by root systems in R-fed plants from the

total ^{15}N retained within RP-fed plants and then used only the N supplied to pitchers to calculate the uptake efficiency for plants in the RP-fed treatment.

To assess the influence of each treatment on relative growth change in Experiment One, we regressed the natural log of pitcher mass on pitcher length for all pitchers that had been harvested throughout the entire experiment (pitchers with any sign of senescence were removed from the data set). Relative growth change was then calculated as:

$$100 \times ((\text{mass}_{2005} - \text{mass}_{2004}) / \text{mass}_{2004})$$

Table 1. N uptake efficiency in Experiment One (treatment $P < 0.0001$, harvest $P = 0.0391$, treatment \times harvest interaction $P = 0.338$)

Treatment	^{15}N uptake efficiency (%)		
	Mid-season 2004	End of season 2004	End of season 2005
Root-fed	3.03 (0.73) ^{a,1}	7.10 (1.06) ^{a,2}	14.01 (3.31) ^{a,2}
Pitcher-fed	54.59 (5.78) ^{b,1}	69.24 (1.94) ^{b,1}	67.98 (6.99) ^{b,1}
Root + pitcher-fed	65.92 (2.99) ^{b,1}	66.44 (4.53) ^{b,1}	68.72 (2.52) ^{b,1}

Values are the mean \pm 1 SE from five replicates. Letters indicate significant differences between treatments within a harvest; numbers indicate significant differences within treatments ($P < 0.05$, Tukey–Kramer post-hoc test for multiple comparisons among means).

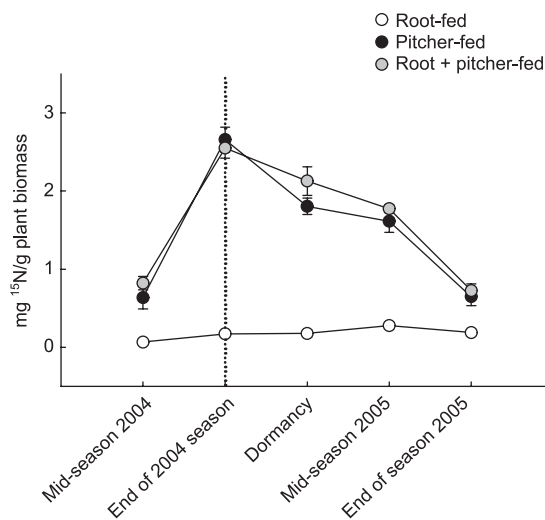


Fig. 1. Concentration of ^{15}N tracer within plants across all harvest periods in Experiment One. ($P < 0.001$ for treatment, harvest and treatment \times harvest interaction). Values include 2004 and 2005 plant parts and are the mean \pm 1 SE from five replicates for the first two harvests, three replicates for the third harvest, and two–four replicates for the last two harvests. Dashed vertical line indicates the end of ^{15}N labelling.

where mass_{2005} and mass_{2004} refer to mass of pitchers in the 2005 and 2004 growing seasons, respectively.

All statistical analyses were performed using SAS version 9.1 (SAS Institute 2002). When necessary, data were log-transformed to meet normality assumptions. Data were analyzed using two-way ANOVAs with fixed effects of treatment and harvest in the first experiment, and harvest and plant part in the second experiment. When significant interactions were identified, the ‘slice’ command in SAS was used to evaluate each level of both factors. When there were no significant interactions, main effects were assessed with one-way ANOVAs followed by Tukey–Kramer’s multiple comparisons test among means. In the first experiment, relative growth change was assessed using a one-way ANOVA with treatment as the fixed effect.

Results

EXPERIMENT ONE: DYNAMICS OF ROOT AND PITCHER ASSIMILATED N

Across all harvests, *S. purpurea* had a higher uptake efficiency of $^{15}\text{N-NH}_4\text{NO}_3$ by pitchers than root systems (Table 1). At the first harvest, uptake of labelled N by pitchers that also received N to their root systems was higher (65.9%), although not significantly, than plants receiving N only to their pitchers (54.6%). However, by the end of labelling (End of season 2004 harvest), P-fed and RP-fed plants had assimilated similar amounts of ^{15}N supplied during 2004 (69.2% and 66.5%, respectively); this value remained near 68% for P-fed and RP-fed plants harvested at the end of 2005 (Table 1). By mid-2004, R-fed plants had assimilated c. 3.0% of the ^{15}N provided; however, uptake increased significantly to 7.1% for plants harvested at the end of 2004 (Table 1). Although consistently low relative to pitchers, uptake efficiency of ^{15}N by roots was variable and reached 20% in one plant that was harvested at the end of 2005.

Overall, root uptake of labelled N contributed little to the plant’s N budget; this ^{15}N pool represented only 0.19 mg $^{15}\text{N g}^{-1}$ of plant biomass at the end of the ^{15}N labelling period. In contrast, P-fed and RP-fed plants had over 2.5 mg ^{15}N tracer g^{-1} of plant biomass at the end of the ^{15}N labelling period (Fig. 1). Once the labelling period ended, P-fed and RP-fed plants exhibited a steady decline in mg of ^{15}N tracer g^{-1} of biomass as assimilation of unlabelled N diluted the ^{15}N pool (Fig. 1).

In all three treatments, pitchers represented the largest sink for the assimilated ^{15}N (Fig. 2a). P-fed and RP-fed plants retained at least 90% of the assimilated ^{15}N within their pitchers (2004 and 2005 pitchers combined in Fig. 2a); this did not change significantly throughout the entire experiment. However, during 2005, ^{15}N was translocated out of senescing 2004 pitchers and into newly formed 2005 pitchers. By the end of the experiment, pitchers produced in 2005

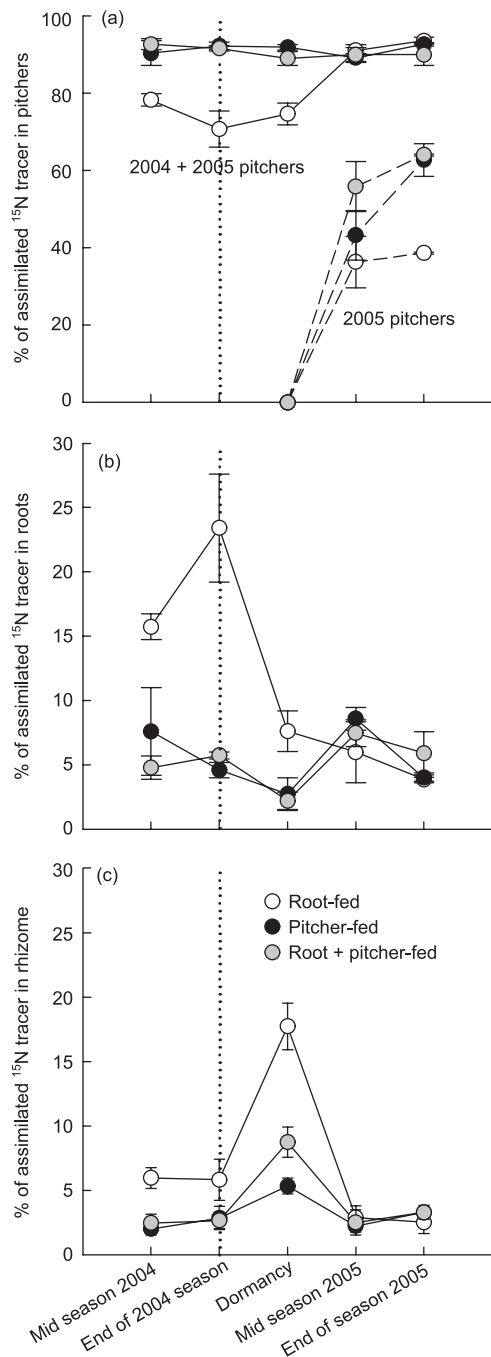


Fig. 2. Distribution of assimilated ^{15}N tracer across all harvest periods in Experiment One in (a) 2004 and 2005 pitchers combined (solid lines) (treatment $P < 0.0001$, harvest $P = 0.005$ and treatment \times harvest interaction $P < 0.0001$) and 2005 pitchers alone (dashed lines) (treatment $P = 0.002$, harvest $P = 0.053$ and treatment \times harvest interaction $P < 0.310$; ANOVA did not include zero values at the dormancy harvest), (b) roots (harvest, treatment and treatment \times harvest interaction $P < 0.0001$) and (c) rhizomes (treatment $P = 0.0002$, harvest $P < 0.0001$ and treatment \times harvest interaction $P = 0.1425$). Values represent the mean ± 1 SE; sample sizes as in Fig. 1. Dashed vertical line indicates the end of ^{15}N labelling.

contained 62.7% and 64.0% of the ^{15}N assimilated during 2004 in P-fed and RP-fed plants, respectively. Root-fed plants allocated between 70% and 80% of root-assimilated ^{15}N to pitchers in 2004. During 2005, however, remo-

bilization increased the percentage of ^{15}N contained within pitchers to *c.* 90%; this was the result of ^{15}N remobilization out of roots and rhizomes and into 2005 pitchers (Fig. 2a–c). By the end of 2005, new pitchers contained 38.0% of the ^{15}N assimilated during 2004.

Roots represented the second largest sink of assimilated ^{15}N . Root-fed plants initially retained the largest amount of assimilated ^{15}N within roots and also experienced the most dynamic changes through time (Fig. 2b). By the end of 2004, R-fed plants retained an average of 23.4% of the assimilated ^{15}N tracer in roots; this value declined significantly throughout the remaining harvests and by the end of 2005, only 3.9% of the assimilated ^{15}N was retained within roots. Pitcher-fed and RP-fed plants shared similar patterns of ^{15}N retention within roots. By the end of 2004, roots of P-fed and RP-fed plants had each retained $\leq 5\%$ of the assimilated ^{15}N . During dormancy, plants from both treatments translocated ^{15}N out of roots; only *c.* 2.5% of assimilated ^{15}N was retained within the roots in this period. In early 2005, however, ^{15}N was remobilized back into roots and the amount of ^{15}N in this pool reached 8%. By the end of the experiment, roots retained similar amounts of assimilated ^{15}N (*c.* 5%) across all three treatments; this represented *c.* 1% of the root N pool in R-fed plants and $\geq 5\%$ of the N pool in P-fed and RP-fed plants.

Across all three treatments, rhizomes retained the least amount of assimilated ^{15}N , with the exception of the dormancy harvest when there was substantial translocation of ^{15}N into rhizomes (Fig. 2c). In the first two harvests, R-fed plants allocated *c.* 6% of the assimilated ^{15}N to rhizomes, while P-fed and RP-fed plants allocated between 2% and 3%. During dormancy, however, ^{15}N retention in rhizomes increased to 18%, 9% and 5% in R-fed, P-fed and RP-fed plants, respectively. By the end of the experiment, however, ^{15}N was remobilized back out of the rhizomes and only 3% of the assimilated ^{15}N was found in the rhizomes across all three treatments. On a mass basis, this 3% represented *c.* 5% of the rhizome N pool in P-fed and RP-fed plants, but only *c.* 1% of the rhizome N pool in R-fed plants.

By deriving 2004 pitcher mass from pitcher height for the plants harvested at the end of the experiment ($Y = 0.252X - 3.58$; $r^2 = 0.834$, on log-transformed data; $P < 0.0001$), we compared relative growth change of pitcher biomass between the 2004 and 2005 growing season. Except for one unfed control plant, plants in all treatments grew. Increased growth resulted from the production of larger and in most cases, more pitchers. A greater positive growth change was observed in P-fed and RP-fed plants (mean = 148.1 ± 53.39 [SE] and 154.2 ± 5.6 , respectively) relative to R-fed and unlabelled control plants (61.3 ± 41.63 and 46.6 ± 34.29 , respectively). With the small sample size and high variance, especially among P-fed plants, however, differences were not significant among treatments ($P = 0.245$).

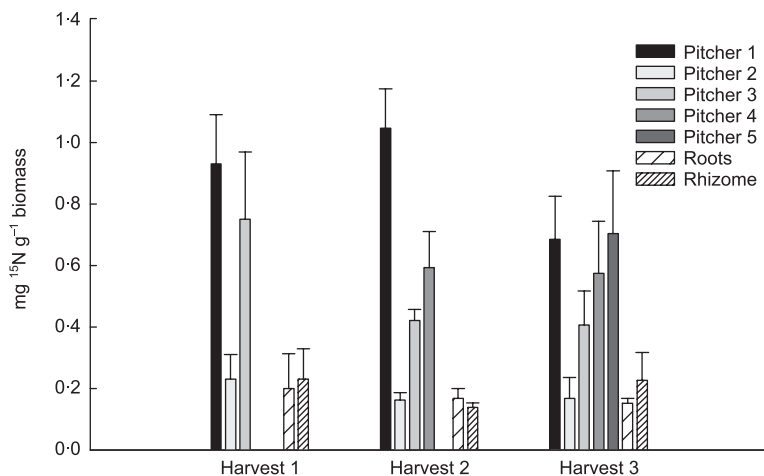


Fig. 3. Concentration of ¹⁵N tracer within pitchers, roots and rhizomes in each harvest of Experiment Two. Values are the mean ± 1 SE for four replicates (harvest $P = 0.078$, plant part $P < 0.0001$, harvest × plant part interaction $P = 0.258$).

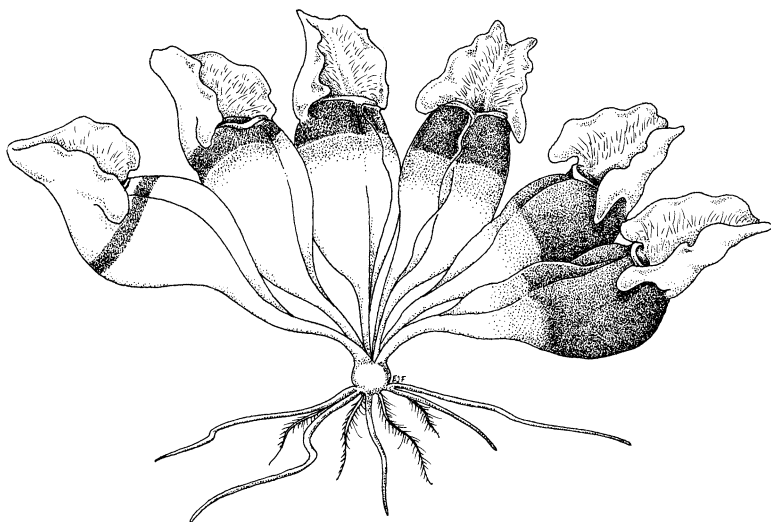


Fig. 4. Distribution of N remobilized from storage and newly assimilated N at the end of the 2004 growing season (end of ¹⁵N labelling). Going from left to right, pitchers appear in the order they were produced. The white, unshaded part refers to the proportion of the pitcher N pool coming from N stored in senesced pitcher produced the previous year. The shading refers to N assimilated in the current growing season, with the light shading representing the minimum portion of the pitcher N pool coming from N translocated from previously produced pitchers and the darker shading representing a combination of additional N translocated from previous pitchers, and N assimilated by and retained within individual pitchers. For simplicity, the diagram shows all pitchers on the plant to be similar in size. This was generally the case in Experiment One; however, in Experiment Two pitchers produced later in the season were generally smaller than those produced earlier. Illustration by E.J. Farnsworth.

EXPERIMENT TWO: FATE OF NITROGEN ASSIMILATED BY FIRST PITCHER

First pitchers assimilated on average 58.3%, 58.5% and 55.8% of the added ¹⁵N-NH₄NO₃ in the first, second and third harvest periods, respectively. In the first two harvest periods, first pitchers were the largest sink for assimilated ¹⁵N (Fig. 3). By Harvest 3 the strength of

the sink of the target pitcher (fifth pitcher) equalled that of the first pitcher. There was no significant effect of harvest on mg of ¹⁵N g⁻¹ pitcher biomass ($P = 0.07$); however, as the growing season progressed from Harvest 1 to Harvest 3, the proportion of ¹⁵N retained within the first and third pitchers declined (Fig. 3). Within each harvest period there was a significant difference between the mg ¹⁵N g⁻¹ biomass in individual plant parts ($P < 0.0001$), with second pitchers, roots and rhizomes having significantly lower mg of ¹⁵N g⁻¹ biomass relative to all other pitchers (Fig. 3).

Although the ¹⁵N assimilated by first pitchers provided a greater proportion of the pitcher N pool to pitchers produced later in the season, these pitchers were also slightly smaller than those produced earlier in the season. By the final harvest, third, fourth and fifth pitchers retained a similar percentage of the ¹⁵N (15%–20%) assimilated by first pitchers. First pitchers were the largest sink for N and retained *c.* 30% of the ¹⁵N they assimilated. In contrast, second pitchers, which had already formed when the ¹⁵N labelling commenced, obtained only *c.* 6%, whilst roots and rhizomes combined obtained < 10% of the ¹⁵N assimilated by first pitchers.

Discussion

In two experiments using isotopically labelled ¹⁵N-NH₄NO₃, we examined in detail the assimilation, translocation, storage and remobilization of N by *S. purpurea*. In nutrient-poor habitats such as bogs, the ability to store nutrients for use in subsequent seasons is an important nutrient conservation strategy for perennial plants (Small 1972). Our data support the hypothesis that pitchers take up vastly more N than do roots, and that N storage in over-wintering above-ground tissues (i.e. senescent pitchers) contribute significant resources to new pitcher production in the subsequent growing season. A predilection for storing nutrients, rather than using them immediately, may be one cause for the observation that photosynthetic rates of many wetland plants, including *S. purpurea*, are lower than is expected, given their observed foliar N concentrations (Shipley & Lechowicz 2000; Ellison 2006).

We summarize whole-plant and individual-leaf level N dynamics in *S. purpurea* simultaneously in a generalized conceptual diagram (Fig. 4). As subsequent pitchers are produced throughout the growing season, they rely less on N remobilized from the previous season's pitchers (unshaded areas in Fig. 4) and rely more on N translocated from new pitchers preceding them (shaded areas in Fig. 4). They also retain a greater proportion of the N they assimilate (dark shading in Fig. 4). Because uptake of N by roots contributes so little to the plant's overall N budget, Fig. 4 depicts the dynamics of N supplied to pitchers only.

The first pitcher is produced exclusively from N remobilized from the previous year's pitchers. Once established, this first pitcher can assimilate N from

prey and N deposition. The first pitcher retains a small fraction of its newly assimilated N (*c.* 8% of its N pool; dark shading in leftmost pitcher in Fig. 4); most is translocated to newly-forming pitchers. Because the second pitcher begins to form and grow before the first pitcher is fully developed, most of the second pitcher's N pool also comes from remobilization; only *c.* 2% comes from N assimilated by the first pitcher. The data from Experiment Two suggest that, with the exception of the second pitcher, the first pitcher supplies *c.* 6% of each new pitcher's N pool (light shading in pitchers 2–6 in Fig. 4). Assuming subsequent pitchers retain and translocate newly-acquired N in the same way as does the first pitcher, our data suggest that the *i*th pitcher in turn provides only a small amount of newly assimilated N to pitcher *i* + 1, but provides at least 6% of the N pool to pitchers *i* + 2, *i* + 3, Thus, at least 26% of the N pool of the sixth pitcher has come from translocation from previous pitchers (*c.* 6% from pitchers 1–4, and *c.* 2% from pitcher 5; denoted by the light shading on the rightmost pitcher in Fig. 4).

Each pitcher also retains a portion of the N it assimilates (dark shading in Fig. 4). The data from Experiment One do not allow us to determine precisely the quantity of N assimilated, translocated and retained by any individual pitcher other than the first one (hence the fuzziness in Fig. 4 of the distinction between the proportion of N from translocation [light shading] and the proportion of assimilated N retained [dark shading]). However, we hypothesize that the amount of assimilated N retained within a pitcher increases throughout the growing season. It is likely that by the time the fifth and sixth pitchers develop, there is little demand for the N assimilated by these pitchers (i.e. as pitcher production has ended for the season); therefore these pitchers would not only receive the largest amount of N translocated from previous pitchers, but would also retain the bulk of the N they assimilate. Experiment Two provides some evidence suggesting that these later pitchers are more efficient at assimilating available N. In that experiment, first pitchers assimilated *c.* 57% of N provided throughout the experiment. Similarly, in Experiment One, the first three pitchers assimilated an average of 55% of provided N (mid-season harvest). However, by the end of 2004, average pitcher uptake efficiency in Experiment One had increased to *c.* 69%, suggesting that these later pitchers (fourth through sixth) had assimilated much more than 55% of the N provided. In the field, high prey capture by late pitchers could greatly enhance the amount of N available for remobilization the following season.

Overall, our data suggest that *c.* 35% of the 2004 pitcher N pool came from N assimilated during that same season. The remaining *c.* 65% was remobilized from N in senescent pitchers produced during 2003. By the end of the following season, senesced 2004 pitchers had remobilized *c.* 67% of the ¹⁵N within them into new pitchers, while retaining the remaining *c.* 33%.

This high N reutilization efficiency represents a conservative strategy for *S. purpurea* by decreasing the rate of N turnover and increasing its mean residence time (MRT). The MRT of N tends to be long for plants growing in nutrient-poor habitats (Eckstein & Karlsson 1997). In our experiment, the MRT of N for P-fed plants was 2.7 years (calculated according to Rothstein & Zak 2001), which is on the high end of values reported for non-carnivorous perennial herbs (reviewed by Eckstein, Karlsson & Weih 1999). Similarly, in the only other report on MRT of N in carnivorous plants, Eckstein & Karlsson (2001) found an average MRT of 2.2 years for three carnivorous species in the genus *Pinguicula*, only slightly lower than our report for *S. purpurea*.

All pitchers translocate similar amounts of recently assimilated N below-ground. In Experiment Two, N assimilated by first pitchers that was translocated below-ground represented *c.* 2.4% of the below-ground N pool. Similarly, by the end of 2004 in Experiment One, N translocated below-ground by all six pitchers combined represented *c.* 17% of the below-ground N pool (equivalent to *c.* 8% of the total N assimilated by pitchers), suggesting that N translocation from each pitcher represented between 2% and 3% of the below-ground N pool.

Sarracenia purpurea clearly does not rely on its root system for N acquisition, even when nutrients are unavailable to pitchers. Furthermore, our findings likely over-estimate root uptake by *S. purpurea*, as it only experienced competition from soil micro-organisms in our experimental setting. We did observe a somewhat greater uptake efficiency of ¹⁵NH₄¹⁵NO₃ by RP-fed plants relative to P-fed plants (65.9% vs 54.6%) at mid-season 2004, and it is possible that N addition to pitchers stimulated root uptake of N (cf. Hanslin & Karlsson 1996; Adamec 2002). However, even if roots had taken up additional N early in the experiment, this did not continue throughout the growing season: uptake efficiencies were similar in P-fed and RP-fed plants by the end of the growing season. Furthermore, P-fed and RP-fed plants had very similar ¹⁵N allocation patterns that differed from R-fed plants (Fig. 2), suggesting that pitchers were responsible for assimilating the majority of the ¹⁵N within RP-fed plants.

Despite being starved of N, control plants also grew somewhat. Furthermore, there was no detectable relationship between N feeding and N concentration within plant parts. Thus it is likely that an undocumented N source was available to these plants. Bledzki & Ellison (1998) showed that the bdelloid rotifer *Habrotricha rosa* Donner, a common inhabitant of pitcher plants, excretes measurable N (as well as phosphorus), even in the absence of captured prey, but we are confident that rotifers had not colonized our plants. Prankevicus & Cameron (1991) observed N-fixing bacteria in the pitcher fluid of *S. purpurea*. Although we used dd-H₂O as the pitcher liquid in our control plants, the pitchers were by no means sterile

and could have contained N-fixing bacteria. However, the significance of N-fixation to the overall N budget of *S. purpurea* remains to be quantified.

Studies using stable isotopes have revealed a wide range of strategies that plants use to acquire, retain, allocate and (re)translocate scarce nutrients (reviewed by Dawson *et al.* 2002). These strategies reflect the coordination and trade-offs among physiological properties, leaf characteristics and life-history traits that are common to all plants (Westoby & Wright 2006). Like other plants growing in nutrient-poor environments such as wetlands (Shipley & Lechowicz 2000), many carnivorous plants, including *S. purpurea*, are long-lived, have leaf characteristics at the extreme lower end of the 'universal spectrum of leaf traits' (Wright *et al.* 2004), and are very conservative not only in photosynthetic nutrient use efficiency (Ellison 2006) but also in the retention and subsequent reuse of available nutrients (data presented herein).

Our work with a broad range of pitcher plants has shown that *Sarracenia* does not shift its position on the universal spectrum of leaf traits following addition of prey or inorganic nutrients (Ellison 2006; Farnsworth & Ellison, in review). The detailed mechanistic analysis of nitrogen cycling dynamics of *S. purpurea* presented here suggests why previous studies have failed to observe allometric changes in photosynthetic rates and other leaf traits to N additions. Excess nitrogen is stored for future use and production of new biomass – primarily new pitchers – is aimed at enhancing prey capture rather than increasing photosynthetic tissue. This is in accord with the physiological mechanism underlying the universal spectrum of leaf traits that was posited by Shipley *et al.* (2006): trade-offs in allocation to structural tissues (here, pitchers) *vs* liquid-phase (e.g. photosynthetic) processes. New research continues to reveal the range of conditions under which plants depart from the universal spectrum of leaf traits (e.g. Santiago & Wright 2007). Our study suggests that analyses of nutrient cycling dynamics should prove fruitful in developing a mechanistic understanding of how leaf traits and physiological characteristics are coordinated in extreme environments.

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