W. Schulze · E.D. Schulze · J.S. Pate · A.N. Gillison

The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis, Cephalotus follicularis* and *Darlingtonia californica*

Abstract This study investigated the nitrogen (N) acquisition from soil and insect capture during the growth of three species of pitcher plants, *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. $^{15}N/^{14}N$ natural abundance ratios ($\delta^{15}N$) of plants and pitchers of different age, non-carnivorous reference plants, and insect prey were used to estimate proportional contributions of insects to the N content of leaves and whole plants. Young *Nepenthes* leaves (phyllodes) carrying closed pitchers comprised major sinks for N and developed mainly from insect N captured elsewhere on the plant. Their $\delta^{15}N$ values of up to 7.2‰ were higher than the average $\delta^{15}N$ value of captured insects (mean $\delta^{15}N$ value = 5.3‰). In leaves carrying old pitchers that are acting as a N source, the $\delta^{15}N$ decreased to 3.0‰ indicating either an increasing contribution of soil N to those plant parts which in fact captured the insects or N gain from N_2 fixation by microorganisms which may exist in old pitchers. The $\delta^{15}N$ value of N in water collected from old pitchers was 1.2‰ and contained free amino acids. The fraction of insect N in young and old pitchers and their associated leaves decreased from 1.0 to 0.3 mg g^{-1}. This fraction decreased further with the size of the investigated tiller. *Nepenthes* contained on average 61.5 ± 7.6% (mean ± SD, range 50–71%) insect N based on the N content of a whole tiller. In the absence of suitable non-carnivorous reference plants for *Cephalotus*, $\delta^{15}N$ values were assessed across a developmental sequence from young plants lacking pitchers to large adults with up to 38 pitchers. The data indicated dependence on soil N until 4 pitchers had opened. Beyond that stage, plant size increased with the number of catching pitchers but the fraction of soil N remained high. Large *Cephalotus* plants were estimated to derive 26 ± 5.9% (mean ± SD of the three largest plants; range: 19–30%) of the N from insects. In *Cephalotus* we observed an increased $\delta^{15}N$ value in sink versus source pitchers of about 1.2‰ on average. Source and sink pitchers of *Darlingtonia* had a similar $\delta^{15}N$ value, but plant N in this species showed $\delta^{15}N$ signals closer to that of insect N than in either *Cephalotus* or *Nepenthes*. Insect N contributed 76.4 ± 8.4% (range 57–90%) to total pitcher N content. The data suggest complex patterns of partitioning of insect and soil-derived N between source and sink regions in pitcher plants and possibly higher dependence on insect N than recorded elsewhere for *Drosera* species.

Key words Carnivorous plants · Pitcher plants · Insect nitrogen · Nitrogen partitioning

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Introduction

The relationship between insect capture, nitrogen (N) nutrition and plant growth has been of continued botanical interest since the pioneer studies of Charles Darwin (1875). The species most investigated with respect to dependence on insects are of the genera *Drosera* and *Pinguicula*, which catch insects with glandular leaves (Dixon et al. 1980; Thum 1989; Schulze and Schulze 1990; Karlsson et al. 1991; Karlsson and Pate 1992; R. Zamora, J.M. Gomez, J.A. Hodar, submitted). In contrast, few studies have focussed on pitcher plants which catch insects in highly specialized leaves (Troll 1939), and these studies have concentrated on the biochemistry of insect digestion (Lösch 1990) or the biology of mosquito larvae and ants living in the pitchers (e.g., Clarke

and Kitching 1995). Only recently have some studies assesed the nutrient status (Chapin and Pastor 1995) and the rate of prey capture (Newell and Nastase 1997) in *Sarracenia purpurea*.

Studies using ^{15}N natural abundance in Australian species of *Drosera* have demonstrated that the contribution of insect N varies with growth form from as little as 24% in rosette species to 64% in the shrub-like erect *Drosera gigantea* (Schulze et al. 1991). To our knowledge, no such quantification exists for pitcher plants. Growth forms of pitcher plants range from small rosettes as in *Cephalotus* to the vine-like *Nepenthes*, which may vary from a rosette to scrambling shrub or, as is often the case in Indo-Malaysia, may climb to reach the canopy of tropical rain forests where it may also exist as an arboreal epiphyte (W. Schulze, personal observation, Tschibodas, Indonesia). Insect capture in all cases involves pitchers representing modified leaves which attract insects by their bright, flowerlike colors and perhaps also by smell and sugar-rich secretions (Raven and Johnson 1986). We tested the hypothesis that the contribution of insect N to total plant N might be generally higher for the complex capturing organs of pitcher plants than for the simpler capturing devices such as the glandular leaves of *Drosera* and *Byblis*.

Considering that pitcher plants are among the largest carnivorous plants, we contend that complex systems might have developed for partitioning N between current source pitchers (capturing insects) and sinks (newly growing leaves and pitchers). Within such systems, organs would share resources of soil and insect N at possibly different rates depending on the availability of N with age or season. Taking into account that source pitchers may discriminate against ^{15}N during their own metabolism and thus allocate enriched N to sink organs via the phloem, we hypothesized that growing tissues (including young pitchers acting as a sink) might be ^{15}N enriched (higher δ^{15}N values) relative to older tissues. The ^{15}N depletion of leaves and pitchers would be enhanced by import of ^{15}N-depleted soil N supplied in the transpiration stream in larger quantity to fully expanded than to young leaves. We therefore hypothesized that an appreciable δ^{15}N gradient might exist between sink and source organs in addition to overall differences between small (soil-dependent) and large (insect-supported) plants.

We investigated the above hypotheses using three pitcher plants, one plant a vine, *Nepenthes mirabilis* (Australia), another a rhizomatous species, *Darlingtonia californica* (California, USA), the third a rosette species, *Cephalotus follicularis* (Australia). The contributions of insect N versus soil N to total plant N content were quantified by using the ^{15}N natural abundance technique, as previously applied to carnivorous Australian *Drosera* species by Schulze et al. (1991).

Materials and methods

Plant material was collected in strict accordance with the official permit conditions of the respective land management and conservation authorities.

N. mirabilis

N. mirabilis was obtained in September 1993 from grazed coastal peatland in the lowlands of the Atherton Tablemountains, Queensland, Australia. The vegetation was dominated by monocotyledonous Restionaceae and Cyperaceae, ferns (*Blechnum* species), and the shrubs *Melastoma malabatrica*, *Phylidrum lanuginosum*, and *Melaleuca* spp. In *Nepenthes*, leaves form cup-like
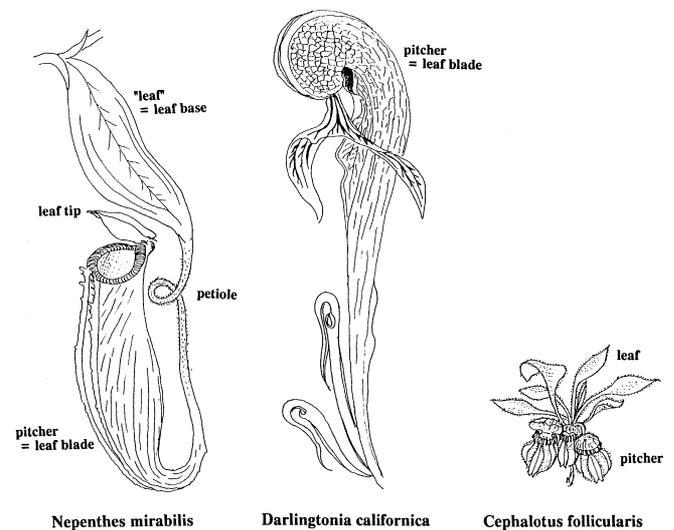

Fig. 1 Pitchers of *Nepenthes*, *Cephalotus*, and *Darlingtonia*, according to Troll (1939)

Table 1 Size of sample tillers and the size of the corresponding whole plants of *Nepenthes*

Plant	Sample tiller						Whole plant					
	Length (m)	Leaves		Pitchers			Number of tillers	Leaves		Pitchers		
		Living	Dead	Closed	Open	Dead		Living	Dead	Closed	Open	Dead
1	1.22	19	3	2	9	0	4	77	17	7	41	6
2	1.53	19	5	2	5	2	15	220	80	15	86	16
3	1.19	18	3	2	3	2	4	54	6	5	13	3
4	0.75	10	3	1	3	0	7	73	23	3	20	2
5	0.59	11	4	1	2	0	2	22	6	1	6	0

pitchers with the leaf tip affording a cover protecting the inlet of the trap, while the petiole is phyllodineous. This will be referred to hereafter as the *Nepenthes* leaf (Fig. 1). Different-sized tiller-like shoots were taken as samples from five individual plants differing in total size (Table 1). The length of tillers ranged from 0.6 m to 1.5 m, each carrying from 10 to 19 leaves, and from 2 to 9 open pitchers which were potentially capable of capturing insects. The whole plants studied each carried between 6 and 86 active pitchers. Leaves along a tiller were numbered from the bottom to the tip, with number 1 designated the oldest leaf. Pitchers that were still closed, and thus not catching insects, were referred to as "young pitchers" or "leaves." The growing tip of the vine and the very young leaves with their associated pitchers (sink organs) were presumed to comprise organs importing N from older plant parts or roots. Old pitchers actively capturing insects and presumably exporting N were referred to as source pitchers and leaves. Our model of N partitioning assumes that N was also being imported via xylem water from soil uptake with the transpiration stream. The most common prey for *Nepenthes* were Diptera, Hemiptera, and Formicidae.

C. follicularis

C. follicularis was collected in November 1993 at three different sites in southwestern Australia, one site a ditch following an old railway line close to Denmark (site 1), the second a wetland area also close to Denmark (site 2), and the third a wetland area between Denmark and Albany (site 3). All sites carried heathland vegetation dominated by Restionaceae, Cyperaceae, Myrtaceae, Proteaceae and Fabaceae. *Cephalotus* (Cephalotaceae) is distantly related to *Nepenthes* (Nepenthaceae) (see Schulze 1991), and it produces pitchers superficially resembling those of Nepenthaceae, but without a leaf-like phyllode. Normal leaves form in addition to pitchers (Fig. 1) and, as shown by Troll (1939), transitions between normal leaves and pitchers are encountered. At each site, 10–12 plants of different size (juveniles to fully grown plants of different sizes) were collected (Table 2). Some plants had only 1 leaf and no pitchers, others were much larger and had up to 40 leaves and 38 pitchers. Most common insect prey were Diptera, Coleoptera, Formicidae, and Arachnidae.

D. californica

D. californica was collected in June 1996 in a *Sphagnum* bog in a *Pinus contorta* forest in north California with *Sisyrinchium*, *Camassia*, *Sanguisorba*, *Ledum*, and *Carex* species as major understorey species. *Darlingtonia* is a rhizomatous carnivorous plant in which all leaves take the form of tube-like pitchers with a dome-shaped top protecting the pitcher mouth and extending the leaf tip into characteristically shaped appendages (Fig. 1). The appendages serve as landing platforms for insects and the dome-shaped top has characteristic chlorophyll-free patches which appear to guide prey into the tube. The height of the pitchers averages 0.38 m. *Darlingtonia* is more closely related in evolutionary terms to *Nepenthes* than to *Cephalotus* (Schulze 1991) and its prey consisted mainly of Diptera species.

Sampling procedure and analyses

Plant material of all species was separated into leaves and pitchers and the contents of the pitchers collected separately. This was done most thoroughly for pitchers of *Cephalotus* from which we separated digested insect debris, Diptera larvae living in the pitcher and, in one instance, even a live frog. Dry weights, N concentrations and isotopic composition of all plant and animal components were determined on oven-dried material (80°C). For *Nepenthes*, the concentration of amino acids, potassium, sodium, ammonium, and nitrate as well as the N content and $\delta^{15}N$ value of the liquid in the open pitchers was analyzed. N concentrations were measured in a

Table 2 Size of *Cephalotus* sample plants at the different sites

Site	Plant (no.)	Leaves			Pitchers	
		Living	Dead	Closed	Open	Dead
1	1	2	0	0	0	0
1	2	2	0	2	0	0
1	3	3	0	1	0	0
1	4	4	0	1	0	0
1	5	2	0	2	2	0
1	6	3	0	1	3	0
1	7	10	0	7	4	0
1	8	7	0	1	6	0
1	9	4	0	4	7	0
1	10	6	0	2	8	0
2	1	1	0	0	0	0
2	2	2	0	0	0	0
2	4	3	0	1	0	1
2	3	5	0	1	0	1
2	5	2	2	1	3	1
2	6	3	2	1	3	0
2	7	4	0	0	3	0
2	8	5	0	0	4	0
2	9	8	0	0	5	0
2	10	7	2	2	6	0
2	11	3	0	1	7	0
2	12	6	0	5	11	0
3	1	3	0	2	0	0
3	2	7	0	2	0	0
3	3	3	0	0	0	0
3	4	5	0	0	0	0
3	5	3	0	1	1	0
3	6	0	0	0	3	0
3	7	0	0	0	3	0
3	8	5	1	1	9	1
3	9	8	1	1	9	1
3	10	6	1	0	10	0
3	11	23	0	3	13	0
3	12	47	0	1	38	0

C/N analyzer (Carlo Erba, Milan, Italy), isotope composition was measured in an on-line system connecting the elementary analyzer to an isotopic mass spectrometer (deltaS, Finnigan MAT, Bremen, Germany). The isotopic composition was expressed in delta notation with atmospheric N_2 as the standard.

Plant samples from the dominant non-carnivorous vegetation were collected as reference plants to compare with the isotopic composition of *Nepenthes* and *Darlingtonia*. Suitable reference plants were not available in the *Cephalotus* habitat. Newly captured insects that were still living were taken as reference for insect isotopic composition in the case of all three pitcher plant species. At each site, 10–20 individual insects of three to eight genera, some of which were also found as prey, were collected. Species were analyzed for $\delta^{15}N$ separately, and the mean $\delta^{15}N$ of insects at each site was taken as reference. $\delta^{15}N$ values of insects ranged from 4 to 8‰, an exception being the live Diptera larvae inside the pitchers of *Cephalotus* with a $\delta^{15}N$ of almost 12‰. Differences between insects and insect debris were not significant.

Results

Nitrogen relations in N. mirabilis

Leaf weight increased initially with increasing leaf position and along the tiller (Fig. 2A). Leaf weight then became more or less constant and decreased towards younger sink leaves at the tip of each tiller. This pattern
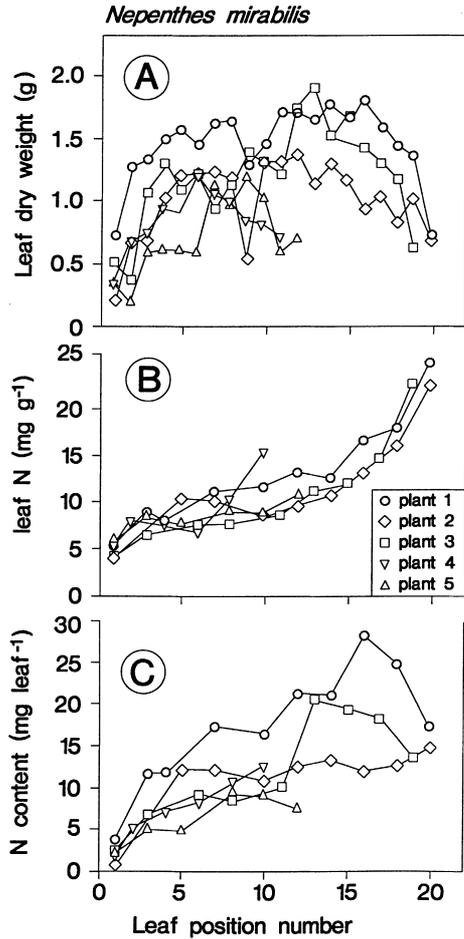
Fig. 2 Biomass (**A**), N concentration (**B**), and N content (**C**) at the different leaf positions for tillers of different *Nepenthes mirabilis* plants. Young leaves are leaves with high leaf position numbers
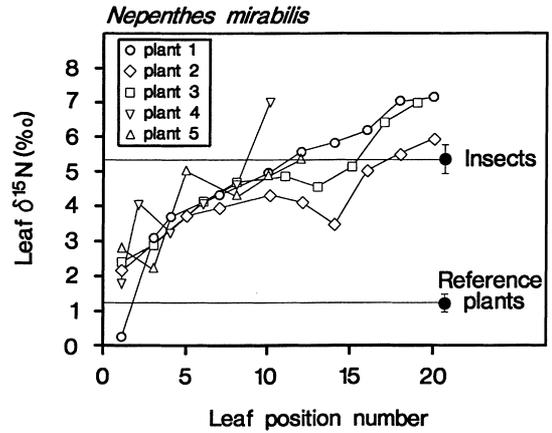

Fig. 3 $\delta^{15}N$ values at different leaf positions for tillers of different *N. mirabilis* plants
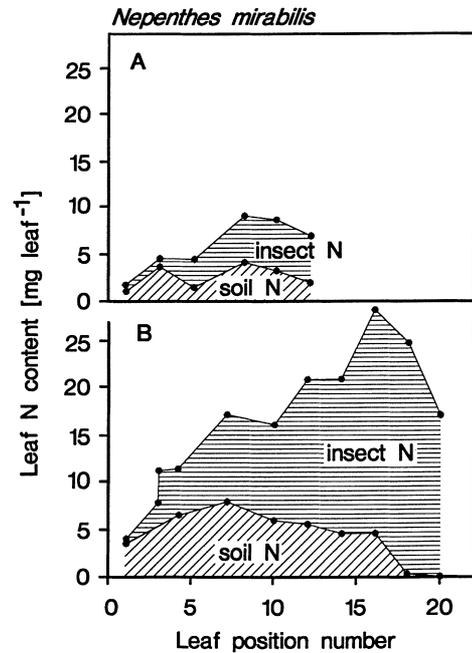

Fig. 4 The contribution of soil and insect N to total leaf N content with respect to leaf position number for a small (**A**) and a large (**B**) *N. mirabilis* plant

was consistent regardless of overall tiller size. However, there were obvious differences in the maximum leaf weight between tillers. Smaller tillers had fewer leaves and lower maximum leaf dry weights than larger tillers.

The N concentration increased in a sigmoid pattern with leaf position number such that youngest leaves had an N concentration twice that of source leaves (Fig. 2B). The oldest leaves (position 1) which were obviously se-nescent contained N concentrations only half those of younger source leaves. Again this pattern was manifest irrespective of tiller size. Leaf N content exhibited a more complex pattern (Fig. 2C). Generally, N contents increased with leaf position towards the tiller apex, but the very youngest leaf usually showed a lower N content.

$\delta^{15}N$ values increased with leaf position irrespective of tiller size (Fig. 3). Youngest sink pitchers of large tillers had $\delta^{15}N$ values (6.66 ± 0.15‰; mean ± SD) that were significantly ($P = 0.05$) higher than those of the insects caught by the pitchers (5.33 ± 1.58‰). $\delta^{15}N$ values of oldest pitchers (3.28 ± 0.86‰) were signifi-cantly ($P = 0.01$) lower than in young pitchers, but higher than those of non-carnivorous reference plants (1.20 ± 0.53‰).

When calculating the contribution of insect N to total plant N in *Nepenthes*, a problem arose upon finding that sink organs showed higher $\delta^{15}N$ values than insects captured by source pitchers. This was interpreted as being due to some form of discrimination during di-gestion of the insect and subsequent preferential allo-cation of ^{15}N to sink organs. We assumed that the young leaves or pitchers of highest $\delta^{15}N$ value were composed of totally insect-derived N. Based on this assumption, we estimated the contribution of insect N to total plant N using this highest leaf $\delta^{15}N$ value and the $\delta^{15}N$ values of accompanying non-carnivorous vegetation as reference values for the calculation.

Figure 4 shows the values for dependence on soil versus insects for leaves of a small (Fig. 4A) and large

tiller (Fig. 4B), with the leaf at position 1 being the oldest and first-formed leaf on a newly grown tiller. Our supposition was that this leaf had no access to insect N and depended totally on soil N in having no donor pitchers from which it could gain insect N. The proportion of insect-derived N was then found to increase with leaf position and presumed capacity of the whole tiller to capture insects. The total proportion of N from soil was larger at the same relative leaf position on a large tiller versus a small tiller. This indicated that the large tiller was still gaining N from the soil or other sources during growth, despite the fact that the total fraction of insect N was increasing exponentially relative to soil inputs. The assumption underlying this approach was of course that soil was the main source of N which led to decreased $\delta^{15}N$ values of older leaves. Using the above approach, we concluded that the mean proportion of insect N acquired was $61.53 \pm 7.56\%_{oo}$ of the total N averaged for the whole tiller. However, it is also possible that the water resting in old pitchers might be a suitable habitat for microorganisms fixing N_2. Such biological interactions have been described for *Sarracenia* (Prankevicius and Cameron 1991). To test for possible N_2 fixation in water of old pitchers, we investigated the $\delta^{15}N$ value and the amino acid composition of this water. The liquid in the open pitchers had a $\delta^{15}N$ value of $1.19 \pm 1.0\%_{oo}$. The concentration of ammonium was 235 μmol l^{-1}, nitrate could not be detected. The concentration of amino acids ranged between 0.26 μmol l^{-1} for lysine and 3.11 μmol l^{-1} for arginine. The total N content was 1.1 ± 0.23 mmol l^{-1}. Soil $\delta^{15}N$ was $0.67 \pm 0.03\%_{oo}$. The dominant cation was potassium (181 μmol l^{-1}). The low $\delta^{15}N$ value would indicate that the N content of this water was not derived from insects.

Fig. 5 Total plant biomass (**A**), leaf N concentration (**B**), and total plant N content (**C**) in relation to the number of open pitchers (= plant size) for *Cephalotus follicularis* at different sites

N. relations in *C. follicularis*

In *Cephalotus* we concentrated on comparing plants of different age and size rather than N relations within plants. Whole plant dry weight and leaf N content were found to increase with total number of open pitchers (Fig. 5A, C) but the rate of such increase differed between collection sites.

Somewhat surprisingly, leaf N concentrations in younger plants differed by a factor of two between sites and decreased rather than increased with plant size, as was observed in *Nepenthes* (Fig. 5B). Large, presumably older plants, had very similar N concentrations across sites and the site showing the lowest growth rate also carried young plants of lowest leaf N concentration.

As shown in Fig. 6, $\delta^{15}N$ values increased with the number of pitchers present on a plant, so small plants tended to have generally lower $\delta^{15}N$ values than large plants. The smallest *Cephalotus* plants, with no open pitchers and thus unable to acquire insect N, showed highly variable $\delta^{15}N$ values, but these were significantly ($P = 0.01$) higher than those of neighboring non-

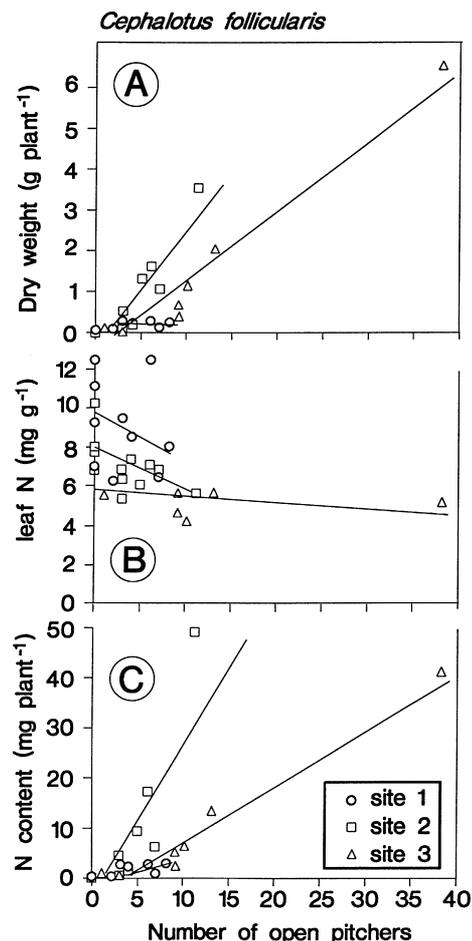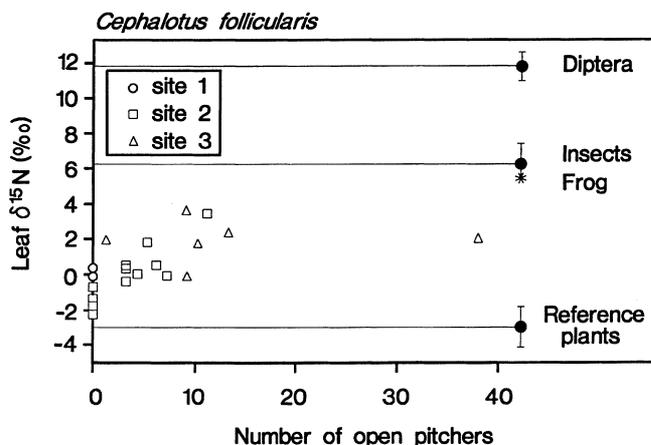**Fig. 6** $\delta^{15}N$ values with respect to the number of open pitchers for *C. follicularis* at different sites

carnivorous plants. It was therefore not appropriate to use neighboring flora as a reference for calculating the fraction of insect N in *Cephalotus*. Instead, we used mean $\delta^{15}N$ values of plants without pitchers as reference points for plant N derived exclusively from the soil. In

contrast to *Nepenthes*, $\delta^{15}N$ values in *Cephalotus* never reached the values of captured insects. Thus, the insect values could be used meaningfully as the second reference point when calculating dependence on insect N.

Young pitchers of *Cephalotus* appeared to have a higher $\delta^{15}N$ value (0.28 ± 1.05‰) than old pitchers (0.94 ± 1.76‰). However, the large variation in $\delta^{15}N$ values and the small differences between means made this result less obvious than in *Nepenthes*. Insect debris had 0.5‰ higher $\delta^{15}N$ values than live insects, but the difference was not significant. Interestingly, and in analogy to the high $\delta^{15}N$ values in young *Nepenthes* leaves, the flowers of *Cephalotus* showed the highest $\delta^{15}N$ values (1.73 ± 1.40‰) and only the largest plants produced flowers. Living Diptera larvae had a 4‰ higher $\delta^{15}N$ value than the captured insects (i.e., they accumulate ^{15}N). The pH of pitcher fluid was 5.1 and ammonium (0.35–3.50 mM) was the main inorganic form of N.

Calculation of the contribution of soil and insect N to total plant N content (Fig. 7) showed a very large
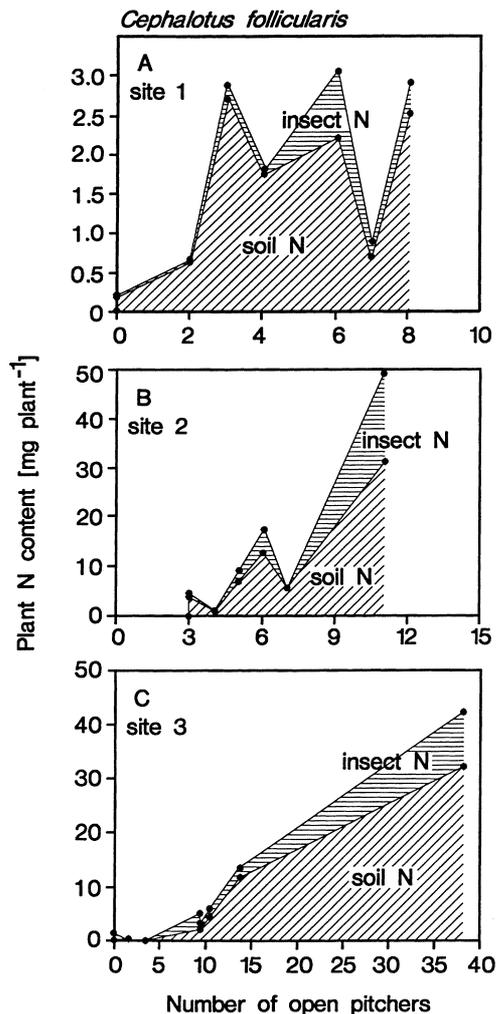

Fig. 7 The contribution of soil and insect N to total plant N for different-sized *C. follicularis* plants at different sites

fraction of soil N even in large plants. Insect N contributed to total plant N only in plants with more than four open pitchers. The total amount of soil N continued to increase with plant size and the fraction of insect N remained low, i.e., insect N was not a prerequisite for plants to reach a large size and accordingly develop many pitchers. The average contribution of insect N to total plant N varied from 19.6% at site 1 to 30.9% at site 2 (based on the three largest individuals). The site which had the highest insect capture also exhibited the highest plant weight, despite a relatively small number of pitchers in those plants (Fig. 5A). At site 1, where insect capture was negligible and where soil N was not as available, plants remained very small.

N. relations in *D. californica*

In the case of *Darlingtonia*, individual young and old pitchers were investigated rather than comparing age sequences or sequence of pitchers from differently sized plants. As shown in Table 3, biomass and total N content increased with pitcher age, but N concentrations remained closely similar for both age classes (Table 3).

$\delta^{15}N$ values for *Darlingtonia* were generally higher than for *Cephalotus* and lay mostly within the range of the $\delta^{15}N$ values found for *Nepenthes*. The $\delta^{15}N$ values of plant material lay very close to those of insects while the $\delta^{15}N$ values in non-carnivorous reference plants were much lower. In this case we do not have an independent control for a $\delta^{15}N$ value for plants without insect capture as was the case for young *Cephalotus* plants or old leaves of *Nepenthes*. However, based on simple comparisons of $\delta^{15}N$ of *Darlingtonia*, insects and reference flora, we estimate the apparent contribution of insect N to total N to be very high (76.4 ± 8.4‰). Although there was great variation in $\delta^{15}N$ values for the five reference plants, ranging from −14.2 to 3.7‰, the mean $\delta^{15}N$ of −5.1‰ does represent a close estimate of the $\delta^{15}N$ values, since three reference plants ranged between −3 and −6‰ and the mean value is identical to the median value of all the reference plants collected.

Table 3 Biomass, N content, N concentration and the amount and percentage of N derived from insects for young and old pitchers of *Darlingtonia californica*. All values are means ± SD of five samples. Different letters indicate significant differences among the age groups

	Age of pitcher	
	Young	Old
Biomass (g)	0.22[a] ± 0.11	4.74[b] ± 1.28
N concentration (mg g^{-1})	9.60[a] ± 1.80	9.40[a] ± 1.30
N content (mg)	2.02[a] ± 1.03	45.77[b] ± 18.49
$\delta^{15}N$ *Darlingtonia*	3.88[a] ± 1.04	4.24[a] ± 0.81
$\delta^{15}N$ reference plants	−5.02[b] ± 6.44	
$\delta^{15}N$ insects	6.30[c] ± 1.17	
Contribution of insect N		
N from insects (mg)	1.54[a] ± 0.88	36.71[b] ± 18.55
insect N of total N (%)	74.76[a] ± 9.73	78.12[a] ± 7.58

Table 4 The contribution of insect N to total N content for carnivorous plants of different life forms with different capturing organs. Values are means ± SD

Capturing organ	Genus	Growth form	Insect N per total N (%)	n
Sticky leaf	*Drosera*	Rosette[a]	19.6 ± 11	3 populations
		Vine[a]	52.9 ± 24	4 species
		Erect low[a]	48.5 ± 11	3 species
		Erect high[a]	53.7 ± 10	3 species
Pitcher	*Cephalotus*	Rosette	26.1 ± 6	3 sites
	Nepenthes	Vine	61.5 ± 7	5 plants
	Darlingtonia	Rhizome	76.4 ± 9	5 plants
	Heliamphora	Rhizome[b]	79.3 ± 4	5 plants
	Brocchinia	Erect rosette[b]	59.8 ± 2	3 sites

[a] Schulze et al. (1991)
[b] G. Gebauer et al. (unpublished data)

Discussion

A major aim of the present study was to compare the effect of insect capture by pitcher plants with non-pitcher-bearing carnivorous species. Such a comparison is possible between the three species presented here and *Drosera* species studied earlier (Table 4). It emerges that the pitcher plants generally contain higher percentages of insect-derived N than *Drosera* with glandular leaves. Furthermore, in rosette life forms, the contribution of insect N is smaller than in vines and erect life forms. The high fraction of insect N in the rhizomatous species may result from storage processes which accumulate and carry over insect N across several seasons. Supporting this contention, young and old pitchers of *Darlingtonia* exhibit closely similar δ^{15}N values.

At the same time, we must admit that the calculation for quantifying the fraction of insect N is most uncertain when applied to genera such as *Heliamphora*, *Darlingtonia*, and *Brocchinia*. In the case of *Darlingtonia*, it becomes obvious that the estimation of the contribution of insect N to total N as calculated in Schulze et al. (1991) and in this study strongly depends on the δ^{15}N values of the non-carnivorous reference flora. We found very low δ^{15}N values in the reference flora for *Darlingtonia*, ranging from 14.2 to 3.7‰. It has been shown that δ^{15}N values of plants increase with increasing rooting depth (Gebauer and Schulze 1991). However, for this study, all five species were among the dominant herbaceous species found at the site and growing in the same rooting horizon. The calculated value of a contribution of insect N to total N of 76% thus only represents a rough estimate. Furthermore, problems when using non-carnivorous neighboring plants and insects as reference became apparent in the present study with respect to both *Cephalotus* and *Nepenthes*.

The beneficial effects of insect capture on growth of insectivorous plants have been demonstrated repeatedly for a number of species (e.g., Thum 1989; Schulze and Schulze 1990; Karlsson et al. 1991; Karlsson and Pate 1992; Hanslin and Karlsson 1996; R. Zamora, J.M. Gomez, J.A. Hodar, submitted). However, none of these investigations specifically studied internal processes of N distribution from soil and insects. Judging from this study, *Nepenthes* is the most suitable for such investi-

gations since its tillers carry organs of different age embodying progressive changes in N supply with tiller development. Our data suggest that each tiller operates independently from the rest of the plant, with almost total reliance on soil N at a small, young stage and on progressively increasing fractions of insect N as tillers become larger and carry more pitchers.

A complication encountered in the study was that δ^{15}N values of young sink tissue were higher than that of the presumed N sources from insects or soils. To our knowledge, this observation has not been made before and deserves special attention in terms of possible sources of discrimination. For the present we can only speculate about the mechanisms involved. Older source pitchers digest insects by extracellular processes. This leads to a decrease in plant δ^{15}N rather than an increase as is obvious from high δ^{15}N values for insect debris. One possibility is that uptake of digested amino acids might discriminate against ^{15}N-enriched material. However, this cannot explain the apparent N enrichment of young tissues. The critical step of isotopic discrimination might conceivably occur at that point where source leaves preferably use ^{15}N-depleted amino acids for their own protein synthesis. This would lead to an enrichment of the N remaining for export via the phloem and support of growth. Thus, the young organs of *Nepenthes* consist in fact of totally insect-derived N. With an increasing rate of transpiration in older leaves, soil N would also enter source organs, leading to a dilution of the initially high levels of insect N. In the present study, this effect is apparent from the increasing N content of leaves with leaf position. Thus, *Nepenthes* is a unique object to study the uptake of N from soil and atmospheric sources which, *inter alia*, might well solve important questions related to N air pollution (Schulze, 1989). However, the increasingly low δ^{15}N values in leaves and pitchers with increasing age could also result from an increasing uptake of N from N-fixing bacteria living in the pitchers. It has been reported for *S. purpurea* (Prankevicius and Cameron 1991) that N-fixing bacteria live in the pitchers and fix enough N to also feed the plant. However, Prankevicius and Cameron could not demonstrate that *Sarracenia* takes up fixed N. Similarly, older *Nepenthes* leaves could contain increasing amounts of N-fixing bacteria flourishing in the anoxic water resting in old pitchers. Considering the low

$\delta^{15}N$ (1.19‰) values of the liquid in the pitchers of *Nepenthes*, we cannot exclude that N_2 fixation (operating at a $\delta^{15}N$ value of −2 to 2‰, Shearer and Kohl 1988) might contribute significantly to the N pool in the pitcher. At this stage we cannot separate between soil-derived N and N_2 fixation. Thus the fraction which is labelled as soil-derived N in Fig. 4 may also contain an unknown amount of N from N_2 fixation.

The present study provides the first investigation of N relations from juvenile plants to the flowering stages for a pitcher plant (*Cephalotus*). The data suggest that before plants can reach a large size they subsist for a long period mainly on soil N. Insect N may then be viewed as an additional benefit towards maintaining fast growth as adults, but certainly not a prerequisite for early growth. Nevertheless, the beneficial effect of insect N might be greater on poor soils (Givnish 1989). However, the critical stage for survival of species is often regarded as the seedling stage and since plants of *Cephalotus* appear to have to reach quite a large size before starting to catch insects, one might argue that selection pressure would favor individuals of greater ability to use soil N, a conclusion contradictory to that proposed by Givnish (1989). Conversely, the chance of reaching reproductive maturity and producing seeds would be expected to increase with insect capture and in this respect carnivory would improve fitness of the species. This is supported by the relatively high $\delta^{15}N$ values found in *Cephalotus* flowers, and the fact that only the large plants, which capture insects, produce flowers.

It has been shown in other studies (Friday and Quarmby 1994) that not only N but also P can readily be taken up by carnivorous plants and contribute to plant nutrient status. In this study we have focussed on the N budget of carnivorous plants in their natural environment. However, insects are not only a source of N for the plant, but also a source of P, as has been demonstrated in double-labeling feeding experiments by Friday and Quarmby (1994). Thus, we can assume that in this study, plants receiving high amounts of insect N will also take up P from the insects in addition to P from the soil. This additional source of P is a further benefit for growth and reproduction in plants capturing insects.

The present study confirms yet again the importance of insect capture to carnivorous plants in N- and P-deprived environments. It also provides tentative evidence of the evolutionary advantage of the sophisticated capturing organs of the pitcher plants over carnivores using glandular leaves. Our study also shows that the conventional way to calculate the contribution of insect N must be used with care. Large gradients in $\delta^{15}N$ values can exist within the same organism depending on growth history.

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