Feeding enhances photosynthetic efficiency in the carnivorous pitcher plant
*Nepenthes talangensis*

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**Background and Aims** Cost–benefit models predict that carnivory can increase the rate of photosynthesis ($A_N$) by leaves of carnivorous plants as a result of increased nitrogen absorption from prey. However, the cost of carnivory includes decreased $A_N$ and increased respiration rates ($R_D$) of trapping organs. The principal aim of the present study was to assess the costs and benefits of carnivory in the pitcher plant *Nepenthes talangensis*, leaves of which are composed of a lamina and a pitcher trap, in response to feeding with beetle larvae.

**Methods** Pitchers of *Nepenthes* grown at 200 $\mu$mol m$^{-2}$ s$^{-1}$ photosynthetically active radiation (PAR) were fed with insect larvae for 2 months, and the effects on the photosynthetic processes were then assessed by simultaneous measurements of gas exchange and chlorophyll fluorescence of laminae and pitchers, which were correlated with nitrogen, carbon and total chlorophyll concentrations.

**Key Results** $A_N$ and maximum ($\Phi_{PSII}$) and effective quantum yield of photosystem II ($\Phi_{PSII}$) were greater in the fed than unfed laminae but not in the fed compared with unfed pitchers. Respiration rate was not significantly affected in fed compared with unfed plants. The unfed plants had greater non-photochemical quenching (NPQ) of chlorophyll fluorescence. Higher NPQ in unfed lamina did not compensate for their lower $\Phi_{PSII}$, resulting in lower photochemical quenching (QP) and thus higher excitation pressure on PSII. Biomass and nitrogen and chlorophyll concentration also increased as a result of feeding. The cost of carnivory was shown by lower $A_N$ and $\Phi_{PSII}$ in pitchers than in laminae, but $R_D$ depended on whether it was expressed on a dry weight or a surface area basis. Correlation between nitrogen and $A_N$ in the pitchers was not found. Cost–benefit analysis showed a large beneficial effect on photosynthesis from feeding as light intensity increased from 200 to 1000 $\mu$mol m$^{-2}$ s$^{-1}$ PAR after which it did not increase further. All fed plants began to flower.

**Conclusion** Feeding pitchers with insect larvae increases $A_N$ of leaf laminae, due to higher nutrient acquisition, with strong correlation with nitrogen concentration, but $A_N$ of pitchers does not increase, despite increased nitrogen concentration in their tissue. Increased $A_N$ improves growth and reproduction and is likely to increase the competitive advantage of carnivorous over non-carnivorous plants in nutrient-poor habitats.

**Key words:** carnivorous plants, chlorophyll fluorescence, *Nepenthes talangensis*, nitrogen, pitcher plant, photosynthetic rate, photosystem II, respiration rate.

INTRODUCTION

Carnivorous pitcher plants of the genus *Nepenthes* are largely found in south-east Asia, principally Borneo, Sumatra, Java and peninsular Malaysia, with scattered populations in India, Sri Lanka, Australia, New Caledonia, Madagascar and the Seychelles. *Nepenthes* leaves are differentiated into a photosynthetically active lamina and a pitcher trap, which has evolved to attract, trap and digest prey. The pitchers usually consist of different structural and functional zones: lid, peristome, and upper waxy and lower glandular zones within the pitcher (Clarke, 1997, 2001).

Carnivorous plants grow terrestrially in sunny, nutrient-poor and permanently moist habitats. Cost–benefit models of carnivory predict that in a well-lit environment the nutritional benefits gained from captured prey exceed the costs of modifying leaves into photosynthetically inefficient traps (Givnish et al., 1984). Generally, costs of carnivory include energetic demands for growth of traps and their function: either increased rate of dark respiration ($R_D$) as a result of extra energy requirements for attracting, capturing and digesting the prey, or decreased photosynthetic rate ($A_N$) as a result of leaf adaptation for carnivory, or both. Three potential benefits resulting from increased mineral absorption from prey have been proposed. First, carnivory may increase a plant’s rate of photosynthesis ($A_N$) through improved nutrient supply, particularly nitrogen status, although other nutrients (principally phosphate and potassium) may be important. Second, carnivory may result in an increased seed production through improved mineral acquisition; and third, carnivory may replace autotrophy partly by heterotrophy (Givnish et al., 1984). Givnish et al. (1984) considered the second benefit as a part of the first, as increased $A_N$ should lead to increased seed production. Several authors have dismissed the third benefit, as experimental findings suggest that carnivorous plants do not obtain substantial amounts of carbon from prey and carnivory could not replace autotrophy at low light intensity (Chandler and Anderson, 1976). However, Risch et al. (2002) found that *Nepenthes* incorporated carbon from carnivory into organic substances, which raises a question about the importance of facultative heterotrophy. The growth of some

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species of carnivorous plants is partly dependent on organic carbon uptake from prey, as revealed by increased growth without increasing $A_N$ (Adamec, 1997, 2008).

With regard to the costs of carnivory, it has been observed that photosynthetic rates of traps are lower than those of leaves (Knight, 1992; Adamec, 2006; Pavlović et al., 2007). With regard to the benefits, around 30 studies have tested whether the growth of carnivorous plants is enhanced by carnivory. Ellison (2006) concluded that there is a significant positive effect ($P = 0.02$) of adding prey on plant growth among different carnivorous genera, supporting the hypothesis that there is a benefit to carnivory. However, he pointed out that this is only indirect evidence, because the cost–benefit model expresses benefits in terms of photosynthetic rates, not in terms of growth. Only three studies have examined the effect of prey capture on $A_N$ of terrestrial carnivorous plants directly, and these gave very different results. Convincing evidence that prey availability increased absolute $A_N$ in Sarracenia has been provided by Farnsworth and Ellison (2008). However, Méndez and Karlsson (1999) and Wakefield et al. (2005) did not find any changes in $A_N$ in Pinguicula vulgaris and Sarracenia purpurea in response to the capture of prey.

Either of two possible results can be expected from feeding prey to carnivorous plants. First, fed plants will have greater biomass but nitrogen (N) concentration (mg N g$^{-1}$ d. wt) will not be affected, despite total N (mg) per plant increasing. This was observed by Moran and Moran (1998) in Nepenthes rafflesiana. In extreme cases, N concentrations per unit dry matter might even decrease due to dilution by increased growth (Karlsson and Carlsson, 1984; Adamec, 2008). Because N concentration is positively correlated with $A_N$ in carnivorous plants (Ellison and Farnsworth, 2005; Pavlović et al., 2007), it can be expected that $A_N$ will not be enhanced, as found by Méndez and Karlsson (1999) in Pinguicula vulgaris. In this case, the cost–benefit model must be considered in terms of growth rate partly due to direct uptake of organic compounds from prey. The second possibility is that fed plants will have higher leaf N concentrations and, therefore, higher $A_N$ as predicted by Givnish et al. (1984). This was found by Farnsworth and Ellison (2008) in Sarracenia.

The principal aim of the present study was to assess the costs and benefits of carnivory in Nepenthes, which provides a good experimental model for the study of the cost–benefit model of carnivory because the leaves are divided into photosynthetically active laminae and a pitcher trap. We assumed that increased photosynthesis is the most important benefit of carnivory, and tested the hypothesis that prey availability would result in increased $A_N$, photosystem II (PSII) efficiency, biomass, and nutrient and chlorophyll concentrations and that the cost of carnivory would include increased R$D$ and decreased $A_N$ and PSII efficiency in the pitcher sensu stricto according to the Givnish hypothesis (Givnish et al., 1984). Tests were made on the pitcher plant Nepenthes talangensis, a rare, endemic species from Sumatra. This is the first detailed study of photosynthesis in a carnivorous plant, with and without experimental addition of prey, using simultaneous measurements of gas exchange and chlorophyll fluorescence by the saturation pulse method.

### MATERIALS AND METHODS

#### Plant material and culture conditions

The pitcher plant Nepenthes talangensis Nerz and Wistuba (1994) grows in mossy forest and stunted upper mountain forest near the summit of Gunung Talang (1800–2500 m alt.) in Sumatra (Nerz and Wistuba, 1994; Clarke, 2001). Its pitchers are light green to yellow in colour with red spots, lack a waxy zone (glandular region covers entire inner surface) and the pitcher fluid is extremely viscous (Clarke, 2001). Five-year-old plants, propagated from seeds, were 20–30 cm tall, with three or four pitchers up to 5 cm long. During the experiments, ten plants were grown under controlled conditions in a growth chamber with a photoperiod of 12 h dark/12 h light [200 μmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation (PAR), day/night temperatures of 25/17 °C and high humidity (80–100 %)]. They were grown in a Sphagnum/perlite/bark/moss mixture substrate. To prevent entry of prey into pitchers they were plugged, without damaging them, with wads of cotton wool moistened in distilled water. Any newly opened pitchers during experiments were treated in the same way. The wads were removed from five plants after 6 months. The remaining five plants served as unfed controls. The fed plants were supplied with one live meal worm (Tenebrio molitor) for each pitcher each week for 8 weeks (total 2.46 ± 0.05 mg f. wt worms per plant over 8 weeks, N concentration in worms = 8.7 %, 78 mg N per plant over 8 weeks). Fed plants were also able to catch natural prey, mostly sciarid flies, but the contribution of these to the nutrition of fed plants was negligible (<2 % of a worm’s weight).

#### Simultaneous measurement of CO$_2$ assimilation and chlorophyll fluorescence

To assess whether feeding enhanced photosynthetic efficiency, we analysed five youngest fully developed laminae (one per plant) with un-formed pitchers that had developed during the 8-week feeding period (‘young lamina without pitcher’), and five older laminae carrying the pitcher (separated into ‘older lamina with pitcher’ and ‘pitcher’), which had developed before the feeding experiment had started. Rates of photosynthesis ($A_N$) and chlorophyll fluorescence were measured simultaneously with a CIAS-2 (PP-Systems, Hitchin, UK) and a fluorocam FC 1000-LC (Photon Systems Instruments, Brno, Czech Republic) attached to an infrared gas analyser. Prior to measurements, the plants were dark-adapted overnight to achieve fully relaxed non-photochemical quenching (NPQ). Thereafter, the middle part of the lamina and the lid in the case of the pitcher (2.5 cm$^2$) were enclosed in the leaf cuvette (PLC6, PP-Systems). Once stabilization (15 min) was achieved the respiration rate ($R_D$) was recorded. Then the chlorophyll fluorescence was measured. Minimal fluorescence ($F_{0}$) and then maximal fluorescence ($F_{m}$) were measured using a saturation pulse (4000 μmol m$^{-2}$ s$^{-1}$ PAR, 800-ms duration): maximal quantum yield of PSII ($F_{m}/F_{m}$) was calculated as $F_{m} - F_{0}/F_{m}$. An induction curve of 15 min duration was then obtained by switching on an actinic light of 200 μmol m$^{-2}$ s$^{-1}$ PAR. For analysis of the quenching mechanism, ten saturation pulses were triggered.
Simultaneously, stable \( A_N \) was recorded at a \( CO_2 \) concentration of 360 \( \mu mol \ mol^{-1} \), leaf temperature 23 ± 1°C, relative air humidity 65–70% and water vapour deficit 700–1000 Pa. Effective quantum yield of photosystem II (\( \Phi_{PSII} \)), photochemical quenching (QP) and NPQ were calculated (Maxwell and Johnson, 2000). The saturation irradiance (1200 \( \mu mol \ m^{-2} \ s^{-1} \) PAR) was applied for 15 min to allow adaptation, and light response curves were determined. The light intensity was decreased stepwise with irradiation periods of 3 min and subsequent saturation pulses were applied until 40 \( \mu mol \ m^{-2} \ s^{-1} \) PAR was reached. The apparent quantum yield of \( CO_2 \) fixation (\( \Phi_{CO2} \)) was determined as the slope of the light response curve between 40 and 150 \( \mu mol \ m^{-2} \ s^{-1} \) PAR (Farquhar et al., 1980). Light response curves of \( A_N \), \( \Phi_{PSII} \) and NPQ were recorded simultaneously. All measurements were taken between 0900 and 1200 h.

**Chlorophyll, nitrogen and carbon determination**

The leaves from five fed and unfed plants were removed. Parts of the leaves were dried at 70°C for 5 days to determine percentage dry weight. Chlorophyll concentrations were determined on the same types of leaves on which \( A_N \) had been measured. Samples of leaves from young laminae without pitchers, older laminae carrying pitchers and pitchers themselves were ground in a mortar and pestle with small amount of sand and extracted with 80% (v/v) chilled acetone with MgCO\(_3\) to avoid acidification and phaeophytinization of pigments. The samples were centrifuged at 8000 g for 5 min at 4°C. Chlorophyll \( a + b \) (chl \( a + b \)) in supernatant were determined spectrophotometrically (denway 6400, London, UK): chl \( a \) at 663.2 nm, chl \( b \) at 646.8 nm. Chlorophyll concentration (mg L\(^{-1}\)) was calculated according to Lichtenthaler (1987) and re-expressed as mg chl \( a + b \) g\(^{-1}\) d. wt.

Leaf tissues from photosynthetic measurements were dried at 70°C for 5 days and N and C were determined using an EA 1108 CHN analyser (Fisons Instruments, Milan, Italy). *Nepenthes* pitchers were washed using distilled water before drying and analysing to avoid contamination with nitrogen from prey. After N determination, photosynthetic nitrogen use efficiency (PNUE) was calculated for each type of leaf as: PNUE (\( \mu mol \ CO_2 \ mol^{-1} \ N^{-1} \ s^{-1} \)) = \( A_{Nmax} \) (\( \mu mol \ CO_2 \ g^{-1} \ d. \ wt \) s\(^{-1}\)/N (mol N g\(^{-1}\) d. wt).

**Statistical analysis**

Prior to statistical tests, data were analysed for normality and homogeneity of variance. When non-homogeneity was present, a t-test was employed with the appropriate corrected degrees of freedom. To evaluate the significance of the data between fed and unfed plants [leaf dry weight, \( K_D \), \( A_{Nmax} \), stomatal conductance (\( g_s \)), \( F_v/Fm \), \( \Phi_{PSII} \), \( \Phi_{CO2} \), QP, NPQ, C, N, PNUE, chl \( a + b \), chl \( a/b \)] a t-test was used. Paired data (comparison between the lamina and the pitcher within the same old leaf carrying the pitcher) were statistically evaluated by a two-tailed paired t-test. The results are expressed as the mean of five replicated measurements. ANCOVA (StatistIXL ver. 1.7 for Microsoft Excel) was used to test the homogeneity of slopes of the relationships between \( A_N \) and N content for lamina and pitcher.

**RESULTS**

Feeding the pitchers of *Nepenthes* with beetle larvae increased the dark and light reactions of photosynthesis. In the laminae, \( A_N \) increased almost linearly with increasing irradiance at irradiances less than about 160 \( \mu mol \ photon m^{-2} s^{-1} \) PAR and reached saturation under an irradiance of about 1000 \( \mu mol \ photon m^{-2} s^{-1} \) PAR (Fig. 1A, B). The \( A_N \) of the young fed lamina without pitcher was significantly higher than the unfed control (Table 1). The \( A_{Nmax} \) of laminae from unfed plants was about 50% that of fed lamina at saturating irradiance (Fig. 1A). Consistent with this, effective quantum yield of PSII (\( \Phi_{PSII} \)) and apparent quantum yield of \( CO_2 \) fixation (\( \Phi_{CO2} \)) were also higher in young laminae from plants that had been fed (Table 1). \( \Phi_{PSII} \) decreased with increasing irradiance (Fig. 1D). Fed laminae had \( F_v/Fm \) values of about 0.800, significantly greater than those of laminae from unfed plants. The primary electron acceptor from PSII (plastoquinone A, \( Q_A \)) was more reduced in unfed plants, based on the higher value of QP in fed plants (Table 1). NPQ increased with increasing irradiance: the higher NPQ in laminae from unfed plants suggests greater heat dissipation via the xanthophyll cycle (Fig. 1G). Chlorophyll concentrations and chlorophyll \( a/b \) ratios were greater in fed plants than in unfed plants (Tables 1 and 2), indicating an increased proportion of light-harvesting complexes (LHC II) to reaction centres (RC II) in PSII in unfed plants. This is consistent with higher values of \( F_v \) in unfed plants (data not shown). Lamina dry weight, nitrogen concentration and PNUE were significantly higher in fed plants. Respiration rate was not significantly different, but there was a trend towards slightly greater \( R_D \) in fed plants in all tissues studied (Table 1).

Differences in measured photosynthetic characteristics between fed and unfed plants in the older laminae carrying the pitcher were similar to those of young laminae except their dry weight (Table 2, Fig. 1B, E, H). This is not surprising given that the older laminae were fully developed before the feeding experiment started, whereas the young laminae were developing during the feeding experiment. There were no significant differences in PNUE between the older laminae of fed and unfed plants.

In the pitchers, \( A_N \) was very low and increased linearly with increasing irradiance at irradiances less than about 50 \( \mu mol \ photon m^{-2} s^{-1} \) PAR and reached saturation only at 100 \( \mu mol \ photon m^{-2} s^{-1} \) (Fig. 1C). In contrast to laminae, there were no statistical differences between pitchers of fed and unfed plants in \( A_{Nmax} \), \( F_v/Fm \), \( \Phi_{PSII} \), \( \Phi_{CO2} \), QP, \( g_s \), or chlorophyll concentration (Table 2). However, unfed pitchers had higher NPQ than fed pitchers and lower nitrogen concentrations, similar to the pattern in young and old laminae. Almost all photosynthetic parameters were significantly lower in pitchers than in laminae (Table 2). The primary acceptor of PSII, \( Q_A \), was maintained at more than 70% oxidized in the lamina, but in the pitcher it was only 25–35% oxidized. NPQ was similar in pitchers and laminae, but was strongly dependent on whether the plants were fed or unfed. However, at higher irradiance, laminae had higher NPQ.
The NPQ was saturated at 300 μmol photon m⁻² s⁻¹ PAR in the pitcher (Fig. 1H). Stomatal conductance, P sucrose, nitrogen, carbon and chlorophyll concentrations were also significantly lower in the pitcher. The RD per unit surface area was higher in the lamina. By contrast, RD per unit mass was higher in the pitcher (Table 2).

Figure 2 summarizes the relationship between nitrogen concentration and Aₕ in laminae (P < 0.01) but not in pitchers (P = 0.06). Furthermore, the relationships do not have similar slopes (P = 0.013).

One year after the feeding experiment all fed plants, but no unfed plants, had begun to flower.

**DISCUSSION**

The effects of feeding pitchers with beetle larvae on the photosynthetic activity of the pitcher plant *Nepenthes talangensis* was investigated, using simultaneous measurement of gas exchange and chlorophyll fluorescence, and relating them to nitrogen and chlorophyll content of the laminae and pitchers. *Nepenthes* is a good experimental genus for studying the
cost–benefit model of carnivory with leaves composed of photosynthetically active laminae and a pitcher trap. The laminae of fed *N. talangensis* had a greater N concentration as a result of nitrogen absorption from prey (Tables 1 and 2). In their natural environment, *Nepenthes* species are N-limited, and have evolved the pitcher to assist in their uptake of N (Osunkoya et al., 2007). The average N acquired from insects is high: 61.5, 53.8 and 68.1% of the total for *N. mirabilis*, *N. rafflesiana* and *N. albomarginata*, respectively (Schulze et al., 1997; Moran et al., 2001). Chlorophyll concentration, A₅ and maximum and effective quantum yield of PSII were higher in fed plants. Two consequences of this are (1) an increase in biomass of new formed laminae and (2) flowering of plants after feeding (Table 1). Because about 50–80% of foliar N is incorporated in photosynthetic proteins (Evans, 1989), we suggest that the lower A₅ of unfed plants is due to lower N and Rubisco concentrations and thus lower capacity for CO₂ fixation. The smaller A₅ was accompanied by a smaller stomatal conductance (gₛ) in unfed compared with fed plants but intercellular CO₂ concentration (Cᵢ) was statistically unchanged (data not shown). This indicates that reduced A₅ was due to reduced carboxylation efficiency rather than to stomatal limitation. Lower Ψₛ was a secondary consequence of impaired CO₂ assimilation. When carbon fixation is inhibited, Ψₛ is often down-regulated to match the reduced requirement for electrons and to minimize the production of reactive oxygen species (Golding and Johnson, 2003). Maximum quantum yield of PSII of dark-adapted leaves, which is proportional to the quantum yield of O₂ evolution, was slightly lower in unfed plants, reflecting that potential quantum yields for photochemistry in PSII were also negatively affected in prey-deprived plants (Tables 1 and 2). When nutrient stress restricts carboxylation, even moderate light may become excessive and may result in destructive photo-oxidative reactions. In the first line of defence against photo-oxidation, xanthophylls transform excessive excitation energy to heat, measured as NPQ of chlorophyll fluorescence (Krause and Jahns, 2004). In laminae, NPQ values were higher in unfed plants as a consequence of less light energy being used in photochemistry and through greater heat dissipation by the xanthophyll cycle. This suggests that increased thermal dissipation by the xanthophyll cycle slightly compensates for the lower Ψₛ in unfed lamina, but probably not sufficiently. The decline of Ψₛ in unfed lamina was not offset by thermal dissipation, leading to a lower QP and higher excitation pressure on PSII and thus higher susceptibility to photoinhibition in unfed plants. The unfed *N. talangensis* plants exhibited similar symptoms to plants under nitrogen stress. Huang et al. (2004) found lower A₅, gₛ, Fₛ/Fₘₜ, Ψₛ, QP, chl and chl a/b in nitrogen-deprived rice.
All photosynthetic parameters were significantly lower in pitchers than in laminae (Table 2). *Nepenthes* pitchers have digestive functions and the absence of a positive correlation between $N$ and $A_N$ (Table 2, Figs 1 and 2) suggests that factors other than $N$ limit $A_N$. Pavlovic et al. (2007) suggest high diffusional resistance for CO$_2$ uptake in the *Nepenthes* pitcher due to very low stomatal density and compact mesophyll. Low PNUE in the pitchers found in this and in our previous study (Pavlovic et al., 2007) indicates either high resistance for CO$_2$ uptake or increased allocation of $N$ to structural materials rather than to photosynthetic machinery (Osunkoya et al., 2007, 2008). Lower $Q_P$ (Table 2) and saturation of NPQ at relatively low irradiance in the pitchers (Fig 1I) result in increase excitation pressure on PSII and higher susceptibility to photoinhibition. This may explain the reduced longevity of pitchers relative to laminae, which is well documented (Osunkoya et al., 2008). All the above demonstrate a strong adaptation of pitchers to the carnivorous, but not to the assimilation, function.

The only study to date that has quantified the effects of nutrient stress in *Nepenthes* is that of Moran and Moran (1998), who examined foliar reflectance in nutrient-starved *N. rafflesiana*. They observed no significant differences in root or leaf $N$ concentration, which is inconsistent with the present results. However, $N$ content (concentration $\times$ biomass) was lower in their prey-deprived plants. They suggested that increased growth upon feeding is a primary adaptation because under conditions of resource limitation, plants are able to maintain critical foliar nutrient concentrations by a reduction in growth rate.

Increased $A_N$ after feeding was found by Farnsworth and Ellison (2008) in the genus *Sarracenia*. The well-fed plants had slightly higher foliar $N$ concentration, chlorophyll content and $F_v/F_m$. However, these differences were only found in young unfed *Sarracenia* leaves that were produced subsequent to feeding. Wakefield et al. (2005) found no changes of $A_N$ measured on fed leaves of *Sarracenia purpurea*. This agrees with the findings of Butler and Ellison (2007), who demonstrated that nutrients captured by older pitchers are rapidly translocated to newly formed leaves. In contrast, we found enhanced $A_N$ in older lamina carrying the fed pitcher, although the pitcher itself did not increase in photosynthetic efficiency (Fig. 2C, F). The results of Schulze et al. (1997) show that not only young developing leaves carrying closed pitchers obtain a high portion of $N$ from captured prey, but also that older fully developed leaves carrying open pitchers also obtain more than 50% of their nitrogen from prey in *Nepenthes*. Ellison and Gotelli (2002) showed an increase in $A_N$ following addition of inorganic nitrogen to *Sarracenia purpurea*, but this response resulted from plants producing non-carnivorous phylloides, which are more efficient in photosynthesis than the carnivorous pitcher. *S. purpurea* produces trapless phylloides only during drought, under shade or with increased nutrient availability. In contrast to *Nepenthes*, *Sarracenia* does not have leaves that are differentiated into a photosynthetically active lamina and a pitcher trap, but usually have only a rosette of pitchers that must function in both photosynthesis and prey capture to achieve positive carbon gain, and their photosynthetic efficiency is closer to *Nepenthes* lamina than to the *Nepenthes* pitcher (Pavlovic et al., 2007).

The rate of photosynthesis was not increased as a result of prey capture in the carnivorous butterwort *Pinguicula vulgaris* (Méndez and Karlsson, 1999). However, supplementary feeding in situ increased both rosette size and reproduction, through an increase in flowering frequency and seed production (Thorén and Karlsson, 1998). Méndez and Karlsson (1999) also concluded that the benefits from capturing prey are larger in reproductive terms than in terms of photosynthesis. We also found accelerated flowering in fed *Nepenthes* plants, but propose that this is an indirect effect of increased $A_N$ rather than a direct effect of feeding. Adamec (2008) observed two different responses to feeding in the aquatic carnivorous plants *Utricularia australis* and *Aldrovanda vesiculosa*. Both species, when fed, produced longer shoots and had smaller $N$ concentrations in comparison with control plants. Photosynthetic rate was higher in fed *Aldrovanda* but lower in fed *Utricularia*. It was suggested that tissue $N$ in fed plants was diluted by growth processes much more than in unfed controls, so the main physiological effect of catching prey was not based on enhancement of $A_N$, as was suggested by Givnish et al. (1984), but on providing $N$ and P (and probably C) for essential growth processes. The present study results contrasted with this, as there was a positive correlation between $N$ and $A_N$ in lamina (Fig. 2), in agreement with the study of Ellison and Farnsworth (2005), but not of Wakefield et al. (2005). The contradictory results concerning feeding experiments discussed above might lie in genotypic differences among plant species.

The Givnish model considers not only the ability of carnivory to enhance $A_N$, but also the costs associated with carnivory. The costs include a reduced $A_N$ and higher $R_D$. The first was confirmed in this and in our previous study of *N. alata* and *N. mirabilis* (Pavlovic et al., 2007), but the second is probably species-specific. Different results were obtained here depending on the units of measurements. Area-based $R_D$ was higher in the lamina, and mass-based $R_D$ was higher in the pitcher. This discrepancy is due to different leaf mass area ($LMA$ was higher in lamina) of these two distinct organs. Differences in leaf thickness between lamina and pitcher are well documented in six *Nepenthes* species (Osunkoya et al., 2007). It appears that the result is influenced more by leaf structure than by specialization for carnivorous or photosynthetic function. Reduced $A_N$ and higher $R_D$ was found in *Utricularia* bladder. Photosynthetic rate in leaves of six aquatic *Utricularia* species exceed that in bladders seven- to ten-fold and $R_D$ of bladders was $75-200\%$ greater than in leaves (Adamec, 2006). The high $R_D$ of bladders in *Utricularia* is consistent with specific amino acid changes (Leu113, Ser114 replaced by Cys113, Cys114) in *Utricularia* cytochrome c-oxidase, the rate-limiting enzyme in the respiratory cycle, which accelerates the rate of respiration. These amino acid changes were not confirmed for *Nepenthes* (Jobson et al., 2004).

According to Givnish, there is a trade-off between photosynthetic costs and benefits that could lead to the evolution of carnivory. Enhancement of $A_N$ resulting from the addition of nutrients as a result of carnivory should be more rapid in high-light than in shady environments (Givnish et al., 1984; Ellison and Gotelli, 2001). However, convincing evidence for this is lacking. From the present data we can calculate the benefit of...
carnivory in terms of $A_N$ and $R_D$ as a difference between fed and unfed lamina ($[A_N\text{ fed lamina }- R_D\text{ fed lamina}]) - ([A_N\text{ unfed lamina }- R_D\text{ unfed lamina}])$. The cost of carnivory was calculated as $[R_D\text{ pitchter }- A_N\text{ pitchter}]$. The result is summarized in Fig. 3. As expected, the benefit from carnivory is higher with increasing irradiance and exceeds the cost at approx. 200 $\mu$mol m$^{-2}$ s$^{-1}$ PAR. At low light intensity the light is more limiting to photosynthesis than nutrient acquisition (the benefit is lower than the cost) and carnivory does not pay. It is known that *Nepenthes* stop producing pitchers and thus decrease the cost of carnivory when they grow at low light intensity (usually below 50 $\mu$mol m$^{-2}$ s$^{-1}$ PAR, our personal observations), in general support of the analysis.

In conclusion, despite contradictory results in this area of research, the present results clearly demonstrate the positive effects of experimental carnivory on increasing the chlorophyll and nitrogen contents of laminae and stimulating the rate of photosynthesis and utilization of absorbed light energy for photochemistry in the carnivorous plant *N. talangensis*. In contrast to the laminae, the pitchers are unable to increase $A_N$ in response to feeding, suggesting that they are highly specialized for nutrient acquisition. The ability of the laminae to increase biomass and photosynthetic efficiency in response to prey capture may provide a competitive advantage for these plants over non-carnivorous plants in sunny and nutrient-poor habitats. Moreover, one year after the feeding experiment all fed plants, but no unfed plants, started to flower, confirming that carnivory may also increase reproductive success in nutrient-poor habitats, probably indirectly via increased $A_N$.

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**LITERATURE CITED**


APPENDIX

Abbreviations used in the text.

$A_N$ Net photosynthetic rate

$A_{Nmax}$ Maximal net photosynthetic rate at saturation irradiance

$F_v/F_m$ Maximal quantum yield of PSII

$g_s$ Stomatal conductance

LHC II Light harvesting complex of PSII

LMA Leaf mass area

NPQ Non-photochemical quenching coefficient

PAR Photosynthetically active radiation

PNUE Photosynthetic nitrogen use efficiency

PSI Photosystem I

PSII Photosystem II

QA Plastochinone A

QP Photochemical quenching coefficient

RC II Reaction centre of PSII

$R_D$ Dark respiration rate

$\Phi_{CO2}$ Apparent quantum yield of CO$_2$ assimilation

$\Phi_{PSII}$ Effective quantum yield of PSII