Research Article

Photosynthesis and Nitrogen Metabolism of *Nepenthes alata* in Response to Inorganic NO_3^- and Organic Prey N in the Greenhouse

Jie He and Ameerah Zain

Natural Sciences and Science Education Academic Group, National Institute of Education, Nanyang Technological University,
1 Nanyang Walk, Singapore 637 616

Correspondence should be addressed to Jie He, jie.he@nie.edu.sg

Received 31 August 2012; Accepted 18 September 2012

Academic Editors: E. Collakova, M. Kwaaitaal, and D. Zhao

Copyright © 2012 J. He and A. Zain. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study investigates the relative importance of leaf carnivory on *Nepenthes alata* by studying the effect of different nitrogen (N) sources on its photosynthesis and N metabolism in the greenhouse. Plants were given either inorganic NO_3^- , organic N derived from meal worms, *Tenebrio molitor*, or both NO_3^- and organic N for a period of four weeks. Leaf lamina (defined as leaves) had significant higher photosynthetic pigments and light saturation for photosynthesis compared to that of modified leaves (defined as pitchers). Maximal light saturated photosynthetic rates ($P_{\max}$) were higher in leaves than in pitchers. Leaves also had a higher light utilization than that of pitchers. Both leaves and pitchers of plants that were supplied with both inorganic NO_3^- and organic prey N had a similar photosynthetic capacity and N metabolism compared to plants that were given only inorganic NO_3^- . However, adding organic prey N to the pitchers enhanced both photosynthetic capacity and N metabolism when plants were grown under NO_3^- deprivation condition. These findings suggest that organic prey N is essential for *N. alata* to achieve higher photosynthetic capacity and N metabolism only when plants are subjected to an environment where inorganic N is scarce.

1. Introduction

Carnivorous plants are restricted to environments with an abundant supply of water and light but are poor in nutrients [1]. Although plants are autotrophic with respect to reduced carbon, they must scavenge nitrogen (N) and other minerals from the environment, usually from the soil through uptake by their roots. On the other hand, plant carnivory is an alternate and efficient means to acquire nutrients in nutrient-poor habitats [2]. For instance, preys caught in the *Nepenthes* pitchers are digested in a pool of digestive enzymes in the pitcher where glands function to perceive chemical stimuli, secrete digestive enzymes, and absorb nutrients for plant growth and development [3].

Nepenthes are tropical pitcher plants, and there are approximately 90 species in the genus *Nepenthes*. Osunkoya et al. [4] suggested that most *Nepenthes* species are N-(but not P or K) limited, and thus have evolved the pitcher to assist in their uptake of N. The leaf morphology of the different

Nepenthes species is similar with a photosynthetic lamina and a tendril to which a pitcher is attached. *N. alata* is a pitcher plant that efficiently captures, retains, and digests predominantly insect prey in highly modified leaves, and pitchers [5]. The pitcher consists of the lid, peristome (upper rim of pitcher) which attracts prey, a waxy zone that is involved in trapping prey, and a digestive zone which digests prey [6]. Ellison and Gotelli [2] reported that both leaves and pitchers of the *Nepenthes* plants can photosynthesize. Clarke [7] found that *Nepenthes* fail to produce pitchers if the light or humidity is too low, or nutrient availability is too high.

In Singapore, *N. alata* is a popular ornamental plant that can be found in many home gardens and has commercial value. In nature, these large pitcher plants usually grow in soils consisting of low N, as the pitchers of these plants can obtain N from organic sources like insects or small animals. Usually, *N. alata* plantlets are obtained from tissue culture stock in the nurseries. Some growers of *Nepenthes* feed the pitchers of this plant with meal worms to provide the

additional source of N. However, there are only a few studies that have examined directly the linkage between inorganic N uptake from the soil by the carnivorous plants and photosynthetic rate [5]. In addition, there is little information available on the overall prey and inorganic nutrient acquisition for the carnivorous plants such as *Nepenthes* [5].

This project focused on the relative importance of leaf carnivory and root nutrition mainly with inorganic NO_3^- on photosynthesis and N metabolism of *N. alata* in the greenhouse. Using NO_3^- and prey-derived organic N source, this project aimed to compare the photosynthetic characteristics and light utilization between leaf and pitcher and to study the effects of NO_3^- and prey on the photosynthesis and N metabolism of leaf and pitcher. The parameters studied were photosynthetic O_2 evolution, chlorophyll (Chl) fluorescence, photosynthetic pigments, total reduced N content, and soluble protein content. Understanding the contributions of inorganic NO_3^- and organic prey N to photosynthesis and N metabolism, horticulturalists can select the optimal fertilizer required for cultivation of *N. alata*.

2. Materials and Methods

2.1. Plant Material. *N. alata* plants with 7–8 leaves and 4–5 pitchers were obtained from a commercial nursery. They were transplanted to pots (15 cm diameter) containing sand and vermiculite (1:1), and each pot had only one plant. The pitchers were emptied and washed with distilled water. An amount of 10 mL of distilled water was added to each of the pitchers which were then plugged with glass wool to prevent colonization by common pitcher inhabitants and capture of prey. All plants were acclimatized for one month in the greenhouse under a maximal photosynthetic photon flux density (PPFD) of $600\text{--}700\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. The daily ambient temperature ranged from 24 to 33°C. All plants were watered daily with tap water and supplied with nutrient solution based on full-strength Netherlands Standard Composition every alternate day. This nutrient solution contains full NO_3^- .

2.2. Experimental Design for Different N Treatments. After the plants were acclimatized under the previously stated conditions for one month, all yellow leaves and dead pitchers were removed. Each plant had 6 fully expanded leaves with fully developed pitchers and two young leaves without pitchers. The pitchers were emptied and washed with distilled water. An amount of 10 mL of distilled water was again added to each of the pitchers which were then plugged with glass wool to prevent colonization by common pitcher inhabitants and capture of prey. In order to study the photosynthetic characteristics and N metabolism in response to organic prey and inorganic NO_3^- , for each plant, six alive meal worms (*Tenebrio molitor*) ($6 \times 0.4\text{ g}$) were added to each of the three pitchers, while the other three pitchers of the same plant did not receive any prey. After adding the meal worms, plants were divided into two groups: one group was watered nutrient solution with full NO_3^- , while the other group was watered with nutrient solution without NO_3^- every alternate day. Therefore, there are four treatments: (1) NO_3^- ,

(2) $\text{NO}_3^- + \text{prey}$, (3) prey, and (4) no NO_3^- , no prey. The durations of different treatments were two, three, and four weeks, respectively. Significant differences in responses to different treatments were observed after four weeks. Thus, only data obtained after four weeks were presented.

2.3. Measurement of Chl Fluorescence. Electron transport rate (ETR), photochemical quenching (qP), and nonphotochemical quenching (qN) of Chl fluorescence were determined from both leaves and pitchers using the Imaging PAM Chl Fluorometer (Waltz, Effeltrich, Germany) at 25°C under different PPFDs in the laboratory as described by He et al. [8].

2.4. Measurement of Photosynthetic O_2 Evolution. The photosynthetic O_2 evolution of leaf and pitcher were determined with a Hansatech leaf disc O_2 electrode (King's Lynn, Norfolk, UK). Each leaf and pitcher section was placed in saturating CO_2 conditions (1% CO_2 from 1 M carbonate/bicarbonate buffer, pH 9). Leaf or pitcher section was illuminated, starting from the lowest photosynthetic photon flux density, PPFD ($34\ \mu\text{mol m}^{-2}\text{ s}^{-1}$), to the highest ($1000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). The photosynthetic light response curve was obtained by plotting the O_2 evolution rates against respective light intensity. Maximal photosynthetic O_2 evolution rates (P_{max}) of both leaf and pitcher were measured after two weeks of treatments under a PPFD of $1000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ at 25°C.

2.5. Measurement of Photosynthetic Pigments. Fresh samples of leaf or pitcher of 0.05 g were weighed and cut into smaller pieces. Total Chl and carotenoid were extracted from these samples with dimethylformamide and quantified using a spectrophotometer following the procedure of Wellburn [9] at wavelengths of 480, 647, and 664 nm.

2.6. Measurement of Total Reduced N Concentration (TRN). Dry samples of 0.05 g of leaf and pitcher were placed into a digestion tube with a Kjeldahl tablet and 5 mL of concentrated sulphuric acid according to Allen [10]. The mixture was then digested about 60 min until clear. After the digestion was completed, the mixture was allowed to cool for 30 min, and TRN concentration was determined by a Kjeltac 2030 analyser unit (Höganäs, Sweden).

2.7. Total Soluble Protein (TSP) Extraction and Determination. Samples of 1 g were rapidly frozen in liquid nitrogen after weighing and stored at -80°C until used. Each leaf and pitcher sample was ground to fine powder in liquid N with pestle and mortar. After which, 1 mL of 100 mM Bicine-KOH (pH 8.1), 20 mM MgCl_2 , and 2% PVP buffer were added [11]. After centrifugation (100,000 g, 30 min at 4°C), 4 mL of acetone was then added to 1 mL of the supernatant collected and centrifuged further for 10 min at 4000 rpm. Total soluble protein was extracted using the method described by Lowry et al. [11].

2.8. Statistical Analysis. For Table 1 and Figures 1 and 2, a *t*-test was used to test for differences between leaves and

TABLE 1: Total Chl content, Chl *a/b* ratio, total carotenoid content, and Chl/carotenoid ratio of leaf and pitcher of *N. alata*. The means and standard errors of four readings are given for each pigment. Letters represent comparison between leaf and pitcher. Any two means having a common letter are not significantly different.

Photosynthetic pigments	Leaf	Pitcher
Total Chl content ($\mu\text{g/g FW}$)	1273.12 ± 46.80^a	266.39 ± 29.42^b
Chl <i>a/b</i> ratio	2.95 ± 0.04^a	2.01 ± 0.06^b
Total carotenoid ($\mu\text{g/g FW}$)	207.11 ± 8.28^a	44.80 ± 5.00^b
Chl/Carotenoid ratio	6.15 ± 0.11^a	5.95 ± 0.04^a

pitchers. For Figures 3 and 4, ANOVA was used to discriminate means across all four treatments, followed by using Tukey's multiple comparison test. The difference between treatment means was considered significant at $P < 0.05$. All statistical analyses were carried out using Minitab software (Minitab, Inc., release 15, 2007).

3. Results

3.1. Comparative Studies on Photosynthetic Characteristics between Leaves and Pitchers. To compare the photosynthetic characteristics between leaves and pitchers, all plants were grown under the conditions described in Section 2.1 for one month. The leaves had significant higher total Chl content, Chl *a/b* ratio, and total carotenoid content compared to that of the pitchers (Table 1, $P < 0.05$). However, there was no significant difference in the Chl/carotenoid ratio between leaves and the pitcher. Light saturation point of photosynthetic O_2 evolution for leaf was achieved at PPFDs of about $600\text{--}800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1). For the pitchers, however, light saturation point was much lower, at PPFDs of about $200\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$. These results indicate that the leaves have higher photosynthetic capacities compared to those of pitchers. Light utilization of leaf and pitcher was determined by qP, qN, and ETR. The leaves had qP values of about 0.9 to 0.6 under PPFD of $15\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$. A drastic decrease of qP in the leaf was observed at PPFD higher than $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ with values reaching zero at $1585 \mu\text{mol m}^{-2} \text{s}^{-1}$. The pitcher had lower qP values compared to the leaves of 0.88 to 0.42 at PPFD of $50\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$. The qP of zero was observed at about PPFD of $900 \mu\text{mol m}^{-2} \text{s}^{-1}$. The ETR values of the leaves increased sharply to a maximum of $60 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ at a PPFD of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ after which the ETR values decreased gradually (Figure 2(b)). Similarly, the ETR of the pitcher increased rapidly to a maximum of $22 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ at a PPFD of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ after which it decreased gradually. These results show that the pitchers have a lower light utilisation compared to that of the leaves. On the other hand, the leaves had a gradual increase in qN from PPFD of $25\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$ after which it plateaued to about 0.8. Similarly, the pitcher had a rapid increase in qN from PPFD of $25\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$ after which it plateaued to about 0.6 (Figure 2(a)). Compared to that of pitcher, the higher qN levels in leaves indicate that higher amount of light energy could be dissipated as heat.

3.2. Responses of Photosynthesis and N Metabolism of Leaves and Pitchers to Inorganic NO_3^- and Organic Prey. Four weeks after different N treatments, there were no significant

FIGURE 1: Photosynthetic light response curve of leaf and pitcher of *N. alata* under different PPFDs. Means of 4 measurements from 4 different leaves and 4 different pitchers. Vertical bars represent standard errors. When the standard error bars cannot be seen, they are smaller than the symbols.

differences in $P_{\max}$ and Chl content in the leaves of plants supplied with only NO_3^- to the roots and those supplied with both NO_3^- to the roots and prey added to the pitcher (Figures 3(a) and 3(c)). However, leaves that were supplied with prey to their pitchers only but without NO_3^- to the roots had significant lower $P_{\max}$ and Chl content ($P < 0.05$). Leaves that had neither NO_3^- supplied to the roots nor prey added to the pitchers exhibited the lowest $P_{\max}$ and Chl content (Figures 3(a) and 3(c), $P < 0.05$). After different N treatments, the changes of $P_{\max}$ in pitchers were very similar to those of leaves but the values of $P_{\max}$ were much lower compared to those of leaves (Figure 3(b)). However, lower total Chl content was only observed in pitchers of those plants without NO_3^- and prey (Figure 3(d), $P < 0.05$). Changes in TRN and TSP determined from the same leaves that were used to measure $P_{\max}$ and total Chl content are shown in Figure 4. The differences in TRN concentrations of both leaves and pitchers were very similar to those of $P_{\max}$ after different N treatments for 4 weeks (Figures 4(a) and 4(b)). It is also interesting to see that TSP concentrations of both leaves and pitchers showed the same patterns of Chl content after different N treatments for 4 weeks (Figures 4(c) and 4(d)).

4. Discussion

Carnivorous plants normally grow in moist, nutrient-poor soils. In order to adapt to an environment where critical

FIGURE 2: Changes in qP and qN (a) and ETR (b) of leaf and pitcher of *N. alata* under different PPFDs. Means of 4 measurements from 4 different leaves and 4 different pitchers. Vertical bars represent standard errors. When the standard error bars cannot be seen, they are smaller than the symbols.

nutrients are scarce and where light is not limiting, carnivorous plants have evolved modified leaves specialized for capturing animals and digesting the preys to acquire nutrients [1, 12–15]. In this study, using the carnivorous tropical pitcher plant, *N. alata*, it was demonstrated that the leaves have much higher $P_{\max}$ compared to that of the pitchers of the same plants (Figure 1). It was also reported by others that $P_{\max}$ of traps is usually lower than that of other noncarnivorous leaves of the same plants [1]. This could be due to the fact that leaves have higher levels of photosynthetic pigments compared to the pitcher (Table 1) and high efficiency of light energy utilisation and heat dissipation measured by qP , qN , and ETR (Figure 2). Pavlovič et al. [16] also reported that Chl content in two *Nepenthes* species, *N. alata* and *N. mirabilis*, was higher in the lamina (leaves) than in the pitcher. The red tint of *Nepenthes* pitchers suggests that they might not have much Chl, and this might lead to low $P_{\max}$. Because $P_{\max}$ is positively correlated with N concentration and stomatal conductance, it is hypothesized that there is lower N concentration (Figure 4) and lower stomata density in pitchers than in the leaves. Most carnivorous plants exhibit very low rates of photosynthesis [5]. In the present study, although $P_{\max}$ was significantly higher in leaves than in pitchers, the value of $P_{\max}$ about $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ was much lower compared to that of most C_3 plants. According to Ellison [5], photosynthetic rate of carnivorous plants was about 2 to 5 times lower than that of other noncarnivorous plants. Our finding of $P_{\max}$ of *N. alata* agrees with Ellison's report [5]. Low photosynthetic rate reflects the relatively low growth rate of *N. alata* plants (data not shown). Most carnivorous plants are at a competitive disadvantage in their

habitats due to a lower net photosynthetic rate when light is limiting, and the availability of soil nutrients is poor based on the cost-benefit model [1]. However, the relationship between photosynthetic performance of carnivorous plants and their carnivory is complex and ambiguous [3].

According to the ecological cost-benefit relationships, carnivory of carnivorous plants grown under their natural habitat of limited light and poor nutrients could lead to increasing photosynthetic rate if they were provided with a greater mineral nutrient availability [1]. *N. alata* is one of the most popular *Nepenthes* species in cultivation. According to our observation, *N. alata* plants that are cultivated in the local nursery under high light supplied with fertilizer grow well with numerous pitchers. These observations lead to the question of the relative importance of leaf carnivory and root nutrition mainly with inorganic NO_3^- on photosynthesis and N metabolism. In the present study, when full inorganic NO_3^- was supplied to the roots of *N. alata* plants, adding preys to the pitchers did not increase $P_{\max}$ and total Chl content of both leaves and pitcher (Figure 3). These results indicate that to improve plant growth, it seems sufficient to provide N in the form NO_3^- to the roots. This confirms the idea that carnivory is not indispensable for greenhouse growing carnivorous plants, but it is almost indispensable for carnivorous plants in natural habitats [17]. Prey captured in the pitcher could contribute 10–90% of the N budget of *Nepenthes* plants [16]. N in the end can be considered as limiting primary productivity [18]. For instance, when *N. alata* plants were grown under NO_3^- deprivation condition, adding preys to the pitchers increased $P_{\max}$ of both leaves and pitchers compared to those without preys. However, the

FIGURE 3: Changes in $P_{\max}$ (a), (b) and Chl content (c), (d) of leaves (a), (c) and pitchers (b), (d) of *N. alata* after 4 weeks of different N treatments. Means of four measurements were obtained from four different leaves from four different plants. Means with different letters above the columns are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.

$P_{\max}$ values of plants supplied with only preys were significantly lower than those plants supplied with NO_3^- or both NO_3^- and preys (Figure 3). These findings suggest that in the event of low inorganic NO_3^- availability, the feeding of prey to the pitchers would bring about the same positive effect on growth in the presence of insects at low NO_3^- concentration in *Drosera whittakeri* and *Drosera binata* over a period of one growing season. In most *Sarracenia* species and in *Darlingtonia californica*, prey addition significantly increases $P_{\max}$ [1]. Ellison and Gotelli [20] concluded that there was an increase in photosynthetic rates followed by an addition of organic N in *Sarracenia*

purpurea. The result is also consistent with another study conducted by Wakefield et al. [21] on *Sarracenia purpurea*. However, benefits of carnivory including an increased rate of photosynthesis are often conflicting [20–22]. Obviously, the photosynthetic effect of prey addition is quite different in different carnivorous plant species [20–25]. It was reported that the total Chl content of the younger *Sarracenia* pitchers was positively and significantly correlated with the feeding level of prey. However, Chl content in *Sarracenia* was not correlated with either foliar N [23]. In the present study, even with additional prey N, leaves of *N. alata* plants had much higher $P_{\max}$ and total Chl content compared to their pitchers; feeding preys to the pitcher of plants that

FIGURE 4: Changes in TRN (a), (b) and TSP (c), (d) concentration of leaves (a), (c) and pitchers (b), (d) of *N. alata* following 4 weeks of different N treatments. Means of 4 measurements were obtained from 4 different leaves from 4 different plants. Means with different letters above the columns are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.

were supplied with full NO_3^- did not enhance Chl further (Figures 3(c) and 3(d)). These results may suggest that the function of the pitchers is mainly for prey capture and not for photosynthesis, although they have photosynthetic pigments [2]. This could therefore explain why the leaves utilize light much more efficiently compared to the pitchers as demonstrated by the higher ETR, qP, and higher qN values of the leaves compared to that of the pitcher (Figure 2, Table 1).

It is well known that in noncarnivorous plants, with increasing inorganic NO_3^- content, the photosynthetic capacity of leaves increases [26, 27]. However, there is very little information on the relationship between the amount

of organic N (prey), the rate of photosynthesis, and N metabolism in carnivorous plants. Typically, a reduction of TRN concentration in plants would lead to lower photosynthetic rates and vice versa. In this study, TRN was significantly higher in leaves than in pitchers (Figure 4). This could further explain why leaves had higher photosynthetic capacity and photosynthetic pigments compared to pitchers. In addition, differences in TRN concentrations of both leaves and pitchers among the different N-treated plants were very similar to those of $P_{\max}$, indicating the close relationship between TRN and $P_{\max}$. N availability obviously directly affects the amount of soluble proteins available in the plant, since N is a major element which makes up protein

compounds [26, 27]. Plants require N (in the form of NO_3^-) to synthesize soluble proteins which in turn is required for the synthesis of RuBisCo that is the key enzyme in photosynthetic CO_2 fixation [27, 28]. This could explain why leaves, which had significantly higher TSP compared to the pitcher (Figure 4), had higher photosynthetic capacities (Figure 2). However, how much of the prey contributes to the N actually present in the foliage of *Nepenthes*? Studies like Ellison and Gotelli [20] suggest that in the field, the majority of foliar N is derived from substrate sources rather than carnivory. There are other conflicting findings like Moran et al. [18] who estimated that prey contributed from 54% to 68% of the total foliar N in *Nepenthes*. Schulze et al. [29] concluded that the total N concentration in leaves of *Dionaea muscipula* growing in areas where there was little insect availability was much lower than in plants which received a large amount of insect prey. When measuring NO_3^- reductase (NR) activity, it was shown that negative interactions can exist between organic and inorganic N sources. This was exhibited when insect feeding of greenhouse *Drosera binata* grown in complete solution led to 30–50% lower shoot NR activity compared to unfed plants [18]. Effects of organic prey on NR activity merit our future study.

5. Conclusion

The present study showed that the leaves of *N. alata* had higher photosynthetic capacity and light utilization compared to the pitchers. *N. alata* plants with matured pitchers supplied with both inorganic NO_3^- and organic prey N had a similar $P_{\max}$ compared to plants supplied with only inorganic NO_3^- , suggesting that carnivory is not indispensable for greenhouse growing carnivorous plants.

Abbreviations

Chl:	Chlorophyll
ETR:	Electron transport rate
N:	Nitrogen
NO_3^- :	Nitrate
$P_{\max}$:	Maximal light saturated photosynthetic rates
PPFD:	Photosynthetic photon flux density
qN:	Nonphotochemical quenching
qP:	Photochemical quenching
TRN:	Total reduced N
TSP:	Total soluble protein.

Acknowledgment

This project was supported by teaching materials' vote of National Institute of Education, Nanyang Technological University, Singapore.

References

- [1] T. J. Givnish, E. L. Burkhardt, R. E. Happel, and J. D. Weintraub, "Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats," *American Naturalist*, vol. 124, no. 4, pp. 479–497, 1984.
- [2] A. M. Ellison and N. J. Gotelli, "Evolutionary ecology of carnivorous plants," *Trends in Ecology and Evolution*, vol. 16, no. 11, pp. 623–629, 2001.
- [3] B. E. Juniper, R. J. Robins, and D. M. Joel, *The Carnivorous plants*, Academic Press, London, UK, 1989.
- [4] O. O. Osunkoya, S. D. Daud, B. Di-Giusto, F. L. Wimmer, and T. M. Holige, "Construction costs and physico-chemical properties of the assimilatory organs of *Nepenthes* species in Northern Borneo," *Annals of Botany*, vol. 99, no. 5, pp. 895–906, 2007.
- [5] A. M. Ellison, "Nutrient limitation and stoichiometry of carnivorous plants," *Plant Biology*, vol. 8, no. 6, pp. 740–747, 2006.
- [6] E. Gorb, V. Kastner, A. Peressadko et al., "Structure and properties of the glandular surface in the digestive zone of the pitcher in the carnivorous plant *Nepenthes ventrata* and its role in insect trapping and retention," *Journal of Experimental Biology*, vol. 207, no. 17, pp. 2947–2963, 2004.
- [7] C. M. Clarke, *Nepenthes of Borneo*, Natural History, Kota Kinabalu, Malaysia, 1997.
- [8] J. He, B. H. G. Tan, and L. Qin, "Source-to-sink relationship between green leaves and green pseudobulbs of C3 orchid in regulation of photosynthesis," *Photosynthetica*, vol. 49, no. 2, pp. 209–218, 2011.
- [9] A. R. Wellburn, "The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution," *Journal of Plant Physiology*, vol. 144, no. 3, pp. 307–313, 1994.
- [10] S. E. Allen, "Analysis of vegetation and other organic materials," in *Chemical Analysis of Ecological Materials*, S. E. Allen, Ed., pp. 46–61, Blackwell, Oxford, UK, 1989.
- [11] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.
- [12] V. A. Albert, S. E. Williams, and M. W. Chase, "Carnivorous plants: phylogeny and structural evolution," *Science*, vol. 257, no. 5076, pp. 1491–1495, 1992.
- [13] R. J. Bayer, L. Hufford, and D. E. Soltis, "Phylogenetic relationships in Sarraceniacae based on rbcL and ITS sequences," *Systematic Botany*, vol. 21, no. 2, pp. 121–134, 1996.
- [14] K. M. Cameron, K. J. Wurdack, and R. W. Jobson, "Molecular evidence for the common origin of snap-traps among carnivorous plants," *American Journal of Botany*, vol. 89, no. 9, pp. 1503–1509, 2002.
- [15] A. M. Ellison and N. J. Gotelli, "Energetics and the evolution of carnivorous plants—Darwin's "most wonderful plants in the world,"" *Journal of Experimental Botany*, vol. 60, no. 1, pp. 19–42, 2009.
- [16] A. Pavlovič, E. Masarovičová, and J. Hudák, "Carnivorous syndrome in Asian pitcher plants of the genus *Nepenthes*," *Annals of Botany*, vol. 100, no. 3, pp. 527–536, 2007.
- [17] L. Adamec, "Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*," *Aquatic Botany*, vol. 59, no. 3–4, pp. 297–306, 1997.
- [18] J. A. Moran, M. A. Merbach, N. J. Livingston, C. M. Clarke, and W. E. Booth, "Termite prey specialization in the pitcher plant *Nepenthes albomarginata*—evidence from stable isotope analysis," *Annals of Botany*, vol. 88, no. 2, pp. 307–311, 2001.
- [19] G. E. Chandler and J. W. Anderson, "Studies on the nutrition and growth of *Drosera* species with reference to the carnivorous habit," *New Phytologist*, vol. 76, pp. 129–141, 1976.
- [20] A. M. Ellison and N. J. Gotelli, "Nitrogen availability alters the expression of carnivory in the northern pitcher plant,

- Sarracenia purpurea*,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 7, pp. 4409–4412, 2002.
- [21] A. E. Wakefield, N. J. Gotelli, S. E. Wittman, and A. M. Ellison, “Prey addition alters nutrient stoichiometry of the carnivorous plant *Sarracenia purpurea*,” *Ecology*, vol. 86, no. 7, pp. 1737–1743, 2005.
- [22] P. S. Karlsson, K. O. Nordell, B. Å. Carlsson, and B. M. Svensson, “The effect of soil nutrient status on prey utilization in four carnivorous plants,” *Oecologia*, vol. 86, no. 1, pp. 1–7, 1991.
- [23] E. J. Farnsworth and A. M. Ellison, “Prey availability directly affects physiology, growth, nutrient allocation and scaling relationships among leaf traits in 10 carnivorous plant species,” *Journal of Ecology*, vol. 96, no. 1, pp. 213–221, 2008.
- [24] A. M. Ellison and E. J. Farnsworth, “The cost of carnivory for *Darlingtonia californica* (Sarraceniaceae): evidence from relationships among leaf traits,” *American Journal of Botany*, vol. 92, no. 7, pp. 1085–1093, 2005.
- [25] M. Méndez and P. S. Karlsson, “Costs and benefits of carnivory in plants: insights from the photosynthetic performance of four carnivorous plants in a subarctic environment,” *Oikos*, vol. 86, no. 1, pp. 105–112, 1999.
- [26] C. Field and H. A. Mooney, “The photosynthesis-nitrogen in wild plants,” in *The Economy of Plant Form and Function*, T. J. Givinis, Ed., pp. 25–55, Cambridge University Press, Cambridge, UK, 1986.
- [27] J. R. Evans, “Photosynthesis and nitrogen relationships in leaves of C_3 plants,” *Oecologia*, vol. 78, no. 1, pp. 9–19, 1989.
- [28] D. Pankovic, M. Plesnicar, I. Arsenijevic-Maksimovic, N. Petrovic, Z. Sakac, and R. Kastori, “Effects of nitrogen nutrition on photosynthesis in cd-treated sunflower plants,” *Annals of Botany*, vol. 86, pp. 841–847, 2000.
- [29] W. Schulze, E. D. Schulze, I. Schulze, and R. Oren, “Quantification of insect nitrogen utilization by the venus fly trap *Dionaea muscipula* catching prey with highly variable isotope signatures,” *Journal of Experimental Botany*, vol. 52, no. 358, pp. 1041–1049, 2001.