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Root nutrient uptake enhances photosynthetic assimilation in prey-deprived carnivorous pitcher plant *Nepenthes talangensis*

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Abstract

Carnivorous plants grow in nutrient-poor habitats and obtain substantial amount of nitrogen from prey. Specialization toward carnivory may decrease the ability to utilize soil-derived sources of nutrients in some species. However, no such information exists for pitcher plants of the genus *Nepenthes*, nor the effect of nutrient uptake *via* the roots on photosynthesis in carnivorous plants is known. The principal aim of present study was to investigate, whether improved soil nutrient status increases photosynthetic efficiency in prey-deprived pitcher plant *Nepenthes talangensis*. Gas exchange and chlorophyll (Chl) fluorescence were measured simultaneously and were correlated with Chl and nitrogen concentration as well as with stable carbon isotope abundance ($\delta^{13}C$) in control and fertilized *N. talangensis* plants. Net photosynthetic rate (P_N) and maximum- (F_v/F_m) and effective quantum yield of photosystem II (Φ_{PSII}) were greater in the plants supplied with nutrients. Biomass, leaf nitrogen, and Chl (*a*+*b*) also increased in fertilized plants. In contrast, $\delta^{13}C$ did not differ significantly between treatments indicating that intercellular concentration of CO_2 did not change. We can conclude that increased root nutrient uptake enhanced photosynthetic efficiency in prey-deprived *N. talangensis* plants. Thus, the roots of *Nepenthes* plants are functional and can obtain a substantial amount of nitrogen from the soil.

Additional keywords: carnivorous plant; chlorophyll fluorescence; gas exchange; *Nepenthes talangensis*; nitrogen supply; pitcher plant; rate of photosynthesis.

Introduction

Carnivorous plants grow in nutrient-poor, wet and sunny habitats (Givnish *et al.* 1984). They have evolved modified leaves called traps that attract, catch, and digest an animal prey, mainly insects. Nutrient uptake from prey stimulates growth and reproduction, as was first observed by Darwin (1875) and his son (Darwin 1878). Recently, Ellison (2006) summarized results from many studies and concluded that indeed, there is a significant positive effect of prey addition on carnivorous plants growth. It seems that carnivory is important but is not indispensable. Many, if not all carnivorous plants can survive without animals as prey, however with a reduced growth rate and reproduction. On the other hand, foliar uptake of nutrients from prey alone may not be sufficient for normal growth (Adamec 1997).

Carnivorous pitcher plants of the genus *Nepenthes* mostly grow in southeast Asia, principally Borneo, Sumatra, Java and peninsular Malaysia, with scattered populations in India, Sri Lanka, Australia, New Caledonia, Madagascar, and the Seychelles. The majority of *Nepenthes* species grow in typical carnivorous plant habitats, which are sunny, permanently moist and poor in nutrients (Clarke and Moran 2001). The average foliar N concentrations in the *Nepenthes* species are significantly lower than those in the leaves of noncarnivorous plants cohabiting with them. *Nepenthes* species are N-limited

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Abbreviations: C_a – ambient CO_2 concentration, C_i – intercellular CO_2 concentration; Chl – chlorophyll; F_0 – minimal fluorescence; F_0' – F_0 of the light-adapted state; F_v/F_m – maximal quantum yield of PSII; g_s – stomatal conductance; NPQ – non-photochemical quenching; PAR – photosynthetic active radiation; P_N – net photosynthetic rate; P_{Nmax} – maximum net photosynthetic rate at saturation irradiance; PNUE – photosynthetic nitrogen use efficiency; PSII – photosystem II; q_P – photochemical quenching coefficient; R_D – respiration rate; WUE – water use efficiency, $\delta^{13}C$ – carbon stable isotope abundance; Φ_{PSII} – effective quantum yield of PSII.

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and have thus evolved the pitcher to assist in uptake of N from prey (Osunkoya *et al.* 2007). Prey captured in the pitchers contributes 50–70% of N budget of *Nepenthes* plants and they benefit from it (Schulze *et al.* 1997, Moran *et al.* 2001). Only recently, the positive effect of prey addition on photosynthetic processes was documented. In our previous study we showed that prey-deprived *N. talangensis* plants had a significantly lower N concentration, photosynthetic rate (P_N) and maximum (F_v/F_m) and effective quantum yield of photosystem II (Φ_{PSII}) than the fed plants (Pavlovič *et al.* 2009). Also Farnsworth and Ellison (2008) documented that photosynthetic stress in carnivorous *Sarracenia* was alleviated by prey additions. Carnivorous plants inhabiting nutrient-poor habitats are chronically stressed when prey capture is low (Moran and Moran 1998). They benefit from animal-derived nutrients and the traps may to some extent take over functions that are usually restricted to the roots of non-carnivorous plants. Therefore, the role and importance of their roots is a matter of debate: whether prey capture supplements or replaces conventional root uptake (Adlassnig *et al.* 2005). Normal functioning of carnivorous plant roots is dampened by low nutrient avaibility in waterlogged and anoxic soils (Adamec 1997). Brewer (2003) found that pitcher plant *Sarracenia alata* invest relatively little biomass into roots compared to common co-occurring non-carnivorous plants. Different studies found different responses of the roots to nutrient uptake from the soil. On one hand, Karlsson and Pate (1992) found lack of response to soil nutrient supply of nitrate in the pygmy sundew *Drosera closterostigma*. Specialization toward carnivory may result in a decline in the ability to utilize soil-derived sources of nutrients in this species. In this respect, either the specialization to carnivory or soil hypoxia may be so strong that for instance, terrestrial carnivorous genera like *Genlisea* and *Utricularia* have never developed roots (Juniper *et al.* 1989). On the other hand, *Pinguicula vulgaris* benefits from root nutrient uptake and increased biomass and N concentration as a result of increased nutrient supply (Aldenius *et al.* 1983). Adamec (1997) subdivided carnivorous plants into three groups according to their ability to produce new biomass and accumulate mineral nutrients on the account of nutrients taken up by roots and leaves. No data concerning the genus *Nepenthes* have been published. The *Nepenthes* roots are generally brown or black in colour, less than 2 mm in diameter and form network in the substrate. However some epiphytic species (*e.g. N. inermis*) may have few or even no apparent roots and probably derive nutrients only through the pitchers (Clarke and Moran 2001). With the present stage of knowledge it is difficult to draw any conclusions about the role of roots in terrestrial species of *Nepenthes*.

It is well known that non-carnivorous plants increase their photosynthetic rate as a result of increased nitrogen uptake from the soil and their foliar nitrogen correlates with P_N across thousands of plant species (Wright *et al.* 2004). Carnivory stimulates photosynthesis, however no data concerning root nutrient uptake and its effect on photosynthesis in carnivorous plants have been published up to date. Therefore, the aim of the present study was to investigate the effect of soil nutrient status on photosynthetic processes in prey-deprived terrestrial carnivorous plants *N. talangensis*. We tried to find out the answer to the following question: Can increased nutrient uptake by roots stimulate photosynthesis in prey-deprived carnivorous pitcher plant *N. talangensis*, or is specialization toward carnivory in *Nepenthes* so strong that roots partially lost their function in nutrient-poor habitat? We simultaneously measured Chl fluorescence and gas exchange and analyzed Chl and N concentration and stable carbon isotope abundance ($\delta^{13}C$) in endemic terrestrial carnivorous plants *N. talangensis* from Sumatra in response to soil nutrient uptake.

Materials and methods

Plant material and culture condition: The pitcher plant *N. talangensis* Nerz and Wistuba (1994) grows in mossy forest and stunted upper mountain forest near the summit of Gunung Talang (1,800–2,500 m. a.s.l.) in Sumatra (Nerz and Wistuba 1994, Clarke and Moran 2001). We used 2-year-old, vegetatively propagated plants that were grown in a collection of carnivorous plants at the Department of Plant Physiology in Bratislava. The plants produced rosettes of leaves and did not reach the climbing stage. During the experiments, 10 plants were grown under controlled conditions in pots (10 × 10 × 10 cm) in a growth chamber with a photoperiod of 12 h dark/12 h light (250 μmol m^{-2} s^{-1} PAR, day/night temperatures of 25/17°C and high humidity (80–100%). They were grown in *Sphagnum*/perlite/bark/moss mixture substrate. To prevent entry of prey into pitchers they were plugged with wads of cotton wool moistened in distilled water. After six months of starvation, the plants were randomly grouped into two groups to ensure that at the start of the experiment the plants had similar size and developmental stage. Five plants served as control and did not obtain any nutrient solution (relying only on nutrients in the substrate) and five plants were watered with nutrient solution. The plants were watered with 50 ml of nutrient solution every week for 16 weeks. During the 16-week period, each plant obtained the following amount of nutrients dissolved in 800 ml (50 ml × 16-week) of distilled water: [175 mg NH_4NO_3; 21 mg KH_2PO_4; 91 mg $MgSO_4$; 92 mg KCl; 1.5 mg $FeCl_3$; 1.9 mg H_3BO_3; 1.2 mg $MnCl_2 \cdot 4\ H_2O$; 0.13 mg $ZnSO_4 \cdot 7\ H_2O$; 0.05 mg $CuSO_4 \cdot 5\ H_2O$; 0.06 mg $(NH_4)_4Mo_7O_{24} \cdot 4\ H_2O$]. During the experiment, the plants had no occasion to catch any animal prey.

Simultaneous measurement of CO_2 assimilation and Chl fluorescence: To assess whether increased nutrient supply in prey-deprived pitcher plants enhances photosynthetic efficiency, we analysed five young fully developed laminae from five plants with unformed pitchers that had developed during the 16-week experimental period. Rates of net photosynthesis (P_N) and chlorophyll fluorescence were measured simultaneously with a *CIRAS-2* (*PP-Systems*, Hitchin, UK) and a fluorcam *FC 1000-LC* (*Photon Systems Instruments*, Brno, Czech Republic) attached to the 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 (2.5 cm^2) was enclosed in the leaf cuvette (*PLC6, PP-Systems*, Hitchin, UK). After stabilization (15 min) the respiration rate (R_D) was recorded. Then the Chl fluorescence was measured. Minimal fluorescence (F_0, 0.1 μmol m^{-2} s^{-1} PAR, 5 s) and thereafter maximal fluorescence (F_m) were measured using a saturation pulse (4,000 μmol m^{-2} s^{-1} PAR, 800-ms duration) and maximal quantum yield of PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$. An induction curve of 15-min duration was then obtained by switching on the actinic light of 250 μmol m^{-2} s^{-1} PAR. The light was provided by blue (455 nm) and red (620 nm) LED diodes. For analysing the quenching mechanism, 10 saturation pulses were triggered. After 15 min the actinic light was switched off and F_0' was measured. Effective quantum yield of photosystem II (Φ_{PSII}), photochemical (q_P) and non-photochemical quenching (NPQ) were calculated (Maxwell and Johnson 2000). Then the saturation irradiance (1,500 μmol m^{-2} s^{-1} PAR) was applied for 15 min for adaptation, and light-response curves were determined. P_N was recorded at CO_2 concentration 360 μmol mol^{-1}, leaf temperature 23 ± 1°C, relative air humidity 65–70% and leaf-air vapour pressure difference 700–1,000 Pa. The light intensity was decreased stepwise with irradiation periods of 3 min and subsequent saturation pulses were applied until 60 μmol m^{-2} s^{-1} PAR was reached. Light-response curves of P_N, Φ_{PSII}, and NPQ were recorded simultaneously. Relaxation analysis showed that the irradiance 1,500 μmol m^{-2} s^{-1} PAR did not cause photoinhibition, because 95% of F_m recovered within 20 min in the dark. Thus the major contributor to NPQ was high-energy-state quenching (q_E). All measurements were taken between 9:00–12:00 h (one plant per day).

Chl extraction and quantification: The young laminae from five plants supplied with fertilizer and five control plants were removed. A part of the each lamina was dried at 70°C for 5 days to determine % of dry mass (DM). Remaining parts of laminae 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 phaeophytinisation of pigments. The samples were centrifuged at 8,000 × *g* for 5 min at 4°C. Chl (*a* + *b*) in supernatant was determined spectrophotometrically (*Jenway 6400*, London UK): Chl *a* at 663.2 nm, Chl *b* at 646.8 nm. Chl concentration was calculated according to Lichtenthaler (1987).

N and $\delta^{13}C$ analysis in dry mass: The leaves from gas exchange and Chl fluorescence measurements were ground and 1-mg samples packed into tin capsules. Nitrogen concentration was determined in elemental analyser *NC2100* (*Thermo Quest*, Rodano, Italy). After N determination, photosynthetic nitrogen use efficiency (PNUE) was calculated as: PNUE [μmol(CO_2) mol^{-1}(N) s^{-1}] = P_{Nmax} [μmol(CO_2) g^{-1}(DM) s^{-1}]/N [mol(N) g^{-1}(DM)]. Samples were combusted in oxygen and the $^{13}C/^{12}C$ ratio in the resultant CO_2 was determined by mass spectrometer Delta^{plus} XL (*ThermoFinnigan*, Bremen, Germany). The obtained $^{13}C/^{12}C$ ratios of plant samples, Rp, were referenced to $^{13}C/^{12}C$ ratio of standard V-PDB (Vienna-Pee-Dee-Belemnite), Rs, and expressed as $\delta^{13}C = [(Rp/Rs) - 1] \times 1000$ in ‰. The standard deviation of $\delta^{13}C$ determination in standard samples was lower than 0.1‰.

Statistical analysis: Prior to statistical tests, data were analysed for normality and homogeneity of variance. When nonhomogeneity was present, the *t*-test was employed with the appropriate corrected degrees of freedom. To evaluate the significance of the data between fertilized and control plants [leaf dry mass, R_D, P_{Nmax}, g_s, F_0, F_v/F_m, Φ_{PSII}, q_P, NPQ, C, N, PNUE, Chl (*a*+*b*), and $\delta^{13}C$] the *t*-test was used. The results are expressed as the mean of five replicates. The significance of relationships between N and P_N as well as $\delta^{13}C$ and P_N was tested by simple regression (*StatistiXL ver. 1.7 for Microsoft Excel*).

Results

Fertilized *N. talangensis* plants had larger laminae without pitcher and increased the dark- and light reactions of photosynthesis (Fig. 1). The P_N increased almost linearly with increasing irradiance at irradiances less than about 180 μmol(photon) m^{-2} s^{-1} PAR and reached saturation under an irradiance of about 700 μmol(photon) m^{-2} s^{-1} PAR. The plants supplied with nutrients had significantly higher P_{Nmax} in comparison to the plants without them (Table 1). The differences between treatments were more obvious at higher irradiances (Fig. 2*A*). Rate of respiration (R_D) was not significantly different. Maximum quantum yield in dark-adapted state (F_v/F_m) was significantly higher in plants supplied with fertilizer. The lower F_v/F_m ratio is due to

Fig. 1. *Nepenthes talangensis* without nutrients (*A*) and with nutrient supplied into the soil (*B*). The pictures were taken after gas-exchange and chlorophyll fluorescence measurements by the end of 18-week study period (16-week fertilizer application + 2-week measurements).

Table 1. Leaf biomass, chlorophyll fluorescence, gas exchange, chlorophyll (Chl), nitrogen (N) and carbon (C) concentration, photosynthetic nitrogen use efficiency (PNUE) and stable carbon isotopes abundance (δ^{13}C) in *Nepenthes talangensis* in response to nutrient supply. Chl fluorescence parameters were measured at actinic light intensity 250 µmol m^{-2} s^{-1} PAR (except F_0 and F_v/F_m), PNUE and P_{Nmax} at saturation irradiance. Values shown are means ± SE, *n* = 5, significantly different values (*t*-test) are indicated: $P<0.05$ (*), $P<0.01$ (**); ns – nonsignificant differences; R_D – rate of respiration; P_{Nmax} – maximum net photosynthetic rate at saturation irradiance; g_s – stomatal conductance; F_0 – minimal fluorescence; F_v/F_m – maximal quantum yield of PSII; Φ_{PSII} – effective quantum yield of PSII; q_P – photochemical quenching coefficient; NPQ – non-photochemical quenching; Chl (*a*+*b*) – chlorophyll (*a* + *b*); PNUE – photosynthetic nitrogen use efficiency; δ^{13}C – carbon stable isotope abundance.

Parameter	– nutrient solution	+ nutrient solution
Leaf biomass [mg(DM)]	54.6 ± 8.9	95.4 ± 12.5 *
R_D [µmol(CO_2) m^{-2} s^{-1}]	0.56 ± 0.07	0.57 ± 0.06 ^{ns}
R_D [nmol(CO_2) g^{-1}(DM) s^{-1}]	5.8 ± 0.7	8.0 ± 0.9 ^{ns}
P_{Nmax} [µmol(CO_2) m^{-2} s^{-1}]	3.8 ± 0.2	4.7 ± 0.1 **
P_{Nmax} [nmol(CO_2) g^{-1}(DM) s^{-1}]	41.3 ± 5.7	67.9 ± 7.0 **
g_s [mmol m^{-2} s^{-1}]	61 ± 10	73 ± 8 ^{ns}
F_0	264.8 ± 7.7	217.9 ± 7.8 **
F_v/F_m	0.790 ± 0.008	0.830 ± 0.004 **
Φ_{PSII}	0.45 ± 0.01	0.49 ± 0.01 *
q_P	0.72 ± 0.01	0.73 ± 0.01 ^{ns}
NPQ	1.31 ± 0.16	1.46 ± 0.11 ^{ns}
Chl (*a*+*b*) [mg g^{-1}(DM)]	1.79 ± 0.41	2.72 ± 0.10 *
C [mg g^{-1}(DM)]	438.1 ± 2.1	420.9 ± 2.4 **
N [mg g^{-1}(DM)]	7.0 ± 0.5	23.0 ± 1.5 **
PNUE [µmol(CO_2) mol^{-1}(N) s^{-1}]	82.9 ± 7.9	41.4 ± 4.1 **
δ^{13}C [‰]	–28.75 ± 0.27	–29.21 ± 0.33 ^{ns}

significantly higher F_0 in plants without fertilizer (Table 1). Effective quantum yield of PSII (Φ_{PSII}) progressively decreased with increasing irradiance from between 0.6–0.7 at low PAR to approximately 0.05 at 1,500 µmol photon m^{-2} s^{-1} PAR (Fig. 2*B*). Effective quantum yield of PSII was significantly higher in plant supplied with nutrients (Table 1). However, q_P and NPQ were not significantly different between treatments. NPQ increased with increasing irradiance and numerically higher differences in NPQ between treatments were found at higher irradiances (Fig. 2*C*). The values of NPQ reached 0.6–0.7 at 60 µmol(photon) m^{-2} s^{-1} PAR and 4.2–4.5 at 1,500 µmol(photon) m^{-2} s^{-1} PAR. Chl (*a*+*b*), N concentration, and biomass were significantly higher in the plants supplied with fertilizer. The plants with lower N leaf concentration used N for CO_2 assimilation more effectively as indicated by higher values of PNUE (Table 1). Fig. 3*A* summarizes the relationship between N and P_{Nmax} in laminae. It is obvious that there is significant correlation of the relationship ($P = 0.002$). The values $\delta^{13}C$ ranged from –28.4 to –30.1‰ and –28.1 to –29.4 ‰ in fertilized and control plants, respectively, and are not significantly different. The relationship between P_N and $\delta^{13}C$ is not significant either ($P = 0.157$, Fig. 3*B*).

Discussion

We investigated the role and importance of roots in carnivorous pitcher plant *Nepenthes talangensis*. Prey-deprived carnivorous *N. talangensis* pitcher plant supplied with fertilizer had higher F_v/F_m. F_v/F_m in plants supplied with fertilizer was 0.83, a value similar to that found in healthy leaves of a wide range of C_3 species by Björkman and Demmig (1987). Similar values were found in carnivorous *Sarracenia purpurea* by Bott *et al.* (2008). The F_v/F_m values are used as sensitive indicator of plant photosynthetic performance. The values lower than 0.83 are seen when plants were exposed to stress, indicating in particular the phenomenon of photoinhibition (Maxwell and Johnson 2000). Our prey-deprived *N. talangensis* plants had F_v/F_m ratio lower than 0.80, indicating a mild stress (Table 1). Huang *et al.* (2004) demonstrated that nitrogen deficiency significantly reduced F_v/F_m ratio in rice. In our previous study we showed that feeding pitchers with insect larvae increased the F_v/F_m ratio and thus alleviated the nutrient stress in pitcher plants (Pavlovič *et al.* 2009). Here we found the same effect - the root nutrient uptake significantly reduced stress in prey-deprived *N. talangensis* plants (Table 1), which supports the hypothesis that roots of *Nepenthes* are functioning and their function is not completely offset by digestive glands in the pitcher. However, in the natural habitat with poor soil nitrogen content, the plants may obtain significant amount of nitrogen from prey. On average, more than 60% of nitrogen is taken up from prey (Moran *et al.* 2001, Schulze *et al.* 1997). Similar to our study, Moran and Moran (1998) have shown using foliar reflectance that prey-deprived pitcher plants in natural habitat are nutrient stressed. This indicates the importance of carnivory in natural habitat with low nutrient content in the soil.

Several other photosynthetic parameters also indicate that nutrient stress in prey-deprived plants was eliminated by root nutrient uptake. Effective quantum yield of PSII (Φ_{PSII}) measures the proportion of the light absorbed by chlorophylls associated with PSII that is used in photochemistry (Maxwell and Johnson 2000). Both P_N and Φ_{PSII} were significantly higher in the plants supplied with fertilizer (Fig. 2*A*,*B*). Higher P_N is probably due to higher leaf N concentration because a positive correlation between P_N and N was found (Fig. 3*A*). Non-photochemical quenching (NPQ) quenches singlet-excited chlorophylls and harmlessly dissipate excess excitation energy as heat *via* xantophyll cycle (Müller *et al.* 2001). The NPQ was not significantly different at irradiance 250 μmol(photon) m^{-2} s^{-1} PAR at which the plants were growing (Table 1). At higher irradiance the differences in NPQ between plants with and without fertilizer were more obvious (Fig. 2*C*).

Analysis of carbon stable isotope abundance has practical advantage over gas exchange measurements. It provides a time-integrated analysis of plant response to environmental condition. Carbon isotope discrimination and $\delta^{13}C$ is highly correlated with plant water use efficiency (WUE) and ratio of intercellular to leaf ambient CO_2 concentration, C_i/C_a. Smaller C_i/C_a values are equivalent to an increase in WUE and an increase (less negative values) in $\delta^{13}C$ (Farquhar *et al.* 1989). The range of foliar $\delta^{13}C$ values identified *N. talangensis* as C_3 plant. The nonsignificant differences in $\delta^{13}C$ indicate that

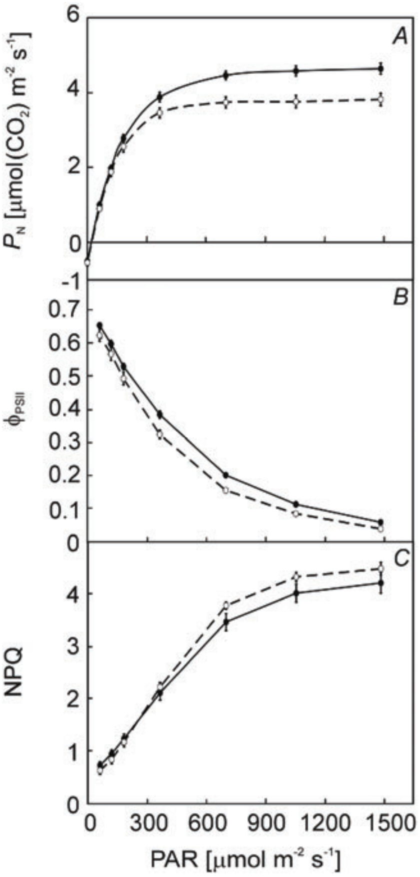

Fig. 2. Rate of net photosynthesis (P_N) (*A*), effective quantum yield of PSII (Φ_{PSII}) (*B*) and non-photochemical quenching (NPQ) (*C*) in response to irradiance. Plant supplied with fertilizer (●, *solid line*) and control plants (○, *dashed line*). Means ± SE, $n = 5$.

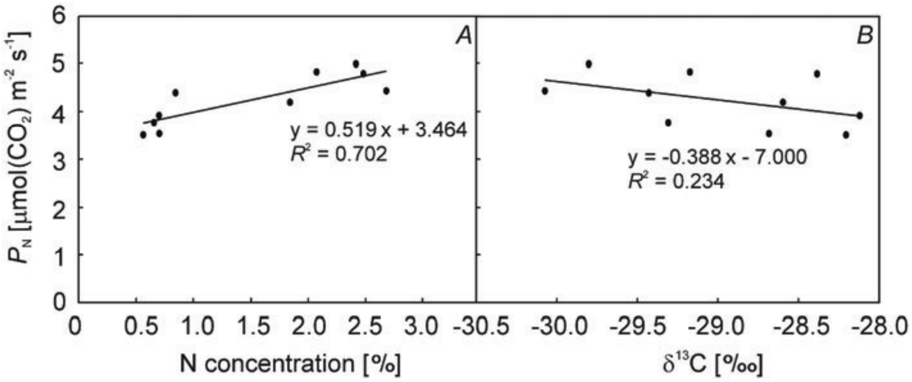

Fig. 3. Net photosynthetic rate (P_N) in relation to leaf nitrogen concentration (*A*) and to carbon stable isotope abundance ($\delta^{13}C$) (*B*). The relationship is highly significant between P_N and N (P = 0.002) and not between P_N and $\delta^{13}C$ (P = 0.157).

enhanced P_N in plants supplied with nutrients was accompanied by a higher stomatal conductance (g_s) which kept the same C_i. Short-term measurements of g_s by infrared gas analyser showed slightly higher g_s, though the difference was not significant (Table 1). Relative invariability in C_i/C_a ratio in response to environmental conditions including nitrogen nutrition was observed formerly (*e.g*. Wong *et al.* 1985) and indicates existence of a stomata-photosynthesis coupling mechanism. Carnivorous plants, growing in permanently moist habitat, are usually not water-stressed, which allows fully open stomata. Minimal stomatal limitation of photosynthesis is very important, because benefits from carnivory, in term of increased P_N as a result of increased N absorption from prey, is significant only in sunny and wet environments where P_N is not limited (Givnish *et al.* 1984, Brewer 2003).

Adamec (1997) subdivided carnivorous plants into three groups according to their ability to produce new biomass and accumulate mineral nutrients on the account of nutrients taken up by roots and leaves. He named the first group "nutrient-requiring species", which markedly increase their growth due to both soil and leaf nutrient supply. The second group is "root-leaf nutrient competitors" in which competition between root and leaf nutrient uptake occurs and root nutrient uptake is limited. The third group named "nutrient-modest species" has very low nutrient uptake capacity and relies on leaf nutrient uptake. According to this and our previous study (Pavlovič *et al.* 2009), *N. talangensis* belongs to the first group of carnivorous plants in which nitrogen concentration, photosynthesis, and new biomass increase as a result of both leaf and root nutrient uptake. The dose of nutrient used in our experiment corresponds to 7 g(N) m^{-2}. Similar low dose of nitrogen [4 g(N) m^{-2}] in *Drosera rotundifolia* led to a moderate increase in stem height, leaf thickness, leaf number and leaf DM per plant (Svensson 1995). On the other hand high dose of N [170 g(N) m^{-2}] led to reduction of total plant biomass in *D. rotundifolia* (Stewart and Nielsen 1992). Eleuterius and Jones (1969) studied the growth of *Sarracenia alata* and found also the decrease growth in fertilized bog soil [37.1 g(N) m^{-2}]. Production of carnivorous organs is a phenotypically plastic trait and carnivory is not favoured under an excess supply of N. Ellison and Gotelli (2002) found that nitrogen addition dramatically altered leaf morphology in *Sarracenia purpurea*. The plants supplied with N produced leaves with large non-carnivorous keels and small carnivorous tubes (phyllodia). In extreme cases, no tubes were produced and the leaves were entirely non-carnivorous. Our fertilized plants also did not produce any pitchers (Fig 1*B*). These results confirmed the hypothesis that carnivory would not be favoured when there is an excess supply of N (Givnish *et al.* 1984). Leaf N concentration in our fertilized plants far exceeds the concentration found in leaves of various species of *Nepenthes* in their natural habitat (Moran and Moran 1998, Moran *et al.* 2001, Osunkoya *et al.* 2007) and exceeds the threshold 20 mg g^{-1} below which nitrogen is considered to be limiting (Ellison 2006). Also lower PNUE in fertilized plants may indicate that they are saturated with N and P_N is not limited by lack of N, which might explain why the production of the pitchers failed (Fig. 1, Table 1).

In conclusion, prey-deprived carnivorous plants without nutrients added to the roots have significantly lower values in several photosynthetic parameters, compared with the nutrient-fed plants. Because the roots of carnivorous plants developed in nutrient-poor and anoxic soils, their function and significance have been questioned. In this study we clearly demonstrate that root nutrient uptake alleviates "photosynthetic stress" in terrestrial prey-deprived *N. talangensis* plants and that the plants can benefit not only from leaf but also from root nutrient uptake. However, the low nitrogen content in the soil and the abundant prey in the pitchers in their natural habitat suggest that nitrogen uptake may be predominantly through the pitchers in *Nepenthes*. This enables them to more effectively compete with noncarnivorous plants in a nutrient-poor habitat. Moreover, some epiphytic species (*e.g. N. inermis*) are rootless and probably only rely on nutrients derived through the pitchers. In this species pitchers completely take over the functions of roots that results in high specialization toward carnivory.

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