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Research article

A carnivorous sundew plant prefers protein over chitin as a source of nitrogen from its traps

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ABSTRACT

Carnivorous plants have evolved in nutrient-poor wetland habitats. They capture arthropod prey, which is an additional source of plant growth limiting nutrients. One of them is nitrogen, which occurs in the form of chitin and proteins in prey carcasses. In this study, the nutritional value of chitin and protein and their digestion traits in the carnivorous sundew *Drosera capensis* L. were estimated using stable nitrogen isotope abundance. Plants fed on chitin derived 49% of the leaf nitrogen from chitin, while those fed on the protein bovine serum albumin (BSA) derived 70% of its leaf nitrogen from this. Moreover, leaf nitrogen content doubled in protein-fed in comparison to chitin-fed plants indicating that the proteins were digested more effectively in comparison to chitin and resulted in significantly higher chlorophyll contents. The surplus chlorophyll and absorbed nitrogen from the protein digestion were incorporated into photosynthetic proteins – the light harvesting antennae of photosystem II. The incorporation of insect nitrogen into the plant photosynthetic apparatus may explain the increased rate of photosynthesis and plant growth after feeding. This general response in many genera of carnivorous plants has been reported in many previous studies.

1. Introduction

Carnivorous plants have independently evolved several times by the process of convergent evolution (Ellison and Gotelli, 2009). They usually grow in sunny, wet and nutrient-poor habitats where the nutritional benefit gained from captured prey exceeds the costs of modifying leaves into traps (Givnish et al. 1984; Pavlovič et al. 2009). The costs of carnivory represents extra energy costs with prey attraction (production of lures), capture (production of traps) and digestion (production of digestive enzymes) being required. There is also a decreased rate of photosynthesis (A_N) and in some species an increased rate of respiration (R_D) as a result of leaf adaptation for carnivory (Givnish et al., 1984; Ellison, 2006; Pavlovič and Saganová, 2015). On the other hand, a potential benefit from carnivory is an increase in A_N through improved nutrient supply; particularly in foliar/shoot nitrogen and phosphorus contents (Farnsworth and Ellison, 2008; Pavlovič et al., 2009, 2014; He and Zain, 2012; Kruse et al., 2014; Gao et al., 2015). The greatest enhancement in photosynthetic gains from prey capture occurs under conditions of soil nutrient shortage, together with sufficient humidity and light. Such conditions have favoured the evolution of botanical carnivory. It has been suggested that the mechanism accounting for the increased A_N in response to nitrogen uptake from prey is an increased concentration in photosynthetic proteins (mainly Rubisco) (Givnish et al., 1984); however, this has never been tested. This suggestion seems reasonable because Rubisco is present at very high levels in the photosynthesizing cells of C_3 plants and may contribute up to 50% of soluble leaf protein and 20–30% of total leaf N (Evans, 1989; Feller et al., 2008). The second major fraction of nitrogen directly related to photosynthesis consists of the pigment–protein complexes in thylakoid membranes (Evans, 1989).

Carnivorous plants obtain a substantial amount of nutrients from prey capture and have evolved five basic trapping mechanisms (Juniper et al., 1989; Król et al., 2012; Pavlovič and Saganová,

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2015). The most important nutrients, which restrict the carnivorous plant growth in nutrient-poor soils are nitrogen (N), phosphorus (P) and potassium (K) (Ellison, 2006). Insect prey is a rich source of N and P, the contents of which exceed that in leaf tissue by 5–10 times, and these elements are markedly absorbed (Adamec, 2002; Pavlovič et al., 2014). This accounts for the relatively high contribution of insect-derived N to total leaf N content in carnivorous plants which successfully capture insect prey (10–90%, Schulze et al., 1991, 1997; Chapin and Pastor, 1995, 2001; Moran et al., 2001; Millet et al., 2003), and is allocated mainly in the new foliage (Schulze et al., 1997; Gao et al., 2015). Adamec (2002) and Pavlovič et al. (2014) analysed prey carcasses after their digestion and found that a high amount of N (40–60%) in insect carcasses was unavailable for absorption by sundew traps. On the other hand, P and K were absorbed much more effectively. They hypothesized that the less effective N uptake was due to the large proportion of N in insect chitin exoskeletons (as poly-N-acetylglucosamine) which is not available for absorption. Indeed, the exoskeleton of the digested prey does not seem to be significantly affected by the digestive processes (Juniper et al., 1989). However, this seems to be in contrast to the recent molecular findings that several classes of chitinases in the digestive fluid in different species of carnivorous plants have been identified, and are even up-regulated by the presence of prey and/or chitin (Matušíková et al., 2005; Eilenberg et al., 2006; Rottloff et al., 2011; Hatano and Hamada, 2012; Renner and Specht, 2012; Paszota et al., 2014).

In our previous work, we have shown that feeding the sundew plant *Drosera capensis* on fruit flies significantly increased its leaf nitrogen and phosphorus content as well as the photosynthetic rate (Pavlovič et al., 2014). In this work, we focused on the importance of two nitrogen-rich compounds (chitin and protein) in the nutrition of the carnivorous sundew plant *D. capensis*. The sundew plants were fed either on chitin or protein (bovine serum albumin, BSA) to estimate the contribution of both N sources to the total N budget in plants. We measured the biomass, elemental composition, stable N isotopes and chlorophyll content to reveal the uptake and nutritional value of both insect nitrogen-rich compounds. In addition, we did Western blot analyses for important photosynthetic proteins to find the role of N nutrition in the photosynthesis of carnivorous plants.

2. Materials and methods

2.1. Plant material and culture conditions

D. capensis L. (Cape sundew) is a small, erect perennial sundew native to the Cape region of South Africa. Experimental plants were grown from seeds under standard greenhouse conditions at the Department of Plant Physiology in Bratislava, Slovakia. Well-drained acidic peat moss (AGRO, Česká Skalice, Czech Republic) in plastic pots (6 × 6 × 6 cm), placed in a tray filled with distilled water to a depth of 1–2 cm was used. There are indications that this natural, non-fertilised substrate was mineral poor like those used in other similar studies (e.g., Adamec, 2002). The small pot size was chosen to keep the experimental plants under conditions of slight mineral starvation. During the experiments, 26 plants were grown under controlled conditions in 150 L aquarium in a growth chamber with a 14 h photoperiod [100 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), day/night temperatures of 25/18 °C, high air humidity (80–100% RH)]. Daily irradiance was provided by white and violet fluorescent tubes. Young, medium-sized non-flowering plants (5–6 months old, 3–4 cm long leaves) of similar size were used in the experiments. No potential animal prey was present for the plants in the growth chamber.

Seven plants in the chitin treatment were supplied with approximately 15 mg of chitin from shrimp shells (95% acetylated, Sigma Aldrich, St. Louis, USA) every week for 16-week-long feeding period (in total 240 mg of chitin per plant for 16 weeks). We analysed the nitrogen content in chitin and we found 64.8 mg N g^{-1} DW. Thus each fed plant obtained a total of 15.6 mg of nitrogen during the whole feeding period. Another seven plants were fed on protein with approximately 6 mg of BSA (Sigma Aldrich, St. Louis, USA) every week for the same feeding period (in total 96 mg BSA per plant for 16 weeks). The analysed nitrogen content in BSA was 144.2 mg N g^{-1} DW, thus each plant obtained in total 13.8 mg of nitrogen during the whole feeding period. Both substances were applied on two or three fully developed traps covered with sticky tentacles during each feeding. Thus both groups of plants obtained approximately the same amount of N in the form of chitin or BSA (ca. 14–16 mg) to investigate the nutritional value of both substances commonly found in insect prey. Seven plants served as unfed control. After 16 weeks of feeding, the plants were allowed to grow for another 6 weeks without any additional feeding before they were harvested for analysis. This period was necessary for the production of new leaves used in elemental and isotopic analyses which were not contaminated with remaining BSA or chitin applied on trap surface.

2.2. Elemental analysis and isotopic composition of leaves

Plant aboveground biomass was harvested from five plants before the feeding experiment and from seven plants per feeding group after the experiment, dried at 70 °C for a week, weighed for DW determination, and ground to a fine powder. To avoid contamination with chitin or BSA, newly formed unfed leaves developed during the 6-week-long period after feeding were used for elemental analyses. All the fed leaves, which usually senesced within 22 (16 + 6)-week-long period after feeding were not harvested for analysis to avoid contamination from chitin and BSA, including old leaves in control plants and plants before experiments. Samples (1 mg) were packed into tin capsules and the nitrogen content was determined in a Vario MICRO Cube (Elementar, Hanau, Germany) Elemental Analyzer. A connected continuous-flow IRMS Delta plus XL (Thermo Finnigan, Bremen, Germany) was used to analyse the stable isotope ratios of nitrogen to assess the plant's nitrogen origin. The $\delta^{15}N$ values (‰) were measured against a laboratory reference gas (pure N_2, which had $\delta^{15}N$ −2.4‰) calibrated by the N1 standard [$(NH_4)_2SO_4$, IAEA, Vienna, Austria]. The $^{15}N/^{14}N$ ratios of plant samples, R_p, were referenced to the $^{15}N/^{14}N$ ratio of atmospheric N_2 (R_s) and expressed as $\delta^{15}N = [(R_p/R_s) - 1] \times 1000$ in ‰ (Pavlovič et al., 2011). The precision of the IRMS, based on multiple measurements of the N1 standard, was 0.08‰. The N content and $\delta^{15}N$ were also measured in BSA and chitin before their application. After foliar digestion, the BSA was completely dissolved (usually within few hours) and no residue remained (what does not necessarily indicate that all proteins were digested) on the leaves but there was a high amount of undigested chitin which was collected by forceps for analyses. The contribution of leaf chitin/BSA-derived N ($\%N_{chitin/BSA}$) to the total foliar N content was estimated using the two end-member mixing model described by Moran et al. (2003): $\%N_{chitin/BSA} = [(\delta^{15}N_{fed} - \delta^{15}N_{unfed})/(\delta^{15}N_{chitin\ or\ BSA} - \delta^{15}N_{unfed})] \cdot 100$, where $\delta^{15}N_{fed}$, $\delta^{15}N_{unfed}$, $\delta^{15}N_{chitin\ or\ BSA}$ are the $\delta^{15}N$ values for fed or unfed *D. capensis* and chitin or BSA samples, respectively.

The samples were mineralised using concentrated acid, and diluted and analysed for P, K, Ca and Mg contents (for all analytical details see Pavlovič et al., 2014). For P analyses, 1.8–2.4 mg dry weight was mineralised with $HClO_4$ and 6–7 mg with HNO_3 for metallic cation analyses. Analyses of P were performed colourimetrically with an automatic FIAstar 5010 Analyzer (Tecator,

Sweden), while metallic cation concentrations were estimated by atomic absorption flame spectrometer Varian AA240FS (Agilent, Santa Clara, CA, USA). Five to seven biological replicates (one sample from one plant) of foliar material were analysed for each variant. The results of tissue nutrient content are expressed in % of DW.

2.3. Chlorophyll and carotenoid extraction and quantification

The leaves (50 mg FW) from the same plants used for elemental and isotopic analyses were ground in a mortar and pestle with a small amount of sand and extracted with 80% (v/v) chilled acetone together with $MgCO_3$ to avoid acidification and pheophytinization of pigments. The samples were centrifuged at 8000 *g* for 5 min at 4 °C. Chlorophyll *a* + *b* (chl *a* + *b*) and total carotenoids in the supernatant were determined spectrophotometrically (Jenway 6705 UV/Vis, Bibby Scientific Ltd, Essex, UK): chl *a* at 663.2 nm, chl *b* at 646.8 nm, carotenoids at 470 nm. A background measurement at 710 nm was subtracted from the pigment absorbance. Concentrations of assimilation pigments were calculated according to Lichtenthaler (1987). This missing portion of DW from pigment analysis was added to biomass calculation.

2.4. SDS-PAGE and Western blots

Fifty mg of FW leaf tissue from three plants used for above mentioned analyses was harvested and the proteins were isolated using extraction buffer containing 28 mM dithiotreitol, 28 mM Na_2CO_3, 175 mM sucrose, 5% SDS, 10 mM EDTA and protease inhibitors (Set VI, Calbiochem, Darmstadt, Germany). For protein denaturation, the samples were heated to 70 °C for 30 min. The concentration of total soluble proteins in samples was determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma–Aldrich, St. Louis, MO, USA) and the absorbance was measured at 562 nm (Jenway 6705 UV/Vis, Bibby Scientific, Essex, UK). The same amount of protein was electrophoresed in tricine-10% (w/v) SDS-polyacrylamide gel (Schägger, 2006) followed by a transfer to the nitrocellulose membrane (Biorad, Germany) by Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Biorad, Hercules, CA, USA). After blocking in Tris-buffered saline with 0.1% (v/v) Tween (TBS-T) containing 5% (w/v) BSA at 4 °C overnight, the membranes were incubated with the primary antibody for one hour at room temperature. Antibodies against proteins of photosystem II (D1) and light harvesting antennae (Lhcb 4, 5), were purchased from Agrisera (Vännäs, Sweden). After washing, the membrane was incubated with the secondary antibody, the goat antirabbit IgG (H + L)-horseradish peroxidase conjugate (Bio-Rad, Hercules, CA, USA). Blots were visualized using Immobilon Western chemiluminescent HRP substrate (Millipore, Billerica, MA, USA) and by a gel scanner Amersham Imager 600 (GE HealthCare Life Sciences, Japan).

2.5. Statistical analyses

The values shown are means ± s.e (5 for plants at the beginning of experiments and 7 after 22 weeks long period). The significant differences were evaluated by one-way analysis of variance (ANOVA) followed by Tukey test (Origin 8.5.1., Northampton, USA).

3. Results

As a result of nitrogen uptake from chitin and protein (BSA), leaf N content significantly increased in comparison with the control plants (Table 1). The decrease of the foliar N content in control plants in comparison to the plants at the beginning of the experiment indicates that the prey-deprived plants were nutrient-stressed and they were not able to maintain a higher foliar N content (Table 1). The shoots of chitin-fed *D. capensis* plants showed significantly more negative $\delta^{15}N$ values than the unfed plants or plants at the beginning of the experiment (Fig. 1). Given the $\delta^{15}N$ value of chitin ($\delta^{15}N = -4.560‰$), we estimated that *D. capensis* derived on average 49% of the shoot N from chitin. By comparing the N content in the chitin before and after digestion, it was found that 93% of the nitrogen was still in the undigested chitin and only 7% was absorbed. On the contrary, plants fed on proteins (BSA) showed more positive $\delta^{15}N$ values, which is in accordance with the positive $\delta^{15}N$ value of BSA ($\delta^{15}N = +7.570‰$). Using the two mixing model it was estimated that around 70% of the total foliar N in the *D. capensis* plants sampled was derived from BSA. BSA was completely dissolved and it was not possible to determine N content after digestion. It can thus be concluded that soluble protein digestion is very effective.

Given that the same amount of nitrogen was theoretically available for plants from both the chitin and the proteins, the proteins were digested and absorbed much more effectively and this resulted in a significantly higher foliar N content in BSA-fed plants in comparison to the chitin-fed plants (Table 1). As the isotopic leaf composition is only a relative measure of N uptake and does not take into account an increase in the foliar N content and biomass, the N uptake was also calculated in absolute terms from both substances. Based on the results from N content, $\delta^{15}N$ and biomass accumulation [$(\%N_{chitin/BSA}/100) \times$ N content (mg g^{-1}) × biomass (g)], a single *D. capensis* plant obtained 1.03 mg of N from BSA and only 0.31 mg of N from chitin. This is in good agreement with the analyses of total N content in shoots [N content (mg g^{-1}) × biomass (g)] as the total N content in fed plants minus the total N content in control plants (Fig. 2). Based on this approach, a single plant obtained 0.94 mg of N out of 13.8 mg of N from BSA and 0.12 mg of N out of 15.6 mg of N from chitin; however, the biomass of senescent dead leaves and roots were not taken into account, so these values are underestimated. Nevertheless, both approaches indicate that the plants utilized N from chitin 4–8 times less effectively than N from BSA. According to the stoichiometric relationships between different nutrients and their shoot concentrations, the growth of control plants was N- and P-limited and this N limitation was markedly alleviated by feeding on chitin and mainly on proteins. However, fed variants invariably exhibited a strong P limitation, which permanently limited plant growth (Table 2). It is highly probable that in the case of a higher P availability, the efficiency of N utilization from BSA would be even higher.

Plants fed on chitin and proteins had significantly higher chlorophyll concentrations and lower chl *a*/*b* ratios in comparison to unfed plants (Fig. 3), but their growth was not enhanced (Fig. 4) which indicates that the unavailability of other nutrients (mainly P) or low light might limit plant growth. The surplus chlorophyll molecules and nitrogen were incorporated mainly into the light-harvesting antennae of photosystem II and the amount of reaction centre protein D1 did not change (Fig. 5).

4. Discussion

Insects, which are the most important prey of terrestrial carnivorous plants, are a concentrated and diverse source of organic and inorganic materials. The content of N in their body is approximately 5–10 times higher than that found in carnivorous plant leaf tissue (Pavlovič et al., 2009, 2014). The nitrogen in insects is mainly bound in chitin in the form of the linear polymer N-acetylglucosamine and in proteins in the form of aminoacids. The chitin content varies across different insect species from 3 to 16% of the whole insect body dry weight, while protein content ranges

Table 1
Leaf tissue nutrient content at the beginning of the experiment, and 22 (16 + 6) weeks later in control and protein vs. chitin fed plants.

Element (% of DW)	Beginning	Control	Chitin fed	BSA fed
N	1.13 ± 0.13 a	0.50 ± 0.02 b	0.74 ± 0.04 c	1.42 ± 0.06 d
P	0.056 ± 0.006 a	0.041 ± 0.002 b	0.050 ± 0.003 a	0.034 ± 0.001 c
K	1.64 ± 0.04 a	1.15 ± 0.16 a	1.39 ± 0.09 a	1.27 ± 0.12 a
Ca	0.43 ± 0.02 a	0.45 ± 0.02 a	0.44 ± 0.02 a	0.43 ± 0.01 a
Mg	0.38 ± 0.01 a	0.53 ± 0.02 b	0.52 ± 0.03 b	0.47 ± 0.02 b

Values are means ± s.e. (n = 5–7).
Different letters in the rows indicate significant differences at P = 0.05 (ANOVA, Tukey test).

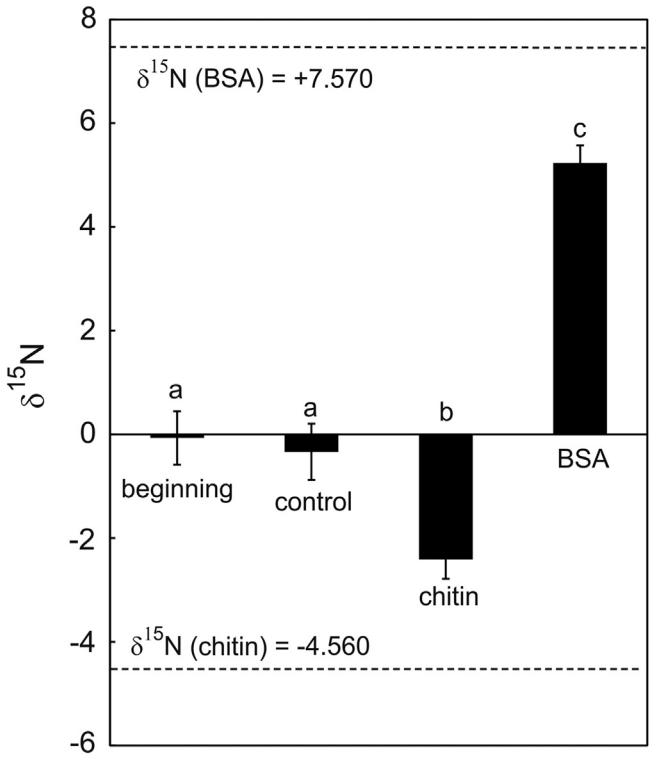

Fig. 1. Isotopic ^{15}N signature of *D. capensis* in plants at the beginning of the experiment, and 22 (16 + 6) weeks later in control and protein *vs*. chitin fed plants. The dashed lines show the ^{15}N signature of nitrogen source: chitin and BSA. Means ± s.e., n = 5–7. Different letters denote significant differences at P < 0.05 (ANOVA followed by Tukey test).

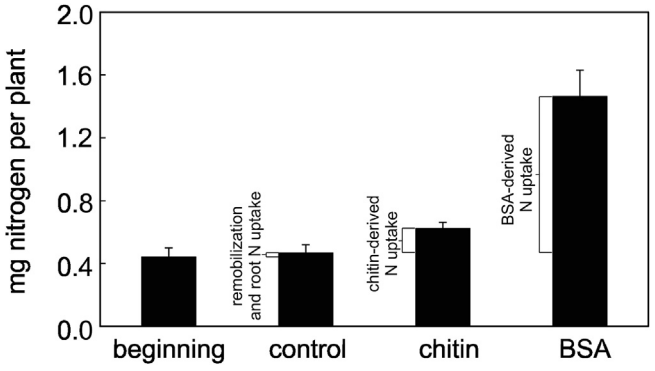

Fig. 2. Nitrogen uptake from chitin and proteins. Nitrogen acquisition was estimated from the increase in plant N content and the plant biomass. Control plants were compared with the plants at the beginning of experiment, and chitin and BSA fed plants were compared with the control plants. The values are underestimated, because the biomass of roots and senesced leaves was not taken into account. Means ± s.e., n = 5–7.

from 13 to 77% (Cauchie, 2002; Xiaoming et al., 2010). These data, and the fact that the chitin molecule contains only a third of the N found in proteins, support the idea that chitin-based nitrogen represents a small fraction of an insect's total nitrogen (Finke, 2007). In spite of this, several classes of chitinases have been identified in the digestive fluid of carnivorous plants (Matušíková et al., 2005; Eilenberg et al., 2006; Rottloff et al., 2011; Hatano and Hamada, 2012; Renner and Specht, 2012; Paszota et al., 2014) indicating that this form of nitrogen is also targeted by them. In this study, the same amount of nitrogen either in the form of protein or chitin was applied to evaluate the efficiency of utilization by carnivorous sundew plant *D. capensis*. It was shown that the proteins were both digested and had their N absorbed more effectively (ca. 4–8 times) than the chitin, and protein digestion and absorption was also more beneficial for the plants (Figs. 2, 3 and 5). One should take into account that some species of carnivorous plant increased root nutrient uptake in response to absorption of leaf nutrients (Adamec, 2002), what might slightly affect the calculation. However, the shift of leaf nitrogen isotopic signature to opposite direction in response to BSA and chitin indicates that the main source of nitrogen was in the food (Fig. 1). For protein digestion, carnivorous plants usually employ two groups of enzymes: aspartic and cysteine endopeptidases (Athauda et al., 2004; Takahashi et al., 2009, 2011, 2012; Schulze et al., 2012; Libiaková et al., 2014). For chitin digestion, class I, III and IV chitinases have been identified in carnivorous plants (Matušíková et al., 2005; Eilenberg et al., 2006; Rottloff et al., 2011; Hatano and Hamada, 2012; Renner and Specht, 2012; Paszota et al., 2014). Based on the percentage contents of both substances in an insect body and the nitrogen content and efficiency of utilization, it can be concluded that the chitin digestion has lower ecological importance for carnivorous plants in the field. However, the principal function of chitin degradation might lie in increasing the ability of other enzymes to gain access to the soft body tissues within. Another important function of chitinases is in the plant defence mechanism. Plant chitinases are considered as pathogen-related (PR) proteins since their activity is induced by fungal, bacterial and viral infection, as well as by more general signals of stress such as wounding (Collinge et al., 1993). The presence of other PR-proteins and low molecular weight compounds with antimicrobial properties in the fluid of carnivorous plants with no obvious hydrolytic function in the digestion of caught prey suggests that these molecules are directed against microorganisms (Hatano and Hamada, 2012; Buch et al., 2013; Rottloff et al., 2013). In this way, the plants can avoid the growth of microbes that compete with the plant for the prey-derived nutrients that are available in the trap.

In many previous studies, it has been found that feeding terrestrial carnivorous plants on insect prey significantly increased A_N and chlorophyll content (Farnsworth and Ellison, 2008; Pavlovič et al., 2009, 2011, 2014; He and Zain, 2012; Kruse et al., 2014). Givnish et al. (1984) suggested that mechanism accounting for increased A_N in response to N uptake from prey is its incorporation into photosynthetic proteins (mainly Rubisco). For some unknown

Table 2
Ratios of element contents (N, P, K) in foliar tissues of *Drosera capensis*.

Element ratio (%/%)	Beginning	Control	Chitin fed	Protein fed
N:P	20.2 ± 0.9 a	12.3 ± 0.3 b	15.0 ± 1.0 b	41.6 ± 1.5 c
N:K	0.69 ± 0.07 a	0.48 ± 0.07 a	0.54 ± 0.02 a	1.16 ± 0.09 b
K:P	30.2 ± 3.2 a	28.6 ± 4.1 a	28.0 ± 1.7 a	37.6 ± 4.2 a

Values are means ± s.e. (n = 5–7, n = 5 for plants at the beginning of experiments, n = 7 for all remaining treatments).
Different letters in the rows indicate significant differences at P = 0.05 (ANOVA, Tukey test).
N limitation is indicated by N < 20 mg g^{-1} and N: P < 14, whereas P limitation by P < 1 mg g^{-1} and N: P > 16 (Ellison, 2006).

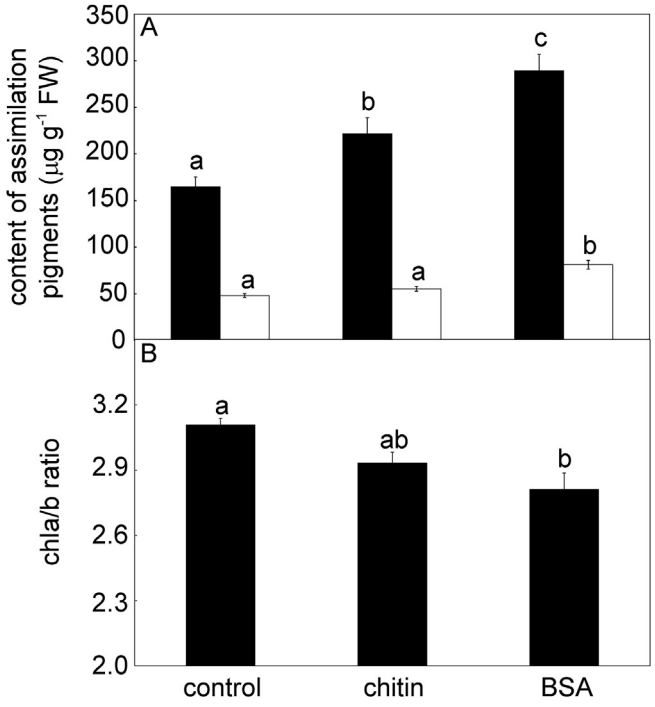

Fig. 3. Assimilation pigment contents (chlorophyll *a*+*b*, black bars, carotenoids, white bars, A) and chl*a*/*b* ratios (B) after feeding on chitin and BSA. Means ± s.e., n = 6–7. Different letters denote significant differences at P < 0.05 (ANOVA followed by Tukey test).

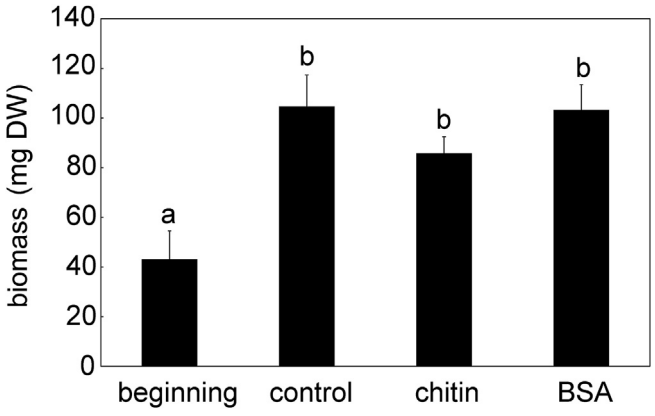

Fig. 4. Biomass of *D. capensis* at the beginning of the experiment, and 22 (16 + 6) weeks later in control and protein-vs. chitin-fed plants. Means ± s.e., n = 4–7. Different letters denote significant differences at P < 0.05 (ANOVA followed by Tukey test).

reason, the immunodetection of Rubisco protein was not successful here. However, we found that the plants fed on proteins accumulated a higher amount of antenna proteins of photosystem II (Fig. 5),

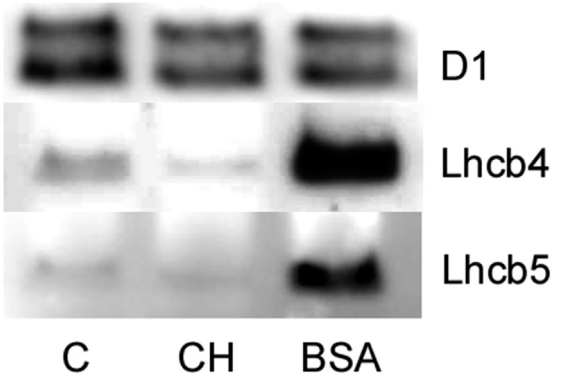

Fig. 5. Protein gel blot analysis of D1, Lhcb4 and Lhcb5 proteins in control plants (C) and after feeding the plants on chitin (CH) and BSA. The same amount of protein samples (10 μg) were electrophoresed and blotted.

which bound the surplus chlorophyll synthesized from BSA feeding (Fig. 3A). This is in accordance with the decreased chl *a*/*b* ratio (Fig. 3B), because chl *b* is bound only in light harvesting antennae (LHC) and not in the reaction center (Hobber and Argyroudi-Akoyunoglou, 2004). It should be emphasized that the plants were growing under shade conditions (100 μmol m^{-2} s^{-1} PAR) but at a high irradiance, the nitrogen could be incorporated into different photosynthetic proteins, e.g. to soluble proteins such that the balance between electron transport and Rubisco activity is maintained (Evans, 1989). It has been documented that under nitrogen-limiting conditions, the photosynthetic organisms are very deficient in chlorophyll *a*/*b* light harvesting complexes (Plumley and Schmidt, 1989). When the rate of chlorophyll *a* synthesis is low, reaction center proteins are the first to bind the limited amount of chl *a* available and the accumulation of light harvesting complexes is a function of the availability of chlorophyll *b* (Hobber and Argyroudi-Akoyunoglou, 2004).

In this study, it was shown for the first time that *D. capensis* absorbs nitrogen from chitin and even benefits from it. However, as the amount of chitin in comparison to proteins is lower in insect body, the chitin contains three times less N per unit weight, and as the efficiency of N utilization from chitin is much lower in comparison with soluble proteins, the chitin digestion has probably much lower ecological importance. The digestion of soluble proteins (such as BSA) is very effective, and nitrogen taken up from prey is incorporated into photosynthetic proteins. This may answer the long-established question about the mechanisms behind the increase in photosynthesis after carnivorous plants feed on prey.

Contributions

AP designed the study, collected samples, carried out Western blots, analysed data and wrote the manuscript, MK measured assimilation pigments and isolated proteins, and LA carried out elemental analyses and edited the manuscript.

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