Reliance on prey-derived nitrogen by the carnivorous plant Drosera rotundifolia decreases with increasing nitrogen deposition

This item was submitted to Loughborough University's Institutional Repository by the/an author.

Citation: MILLETT, J., ..., et al, 2012. Reliance on prey-derived nitrogen by the carnivorous plant Drosera rotundifolia decreases with increasing nitrogen deposition. New Phytologist, 195 (1), pp.182-188.

Additional Information:

- The definitive version is available at www.newphytologist.com.

Metadata Record: [https://dspace.lboro.ac.uk/2134/13427](https://dspace.lboro.ac.uk/2134/13427)

Version: Accepted for publication

Publisher: Wiley (© The Authors / © New Phytologist Trust)

Please cite the published version.
This item was submitted to Loughborough’s Institutional Repository (https://dspace.lboro.ac.uk/) by the author and is made available under the following Creative Commons Licence conditions.

For the full text of this licence, please go to:
http://creativecommons.org/licenses/by-nc-nd/2.5/
Reliance on prey derived nitrogen by the carnivorous plant *Drosera rotundifolia* decreases with increasing nitrogen deposition.

Millett J\(^1\)*, Svensson BM\(^2\), Newton J\(^3\) and Rydin H\(^2\)

\(^1\)Centre for Hydrological and Ecosystem Science, Department of Geography, Loughborough University, Loughborough, LE11 3TU, UK

\(^2\)Department of Plant Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18 D, SE-752 36, Uppsala, Sweden

\(^3\)Life Sciences Mass Spectrometry Facility, Scottish University Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, G75 0QF, UK

*corresponding author: Email: j.millett@lboro.ac.uk, phone: +44 (0)1509222739.

---

**Word count: 4175**

Introduction: 876; Materials & Methods: 1098; Results: 585; Discussion: 1580;

Acknowledgements: 35

**Number of tables: 2**

**Number of figures: 3**
Summary

- Carnivory in plants is presumed to be an adaptation to a low nutrient environment. Nitrogen (N) from carnivory is expected to become a less important component of their N budget as root N availability increases.

- We investigated the uptake of N via roots versus prey of the carnivorous plant *Drosera rotundifolia* growing in ombrotrophic bogs along a latitudinal nitrogen deposition gradient through Sweden, using a natural abundance stable isotope mass balance technique.

- *D. rotundifolia* plants receiving the lowest level of N deposition obtained a greater proportion of N from prey (57%) than plants on bogs with higher N deposition (22% at intermediate and 33% at the highest deposition). When adjusted for differences in plant mass this pattern was also present when considering total prey N uptake (66, 26 and 26 µg prey N plant\(^{-1}\) at the low, intermediate and high N deposition sites respectively). The pattern of mass adjusted root N uptake was opposite to this (47, 75 and 86 µg N plant\(^{-1}\)).

- *D. rotundifolia* plants in this study switched from reliance on prey N to reliance on root derived N as a result of increasing N availability due to atmospheric N deposition.

**Key words:** stable isotope analysis; plant-animal interactions; carnivorous plants; nutrient use; pollution
Introduction

Ever since Darwin's insightful book (1878) plant biologists have been intrigued by carnivorous plants, how and why they attract, capture and digest animal prey and how they use the nutrients once incorporated into their own tissues (Juniper, 1989). Carnivory in plants has evolved independently on at least six occasions (Ellison & Gotelli, 2009) and is widespread, with over 600 carnivorous plant species occurring on every continent except Antarctica. The convergence on this single trait makes carnivorous plants useful model systems for a number of important evolutionary, ecological and ecophysiological questions (Ellison & Gotelli, 2001; Ellison et al., 2003).

Key to understanding the evolution and ecology of carnivorous plants is the interaction between the uptake of nutrients through their roots and the uptake of prey-derived nutrients (Ellison, 2006). The additional source of nitrogen (N) and phosphorous (P) gained by carnivorous plants from their prey is used for growth, photosynthesis and seed production (Darwin, 1878; Aldenius et al., 1983; Thum, 1988; Thorén & Karlsson, 1998; Adamec, 2002). However, carnivory also carries a cost in reduced efficiency of photosynthesis due to the modification of leaves into traps (Givnish et al., 1984; Knight, 1992; Mendez & Karlsson, 1999; Ellison & Farnsworth, 2005). In the cost-benefit model proposed by Givnish et al. (1984) carnivory should be beneficial if the nutrients captured from prey can be used to increase photosynthesis and balance these costs. Givnish et al. (1984) predicted that investment in carnivory should have a net marginal benefit in low nutrient, high light and wet sites. As nutrient availability increases and/or light and water availability decreases the level of investment in carnivory that yields the highest net benefit decreases until being non-carnivorous is the optimal strategy. This is thought to explain differences in trap complexity and resulting differences in prey nutrient acquisition. For example, the percentage of total plant N that is derived from prey (%N_{dfp}) varies considerably, from 10% for Sarracenia...
purpurea to 87% for Drosera pallida (Ellison & Gotelli, 2001). In addition, there can be considerable within species variation in %N_{dfp}. For example, Millett et al. (2003) found that %N_{dfp} for different populations of Drosera rotundifolia was 29-65%. However, no one has yet identified environmental drivers of within species differences in %N_{dfp}.

There is good evidence that prey capture is less beneficial to carnivorous plants when nutrient availability is increased. In a meta-analysis of 26 experimental ‘prey addition’ studies Ellison (2006) showed that prey capture results in increased ‘fitness’ only when root N availability is low. This effect is presumably because increased root nutrient availability means that prey N is a smaller proportion of total plant N and so changes in the amount of this N source have a smaller, or no impact. Additionally, a number of studies have shown that carnivorous species reduce their investment in carnivory when N availability increases. For example, when root N availability is increased S. purpurea pitcher morphology changes, becoming less pitcher-like and more leaf-like (Ellison & Gotelli, 2002); D. rotundifolia traps become less sticky (Thorén et al., 2003); and Utricularia spp. produce fewer traps (Knight, 1991). It seems likely that this phenotypic plasticity will result in a reduction in the amount of N obtained from prey, though this has never been measured. As such, no one has yet shown in-situ, and where there has been no manipulation, that when root N availability increases the amount of N gained from prey decreases and that prey N becomes a less important component of their N budget.

Gradients of anthropogenic N deposition provide a good opportunity to test fundamental questions on responses to N availability. Ombrotrophic bogs and the plants that grow on them are particularly sensitive to changes in atmospheric N deposition because by definition their entire N budget is derived from wet and dry deposition. As a result, plants growing on ombrotrophic bogs show clear responses to increasing levels of N deposition in terms of their N uptake and use (Heijmans et al., 2002; Bragazza et al., 2004). This ultimately results in species loss and community change (e.g., Gunnarsson et al., 2002; Bubier et al., 2007). The
carnivorous plant *D. rotundifolia* grows predominantly on ombrotrophic bogs. It is a relatively common plant with a widespread, circumboreal distribution. Furthermore, *D. rotundifolia* is rooted directly among the living *Sphagnum* shoots, with an intricate competitive balance: increased N availability may have negative direct effects on *Drosera* (Redbo-Torstensson, 1994), but also increase its ability to compete with *Sphagnum* (Svensson, 1995).

In this study we used natural abundance stable isotope ratio measurements (\(\delta^{15}N\)) to quantify the amount of N contained in individual *D. rotundifolia* plants that originated from prey sources and from root uptake. We made these measurements at three ombrotrophic bogs in Sweden, each of which received different amounts of background N deposition. We primarily aimed to test the following hypotheses: 1. that increasing N deposition (and therefore root availability) results in a: increased root N uptake, b: increased growth and therefore plant size and c: plants that are more N replete; 2. that increasing N deposition results in N from prey capture becoming a less important component of the total N budget of the plant and 3. that plants become increasingly N replete as the amount of prey N that incorporated into their tissues increases.
Materials and Methods

Study sites and sampling

Three ombrotrophic mires were identified in Sweden along a latitudinal N deposition gradient (Table 1). Sampling was made in undisturbed, completely open, ombrotrophic parts with a hollow hummock micro topography and typical bog vegetation. The central and northern sites were dominated by *Sphagnum fuscum* and *S. balticum* while the southern site was dominated by *S. fuscum*, *S. magellanicum* and *S. cuspidatum*. At each site ten *S. fuscum* hummocks were chosen as study plots. Sampling was restricted to *S. fuscum* hummocks to avoid differences in hydrology and in *Sphagnum* growth – the effect of N deposition on *Sphagnum* growth differs between hummock and lawn communities (Limpens et al., 2011). Sampling was carried out between 8th and 20th August 2010. At each plot the following samples were removed: 15 *Drosera rotundifolia* L. plants, a sample of *S. fuscum* capitula and a sample of potential prey. All previously captured insects were removed from each *D. rotundifolia* plant and the old growth was removed, leaving current years root, stem, leaves and flowers. These 15 plants were pooled to form a single sample per plot for analysis (n = 10 per site). The *S. fuscum* sample consisted of a total of approx. 10 cm² of capitula (the top 1 cm of the plant) cut from at least three separate places on the hummock. These were pooled to form a single sample. Any other *Sphagnum* species were carefully removed from the sample under a dissecting microscope. The sample of potential prey was collected by placing a 24 × 10 cm yellow sticky insect trap on each plot. This was left for a minimum of 24 hours, after which a sample of insects <2 mm in length (representing the typical prey size for *D. rotundifolia*) was carefully removed using fine tweezers.

Sample analysis
Schulze et al. (1991) pioneered the use of stable isotopes for measuring prey versus root N uptake by carnivorous plants. Using natural abundance measurements of stable isotopes allows estimation of the relative contribution of two distinct N sources to a single pool and can be used in any system where there are only two N sources (or less accurately where they comprise the vast majority of the total N) and where the $\delta^{15}$N value of the two sources is different. The $\delta^{15}$N value of the pool is then a result of the $\delta^{15}$N value of each source and the relative contribution of each source to the pool. This method is used extensively for animals in trophic ecology (Boecklen et al., 2011) and was initially used in plants to determine the relative contribution of N$_2$ from atmospheric fixation and N from root uptake to the N budget of N$_2$ fixing plants (Shearer & Kohl, 1986). Due to discrimination during metabolism, organisms at higher trophic levels tend to become relatively enriched in $^{15}$N, generally by 3-4‰ (Post, 2002), though the entire range of $\delta^{15}$N enrichment factors is far greater (for review see Vanderklift & Ponsard, 2003). As a result, the insect prey of carnivorous plants is expected to be enriched compared to N taken up through the roots. The $\delta^{15}$N of a D. rotundifolia plant is therefore defined by the relative contribution of these two N sources to the total N budget. The technique has been used in a number of studies to measure prey contribution to the total N content of: Drosera spp. (Schulze et al., 1991), D. rotundifolia (Millett et al., 2003, Millett et al., 2012) and Nepenthes spp. (Moran et al., 2001).

All samples were kept cold before being dried for 72 hours at 70 ºC; this was done within 5 days of sample collection. Drosera rotundifolia plants were weighed, and then all plant samples were milled to a fine powder with a ball mill (Retsch MM200). Insect samples were ground to a fine powder in a small pestle and mortar. The $\delta^{15}$N of all tissues was analysed using a Costech ECS 4010 elemental analyser linked to a Thermo Delta XP Plus isotope ratio mass spectrometer. Results are given using the $\delta$ notation expressed in units of per mil (‰).
where $\delta^{15}N = (R_{\text{sample}}/R_{\text{reference}}) - 1 \times 1000$, and $R = ^{15}N/^{14}N$. Data are reported with respect to the primary international reference AIR (i.e. = 0‰), and the measurements are calibrated using internal standards gelatin, alanine and glycine, which are calibrated against secondary international reference materials. %N and %C are determined from the mass spectrometric output using tryptophan as a standard.

The contribution of insect-derived N to the total N content of *D. rotundifolia* was calculated using a simple one-isotope, two-source, end-member mixing model as follows:

$$\%N_{\text{dfp}} = \left[ \left( \delta^{15}N_{\text{DROSERA}} - \delta^{15}N_{\text{SPHAGNUM}} \right) / \left( \delta^{15}N_{\text{INSECT}} - \delta^{15}N_{\text{SPHAGNUM}} \right) \right] \times 100$$

Where, $\%N_{\text{dfp}}$ is the percentage of N derived from insect prey, $\delta^{15}N_{\text{DROSERA}}$ is the $\delta^{15}N$ of the pooled sample of *D. rotundifolia* plants, $\delta^{15}N_{\text{SPHAGNUM}}$ is the $\delta^{15}N$ of the capitula of the *S. fuscum* in which the *D. rotundifolia* is growing, and $\delta^{15}N_{\text{INSECT}}$ is the $\delta^{15}N$ of the sample of the insects available as prey. $\delta^{15}N_{\text{DROSERA}}$ and $\delta^{15}N_{\text{SPHAGNUM}}$ were the values for each plot, while $\delta^{15}N_{\text{INSECT}}$ was the mean value for each site. The mean value was used because it seems likely that the insects form a single population on each site because they are mobile.

**Data analysis**

*D. rotundifolia* dry mass, %N and $\%N_{\text{dfp}}$ were used to calculate the following variables: total N per plant, total prey derived N per plant ($N_{\text{dfp}}$) and total root derived N per plant ($N_{\text{dfr}}$). To test the hypotheses relating to ecophysiological changes in response to N deposition (hypothesis 1 and 2) we used one-way ANOVA and ANCOVA in PASW Statistics (IBM) to test for differences between the three sites. Differences in $N_{\text{dfp}}$ and $N_{\text{dfr}}$ between sites were first analysed with a one-way ANOVA. Due to a strong relationship between plant size and $N_{\text{dfp}}$ and $N_{\text{dfr}}$ we then analysed $N_{\text{dfp}}$ and $N_{\text{dfr}}$ using plant mass as a covariate. Thus, $N_{\text{dfp}}$ and $N_{\text{dfr}}$ are compared between sites at a common mass (the mean of the independent variable).
We present values on an unadjusted basis and adjusted for differences in mass (mass adjusted) where appropriate. Where the data did not confirm to the assumptions of homoscedasticity they were log_{10} transformed. We used linear regression to test our third hypothesis by testing the relationship between the average amount of prey N (N_{dfp} and \%N_{dfp}) in plants on each hummock and average tissue N concentration (\%N). In order to account for differences in plant mass in this analysis we used a ‘size standardised’ measure of prey and root derived N content, RN_{dfp} and RN_{drf} (RN_{dfp}=N_{dfp}/plant mass; RN_{drf}=N_{drf}/plant mass).

**Results**

The concentration of N in *S. fuscum* capitula increased from north to south, i.e., with increasing N deposition (Table 2), being significantly lower at the low and mid deposition sites than in high deposition site. There were large statistically significant differences in the size of *D. rotundifolia* plants between sites (Table 2). Dry mass of plants growing at the south site was nearly three times that of those at the north site, the central site was intermediate. In addition, the total amount of N and the total amount of root derived N (N_{drf}) contained in each plant followed this pattern with an approximately threefold increase in N content from north to south (Table 2). However, while there were statistically significant differences between sites, the total amount of prey derived N (N_{dfp}) did not follow this pattern (Table 2). Plants growing at the south site contained the most prey derived N and those at the central site the least. There was also no clear pattern in the \%N concentration of *D. rotundifolia* plants along the latitudinal gradient. Plants growing at the central site had lower tissue \%N concentrations than those at the north and south sites.
S. fuscum had negative $\delta^{15}$N values (range across sites -4.10‰ to -3.70‰); prey had positive values (1.37‰ to 4.97‰). As a result, there were large and significant ($P<0.001$) differences in $\delta^{15}$N between S. fuscum and the sample of potential insect prey. This difference was 5.07‰ at the North site, 9.07‰ at the Central site and 7.86‰ at the South site. When the $\delta^{15}$N of S. fuscum, insect prey and D. rotundifolia were used to estimate the amounts of prey and root derived N in D. rotundifolia there were clear and statistically significant differences in the source of N for the plants along the sampled gradient. When adjusted for differences in plant mass, N$_{dfp}$ was considerably higher in plants from the low deposition site than from the other sites (Fig. 1a; $P<0.001$). They had also incorporated less root derived N into their tissue, N$_{dfr}$ (Fig. 1b; $P<0.001$) and, as a result of these patterns of prey and root N uptake D. rotundifolia growing at low N deposition obtained on average over half of their N from prey, compared to about 20-30% at the other sites (Fig 1c, $P<0.001$).

When considering differences between plots (both within and between sites), there are some clear relationships between measured variables. Larger D. rotundifolia plants contained more N than smaller plants. This pattern was consistent for root derived, prey derived and total N (Fig. 2, $P<0.001$). When adjusted for these differences in mass, D. rotundifolia plants with higher prey N content (RN$_{dfp}$) tended to have higher tissue %N content (Fig. 3a, $r^2 = 0.19$, $P=0.006$). This relationship was similar but weaker when %N$_{dfp}$ rather than the amount of prey N is considered (Fig. 3b $r^2=0.149$, $P=0.035$). However, when adjusted for differences in plant mass, there were no significant relationships between root N content (RN$_{dfr}$) and %N. ($r^2=0.001$, $P=0.855$). There were weak but significant correlations between the %N of S. fuscum capitula on a hummock and the nutrition of the D. rotundifolia growing on the hummock. D. rotundifolia plants growing on hummocks where S. fuscum contained relatively high tissue %N tended to be larger ($r^2=0.15$, $P=0.033$), had higher tissue %N concentrations ($r^2=0.15$, $P=0.033$), contained more N in their tissues in total ($r^2=0.18$, $P=0.020$) and
contained more root derived N (RN_{dfi}: r^2=0.18, P=0.020). However, there was no relationship
with mass adjusted RN_{dfp} (r^2=0.036, P=0.313) or %N_{dfp} (r^2=0.204, P=0.197).
Discussion

In this first in-situ study without manipulations of root N availability or prey capture rates we show that when N deposition (and therefore availability) increases D. rotundifolia plants incorporate less prey N and more root derived N into their tissues, and therefore obtain a lower percentage of their N from prey. This overall result supports the cost-benefit model proposed by Givnish et al. (1984) in an ecological context.

A somewhat surprising result was that the shift from predominantly prey to root reliance occurred at very low N deposition (<0.4 g N m\(^{-2}\) year\(^{-1}\)). This can be related to the literature on critical loads of N on other peatland processes. A central ecosystem service of peatlands is carbon balance, and a detailed literature review (Limpens et al., 2010) indicated that the key process, Sphagnum growth, starts to decline at a tissue concentration around 10 mg g\(^{-1}\), and this is reached at N depositions (natural or experimental) around 1 g N m\(^{-2}\) year\(^{-1}\). For Sphagnum, the natural low N levels in bogs makes growth N limited (Aerts et al., 1992), a limitation that D. rotundifolia may escape by carnivory (Ellison, 2006).

Prey N uptake was presumably lower when N deposition was higher due to reduced investment in prey capture. Previous studies have shown that carnivorous plants reduce investment in trapping prey when N availability to the roots is increased (Thorén et al., 2003, Ellison & Gotelli, 2007). There is a presumption that this reduces prey capture and the uptake of prey derived nutrient. We provide good evidence that these changes might result in reduced uptake of prey N, at least for D. rotundifolia. The plants at the site receiving the highest level of N deposition took up considerably less prey N than those at the lower N deposition sites, after prey N uptake was adjusted for differences in plant mass. One mechanism might be a reduction in trap stickiness. This response has been shown in a glasshouse study (Thorén et al., 2003) and would presumably reduce prey capture by
allowing more prey to escape once trapped. However, this may also be due to other mechanisms. For example, the red colour of *D. rotundifolia* leaves is thought to be a prey attraction mechanism, and could conceivably respond to N availability. Root N uptake was presumably higher due to increased pore water N concentration resulting in increased uptake, such as was shown by Heijmans (2002).

Our results support the key assumption that when N availability increases, the N nutrition of carnivorous plants shifts away from reliance on prey derived N towards reliance on root derived N. These results also support the suggestion by Thorén *et al.* (2003) that carnivorous plants might switch from investment in nutrient uptake through prey capture, to nutrient uptake through their roots when N availability increases. This impact of increased N availability explains the observed lack of response to prey addition when root N availability is high in previous studies (reviewed by Ellison, 2006), because additional N inputs from prey seem likely to have little benefit if the plants N requirements are met by root uptake.

We also provide some evidence of the nutritional benefit of prey capture. Plants that gained more N from prey (either mass adjusted N$_{dfp}$ or %N$_{dfp}$) had an enhanced nutritional status (higher tissue %N). The relationship with %N$_{dfp}$ was shown by Millett *et al.* (2003), but the relationship with N$_{dfp}$ has not been demonstrated previously. This pattern was absent for root derived N. Conversely, growth (i.e. mass) was closely tied to total plant N content. It is not possible to determine causality but previous studies have shown variable impacts of prey addition and root N addition on the growth and N content of carnivorous plants (Ellison 2006). Prey N uptake and root N uptake are also linked in some carnivorous plant species (Adamec, 2002). We also show that larger plants contain more N$_{dfp}$. Thus, Schulze & Schulze’s (1990) finding that increased leaf area (and presumably plant mass) resulted in higher rates of prey capture in *D. rotundifolia*, translates into an increase in prey N uptake for the plants. We did not measure phosphorous (P) or potassium (K) content or uptake in the
However, these nutrients have both been shown to be important components of carnivorous plant responses to changes in root nutrient availability and prey availability because carnivorous plant growth appear to often be co-limited by these nutrients (Ellison 2006).

Tissue %N content in the *D. rotundifolia* remained remarkably stable at all sites, despite large differences in N deposition and presumably availability. This might indicate that this parameter is well regulated by the plants. This regulation might be caused by the ‘dilution effect’ of increased growth rates and by the reduction in prey N uptake when root N uptake increase. However, this regulation might also be explained by changes in leaf turnover, which can be rapid in *D. rotundifolia* (Schulze & Schulze, 1990). If higher N availability increases the rate of leaf turnover, then N losses from the plant will increase. Furthermore, Butler & Ellison (2007) showed that *S. purpurea* rely to a large extent on stored N for use in subsequent years, but no studies have compared the use of prey derived N with that of root derived N. Our results provide a tentative suggestion that root and prey derived N might be used differently. With both N sources used for growth, but prey N being also allocated to storage processes/mechanisms (resulting in increased %N).

The natural abundance stable isotope method has been used in a number of studies to estimate the contribution of prey N to the N budget of carnivorous plant (see Brearley, 2011 for a summary). However, the method makes a number of assumptions (detailed by Boddey, 2000 for N\textsubscript{2} fixation and outlined by Millett *et al*., 2012 for carnivorous plants). For example, it is assumed that root derived N discrimination between $^{15}$N and $^{14}$N is the same for *S. fuscum* and *D. rotundifolia*. This seems a reasonable assumption. Millett *et al*. (2003) found that the $\delta^{15}$N of *Sphagnum* was almost identical to the lowest value of $\delta^{15}$N for individual *D. rotundifolia* plants. Glandless mutants of *D. erythrorhiza* were used by Schulze et al. (1991) and these would be an ‘ideal’ reference plant, but no such mutants are known for *D.*
rotundifolia. In addition, it is assumed that there is no discrimination between \( ^{15}\text{N} \) and \( ^{14}\text{N} \) during the uptake of prey N. Any derivation from these assumptions will affect the precision of the point estimate for \( \%\text{N}_{\text{dfp}} \). Boecklen et al. (2011) found that the 95\% confidence interval of the point estimate for the percentage contribution of the two end-points spanned on average 33\%. This uncertainty is due to the variability in the \( \delta^{15}\text{N} \) of the two end-points. We believe that our estimates of \( \%\text{N}_{\text{dfp}} \) can therefore probably only provide good qualitative or semi-quantitative estimates. However, the differences in \( \text{N}_{\text{dfp}} \) between our lowest N deposition site and the higher N deposition sites are relatively large as well as being statistically significant. We therefore consider these patterns to be biologically significant.

Our three sites were located on a latitudinal gradient. The purpose of this was to enable the large differences in N deposition to be exploited. There are other differences between the sites that will also clearly affect the plants, but this deposition gradient has been successfully used in studies of nitrogen effects on other peatland processes (e.g. Breeuwer et al., 2009; Granath et al., 2009). That we confined sampling to the S. fuscum hummock microhabitat within truly ombrotrophic peatlands should minimize the latitudinal effects. There is significantly higher precipitation at the southern, highest N deposition site. Higher rainfall might reduce prey capture by washing prey from the traps, but the largest shift from prey reliance was between the central and northern sites with similar precipitation. Differences in plant size, due to increased N deposition are likely to be enhanced by the differences in temperature, and in particular growing season length. However, we cannot conceive a mechanism by which changes in these temperature variables might create the observed differences in prey N uptake. In fact increase temperature and growing season at the central and southern sites would presumably increase prey N uptake due to increased rates of digestion. Furthermore, the large differences in D. rotundifolia plant size and the strong
correlation between size and root and prey N content confirm our use of a mass based
calculations of the tissue content of these two N sources.

In conclusion, understanding the responses of ecosystems to anthropogenically elevated N
deposition is essential if we are to mitigate the impacts. Ombrotrophic bogs are particularly
sensitive to N deposition. We provide good evidence that a key process for the carnivorous
plant *D. rotundifolia* (i.e. prey N use) is significantly impacted by even low rates of N
deposition. We conclude that anthropogenic N deposition results in reduced reliance on
carnivory by the *D. rotundifolia* plants in this study. Carnivory might therefore be a Hobson’s
choice in an ecological sense as well as in an evolutionary sense (as suggested by Ellison,
2006). As such, even for carnivorous plants, high reliance on carnivory might be the strategy
of ‘last resort’ when N availability is very low. Nonetheless, the residual costs of carnivory
likely remain resulting in reduced competitive ability in these higher N deposition sites.

Manipulative experiments are necessary to elucidate the main interactions between foliar and
root nutrient uptake in carnivorous plants, and the investment in carnivory.

**Acknowledgements**

Stable isotope analyses were undertaken at the Life Sciences Mass Spectrometry Facility,
funded by the UK Natural Environment Research Council (EK113-08/07). Many thanks to
two anonymous reviewers for their helpful comments on the manuscript.

**References**

Adamec L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root

Adamec L, Kovářová M. 2006. Field growth characteristics of two aquatic carnivorous


Table 1

Locations, precipitation, temperature, growing season length and N deposition of the three sites used in the study.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Mean annual precip. (mm year$^{-1}$)</th>
<th>Mean temp. Jan./July (°C)</th>
<th>Mean growing season length (days)$^a$</th>
<th>Growing season average temp. (°C)$^b$</th>
<th>N deposition (g N m$^{-2}$ year$^{-1}$)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lappmyran</td>
<td>North (N): 64°10′ N, 19°35′ E</td>
<td>652</td>
<td>-7.5/15.7</td>
<td>152</td>
<td>11.6</td>
<td>0.194</td>
</tr>
<tr>
<td>Åkerlänna</td>
<td>Central (C): 60°01′ N, 17°22′ E</td>
<td>563</td>
<td>-3.4/16.4</td>
<td>206</td>
<td>12.5</td>
<td>0.381</td>
</tr>
<tr>
<td>Römosse</td>
<td>South (S): 56°51′ N, 13°28′ E</td>
<td>1199</td>
<td>-0.6/16.8</td>
<td>224</td>
<td>12.2</td>
<td>1.130</td>
</tr>
</tbody>
</table>

$^a$ Based on data from IVL Svenska Miljöinstitutet (http://luftweb.smhi.se visited 15th October 2011), mean values for 2006-2009 inc.

$^b$ Growing season = number of days with mean temperature ≥5°C

$^c$ Modelled N deposition data from EMEP model (http://www.emep.int visited: 30th September 2011). Data are mean values for 2004-2009 inc.
Table 2

Measured and derived variables for *Drosera rotundifolia*, *Sphagnum fuscum* and a sample of *D. rotundifolia* prey at three ombrotrophic bogs in Sweden.

<table>
<thead>
<tr>
<th></th>
<th>Dros. Mass (mg)*</th>
<th>Dros. N (μg plant⁻¹)*</th>
<th>Dros. Nfr (μg plant⁻¹)*</th>
<th>Dros. Ndp (μg plant⁻¹)*</th>
<th>Sph. δ¹⁵N</th>
<th>Prey δ¹⁵N</th>
<th>Dros. %N</th>
<th>Dros. %N</th>
<th>Sph. %N</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Mean 5.83abc</td>
<td>60abc</td>
<td>26abc</td>
<td>34ab</td>
<td>-3.70</td>
<td>1.37a</td>
<td>0.83a</td>
<td>1.04a</td>
<td>0.66a</td>
</tr>
<tr>
<td></td>
<td>SE 0.35</td>
<td>4.0</td>
<td>3.1</td>
<td>3.1</td>
<td>0.23</td>
<td>0.71</td>
<td>0.23</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Central</td>
<td>Mean 10.0b</td>
<td>90b</td>
<td>71b</td>
<td>20b</td>
<td>-4.10</td>
<td>4.97b</td>
<td>2.14b</td>
<td>0.91b</td>
<td>0.65b</td>
</tr>
<tr>
<td></td>
<td>SE 0.48</td>
<td>4.0</td>
<td>3.6</td>
<td>3.0</td>
<td>0.18</td>
<td>0.81</td>
<td>0.29</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>South</td>
<td>Mean 17.2c</td>
<td>170b</td>
<td>111c</td>
<td>64b</td>
<td>-3.87</td>
<td>2.99b</td>
<td>-1.61b</td>
<td>1.03b</td>
<td>0.91b</td>
</tr>
<tr>
<td></td>
<td>SE 2.5</td>
<td>25</td>
<td>10.9</td>
<td>16.6</td>
<td>0.18</td>
<td>1.06</td>
<td>0.24</td>
<td>0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

ANOVA results: *P* <0.001 <0.001 <0.001 0.002 0.331 0.018 0.002 0.001 <0.001

*Sph. S. fuscum, Dros. D. rotundifolia, Ndp total N from prey (unadjusted for plant size), Nfr total N from root uptake (unadjusted for plant size).

† Comparing differences between sites. Within a variable sites with different letters are significantly different from each other (Fisher’s LSD, *P*<0.05).

*Log₁₀ transformed before analysis
Figure captions

Figure 1

Nitrogen nutrition of *Drosera rotundifolia* plants growing on three ombrotrophic bogs along a latitudinal N deposition gradient in Sweden. Presented are the mean ± SE for: a. prey derived N (N\textsubscript{dfp}), b. root derived N (N\textsubscript{dfr}), both at a common plant mass of 11 mg and corrected for the covariate relationship with mass; and c. the percentage contribution of prey derived N to total plant N content (%N\textsubscript{dfp}). Bars with different letters are significantly different from each other (Fisher’s LSD, *P*<0.05).

Figure 2

Relationship between *Drosera rotundifolia* dry mass and total N content (total N, prey N - N\textsubscript{dfp} and root N - N\textsubscript{dfr}) of plants growing on *Sphagnum fuscum* hummocks at three ombrotrophic bogs along a latitudinal N deposition gradient in Sweden. Each point represents a different hummock (*n* = 15 plants per hummock). Open symbols = prey derived N, grey symbols = root derived N and closed symbols = total N content (root + prey derived). Triangles = North site, squares = central site, circles = south site. Solid lines are the fitted regression lines, dotted lines are 95% CI for the fitted line. Equations for the fitted lines are: N\textsubscript{dfp}, \(y = 0.006x + 0.005\) (\(r^2 = 0.84\)); N\textsubscript{dfr}, \(y = 0.004x - 0.009\) (\(r^2 = 0.67\)); total N, \(y = 0.010x - 0.004\) (\(r^2 = 0.98\)).

Figure 3

The relationship between %N concentration of *Drosera rotundifolia* and a. the amount of N derived from prey (RN\textsubscript{dfp}) and b. %N\textsubscript{dfp} for *D. rotundifolia* in Sweden. Each point is the value for a pooled sample of plants growing on a single *Sphagnum fuscum* hummock (*n*=15 plants per hummock) at three different sites (triangles= north, low N deposition; squares = central,
intermediate N deposition and circles = south, high N deposition). Solid line is the fitted regression line; dashed lines are the 95% CI for the fitted line. A: $y = 22.49x + 0.91$, $r^2 = 0.24$; B: $y = 0.193x + 0.92$, $r^2 = 0.15$. 
Figure 1

[Bar graphs showing adjusted N_{dfb} and %N_{dfb} for North, Central, and South regions with letters indicating statistical differences.]
Figure 3

(a) Graph showing the relationship between \( N_{\text{content}} \) and \( RN_{\text{dfp}} \) (mg N g\(^{-1}\)).

(b) Graph showing the relationship between \( %N_{\text{dfp}} \) and %N_{\text{dfp}}.

- N content (%)
- \( RN_{\text{dfp}} \) (mg N g\(^{-1}\))
- %N_{\text{dfp}}