Nitrogen deposition and prey nitrogen uptake control the nutrition of the carnivorous plant *Drosera rotundifolia*

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1. Abstract

Nitrogen (N) deposition has important negative impacts on natural and semi-natural ecosystems, impacting on biotic interactions across trophic levels. Low-nutrient systems are particularly sensitive to changes in N inputs and are therefore more vulnerable to N deposition. Carnivorous plants are often part of these ecosystems partly because of the additional nutrients obtained from prey. We studied the impact of N deposition on the nutrition of the carnivorous plant *Drosera rotundifolia* growing on 16 ombrotrophic bogs across Europe. We measured tissue N, phosphorus (P) and potassium (K) concentrations and prey and root N uptake using a natural abundance stable isotope approach. Our aim was to test the impact of N deposition on *D. rotundifolia* prey and root N uptake, and nutrient stoichiometry. *Drosera rotundifolia* root N uptake was strongly affected by N deposition, possibly resulting in reduced N limitation. The contribution of prey N to the N contained in *D. rotundifolia* ranged from 20 to 60%. N deposition reduced the maximum amount of N derived from prey, but this varied below this maximum. *Drosera rotundifolia* tissue N concentrations were a product of both root N availability and prey N uptake. Increased prey N uptake was correlated with increased tissue P concentrations indicating uptake of P from prey. N deposition therefore reduced the strength of a carnivorous plant-prey interaction, resulting in a reduction in nutrient transfer between trophic levels. We suggest that N deposition has a negative impact on *D. rotundifolia* and that responses to N deposition might be strongly site specific.

Key words: atmospheric nitrogen deposition; carnivorous plants; Drosera rotundifolia; plant-insect interactions; round-leaved sundew; stable isotopes

2. Introduction

Atmospheric nitrogen (N) deposition is a globally important pollutant (Galloway et al. 2008; Bobbink et al. 2010), which has significant local and regional impacts on ecosystems (Bobbink et al. 2010). N deposition increases N availability which can have direct impacts on individual plants (Tomassen et al. 2003; van Heerwaarden et al. 2003) and can also impact species interactions across trophic levels (Tylianakis et al. 2008). Understanding these impacts is crucial because they can result in altered species abundance, plant community composition (Payne et al. 2013) and reduced biodiversity (Stevens et al. 2004). Ombrotrophic (rain fed) bogs are nutrient poor and obtain their entire N budget from deposition, making them particularly sensitive to N deposition (Bobbink et al. 1998; Lovett et al. 2009). Carnivorous plants are typical of ombrotrophic bogs (Givnish et al. 1984) which makes them sensitive to changes in nutrient availability, for example through the effects of increased atmospheric N deposition (Ellison and Gotelli 2002).

Carnivorous plants supplement nutrients obtained through root uptake by capturing, digesting and assimilating the nutrients in animal prey, usually arthropods (Juniper et al. 1989). The uptake of prey nutrients uncouples growth and reproduction from root nutrient availability and enables carnivorous plants to successfully compete with non-carnivorous plants (Karlsson et al. 1996) when N availability is low. The value of the captured prey is dependent on root nutrient availability; responses to prey addition are normally reduced or absent in more nutrient replete plants (Ellison 2006). Carnivorous plants can also forage for prey, reducing investment in prey capture when the value of that prey is reduced (Ellison and Gotelli 2002; Thorén et al. 2003). It is therefore predicted that prey-derived nutrients become a less important component of their nutrition as root nutrient availability increases; but there is limited evidence to support this basic assumption. This is important because the ecological and evolutionary advantage of carnivory

should be related to the degree to which prey-derived nutrients replace root-derived nutrients. Furthermore, while carnivorous plant growth is generally limited by N availability (Ellison and Gotelli 2001), captured prey contains N, P, K, Ca while atmospheric deposition is mostly N.

Experimental approaches have provided significant insight into carnivorous plant nutrition and the effects of atmospheric nitrogen deposition (*e.g.* see Ellison 2006). There is, however, a need to investigate carnivorous plant nutrition *in situ* and with no experimental manipulation, to avoid experimental artefacts. Many studies add prey to traps and measure plant responses (see Ellison, 2006 for a review of these studies); however, that the plant was not catching that number of prey may itself be significant. Similarly root N additions, *e.g.*, Svensson (1995), are often unrealistic, being less frequent, more regular and at higher concentrations than atmospheric deposition *in situ*. To our knowledge only one study (Millett et al. 2012b) has provided any *in situ* evidence of decreased reliance on prey-derived nutrients when root nutrient availability is increased. The interpretation of this relatively small study is, however, limited due to the potential confounding of temperature and precipitation along the latitudinal N deposition gradient studied. In the present non-manipulative study we investigated *D. rotundifolia* growing *in situ* at 16 sites across Europe. We measured tissue N, P and K content and the relative contributions of root *vs* prey N using a natural abundance stable isotope ratio (δ^{15}N) method (Schulze et al. 1991). We predicted that:

1. *Drosera rotundifolia* N content would be a product of root N availability (*i.e.*, rates of N deposition) and prey N uptake and will be unaffected by temperature or precipitation;

2. prey N uptake and the contribution of prey N to the N content of *D. rotundifolia* would decrease with increasing N deposition across the entire range of N deposition; and

3. tissue concentrations of other nutrients would be unchanged by N uptake (root and prey), but nutrient stoichiometry would reflect decreasing N limitation due to increased (root and prey) N uptake.

3. Materials and methods

3.1 Study sites

Where present we collected *Drosera rotundifolia* plants ($n = 10$ per hummock), *Sphagnum* capitula (*i.e.*, c. 10 cm^2 of the top 0.5 cm of the plant) and potential arthropod prey from *Sphagnum* spp. hummocks ($n = 6 – 10$ hummocks per site, depending on bog area) at 13 ombrotrophic bogs across Europe (details of sampling in SEM1; site details in Fig. A1 and Table A1). These bogs covered a wide geographic range (latitude: 47.2° – 68.3° N, longitude: 5.7° W – 19.6° E) and a wide range of N deposition levels (0.05 – 2.70 g m^{-2} year^{-1}).

The δ^{15}N of all tissues and nitrogen (N) of *D. rotundifolia* tissues was analysed at all sites and phosphorus (P) and potassium (K) content of *D. rotundifolia* tissue were measured at 11 sites (excluding the UK sites) (for details of analytical procedure see SEM1).

For each site, modelled mean annual N deposition, summer temperature (MST) and precipitation (PP) data for the previous five years up to the sampling date were calculated (see SEM1 for details of data sets). A five-year mean was used to represent the likely time scale of impacts on *D. rotundifolia* which has an average life span of 6 years (Crowder et al. 1990), while balancing the likely larger influence of more recent abiotic conditions.

3.2 Data analysis

The relative contributions of root- and prey-derived N to the total N content of the plants were determined by utilising the discrimination of ^{15}N that occurs between trophic levels

(Peterson and Fry 1987; Post 2002). 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:

$$\%N_{dfp} = [(\delta^{15}N_{DROSERA} - \delta^{15}N_{SPHAGNUM}) / (\delta^{15}N_{INSECT} - \delta^{15}N_{SPHAGNUM})] \times 100 \ (1)$$

where $\%N_{dfp}$ is the percentage of N derived from insect prey, $\delta^{15}N_{DROSERA}$ is the $\delta^{15}N$ of the pooled sample of *D. rotundifolia* plants, $\delta^{15}N_{SPHAGNUM}$ is the $\delta^{15}N$ of the capitula of the *Sphagnum* sp. in which *D. rotundifolia* is growing, and $\delta^{15}N_{INSECT}$ is the $\delta^{15}N$ of the sampled insects available as prey.

The data from these sites were combined with the three sites in Millett et al. (2012b) who used an identical sampling protocol. *Drosera rotundifolia* dry mass, $\%N$ and $\%N_{dfp}$ were used to calculate the following variables: total N per plant, total prey-derived N per plant (N_{dfp}) and total root-derived N per plant (N_{dfr}). N_{dfp} and N_{dfr} were also calculated on a per unit of total plant dry mass basis.

The influence of N deposition, PP, MST and where appropriate N_{dfp}/mass on the measured plant characteristics were analysed using multiple linear regression in IBM SPSS Statistics 20.0 (IBM Corp. 2011). Where only one predictor was significant the 'curve fitting' function in IBM SPSS Statistics was used to determine the model with best fit for the appropriate bi-variate relationship (based on the model with the highest r^2). For $\%N_{dfp}$ and N_{dfp} quantile regression was used to estimate the regression slopes and intercepts of the 25th, 50th and 75th percentiles.

4. Results

Drosera rotundifolia plants were present at all sites except for Ringinglow Bog (UK5). MST and PP were not significant predictors for any of the measured or derived variables (See Table A2 for results of multiple linear regressions). There was a clear linear relationship between

the rate of atmospheric N deposition and *Sphagnum* tissue N concentrations and the total amount of N contained in *D. rotundifolia* plants (Fig. 1a, 1b; linear regression: *Sphagnum* N concentration – $F_{1,14} = 34.2$, $P < 0.001$, $r^2 = 0.071$; *D. rotundifolia* total N content - $F_{1,13} = 20.9$, $P = 0.001$, $r^2 = 0.63$). There was no impact of N deposition on *D. rotundifolia* mass (Table A2), but tissue N concentration increased with N deposition (Fig. 3) indicating that changes in total N content are due to increasing tissue N concentrations rather than increasing size. There were also clear relationships between modelled N deposition and the total amount of root-derived N in *D. rotundifolia* plants (Fig. 1c; regression: $F_{1,13} = 35.7$, $P < 0.001$, $r^2 = 0.73$, respectively).

At every site the mean δ^{15}N of *D. rotundifolia* tissue was between that of *Sphagnum* and potential prey. δ^{15}N of *D. rotundifolia* decreased in response to increasing N deposition (Fig. 2a; linear regression: $F_{1,13} = 9.9$, $P = 0.008$, $r^2 = 0.43$), but no significant relationship existed between N deposition and the δ^{15}N of *Sphagnum* or the collected potential prey items. There was a clear, statistically significant nonlinear relationship between N deposition and *D. rotundifolia* prey-N content. Specifically the regression line plotting the 75th percentile of the data was negatively related both to N deposition for N_{dfp} expressed as a proportion of total N content (Fig. 2b) and as a proportion of plant dry mass (Fig. 2c). The slope of the regression line was statistically significant for the former (Quantile regression: slope = –0.163, $P = 0.007$) and showed a clear negative trend for the latter (Quantile regression: slope = –2.22, $P = 0.057$). However, for the 50th and 25th percentiles there was no significant relationship between N deposition and prey-N content (Quantile regression: %N_{dfp}: 50th – slope = –0.150, $P = 0.087$; 25th – slope = –0.045, $P = 0.462$; N_{dfp}: 50th – slope = –1.77, $P = 0.320$; 25th – slope = 0.371, $P = 0.726$).

Drosera rotundifolia tissue N concentration was on average 1.09% (range: 0.62-1.42%) and was a product of both N deposition (*i.e.*, root availability) and prey-N uptake (Fig. 3), showing a positive relationship with both. *Drosera rotundifolia* tissue P concentration was on average 0.11% (range: 0.09-0.16%) and did not change significantly with N deposition (Table A2) but did increase as the prey-N content of *D. rotundifolia* increased (Fig. 4; linear regression: $F_{1,9} = 16.2$, $P = 0.003$, $r^2 = 0.64$). No such relationship existed for tissue K concentrations (Table A2), which were on average 0.57% (range: 0.48-0.71%). Increasing N deposition was significantly related to increasing *D. rotundifolia* tissue N:P ratios, which were on average 9.11 (range: 6.06-12.38; Table A2, Fig. 5; linear regression: $F_{1,9} = 14.9$, $P = 0.004$, $r^2 = 0.62$), but not N:K (average: 1.77; range: 1.16-2.40) and P:K ratios (average: 0.20; range: 0.16-0.29). However, tissue prey-N content did not impact on tissue nutrient stoichiometry.

5. Discussion

Laboratory studies and *in-situ* experimental studies of carnivorous plant nutrition have shown that prey N is less valuable for nutrient replete plants (Ellison 2006). We provide good evidence that prey N is a less important component of the nutrition of *D. rotundifolia* when root N availability increases: the contribution of prey N to the N content of *Drosera rotundifolia* decreases with increasing N deposition. $\delta^{15}N$ of *D. rotundifolia* tissue was strongly negatively related to the amount of modelled N deposition received by a bog. This is the trend that would be expected if they were incorporating less (relatively ^{15}N enriched) prey N into their tissues (Peterson and Fry 1987; Post 2002; Brearley 2011). This pattern was not seen with *Sphagnum* spp. or with a sample of potential prey from each site, suggesting that it is not due to differences in the $\delta^{15}N$ of precipitation or any other site specific factors. We combined the $\delta^{15}N$ of *D. rotundifolia*, *Sphagnum* and potential prey in a simple one-isotope, two-source, end-member

mixing model to quantify the contribution of prey- and root-derived N to the total N contained in *D. rotundifolia*. This approach has been used extensively for food-web studies (Schmidt et al. 2007), in plants for quantifying N_2 fixation (Shearer and Kohl 1986) and to quantify prey N uptake in carnivorous plants (Brearley 2011). The limitations of this approach have been discussed previously (Millett et al. 2012a, 2012b) with the general consensus being that this approach provides a good semi-quantitative estimate of the contribution of the two sources. It is also appreciated that the 'root' N uptake component might also comprise deposited N taken up through the leaves.

There were large differences between populations in the amount of N contained in the plants that was derived from prey (varying between 20.1 and 59.5%). This is consistent with values between different carnivorous plant species (19.6–87.1%; Ellison and Gotelli 2001). It is therefore clear that differences between sites are important for the role of prey nutrients for carnivorous plant nutrition. A large amount of this variability can be attributed to differences in the amount of N deposition received by the bogs. Our data, however, suggest that this is not a simple linear response. The maximum amount of N from prey is determined by N deposition, but the contribution can vary below that maximum, independently of N deposition. This suggests that root N availability (*i.e.*, N deposition) is a major control over prey-N uptake as has been previously suggested (Millett et al. 2003, 2012a, 2012b), but that other factors also have important impacts on prey-N uptake. These factors are particularly important when N deposition is low, becoming less important with increasing N deposition.

In the bogs in our study the scale of differences in the amounts of prey N in *D. rotundifolia* tissues is large. For sites receiving relatively low inputs of atmospheric N deposition, the concentration of prey-N in *D. rotundifolia* tissues varied four-fold. We found no evidence that

meteorological differences were responsible; precipitation and summer temperature had no impact on any measured variables. We can therefore only speculate on the reasons for differences between populations. These may, for example, be due to differences between sites in: prey availability (Zamora 1995); competition with spiders for prey (Jennings et al. 2010); or kleptoparasitism (Thum 1989). Future work should focus on determining which, if any of these, is the reason for these differences in prey-N uptake.

The physiological mechanisms for N controls over the differences in prey-N uptake were not measured. Increasing N deposition was strongly related to root-N uptake potentially 'diluting' the prey-N in the plant and so altering the contribution of each. The evidence, however, in this study also suggests that the plants were able to reduce prey-N uptake as the total concentration in plant tissues decreased with increasing N deposition (rather than only the amount of prey-N relative to the amount of root-N). Gaining nutrients from prey involves four distinct steps: prey attraction (though this is not necessarily essential), capture, digestion and nutrient uptake (Joel et al. 1985; Adlassnig et al. 2010). It might be changes in any or a number of these mechanisms that result in the observed differences in prey capture. There is no evidence as yet that *D. rotundifolia* actually attracts prey (Foot et al. 2014); but investment in prey capture (through the production of sticky mucilage) is reduced when root N availability is increased experimentally (Thorén et al. 2003), which might be the mechanism here. Such a reduction in investment in prey capture has not been demonstrated *in-situ* for *D. rotundifolia* but has been shown for *Sarracenia purpurea* by Ellison and Gotelli (2002). Changes in other parts of the process should, however, not be discounted; the production of digestive enzymes, for example, also carries a metabolic cost and so the plants might reduce enzyme production, thus reducing rates or efficiency of prey digestion.

We predicted that tissue concentrations of nutrients other than N would be unchanged by N uptake (root and prey), but nutrient stoichiometry would reflect decreasing N limitation due to increased (root and prey) N uptake. Our data confirm that plant tissue N concentrations were dependent on both root N and prey N uptake, but tissue P concentrations were also related to prey N uptake. This suggests that the *D. rotundifolia* plants were assimilating P from their prey as has been demonstrated previously (Karlsson and Carlsson 1984; Wakefield et al. 2005; Ellison 2006). As a result of this P uptake from prey, tissue nutrient stoichiometry was affected by atmospheric deposition but not by prey N uptake. What does this mean for plant nutrient limitation? Nutrient limitation can be inferred from tissue nutrient concentrations and nutrient stoichiometry. Our results suggest that *D. rotundifolia* in this study were in general N limited and not P limited (N<2%, P≥0.1%, N:P<14 as indicated by Koerselman and Meuleman 1996; Verhoeven et al. 1996; Aerts and Chapin 2000), despite having the 'option' to augment root-N uptake through prey capture. This supports the suggestion that the most important nutrient gained from prey is P (Chandler and Anderson 1976; Stewart and Nilsen 1993; Wakefield et al. 2005; Ellison 2006), and explains continued N limitation, despite prey N uptake. Both N deposition and prey N uptake reduced N limitation by increasing tissue N concentrations; only N deposition, however, altered the relative importance of N *vs* P limitation. This reflects to some extent the results of experimental studies (summarised by Ellison 2006) which show that inorganic N results in a shift from N limitation towards P limitation and prey addition results in a shift away from P limitation and towards N limitation. These results differ only slightly from those found *in-situ* by Méndez and Karlsson (2005) who found that the nutrient stoichiometry of the carnivorous plant *Pinguicula vulgaris* was affected by root N availability but not by prey

capture; with the lack of a prey capture effect confounded by their limited measure of prey capture.

K limitation is indicated by tissue K concentrations <0.8% and N:K ratio > 3.1 or P:K ratio >0.29 (Olde Venterink et al. 2002). Tissue K concentrations < 0.8% indicate K limitation in all *D. rotundifolia* populations, but N:K and P:K ratios were above those indicating K limitation (3.1 and 0.29 respectively), suggesting that the low tissue concentration might be a result of low tissue nutrient concentrations in carnivorous plants in general rather than a specific limitation of K. These differences in tissue nutrient concentrations and nutrient stoichiometry suggest that prey N uptake is not necessarily comparable to root N uptake because of the additional nutrients gained from prey. As a result, the reduction in prey nutrient uptake in response to atmospheric N deposition might result in nutrient imbalances in the plants.

Our results indicate that, with respect to the key process of prey N uptake, the response to N deposition may differ for plants growing at different bogs. Those which grow on low N deposition bogs and gain a high proportion of their N from prey, might be affected to a greater extent than those (also growing on low N deposition sites) for which prey N represents a relatively small proportion of their N nutrition. For these plants carnivory currently provides less apparent advantage (in terms of additional N), placing them at greater threat because they might lack the flexibility to respond to increased N deposition. So the impact on prey N uptake might be smaller, but the impact on the plant or the population might be larger than at sites where the plants on average obtain more N from prey. Conversely, those which currently gain a large amount of N from prey potentially have more flexibility to direct resources away from prey capture. Interestingly the bog which received the highest level of modelled N deposition (Ringinglow Bog) contained no *D. rotundifolia*. This is despite reported previous presence

(Conway 1947, 1949) an abundance of appropriate habitat and a long search. The species has a downward trend (BRC and BSBI 2014). Could it be that *D. rotundifolia* is unable to persist in these relatively high N conditions? Nitrogen fertilization has been shown to reduce *Sphagnum* growth (after an initial stimulus of growth; Gunnarsson and Rydin 2000). This may affect co-occurring low-growing vascular plant species negatively (such as the carnivorous *Drosera* species) as they rely on *Sphagnum* for support. However, along a Swedish gradient from nitrogen-poor to nitrogen-rich bog ecosystems (stretching from the north to the south-west (Wiedermann et al. 2009) *D. rotundifolia* seems to increase in areas with high nitrogen deposition (Flodin and Gunnarson 2008). Conversely, a negative impact of N on *D. rotundifolia* has been demonstrated; the population size was reduced within one year when the N deposition rate was artificially increased from 10 to 20 kg N ha^{-1} year^{-1} (Redbo-Torstensson 1994). Our suggestion is that as *Sphagnum* incremental growth decreases, incoming light would increase – at least temporarily – before larger, more competitive vascular plants increase in abundance. In this temporal window, resources gained by catching prey could be used by carnivorous plants for reproduction. This alternative is not possible for other low-statured vascular plants, which thus get outcompeted. We therefore hypothesize that the physiological changes shown in our study are indicators of a negative impact of N deposition on *D. rotundifolia* which ultimately results in local extinction, as has been demonstrated for other carnivorous plant species (Gotelli and Ellison 2002).

5.1 Conclusions

We conclude that the differences between populations in the nutrition of the *D. rotundifolia* plants in our study are driven primarily by N deposition, but that other factors are important at low N deposition levels. Global environmental change impacts on biotic

interactions, which might affect the stability of ecosystems (Tylianakis et al. 2008). Atmospheric nitrogen deposition is a key driver of global environmental change and has been shown to affect biotic interactions such as plant-pollinator, plant-fungal and plant-herbivore interactions (Tylianakis et al. 2008). We have shown that N deposition reduces the strength of a carnivorous plant-prey interaction, resulting in a reduction in nutrient transfer between trophic levels. The consequence of this on the carnivorous plant and the whole community is not clear, but that there is a significant change is in itself important. Further research is required to explore the impact of these pollution-driven changes on *D. rotundifolia* biochemistry, interactions with other plants and with the arthropod community (*i.e.*, prey) on the bogs. In particular, further understanding is required of how prey availability varies between bogs and how this variability in prey abundance affects prey capture and plant fitness. In addition, future research should focus on how N deposition affects interactions between carnivorous plants and other plant species and how P uptake from prey affects our interpretation of carnivorous plant ecology and evolution.

6 Acknowledgements

7. References

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Figure legends

Figure 1. Nitrogen (N) content of *Drosera rotundifolia* and *Sphagnum* growing on European

bogs. Presented are: (a) *Sphagnum* tissue N concentrations (b) total N and (c) total N from root

uptake (N_{dfr}). Each symbol is the mean for plants taken from hummocks ($n = 6 – 10$) on a single

bog. Error bars are 1 SEM, based on values for different hummocks. Open symbols are data from

(Millett *et al.* 2012b), filled symbols are data collected for the present study. The black line is the fitted linear regression model.

Figure 2. δ^{15}N and calculated prey nitrogen (N_{dfp}) uptake for *Drosera rotundifolia* growing on European bogs. Presented are: (a) *D. rotundifolia* (circles), prey (triangles) and *Sphagnum* (Squares) δ^{15}N at each bog ; (b) N_{dfp} as a percentage of total plant nitrogen for *D. rotundifolia* and (c) N_{dfp} as a per unit of *D. rotundifolia* plant dry mass. Each symbol is the mean for plants taken from hummocks ($n = 6 - 10$) on a single bog. Error bars are 1 SEM, based on the values for different hummocks, where error bars are not visible they are smaller than the symbol. Open symbols are data used in (Millett *et al.* 2012b), filled symbols are data collected for the present study. Labels identify study sites as detailed in Fig. A1 and Table A1. The black line in (a) is the fitted linear regression model for *D. rotundifolia* and in (b) and (c) the 0.5 quantile. Dashed lines in (b) and (c) are the 0.25 (short dashes) and 0.75 (long dashes) quantiles. NS=not statistically significant ($P>0.05$).

Figure 3. *Drosera rotundifolia* tissue nitrogen (N) concentrations for plants growing on European bogs. Presented are the mean values for plants from hummocks on each bog ($n = 6 - 10$), the modelled N deposition and amount of prey derived N (N_{dfp}) per unit of plant dry mass. The plane represents the fitted equation based on analysis using multiple linear regression. The equation of the fitted plane is: Equation of fitted plane:

$\%N = 0.532 + (0.222 \times _{N \text{ deposition}}) + (0.086 \times _{Ndfp})$

Figure 4. Relationship between the amount of prey derived N in the tissues of *Drosera rotundifolia* plants (N_{dfp}) per unit of plant dry mass and plant tissue P percent concentration for plants growing on European bogs. Each symbol is the mean for plants taken from hummocks ($n = 6 - 10$) on a single bog. Error bars are 1 SEM, based on the values for different hummocks,

where error bars are not visible they are smaller than the symbol. The black line is the fitted

linear regression model. Labels identify study sites as detailed in Fig. A1 and Table A11.

Figure 5. Tissue N:P of *Drosera rotundifolia* plants growing on different bogs in Europe. Each

symbol is the mean for plants taken from hummocks ($n = 6 – 10$) on a single bog. Error bars are

1 SEM, based on the values for different hummocks, where error bars are not visible they are

smaller than the symbol. The black line is the fitted linear regression model.
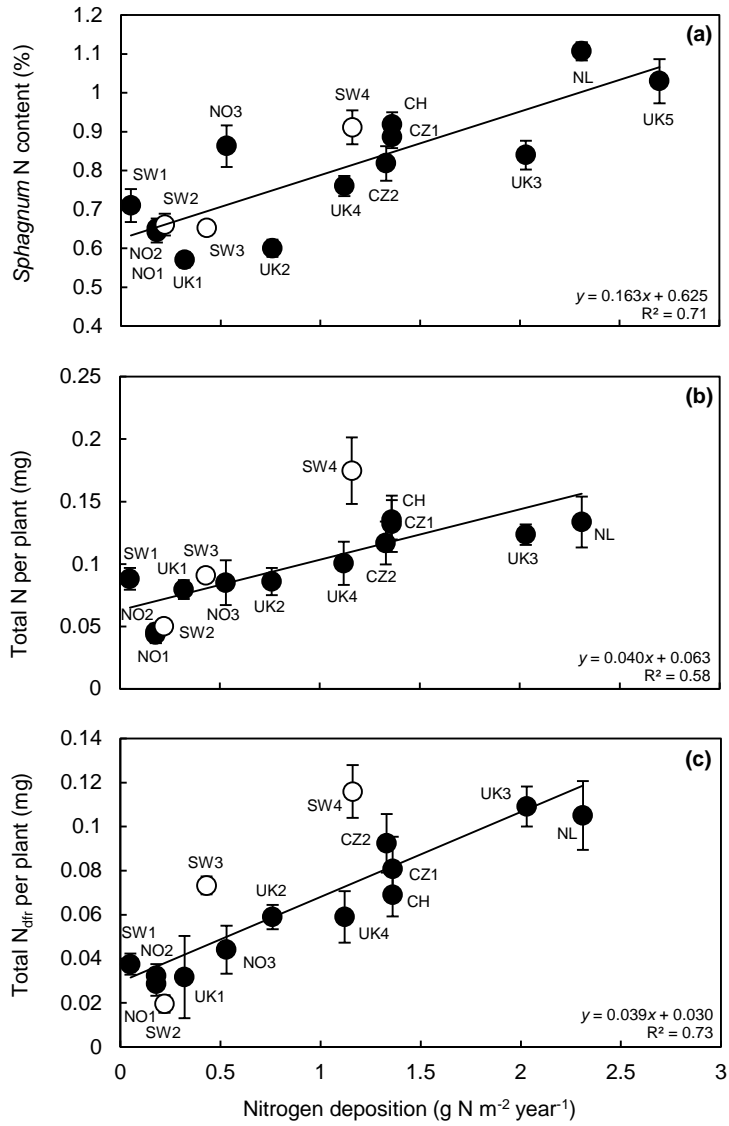
Figure 1

Figure 2

Figure 3

Figure 4

Plot: x-axis "N$_{dfp}$/mass (mg N g dry mass^{-1})" ranging 0 to 8; y-axis "*D. rotundifolia* tissue P content (mg P g dry mass^{-1})" ranging 0.8 to 1.6. Data points labeled SW1, CH, NL, SW2, NO2, NO1, SW3, CZ2, SW4, CZ1, NO3.

$$y = 0.085x + 0.7723$$
$$R^2 = 0.64$$

Figure 5

y = 1.29ln(x) + 10.00
R² = 0.62
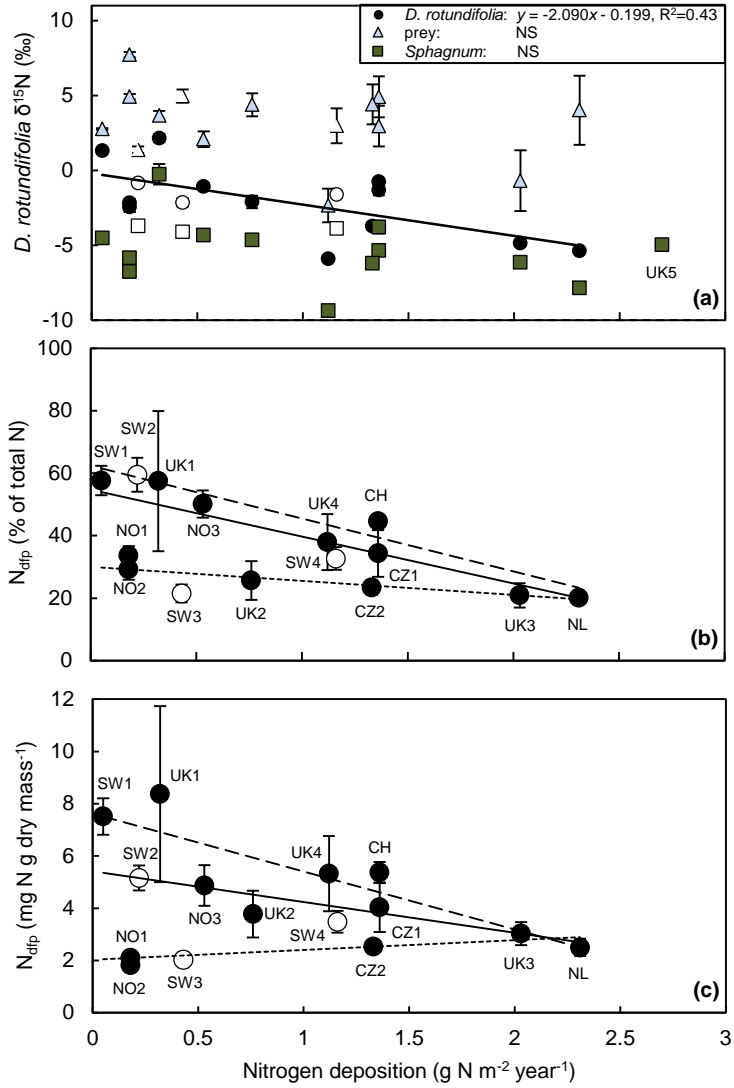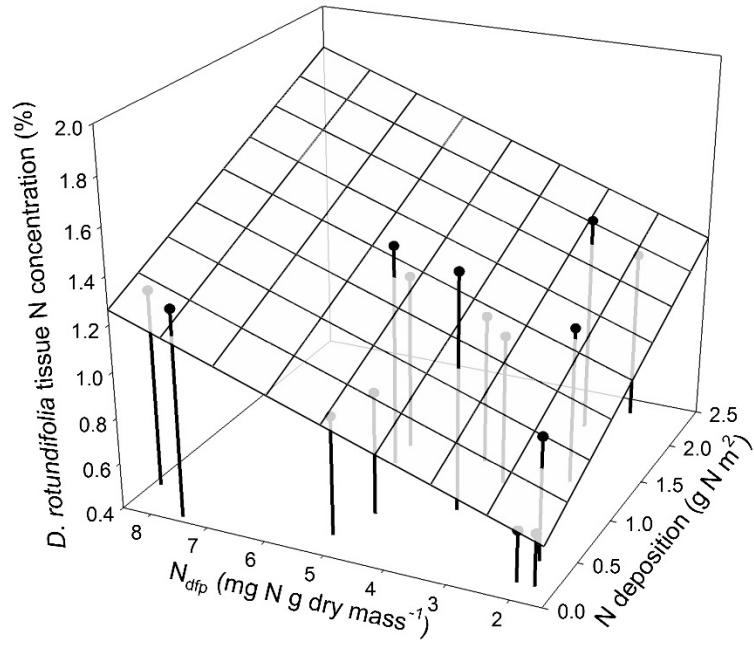
Appendices

Appendix 1: Details of sample collection procedure, plant tissue analysis, environmental variable data sets and approach to regression analysis.

Figure A1: Location of study bogs.

Table A1: Characteristics of study bogs.

Table A2: Results of multivariate linear regressions.

Appendices for: Millett J., Foot G.W., Svensson B.M. Nitrogen deposition and prey nitrogen uptake control the nutrition of the carnivorous plant *Drosera rotundifolia*.

Science of the Total Environment
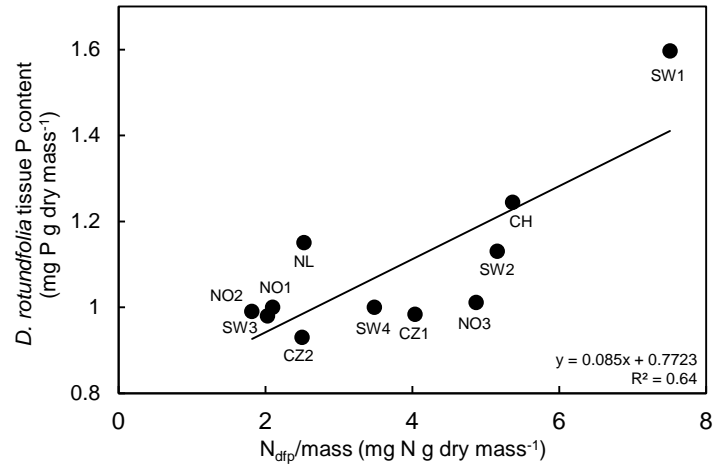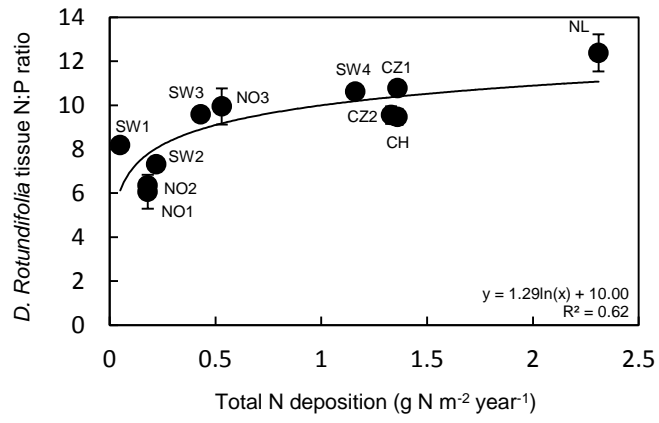
Appendix 1: Details of sample collection procedure, plant tissue analysis, environmental variable data sets and approach to regression analysis.

Sample collection

Where present we collected *Drosera rotundifolia* plants from *Sphagnum* spp. hummocks (n = 6 – 10 hummocks per site, depending on bog area) from 13 ombrotrophic bogs across Europe (Fig. S1, Table S1). We selected these bogs to provide a wide geographic range (latitude: 47.2° – 68.3° N, longitude: 5.7° W – 19.6° E) and a wide range of N deposition levels (0.05 – 2.70 g m^{-2} year^{-1}). Samples were collected in July and August over a two-year period because the amount of work involved meant that this was the only way to collect samples at similar times of the year.

Drosera rotundifolia plants (n = 10 per hummock) were carefully removed from each hummock and the previous years' growth was separated and discarded. A sample (three separate samples of c. 10 cm^2) of the capitula (*i.e.*, the top 0.5 cm of the plant) of the dominant *Sphagnum* species was collected with scissors. A sample of potential prey was collected by placing sticky yellow fly traps on the hummock for at least 24 hours and then carefully removing the captured potential prey (*i.e.*, <3 mm length) with tweezers. All samples were kept cold and transported back to a laboratory before being dried for 72 hours at 70 ºC; this was done within 5 days of 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 by hand to a fine powder in a small mortar.

Stable isotope analysis

either at UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.,

Cheshire, UK) or at OEA Labs, UK, using a CE Instruments EA 1110 elemental analyser coupled to a differentially pumped Europa Scientific 2020 stable isotope ratio mass spectrometer. Results are given using the δ notation expressed in units of per mil (‰) where $\delta^{15}N = [(R_{sample}/R_{reference}) - 1] \times 1000$, and $R = {}^{15}N/{}^{14}N$. Data are reported with respect to the primary international reference AIR (*i.e.*, = 0‰), and the measurements were calibrated at UC Davis using USGS-41 glutamic acid and internal standards: nylon, bovine liver, peach leaves and glutamic acid, and at OEA Labs using IAEA N1, IAEA N2 and an internal bovine liver standard. Precision for both laboratories was 0.2‰.

Analysis of tissue Phosphorous (P) and Potassium (K)

Plant tissue P and K were measured by AGVISE Laboratories (Northwood, ND, USA) using standard ICP spectrometry methods.

Environmental variables data sets

Annual atmospheric N deposition modelled using a higher resolution national model for the UK (Smith et al. 2000, NEGTAP 2001) and for non-UK sites using the lower resolution European Monitoring and Evaluation Program (EMEP)-based Integrated Deposition Model (IDEM) (Pieterse et al. 2007). Meteorological data (mean summer (June–August) temperature – MST and mean annual precipitation – PP) for the previous five years for each site (with a 0.25 degree latitude-longitude resolution) were calculated using an interpolated data set from E-OBS (Haylock et al. 2008) accessed through the KNMI climate explorer (KNMI 2013).

Regression analysis approach

The influence of the abiotic variables: N deposition, PP, MST and where appropriate N_{dfp}/mass on the measured plant characteristics was analysed using multiple linear regression

in IBM SPSS Statistics 20.0 (IBM Corp. 2011). The set of independent variables used in the model were different for different response variables. N deposition, PP and MST were used for: *D. rotundifolia* and *Sphagnum* ^{15}N data and root- or prey-derived N. N_{dfp}/mass was added to the model for *D. rotundifolia* mass, total N and %N concentration. For %P, %K and nutrient ratios a more limited model was used with only N deposition and prey-N content as the independent variables. This was to avoid over-parameterization due to the lower number of sites for these data (*i.e.*, $n = 11$).

Quantile regression

Initial data exploration using scatter plots for %N_{dfp} and N_{dfp} plotted against N deposition indicated substantial heterogeneity and a lack of normality in error distributions. Variance decreased systematically with increasing N deposition. These characteristics of the data indicated that quantile regression would be a more appropriate analysis (Cade and Noon 2003) as quantile regression is robust to heterogeneous variance. In addition, quantile regression enables the analysis of the upper or lower limits of a distribution rather than just the mean or median.

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Table A1. Characteristics of study bogs with modelled atmospheric N deposition (for the five years prior to sampling), mean summer temperature (MST) and mean annual precipitation (PP). The location of the bogs is shown in Fig. S1.

Site	Country	Latitude – longitude	N deposition (g m^{-2} year^{-1})*	MST[†] (° C)	PP (mm year^{-1})
Sites visited in 2010 (Millett *et al*. 2012a)					
SW2: Lappmyran	Sweden	64.16° N – 19.58° E	0.22	13.8	621
SW3: Åkerlänna Römosse	Sweden	60.02° N – 17.35° E	0.43	16.1	611
SW4: Saxnäs mosse	Sweden	68.33° N – 18.78° E	1.16	15.6	952
Sites visited in 2011					
CZ1: Cihadla	Czech Republic	50.68° N – 15.25° E	1.36	17.6	749
CZ2: Murtvy luh	Czech Republic	48.87° N – 13.88° E	1.33	14.9	963
NO1: Lysklettmyrin	Norway	63.32° N – 10.49° E	0.18	13.6	1066
NO2: Skjetnmyra	Norway	63.32° N – 9.96° E	0.18	13.7	1135
NO3: Bervamyr	Norway	58.52° N – 6.75° E	0.53	12.7	2270
CH: Etang de la Gruère	Switzerland	47.24° N – 7.04° E	1.36	16.1	1105
NL: 't Groote Veen	Netherlands	52.83° N – 6.45° E	2.31	16.6	876
Sites visited in 2012					
UK1: Inverasdale Peatlands	Scotland	57.46° N – 3.61° W	0.32	12.2	905
UK2: Moidach More	Scotland	57.85° N – 5.73° W	0.76	12.3	1486
UK3: Butterburn Flow	England	55.07° N – 2.51° W	2.03	12.9	1475
UK4: Bowness Common	England	54.92° N – 3.27° W	1.12	14.4	1168
UK5: Ringinglow Bog	England	53.35° N – 1.60° W	2.70	14.5	956
SW1: Abisko	Sweden	68.33° N –18.78° E	0.05	9.7	471

*N deposition levels were obtained from modelled data from EMEP (www.emep.int) and for the UK from APIS (www.apis.ac.uk). [†]Mean Summer Temperature (MST) and Mean Annual Precipitation (PP) from E-OBS interpolated meteorological dataset (Haylock et al. 2008) accessed through the KNMI Climate Explorer (http://climexp.knmi.nl/). Data are for the five year period prior to sampling.

Table A2. Results of multivariate linear regressions. Presented are the slope and *P* value of each variable and the R^2, F and

P values for the whole regression model. Different sets of independent variables were used for each dependent variable.

Significant ($P < 0.05$) are emboldened

| | Individual variables | | | | | | | | Model | | |
| | N dep. | | N_{dfp}/mass | | MST | | PP | | | | |
	Slope	*P*	Slope	*P*	Slope	*P*	Slope	*P*	R^2	F	*P*
Prey ^{15}N	-1.727	0.158	-	-	0.474	0.256	0.000	0.767	0.215	1.006	0.427
Sph. ^{15}N	-8.370	0.302	-	-	-0.038	0.910	0.000	0.877	0.127	0.583	0.638
Dros. ^{15}N	**-2.000**	**0.047**	-	-	-0.067	0.833	0.000	0.982	0.434	2.810	0.089
Dros. Mass	1.256	0.334	0.126	0.766	0.960	0.066	0.000	0.904	0.549	3.045	0.070
Dros. %Ndfp	-0.095	0.132	-	-	-0.013	0.530	0.000	0.660	0.376	2.209	0.144
Ndfp/mass	-0.131	0.888	-	-	-0.439	0.189	0.000	0.938	0.240	1.158	0.369
Ndfp/plant	0.003	0.754	-	-	0.000	0.910	0.000	0.858	0.024	0.091	0.963
Ndfr/mass	**3.480**	**0.001**	-	-	-0.536	0.057	-0.001	0.471	**0.682**	**7.849**	**0.004**
Ndfr/plant	**0.037**	**0.001**	-	-	0.001	0.722	0.000	0.518	**0.751**	**11.049**	**0.001**
Dros. total N/plant	**0.038**	**0.009**	0.006	0.172	0.005	0.325	0.000	0.677	**0.667**	**5.018**	**0.018**
Sph. %N	**0.144**	**0.001**	-	-	0.016	0.257	0.000	0.610	**0.741**	**11.466**	**0.001**
Dros. %N	**0.302**	**0.001**	**0.070**	**0.011**	-0.054	0.059	0.000	0.173	**0.753**	**7.627**	**0.004**
Dros. %P	-0.001	0.914	**0.008**	**0.023**					0.548	4.842	0.042
Dros. %K	0.062	0.078	0.020	0.135					0.228	2.481	0.145
Dros. N:P	**2.330**	**0.003**	0.144	0.524					**0.704**	**9.503**	**0.008**
Dros. N:K	0.200	0.315	0.079	0.308					0.173	0.838	0.467
Dros. P:K	-0.032	0.141	0.007	0.235					0.469	3.530	0.080

Sph.=*Sphagnum*, Dros.=*Drosera rotundifolia*, N dep.=Nitrogen deposition, N_{dfp}=*D. rotundifolia* N derived from prey, N_{dfr}=*D. rotundifolia* N derived from root, MST=Mean Summer Temperature, PP=Mean Annual Precipitation.

Figure A1. Location of study bogs. Site details are in Table 1.