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Response of *Sphagnum papillosum* and *Drosera rotundifolia* to reduced and oxidised wet N deposition.

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Abstract

We transplanted *Sphagnum* 'turfs' containing abundant *Drosera rotundifolia* into an existing nitrogen deposition experiment at Whim Moss near to Edinburgh. These mesocosms received simulated N deposition in the form of either NH_4^+ or NO_3^-, to give total N deposition rates of approximately 8, 16 or 32, or 64 kg N ha^{-1} $year^{-1}$. Simulated N deposition was added in a realistic way (i.e. with rainfall throughout the year). The $\delta^{15}N$ of this added N was elevated relative to background N. We measured the tissue chemistry and $\delta^{15}N$ of *S. papillosum* and *D. rotundifolia* over two years after transplant. Our aim was to determine uptake of the deposited N and the impact on *S. papillosum* tissue chemistry and *D. rotundifolia* tissue chemistry and ecology.

We found clear, significant impacts of N deposition on *S. papillosum*, with increased capitula N content and reduced C:N ratio. Increased $\delta^{15}N$ indicated uptake of deposited N. The response of *D. rotundifolia* was less clear with impacts only at the highest rate of N deposition. There was no evidence of differential uptake of reduced or oxidised wet N deposition by either *S. papillosum* or *D. rotundifolia*.

Using the natural abundance stale isotope method we estimated the minimum contribution of prey N to the total N in *D. rotundifolia* to be 35%.

The results suggest that differences in the uptake of reduced or oxidised wet N deposition might not be ecologically significant when wet N deposition is added realistically. They also support the suggestion that a model of N dynamics in *Sphagnum* dominated ecosystems that includes the role of *Sphagnum* as a small scale ecosystem engineer, is required to predict accurately vascular plant responses to N deposition.

Keywords

Nitrogen deposition; ombrotrophic bog; carnivorous plants; stable isotopes; nitrogen cycling; global environmental change.

Introduction

Plant and ecosystem productivity is often limited by nitrogen (N) availability (Vitousek and Howarth, 1991). As such, the deposition of anthropogenic N emissions can have important long-term impacts on biodiversity and ecosystem function (Galloway et al. 2008; Sala et al. 2000; Vitousek et al. 1997). The largest impacts are expected to occur in the most N limited ecosystems, because the ability of plants in these ecosystems to respond to increased N availability is often limited (Bobbink et al. 1998). Species that are better able to utilise the increased N availability will outcompete those that are less able to utilise the increased N, resulting in a loss of the less competitive species. Thus in order to understand plant community responses it is important that we understand how different competing species might respond to increased N deposition. Furthermore, these responses must be considered under near natural conditions because other biotic and biotic factors might conceivably interact with plant responses to N availability.

Plant communities on ombrotrophic bogs are particularly threatened by increases in atmospheric N deposition, because atmospheric deposition is the only external N source. As a result, changes in atmospheric N deposition have substantial impacts on total N inputs. In the short term the impacts of N deposition are detectable in plant tissue chemistry and stoichiometry (Skinner et al. 2006). In the longer term these impacts translate into species loss and community change (Bubier et al. 2007). Ombrotrophic bogs are dominated by *Sphagnum* spp., the capitula of which form a tightly interconnected lawn. This *Sphagnum* lawn plays an important role in ombrotrophic bog N dynamics and response to deposition. The *Sphagnum* capitula absorb atmospheric N inputs (Williams 1999). The resulting interception and retention by the *Sphagnum* lawn reduces availability to associated vascular plants (Breeman 1995; Svensson 1995). At higher rates of N deposition the *Sphagnum* is predicted to become N saturated and to no longer retain deposited N (Lamers et al. 2000), though N retention by *Sphagnum* capitula may be reduced even at low levels of N deposition (Bragazza et al. 2005). Nonetheless, the moderation of N availability by *Sphagnum* is predicted to be reduced at higher rates of N deposition (Heijmans et al. 2002). Therefore, it is important to determine individual species responses to N deposition when growing within this *Sphagnum* lawn.

Carnivorous plants capture and extract nutrients from animal prey (Ellison and Gotelli 2001). They are in general restricted to low nitrogen, high light environments due to costs (Carbon) and benefits (N) associated with the carnivorous habit (Ellison and Gotelli 2001; Givnish et al. 1984,). As such, carnivorous plants are common in ombrotrophic bogs. It is predicted that carnivory will become less important as nutrient availability increases, but that due to the cost of carnivory carnivorous plants will be out competed by associated non-carnivorous plants (Ellison and Gotelli 2002). However, there are few studies that have quantified the contribution of prey N to the N budget of carnivorous plants. Those that have, used the natural abundance stable isotope method (e.g. Schulze et al. 1991, 1997; Moran et al. 2001; Millett et al. 2003). This method allows the estimation of the contribution of prey N to the total N content of carnivorous plants *in-situ* and with no manipulation of prey or root N availability. The ^{15}N natural abundance in carnivorous plants is a result of the ^{15}N in root derived N and that in insect derived N. These two sources of N tend to be distinct in their $\delta^{15}N$ signature (i.e. the ratio of $^{14}N/^{15}N$ relative to that in air), due to ^{15}N enrichment at higher trophic levels. The natural abundance stable isotope method uses these differences in a simple 2 end-point mixing model to quantify insect and root N uptake. The $\delta^{15}N$ of *Sphagnum* is also a useful indicator of N availability and because it closely reflects available N in the bog and is therefore sensitive to the $\delta^{15}N$ of atmospheric inputs.

The carnivorous plant *Drosera rotundifolia* has a circumboreal distribution and normally, though not exclusively, grows within the *Sphagnum* lawn in ombrotrophic bogs. Therefore an intricate relationship exists between the responses of these two plants to N deposition. In this study we transplanted mesocosms consisting of *Sphagnum* 'turf' containing *Drosera rotundifolia* from a naturally occurring ombrotrophic bog, into an existing manipulative N deposition experiment set up on an ombrotrophic bog (Whim Moss) near Edinburgh, UK. The mesocosms were in place for two years with levels of N deposition (reduced or oxidised) manipulated under realistic conditions (i.e. added throughout the year and concurrently with each rainfall event) with additional N inputs ranging from 8 to 56 kg N Ha^{-1}. We measured

the response of the *Sphagnum* and the *D. rotundifolia* in terms of $\delta^{15}N$, N content and C:N ratio and aimed to address the following questions: 1. Do *S. papillosum* and *D. rotundifolia* differ in their uptake of deposited N? 2. Do differences in levels of deposited N result in differences in the $\delta^{15}N$ signature of the two species? 3. Is the form of deposited N (reduced or oxidised) important in determining plant responses to deposition? 4. Is there evidence of decreased reliance on prey derived N for *D. rotundifolia* when N deposition increases?

Materials and Methods

Whim Moss (UK Grid ref NT 203532; N55.77º, W-3.27º) is an ombrotrophic blanket bog (NVC M19, Rodwell 1991), which is 280 m a.s.l. and has a gently undulating surface. Annual rainfall is approximately 1000 mm and mean monthly temperatures range from 5ºC to 17ºC. Background N deposition at the site was measured between July 2002 and June 2003. Wet deposition was 5.8 kg N ha^{-1} $year^{-1}$, deposition of NH_3 was estimated to be 4.0 kg N ha^{-1} $year^{-1}$ and other forms of N (NH_4^+ particles, nitric and nitrous acid and NO_x) were estimated to be 1.2 kg N ha^{-1} $year^{-1}$. Therefore total background N deposition at the site is estimated to be 11 kg N ha^{-1} $year^{-1}$ but may vary by 1-2 kg N ha^{-1} $year^{-1}$. The site has received experimental additions of wet N deposition since July 2002. A full description of the experimental set up can be found in Sheppard et al. (2004) but a summary is provided here. Wet N deposition treatments were applied throughout the year in parallel to this background deposition and rainfall. This system provides uniquely realistic patterns of N deposition. Rainfall collected at the study site was mixed with NH_4Cl or $NaNO_3$ and sprayed onto 12.6 m^2 circular plots to achieve total N depositions of approximately 16, 32 and 64 kg N ha^{-1} $year^{-1}$ (based on estimated background N deposition of 8 kg N ha^{-1} $year^{-1}$ prior to the establishment of the experiment). The system was automated and N was only applied when rainfall occurred and when the wind speed was below 5 m s^{-1}. Each plot received the same volume of water (measured using individual water meters), equivalent to an additional 100 mm of rain per year. Additional control plots received only water additions at the same rate as the other plots. Each of these seven treatments was replicated 4 times in a complete replicate block design. N additions were made from a bulk chemical supply to ensure that the $\delta^{15}N$ of added N remained constant. The $\delta^{15}N$ of added NH_4Cl and $NaNO_3$ was -0.68‰ to -0.58‰ and +3.1‰ to +4.3‰ respectively (Skinner et al. 2006).

In September 2007 mesocosms (38 cm x 24 cm and 5 cm deep) consisting of *Sphagnum* 'turfs' dominated by *S. papillosum* with abundant *Drosera rotundifolia* were transplanted into each of the 28 experimental plots. The turfs were removed from a mire with typical NVC M15 vegetation, (Rodwell 1991) next to Glenbrittal Youth Hostel on the Isle of Skye (UK Grid REF: NG409225; N: 57.22º, W: -6.29º). This wet heath exhibits a hummock-hollow-pool topography and is dominated by *Molinia caerulea*, *Scirpus cespitosus*, *Erica tetralix* and *Calluna vulgaris*. *Drosera rotundifolia* was present mainly in the more open areas next to pools, and this is where turfs were collected. Each turf was placed into a hole of the same dimensions on the edge of each plot. All turfs were placed at the eastern side of the plot to remove the potential for differences due to spray drift. A sample of *D. rotundifolia* growing in each mesocosm was taken in May 2008 and in August of 2008 and 2009. This consisted of the shoots and roots (excluding the remains of previous year's growth) of 4 individual *D. rotundifolia* plants per plot. In addition, a sample of *S.*

papillosum capitula consisting of the capitula of at least 10 individual *S. papillosum* plants was taken in August 2008 and August 2009. At the same time that plant material was collected in August a sample of available insect prey was also collected. This is so that an assessment could be made of the ^{15}N natural abundance of the insect prey captured by *D. rotundifolia*. Insects were captured on sticky yellow insect traps left on each plot for 24 hours. Only insects <2 mm were used for this sample, reflecting the likely size of prey.

The plant samples were rinsed in de-ionised water and any contaminating debris was removed. All samples were then dried at 70ºC for 72 hours. *D. rotundifolia* plants were then weighed. All plant material was milled to a fine powder in a ball mill, insects were ground using a pestle and mortar. The $\delta^{15}N$ of all tissues was analysed using a Carlo-Erba elemental analyser linked to a Dennis Leigh Technologies IRMS. Results are given using the δ notation expressed in units of per mil (‰) where $\delta = (R_{sample}/R_{reference}) - 1 \times 1000$, and $R = {}^{15}N:{}^{14}N$. Data are reported with respect to N in air. %N and C:N ratios are determined from the output of this analysis.

The contribution of insect derived N to the total N content of *D. rotundifolia* was calculated in using a simple two end-point mixing model as follows (following Millett et al. 2003):

$$\%N_{dfp} = (\delta^{15}N_{DROSERA} - \delta^{15}N_{REF}) / (\delta^{15}N_{INSECT} - \delta^{15}N_{REF})$$

Where, $\%N_{dfp}$ is the proportion of N derived from insect prey, $\delta^{15}N_{DROSERA}$ is the $\delta^{15}N$ of the *D. rotundifolia* plants, $\delta^{15}N_{REF}$ is the $\delta^{15}N$ of either the capitula of the *Sphagnum* in which the *D. rotundifolia* is growing or the lowest $\delta^{15}N$ of individual *D. rotundifolia* plants in each treatment in 2008, and $\delta^{15}N_{INSECT}$ is the $\delta^{15}N$ of the sample of the insects available as prey. The use of the lowest value of $\delta^{15}N$ for *D. rotundifolia* enables the minimum $\%N_{dfp}$ to be estimated, assuming that the $\delta^{15}N$ of this plant is lowest because $\%N_{dfp}$ is lower than all other *D. rotundifolia* plants (but may not be zero). Two different values for $\delta^{15}N_{REF}$ were used to enable the reliability of using *S. papillosum* to be tested.

Data were analysed using GLM in SPSS (SPSS 2008). A repeated measures model was used for summer 2008 and summer 2009 plant data. Measures of *D. rotundifolia* characteristics in 2008 were analysed using a univariate GLM model. Differences in $\delta^{15}N$ between *D. rotundifolia*, *S. papillosum* and captured insects were tested using a repeated measures GLM.

Results

There was a clear and consistent increase in $\delta^{15}N$ and N content and a decrease in C:N of *S. papillosum* tissues as a result of increasing N deposition (Fig. 1, Table 1). These impacts were statistically significant at N addition rates of more than 16 kg N ha^{-1} $year^{-1}$. This pattern was consistent for both years, though between 2008 and 2009 there was an overall decrease in mean C:N (from 36.1±1.1 to 32.7±1.2) and $\delta^{15}N$ (from -1.78±0.18 to -1.07±0.17) with no significant change in N content. *Sphagnum papillosum* receiving dditional N deposition as NO_3 had slightly higher $\delta^{15}N$ than those receiving additional N deposition as NH_4, while those receiving just

ambient N deposition had lower $\delta^{15}N$ than both these treatments (Fig. 2). However, these differences were not statistically significant.

The response of *D. rotundifolia* to the N addition treatments was different to that of *S. papillosum*. $\delta^{15}N$ of *D. rotundifolia* tissues did not differ between N addition treatments. Furthermore, there was no clear trend in tissue percent N and C:N ratio for plants growing at lower N additions (<64 kg N ha^{-1} $year^{-1}$). However, at N additions of over 32 kg N ha^{-1} $year^{-1}$ there was a significant increase in tissue percent N content and decrease in C:N ratio. This pattern was also consistent in both years, though there was an increase in percent N content (from 1.54±0.05% to 1.78±0.06%) and a decrease in C:N (from 31.4±0.9 to 27.4±0.9) between 2008 and 2009. There was no significant difference between $\delta^{15}N$ of *D. rotundifolia* in 2008 and 2009. $\delta^{15}N$ of *D. rotundifolia* plants was significantly affected by the form of additional wet N deposition added to plots with those receiving NO_3 having a higher $\delta^{15}N$ than those receiving NH_4 (Fig. 2).

On average the dry mass of *D. rotundifolia* plants increased from 5.0±0.4 mg to 15.0±1.0 mg from spring 2008 to summer 2008. The percent content of N in the plant tissue decreased from 2.53±0.10% to 1.56±0.05% and N content increased from 0.13±0.01 to 0.23±0.01 mg N $plant^{-1}$. There was no change in mass between summer 2008 and summer 2009, but there was a relatively small but significant increase in tissue N percent content to 1.76±0.06% and as a result an increase in total plant N content to 0.26±0.02 mg N $plant^{-1}$. Between 2008 and 2009 there was an increase in rosette diameter (from 19.6±0.71 to 22.8±1.22 mm) and the number of leaves per plant (from 3.66±0.13 to 5.69±0.65). Plant mass and total plant N content did not differ between N addition treatments in either 2008 or 2009.

21.4±2.4% and 7.6±1.5% of all *D. rotundifolia* leaves contained trapped prey at the time of sampling in 2008 and 2009 respectively. On average 0.85±0.09 and 0.41±0.09 trapped insects were found on each plant in 2008 and 2009 respectively. Significantly more insects were present in traps and a larger proportion of leaves contained trapped prey in 2008 but there was no significant difference between treatments. The mean $\delta^{15}N$ of collected insects was 4.38±0.33‰. The $\delta^{15}N$ of trapped insects differed significantly in each year but not between treatments (Fig. 3). Considering all the $\delta^{15}N$ data together there were significant differences in the $\delta^{15}N$ of *D. rotundifolia*, *S. papillosum* and trapped insects (GLM: $F=50.135$, $P<0.001$; Fig. 3). The average minimum $\delta^{15}N$ of individual *D. rotundifolia* plants in 2008 was significantly lower than that of *S. papillosum* in 2008 and 2009 and the mean $\delta^{15}N$ of *D. rotundifolia* in 2008 and 2009 (Fig. 3). Insect $\delta^{15}N$ was higher than that of all *D. rotundifolia* and *S. papillosum* plants. When these differences in $\delta^{15}N$ were used to estimate N_{dfp}, the *D. rotundifolia* plants were estimated to have obtained either 10±3.2% and 17±18.9% (using $\delta^{15}N$ of *S. papillosum* as the reference value in 2008 and 2009 respectively) or a minimum of 29±2.2% and 42±5.9% (using the lowest value of $\delta^{15}N$ for *D. rotundifolia* as the reference value in 2008 and 2009 respectively).

Discussion

This is the first field-based experimental N deposition study where N is added in a realistic way (i.e. throughout the year and concurrently with rainfall events). As such

we can be confidant that plant responses represent that expected *in-situ*. There is clear evidence that *S. papillosum* took up and incorporated deposited N into its tissues. This resulted in increased $\delta^{15}N$ due to the distinct $\delta^{15}N$ signature of the added N. This increased N uptake resulted in increased tissue N percent content and reduced C:N ratio of *S. papillosum* plant tissue. This response supports previous studies that have demonstrated the incorporation of experimentally added N along a range of addition rates (e.g. Soares and Pearson 1997) and changes along gradients of N deposition (Bragazza et al. 2005). These impacts were statistically significant at levels of N deposition above 16 kg N ha^{-1} $year^{-1}$. This is consistent with the model proposed by Lamers et al. (2000), who suggested that at N deposition rates between 12-18 kg N ha^{-1} $year^{-1}$ N percent content in the tissue of *Sphagnum* would increase. However, Bragazza et al. (2005) and Jiroušek et al. (2011) found that under long term N deposition of more than 10 kg N ha^{-1} $year^{-1}$ there is no impact of increasing N deposition on *Sphagnum* tissue N content. Our results are not inconsistent with these previous studies. *Sphagnum* capitula N content in our study (1.10 to 1.60%) are not qualitatively different to those of Bragazza et al. (2005) (approx. 1.19%) or Jiroušek et al. (2011) (1.07 to 1.67%) at similar levels of N deposition. It might be expected that there will be a time-lag between the addition of N and the saturation of plant tissues, and that this time-lag will be longer under lower levels of N deposition. Therefore, in our 2-year study the measurements might reflect differences in the rate of change rather than final tissue N concentrations *per se*.

The impact of N deposition on *D. rotundifolia* tissue N percent and C:N ratio was only significant at the highest rates of input (i.e. >32 kg N ha^{-1} $year^{-1}$). Presumably this is due to some N saturation in *S. papillosum* or at least due to uptake by *S. papillosum* being slower than deposition at these higher levels of N deposition. Lamers et al. (2000) suggested that deposited N would only become available to co-occurring plants above 18 kg N ha^{-1} $year^{-1}$. Our findings support this, at least for *D. rotundifolia*. *Sphagnum* has previously been shown to intercept and store added N making the N unavailable to co-occurring *D. rotundifolia* (Svensson 1995). This appears to be the case in the present study and is consistent with the role of *Sphagnum* as a small scale ecosystem engineer. However, *Sphagnum* might not necessarily absorb all deposited N. If this is the case in the present study then additional N availability was not high enough to result in changes in *D. rotundifolia* growth and physiology until N deposition was above 32 kg N ha^{-1} $year^{-1}$.

Differential uptake of NH_4^+ and NO_3^- by *Sphagnum* spp. has been demonstrated in glasshouse studies, with preferential uptake of NH_4^+ (Jauhiainen et al. 1998; Wiederman et al. 2009). The implication is that *Sphagnum* spp. will be differently affected by deposition of NH_4^+ and NO_3^-. The present study provides an ideal opportunity to test the significance of this differential uptake, in a system receiving N deposition in a realistic manner. The patterns of $\delta^{15}N$ of the *S. papillosum* and *D. rotundifolia* followed that of their potential N sources. Those in the controls had lowest $\delta^{15}N$, those receiving NH_4^+ had intermediate tissue $\delta^{15}N$ values and those receiving NO_3^- had the highest tissue $\delta^{15}N$ values. These differences probably reflect the lower $\delta^{15}N$ of rain water (not measured in the present study but generally ^{15}N depleted – Heaton 1986, Freyer 1978), the intermediate $\delta^{15}N$ of the added NH_4Cl (-0.68‰ to -0.58‰) and the higher $\delta^{15}N$ of the added $NaNO_3$ (+3.1‰ to +4.3‰). This indicates little difference in the uptake of N from these different sources, which would have resulted in patterns of $\delta^{15}N$ that differed from that of the added N. This is further

supported by the lack of impact of N type on any of the measurements except for $\delta^{15}N$. The discrepancy in the results of previous studies and the present study may be due to the way in which the N is added in the present study. This may mean that the results from previous *ex-situ* studies do not translate into measurable impacts where simulated N deposition is added in a realistic way. This may be because the differences are short lived as was shown by Pearce and Van der Wal (1999) for *Racomitrium lanuginosum*. However, the uptake and impact of different forms of N by bryophytes is species specific (Paulissen et al. 2005), making it difficult to compare between studies.

There was no significant impact of N addition on $\delta^{15}N$ of *D. rotundifolia*, as would have been expected if the plants took up any of the added N. This could be due to a number of reasons. It might be the case that the additional N provided by the deposition treatments was not available to the *D. rotundifolia* plants because it was intercepted by the *Sphagnum* before they were able to access the N, as has been shown by Svensson (1995). Alternatively the *D. rotundifolia* plants may have taken up the added N but the change in $\delta^{15}N$ was offset by a reduction in prey capture (i.e. the elevated $\delta^{15}N$ of the prey would then contribute less to the $\delta^{15}N$ of the *D. rotundifolia*). There is further evidence to support this second scenario. The differences in $\delta^{15}N$ of *D. rotundifolia* reflected those in the added N. This strongly suggests that the *D. rotundifolia* plants were taking up the added N. A reduction in prey capture when N deposition was higher would be expected because the investment in prey capture has a lower relative benefit at higher N availabilities (Givnish et al. 1986). Additionally, reduced investment in carnivory by *D. rotundifolia* when root N availability is high has been shown by Thorén et al. (2003). Therefore, we tentatively suggest that this was the case for the *D. rotundifolia* plants in our study. However, this cannot be confirmed with the data we collected, in part due to the difficulty in our study in determining the proportion of N derived from prey ($\%N_{dfp}$) using the natural abundance stable isotope method.

Where there are sufficient differences in the $\delta^{15}N$ of root derived N and prey derived N, $\%N_{dfp}$ can be calculated by using the $\delta^{15}N$ signature of the carnivorous plant, a sample of potential prey and associated non-carnivorous plants (e.g. Schulze et al. 1991, Schulze et al. 1997, Millett et al. 2003). This approach is the same as that proposed by Shearer and Kohl (1986) and used widely to estimate the proportion of N_2 derived from atmospheric fixation in N_2-fixing plants. For any use of this natural abundance stable isotope mehod the choice of references for the two end-points is central to the accuracy of the model (Boddey et al. 2000, Unkovich et al. 2008). When estimating N_{dfp} the two end points represent carnivorous plants that have obtained all or none of their N from insect prey. The use of a sample of potential or actual prey has been universally adopted for the former. This assumes that the $\delta^{15}N$ of N taken up from digested prey is the same as the $\delta^{15}N$ of the entire insect. However, carnivorous plants do not take up all N contained in their insect prey. For example, Hanslin and Karlsson (1996) found that *Pinguicula* spp. and *D. rotundifolia* took up between 29 and 42% of the N contained in insect prey. If this uptake is not evenly distributed between insect tissues, or if there is fractionation of ^{15}N during assimilation, the $\delta^{15}N$ of prey derived N may differ from that of the prey. Nonetheless, these differences are likely to be small relative to the large difference between insect $\delta^{15}N$ and the $\delta^{15}N$ of the target carnivorous plant. As such, the use of insects should provide a reasonable approximation of the $\delta^{15}N$ of N taken up from

prey, as long as the species composition used is representative of that of actual prey capture.

Different approaches have been taken to estimating $\delta^{15}N$ of carnivorous plants that obtain no N from prey capture. Schulze et al. (1991) and Moran et al. (2001) used non-carnivorous vascular plants growing close to their target carnivorous plants. Millett et al. (2003) used both the *Sphagnum* that was the substrate for the target carnivorous plants and non-carnivorous vascular plants growing in close proximity. They found differences in $\delta^{15}N$ as large as 1.4‰ between the *Sphagnum* and non-carnivorous vascular plants. Schulze et al. (1997) used non-trapping laves of the target carnivorous plant as references for this end point, though this assumes no transfer of prey derived N between tissues. In the present study, we used two different reference plants: the *S. papillosum* that was the substrate for the *D. rotundifolia* plants and the lowest $\delta^{15}N$ of *D. rotundifolia* plants. It was not possible to use co-occurring non-carnivorous vascular plants because the turfs were transplanted without these. The use of the minimum *D. rotundifolia* $\delta^{15}N$ probably provides an accurate minimum $\%N_{dfp}$. This is because issues regarding the requirements of similarity in root N sources and in ^{15}N fractionation are eliminated by using the same species. Millett et al. (2003) found that the *D. rotundifolia* with the lowest $\delta^{15}N$ had $\delta^{15}N$ values almost identical to that of the *Sphagnum* in which they were growing. This indicates that in their study the use of either of these two methods would give similar values for $\%N_{dfp}$, and shows that both were suitable. However, in the present study the values for $\%N_{dfp}$ calculated using *S papillosum* as the reference species (on average 13%) considerably underestimated $\%N_{dfp}$. The estimate for $\%N_{dfp}$ was lower than the minimum inferred from using the lowest *D. rotundifolia* $\delta^{15}N$ (on average 35%). Therefore, we must conclude that in the present study *S. papillosum* is not a suitable reference species for calculating $\%N_{dfp}$. The reasons for this probably relate to the *ex-situ* nature of this study, resulting in complex ^{15}N sources and interactions between N deposition, *S. papillosum* and *D. rotundifolia* N uptake in a system that had not yet reached equilibrium. We suggest that care should be taken when using the natural abundance method to estimate $\%N_{dfp}$ for carnivorous plants in *ex-situ* studies. As a result we can only conclude that the *D. rotundifolia* plants in our study obtained on average a minimum of 35% of their N from prey. This is consistent with previous studies (e.g. 50% for *D. rotundifolia* in the UK found by Millett et al. 2003).

We conclude that the *D. rotundifolia* in this study obtained a significant proportion of their N budget from their insect prey, but it was not possible to determine the impact of N deposition on this N source. *S. papillosum* in this study intercepted and stored deposited N resulting in increased tissue N concentration and decreased C:N ratio. *Drosera rotundifolia* was relatively unaffected by the increased N deposition, though there was some evidence of increased tissue N concentration at the highest N deposition rates (64 kg N ha^{-1} $year^{-1}$). These contrasting responses may be because of the alternative (prey) N source of *D. rotundifolia* or because *Sphagnum* acts as a small scale ecosystem engineer at lower N deposition rates. Furthermore, this study shows that even after only two years of N addition, wet N deposition with a distinct ^{15}N signature can be traced in *S. papillosum* but not *D. rotundifolia*. Finally, the plants in this study system showed no discernable difference in response to reduced or oxidised forms of wet N deposition. There was also no evidence of differential uptake of deposited N when added in reduced or oxidised form. This suggests that,

414 in the short term at least, it is the amount of wet N deposition and not the form of
415 deposited N that is most important in terms of plant response.
416
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422
423 **References**
424
425 Bobbink R (1998) Impacts of tropospheric ozone and airborne nitrogenous pollutants
426 on natural and semi-natural ecosystems: a commentary. *New Phytologist* 139:161-
427 168

428 Boddey RM, Peoples MB, Palmer B, Dart PJ (2000) Use of the ^{15}N natural
429 abundance technique to quantify biological nitrogen fixation by woody perennials.
430 Nutr. Cycl. Agroecosys. 57:235-270

431 Bragazza L, Limpens J, Gerdol R, Grosvernier P, Hájek M, Hájek T, Hájková P,
432 Hansen I, Lacumin P, Kutnar L, Rydin H, Tahvanainen T (2005) Nitrogen
433 concentration and $\delta^{15}N$ signature of ombrotrophic *Sphagnum* mosses at
434 different N deposition levels in Europe. *Global Change Biology* 11:106-114

435 Breemen N (1995) How *Sphagnum* bogs down other plants. *Trends in Ecology &*
436 *Evolution* 10:270-275

437 Bubier J, Moore T, Bledzki LA (2007) Effects of nutrient addition on vegetation and
438 carbon cycling in an ombrotrophic bog. *Global Change Biology* 13:1168-1186

439 Ellison AM, Gotelli NJ (2001) Evolutionary ecology of carnivorous plants. *Trends in*
440 *Ecology and Evolution* 16:623-629

441 Ellison AM, Gotelli NJ (2002) Nitrogen availability alters the expression of carnivory
442 in the northern pitcher plant, *Sarracenia purpurea*. *Proceedings of the National*
443 *Academy of Sciences* 99:4409-4412

444 Freyer HD (1978) Seasonal trends of NH_4^+ and NO_3^- nitrogen isotope composition in
445 rain collected at Jullich, Germany. *Tellus* 30:83-92

446 Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli,
447 LA, Seitzinger SP, Sutton MA, (2008) Transformation of the nitrogen cycle: recent
448 trends, questions, and potential solutions. *Science* 320:889-892

449 Givnish TJ, Burkhardt EL, Happel RE, Weintraub JD (1984) Carnivory in the
450 bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of
451 carnivorous plants to sunny, moist nutrient-poor habitats. *American Naturalist*
452 124:479-497

453 Hanslin HM, Karlsson PS (1996) Nitrogen uptake from prey and substrate as
454 affected by prey capture level and plant reproductive status in four carnivorous plant

455 species. *Oecologia* 106:370-375

456 Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and
457 atmosphere: a review. *Chemical Geology* 59:87-102

458 Heijmans MMPD, Klees H, de Visser W, Berendse F (2002) Effects of increased
459 nitrogen deposition on the distribution of ^{15}N-labeled nitrogen between *Sphagnum*
460 and vascular plants. *Ecosystems* 5:500-508

461 Jauhiainen J, Wallén B, Malmer N (1998) Potential NH_4^+ and NO_3^- uptake in seven
462 *Sphagnum* species. *New Phytologist* 138:287-293

463 Jiroušek M, Hájek M, Bragazza L (2011) Nutrient stoichiometry in *Sphagnum* along a
464 nitrogen deposition gradient in highly polluted region of Central-East Europe.
465 *Environmental Pollution* 159:585-590

466 Lamers LPM, Bobbink R, Roelofs JGM (2000) Natural nitrogen filter fails in polluted
467 raised bogs. *Global Change Biology* 6:583-586

468 Leith ID, Sheppard LJ, Fowler D, Cape NJ, Jones M, Crossley KJ, Hargreaves S,
469 Tang YS, Theobald M, Sutton MA (2004) Quantification of dry N deposition to an
470 ombrotrophic bog from an automated NH_3 release system. *Water, Air, and Soil*
471 *Pollution* 6:207-218

472 Leith ID, Sheppard LJ, Pitcairn CER, Cape JN, Hill PW, Kennedy VH, Tang YS,
473 Smith RI, Fowler D (2001) Comparison of the effects of wet N deposition (NH_4Cl)
474 and dry N deposition (NH_3) on UK moorland species. *Water, Air, and Soil Pollution*
475 130:1043-1048

476 Millett J, Jones RI, Waldron S (2003) The contribution of insect prey to the total
477 nitrogen content of sundews (*Drosera* spp.) determined *in situ* by stable isotope
478 analysis. *New Phytologist* 158:527-534

479 Moran JA, Merbach MA, Livingston NJ, Clarke CM, Booth WE (2001) Termite prey
480 specialization in the pitcher plant *Nepenthes albomarginata* – evidence from stable
481 isotope analysis. *Annals of Botany* 88:307-311

482 Paulissen MPCP, Besalú LE, Bruijn H de, Van der Ven PJM, Bobbink R (2005)
483 Contrasting effects of ammonium enrichment on fen bryophytes. *Journal of Bryology*
484 27:109-117

485 Pearce ISK, van der Wal R (2002) Effects of nitrogen deposition on growth and
486 survival of montane *Racomitrium lanuginosum* heath. *Biological conservation*
487 104:83-89

488 Rodwell JS (1991) *Mires and Heaths, British Plant Communities*. Cambridge
489 University Press, Cambridge, UK.

490 Sala OE, Chapin III FS, Armesto JJ, Berlow R, Bloomfield J, Dirzo R, Huber-
491 Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge D, Mooney,
492 HA, Oesterheld M, Poff, NL, Sykes MT, Walker BH, Walker M, Wall DH, (2000)
493 Global biodiversity scenarios for the year 2100. *Science* 287:1770-1774

494 Schulze ED, Gebauer G, Schulze W, Pate JS (1991) The utilization of nitrogen from
495 insect capture by different growth forms of *Drosera* from Southwest Australia.
496 *Oecologia* 87:240-246

497 Schulze W, Schulze ED, Pate JS, Gillison AN (1997) The nitrogen supply from soils
498 and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus*
499 *follicularis*, and *Darlingtonia californica*. *Oecologia* 112:464-471

500 Shearer GB, Kohl DH (1986) N_2 - fixation in field settings: estimations based on
501 natural ^{15}N abundance. Aust. J. Plant Physiol. 13:699-756

502 Sheppard LJ, Leith ID, Crossley A, Carfrae JA, Elix J, Tang YS, Blackall T, Jones, M,
503 Theobald M, Hargreaves K, Cape NJ, Fowler D, Raven J (2004) Rapid responses of
504 an ombrotrophic bog community to N enrichment: discrimination between different
505 forms. *Water, Air, and Soil Pollution* 6:197-205

506 Skinner RA, Ineson P, Jones H, Sleep D, Leith ID, Sheppard LJ, (2006) Heathland
507 vegetation as a bio-monitor for nitrogen deposition and source attribution using $\delta^{15}N$
508 values. *Atmospheric Environment* 40:498-507

509 Soares AS, Pearson J (1997) Short-term physiological responses of mosses to
510 atmospheric ammonium and nitrate. *Water, Air, and Soil Pollution* 93:225-242

511 SPSS (2008) SPSS Statistics 17.0. Release 17.0.0 (Aug 23, 2008).

512 Svensson BM (1995) Competition between *Sphagnum fuscum* and *Drosera*
513 *rotundifolia*: a case of ecosystem engineering. *Oikos* 74:205-212

514 Thorén LM, Tuomi J, Kämäräinen T, Laine K. (2003) Resource availability affects
515 investment in carnivory in *Drosera rotundifolia*.

516 Unkovich M, Herridge DF, Peoples MB, Boddey RM, Cadisch G, Giller K, Alves B,
517 Chalk PM (2008) Measuring plant-associated nitrogen fixation in agricultural
518 systems. ACIAR Monograph No.136. Canberra: Australian Centre for International
519 Agricultural Research.

520 Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW,
521 Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle:
522 sources and concequences. *Ecological Applications* 7:737-750

523 Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: How
524 can it occur? *Biogeochemistry* 13:87-115

525 Wiedermann MM, Gunnarsson U, Ericson L, Nordin A (2009) Ecophysiological
526 adjustment of two *Sphagnum* species in response to anthropogenic nitrogen
527 deposition. *New Phytologist* 181:208-217

528 Williams B, Silcock D, Young M (1999) Seasonal dynamics of N in two *Sphagnum*
529 moss species and the underlying peat treated with $^{15}NH_4$ $^{15}NO_3$. *Biogeochemistry*
530 45:285-302

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Tables

Table 1

Significance of Repeated Measures GLM for characteristics of *D. rotundifolia* and *S. papillosum* growing in microcosms transplanted into the Whim Moss N deposition Experiment. Presented are the d.f. and *P* values for the effect of level of N deposition (4 levels) type of N deposition (3 levels) and years of measurement (2 levels). All 2 and 3-way interactions were non-significant and are therefore excluded.

	N level	N type	Year
d.f.	2, 20	1, 20	1, 20
D. rotundifolia			
N%	**0.004**	0.42	**0.02**
C:N	**0.002**	0.49	**0.01**
$\delta^{15}N$	0.38	**0.05**	0.69
Mass/plant	0.17	0.10	1.00
Total N/plant	0.31	0.30	0.39
Rosette diameter	0.38	0.77	**0.01**
Number of leaves/plant	0.34	0.12	**0.003**
Number of leaves with prey/plant	0.14	0.22	**0.02**
% of leaves with prey	0.14	0.22	**0.02**
S. papillosum			
$\delta^{15}N$	**0.04**	0.39	**0.001**
C:N	**0.004**	0.68	**0.007**
%N	**0.007**	0.76	0.35

Legends of Figures

Figure 1

Impact of wet N deposition on $\delta^{15}N$, %N and C:N ratio of *D. rotundifolia* plants and *S. papillosum* capitula growing in *Sphagnum* turfs transplanted into the Whim Moss N deposition experiment. Presented are the mean±SEM for plants growing at four levels of N deposition in two years. Levels of N deposition represent total rates of deposition, taking into account background inputs of approximately 8 kg N ha^{-1} $year^{-1}$, numbers in parenthesis are the rates of additional N applied to plots. Mean values represent reduced and oxidized N deposition. Letters indicate significance of difference between each N deposition rate, regardless of year (there was no significant year×N deposition interaction for any of the measures) (Fisher's LSD, $P<0.05$). The significance of the main effect of year is presented for each measure.

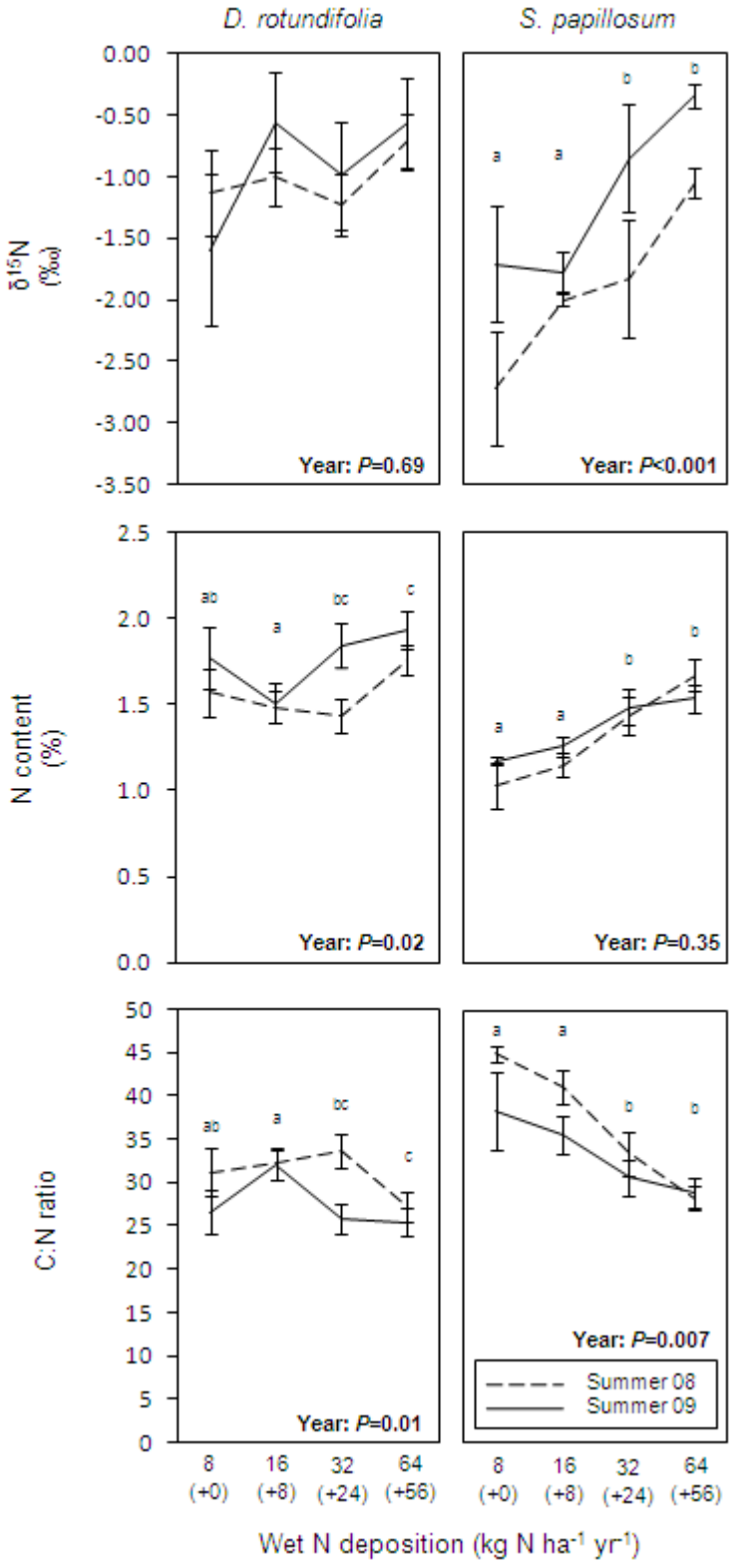
Figure 2

Impact of reduced (as NH_4Cl) or oxidised (as $NaNO_3$) N deposition on $\delta^{15}N$ of *D. rotundifolia* plants growing in *Sphagnum* turfs transplanted into Whim Moss N deposition experiment. Presented are mean±SEM for control (i.e. no additional N added) or the combined mean for each of the two forms of added N. Symbols with different letters are significantly different from each other (Fisher's LSD, $P<0.05$).

Figure 3

$\delta^{15}N$ of *S. papillosum*, *D. rotundifolia* and a sample of potential *D. rotundifolia* insect prey for *S. papillosum* turfs transplanted into the Whim Moss N deposition experiment. Presented are the mean±SEM for *S.* papillosum and insect $\delta^{15}N$ in 2008 (open symbols) and 2009 (closed symbols). The values for *D. rotundifolia* are presented as the mean for all plants in 2008 and 2009 and the mean for the individual plant with the lowest $\delta^{15}N$ in each treatment in 2008. Letters indicate significant differences between bars (Fisher's LSD, $P<0.05$).

570 **Fig. 1**



571

572

Fig. 2

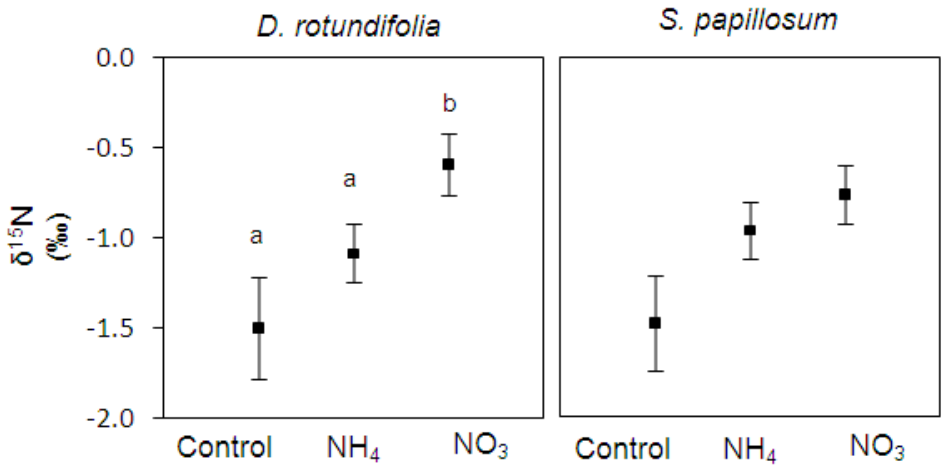
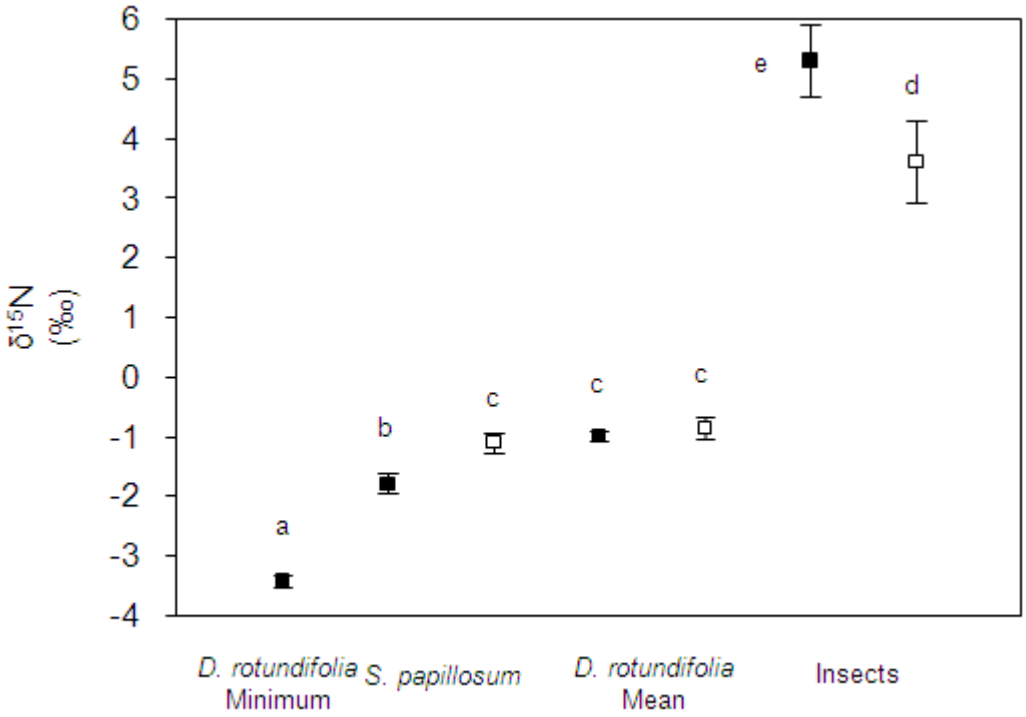

D. rotundifolia
S. papillosum
0.0
-0.5
-1.0
-1.5
-2.0
δ15N (‰)
a
a
b
Control
NH4
NO3
Control
NH4
NO3

Fig. 3


6
5
4
3
2
1
0
-1
-2
-3
-4
δ15N (‰)
a
b
c
c
c
e
d
D. rotundifolia Minimum
S. papillosum
D. rotundifolia Mean
Insects