

**Effect of Increased Atmospheric Nitrogen Deposition and Elevated CO₂ on Traits
Responsible for Carnivory in the Sundews *Drosera rotundifolia* and *Drosera intermedia***

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Abstract

Two experiments were conducted to study the effects of increased atmospheric nitrogen deposition and elevated CO₂ on investment in carnivory by two sundew species, *Drosera rotundifolia* and *D. intermedia*. A field study was conducted with *D. rotundifolia* at Inverness Mud Lake Bog in Inverness Twp., MI where plants were exposed to simulated future increases in atmospheric nitrogen deposition predicted under the IPCC B1 and A1FI emissions scenarios. A second study was conducted with *D. intermedia* using an array of 40 open-topped CO₂ chambers. Plants were exposed to ambient or elevated CO₂ with or without simulated A1FI atmospheric nitrogen deposition. ANOVA did not detect a significant treatment effect for any measured prey-capture trait (percent redness, trichome number, and leaf stickiness). However, paired comparisons within treatments suggested that both sundew species alter investment in at least one prey-capture trait in response to increased nitrogen and/or carbon availability. *Drosera rotundifolia* decreased investment in red tissue and trichomes in response to B1 and A1FI nitrogen addition, respectively. When grown under elevated CO₂, *D. intermedia* displayed a larger increase in redness and produced more sticky dew than when grown under ambient CO₂. Therefore, *D. rotundifolia* and *D. intermedia* appear capable of adjusting investment in prey-capture traits in response to increasing levels of atmospheric carbon and nitrogen, though this ability is far from complete, and will likely be species-specific.

Introduction

In most plant species, leaves serve the sole purpose of capturing carbon, via photosynthesis. However, in some species, leaves have been modified to perform other functions as well. For instance, floral leaves, or bracts, through the process of natural selection, have been modified to resemble showy petals, which attract pollinators. Similarly, the leaves of cacti have been modified into spines that provide protection from herbivores (Levetin, 1999). The leaves of carnivorous plants have been modified to capture both carbon via photosynthesis and nitrogen and other nutrients by attracting, trapping, and digesting invertebrate prey.

Nitrogen is typically the most limiting nutrient for carnivorous plants because they inhabit nutrient poor soils. Thus, the leaves serve the dual function of capturing both carbon (through photosynthesis) and nitrogen (through carnivory). To accomplish both functions, leaves of carnivorous plants have evolved to express both red and green pigments, as red pigmentation has been shown to attract insect prey (Schaefer and Ruxton 2008; Newell and Nastase 1998). Since any given part of a leaf cannot be simultaneously red and green, the leaves of carnivorous plants experience an unavoidable trade-off between using leaf area for photosynthesis versus prey capture. Not surprisingly, the ratio of colors differs between and within species and even among leaves on the same plant (Effinger, 2013).

The optimal ratio of red to green tissue for carnivorous plants will necessarily change with changing availability of carbon and nitrogen. In recent decades, humans have been influencing the carbon and nitrogen cycles in various ways that increase availability of both to plants. Through fossil fuel combustion, deforestation, and the fertilization of agricultural fields, humans have been releasing carbon and nitrogen in many forms into the biosphere and the atmosphere, broadly affecting species and ecosystems.

Atmospheric nitrogen deposition occurs when nitrogenous compounds in the atmosphere enter the biosphere, both during and between precipitation events. The anthropogenic activities that contribute to nitrogen deposition are the burning of fossil fuels and the fertilization of agricultural land, adding NO_x (NO and NO_2) and NH_3 to the atmosphere, respectively. Together these activities account for over 70% of the total nitrogen deposition over the U.S., mostly occurring in urban areas and peaking between May and September (Zhang et al. 2012). NO_x gases are oxidized to form HNO_3 while NH_3 reacts with H_2SO_4 and HNO_3 to produce ammonium sulfate and nitrate aerosols, all of which are returned to the biosphere through dry or wet deposition. This deposition is highest over the Midwest (Figure 1, from Zhang et al. 2012). Due to the rapid nature of these reactions, most nitrogen deposition that occurs in the U.S. originated as emissions from within the U.S. (Zhang et al. 2012).

The burning of fossil fuels releases carbon into the atmosphere in the form of CO_2 , the primary greenhouse gas responsible for climate change. Humans have been the main driver of the increase in CO_2 emissions over the last century. Since 1750, humans have emitted 555 ± 85 PgC into the atmosphere (Ciais et al. 2013). As a result, the atmosphere and ocean have experienced warming resulting in diminished snow and ice cover and a rise in sea level (IPCC, 2013).

The Intergovernmental Panel on Climate Change (IPCC), using emissions predictions, has developed multiple systems of future scenarios, which predict the geophysical effects on the Earth as a result of climate change. Their predictions take changes in demographics, socio-economic development, and technological progress into account. Under all of the IPCC SRES emissions scenarios, the concentration of CO_2 in the atmosphere is predicted to increase, and by

2100 the concentration will likely be between 540ppm and 970ppm (IPCC 2000). The most recent IPCC report predicts that under their four Representative Concentration Pathways (RCP) scenarios, the application of nitrogen for agricultural use will increase by 90 to 190 TgN yr⁻¹ by 2100 (Ciais et al. 2013). This increase in application, together with increased fossil fuel burning, is likely to cause an increase in emissions of NO_x and NH₃, thus increasing atmospheric nitrogen deposition (Figure 2, from Galloway et al. 2008).

Two of the pathways under the SRES grouping are relevant to this study. The A1FI scenario “describes a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies” (IPCC, 2000). Under this scenario the world would continue to be powered by fossil fuels. An alternative pathway, the B2 scenario, “describes a world in which the emphasis is on local solutions to economic, social, and environmental sustainability. It is a world with continuously increasing global population... intermediate levels of economic development, and less rapid and more diverse technological change” (IPCC, 2000). These two scenarios are quite different, and as such, result in different emissions patterns. Under the A1FI scenario, NO_x emissions are predicted to be much higher than in the B2 scenario (Figure 3). Additionally, the amount of CO₂ in the atmosphere is predicted to reach 2,189 GtC by 2100 under the A1FI scenario, which is almost double the 1,164 GtC predicted under the B2 scenario under the same timeline (IPCC, 2000).

Future increases in atmospheric CO₂ and nitrogen deposition are likely to have substantial effects on most or all plant species. One study suggests that elevated CO₂ causes faster growth and increases in photosynthetic rate and yield (Idso et al. 1993). Conversely, other studies provide evidence for a more widely accepted scenario, in which elevated atmospheric

CO₂ decreases the nitrogen concentrations in plant tissues and thus decreases photosynthetic capabilities (Cotrufo et al. 1998). Therefore, it is not yet known how particular plant species, especially those that are carnivorous, will react to increasing CO₂ and nitrogen deposition levels that result in part from climate change.

Two species of carnivorous plants that would likely be affected by changes in carbon and nitrogen availability are the Round-leaved sundew (*Drosera rotundifolia*) (Figure 4) and the Spoonleaf sundew (*D. intermedia*) (Figure 5), which can be found in ombrotrophic bogs around Michigan. Their leaves include both red and green tissue, which allows sundews to photosynthesize efficiently as well as attract insect prey. Additionally, sundew leaves have trichomes that secrete sticky dew and digestive enzymes. When an insect gets caught in the sticky droplets of the trichomes, the leaf slowly rolls up to attach as many trichomes to the insect as possible and begins digesting the insect and absorbing nitrogen. Therefore, red color is essential for the successful attraction of prey and the stickiness of the leaves for capturing prey. Because the leaves of carnivorous plants serve the dual functions of carbon and nitrogen capture, they may be particularly responsive to altered availability of atmospheric carbon and nitrogen.

In Mud Lake Bog and elsewhere, *Drosera* species obtain approximately 50% of their nitrogen from insect prey (Glassman and Karowe in review; Millet et al., 2003). If nitrogen were to become more available through the roots, sundews would not need to obtain as much from insect prey, and could decrease investment in prey capture traits in order to invest more in carbon capture. This would result in a greener, less sticky leaf. In contrast, if carbon availability were to increase, then sundews might decrease investment in carbon capture and increase their investment in nitrogen capture, resulting in redder and stickier leaves. Therefore, future simultaneous increases in atmospheric CO₂ and nitrogen deposition may have opposite effects on

resource allocation in sundews.

Some studies have examined how nitrogen addition affects the amount of nitrogen sundews obtain from insects as compared to the amount they obtain from the soil and through their roots. Millet et al. (2012) found that, when atmospheric nitrogen deposition was increased experimentally, *D. rotundifolia* obtained an increasing amount of their N from roots and a decreasing amount from prey, as indicated by N¹⁵ analysis. Therefore, *D. rotundifolia* decreased its reliance on prey-derived nitrogen and increased its reliance on root-derived nitrogen. It is unknown whether this increase in root-derived nitrogen resulted in decreased investment in traits responsible for prey capture. Consistent with this hypothesis, Thoren et al. (2003) discovered that nitrogen added to the growth medium of *D. rotundifolia* resulted in less sticky leaves. To our knowledge, no study has addressed the effects of nitrogen addition on sundew leaf color; the effects of increased carbon availability, or the effects of simultaneous increases in both on sundew prey capture traits (i.e. on sundew investment in carnivory). Since elevated CO₂ and increased atmospheric nitrogen deposition would likely cause opposite changes in leaf color and stickiness when applied individually, it is not obvious how sundews should respond to simultaneous increases in both.

In this study, I address the following questions:

1. Will *Drosera rotundifolia* and *D. intermedia* decrease investment in carnivory when exposed to simulated future increases in atmospheric nitrogen deposition?
2. Will *D. intermedia* increase investment in carnivory under elevated CO₂?
3. How will simultaneous increases in atmospheric nitrogen deposition and atmospheric CO₂ affect *D. intermedia* investment in carnivory?

Materials and Methods

Study Species

Drosera rotundifolia and *D. intermedia* are carnivorous, flowering plants that occur throughout much of the United States and Canada (Figures 6 and 7). They typically live in nutrient poor habitats and, as a result, the leaves of the plants have been modified to capture insect prey. The composition of prey for the two species is different. For example, *D. rotundifolia* tend to catch Collembola (Thum 1986) and Diptera (Millet et al., 2003) while *D. intermedia* tend to catch Nematocera and Brachycera (Thum 1986).

Study Sites

The first study, a nitrogen deposition experiment, was conducted in the summer of 2014 at Inverness Mud Lake Bog in Inverness Twp., Michigan, an ombrotrophic, *Sphagnum*-dominated bog.

The second study, an elevated CO₂ experiment, was conducted at The University of Michigan Biological Station (UMBS) in Pellston, Michigan using an array of 40 open-topped CO₂ chambers (Karowe and Grubb 2011). Twenty of the chambers received ambient CO₂ while the remaining 20 received elevated (approximately 750ppm) CO₂.

Effect of Increased Atmospheric Nitrogen Deposition on D. rotundifolia Investment in Carnivory

Ninety *D. rotundifolia* plants that exhibited a wide variety of coloration (red to green) were used in this study. Relatively large plants were chosen because they produced better quality pictures for color analysis (see below).

Plants were organized into triplicates based primarily on similar coloration (percent red tissue) and secondarily on similar number of leaves. Within each triplicate, one plant was randomly assigned to each of three treatments: control (no nitrogen addition), low nitrogen addition (0.0339g of $\text{NH}_4\text{NO}_3/\text{m}^2/\text{week}$), and high nitrogen addition (0.0844g of $\text{NH}_4\text{NO}_3/\text{m}^2/\text{week}$). The levels of nitrogen addition corresponds to the difference between current (2011-2013) levels of atmospheric nitrogen deposition at UMBS (National Atmospheric Deposition Program, 2007) and estimated future levels calculated from predicted NO_x emissions in the IPCC Special Report on Emissions Scenarios (IPCC, 2000). Low nitrogen addition corresponds to the B2 scenario and the high nitrogen addition corresponds to the A1FI scenario. Since future atmospheric nitrogen deposition depends on the future trajectories of NO_x and NH_3 emissions, we assumed that the increase in atmospheric nitrogen deposition would be proportional to the increase in NO_x emissions (Ciais et al. 2013). NO_x emissions are predicted to increase by 49% above 1990 levels under the B2 scenario, and by 71.9% above 1990 levels under the A1FI scenario. Atmospheric wet deposition during the summer at UMBS averaged 1.34Kg per hectare per year from 1981- 1991 but decreased to 1.02Kg per hectare per year from 2011-2013 (National Atmospheric Deposition Program, 2007). Therefore, for the B2 scenario, summer atmospheric nitrogen deposition at UMBS in 2100 was calculated as 49% times the 1990 level plus 0.32Kg. We then scaled this to weekly deposition per m^2 to get 0.0339g of $\text{NH}_4\text{NO}_3/\text{m}^2/\text{week}$. For the A1FI scenario, summer atmospheric nitrogen deposition at UMBS in 2100 was calculated as 71.9% times the 1990 level plus 0.32Kg. We then scaled this to weekly deposition per m^2 to get 0.0844g of $\text{NH}_4\text{NO}_3/\text{m}^2/\text{week}$.

Nitrogen was applied with a garden sprayer in the form of NH_4NO_3 dissolved in water. A 1x1m area centered on the plant received 100mL of tap water containing 0g, 0.0339g, or 0.0844g

NH₄NO₃ once a week for five weeks (July 6, 2014 - August 4, 2014). We calibrated each sprayer by timing how many seconds it took the sprayer to fill a 100mL graduated cylinder, and then sprayed each plot for this number of seconds.

To evaluate the effects of nitrogen addition on leaf coloration, each plant was photographed in Mud Lake Bog once a week. Photos were taken between 10AM and 1PM using a shade tent to reduce glare on the leaves. On each plant, one leaf was chosen for color analysis; this was always the leaf that was most directly facing the lens and most in focus. Adobe Photoshop was used to determine that leaf's coloration by first using the Quick Selection tool to select the leaf pad while also deselecting the trichome heads (which we assume not to change color over the duration of the study). The number of trichome heads was recorded as a measure of the number of trichomes per leaf. After noting the total number of pixels of the selected area, Adobe Photoshop's Select a Color Range tool was used to select the red pixels within the selected area, and the leaf's "percent redness" was calculated as $100 * \text{number of red pixels} / \text{total number of pixels}$.

We determined the effect of nitrogen addition on change in percent redness using a mixture of Kruskal-Wallis, ANOVA, paired t-test, and Wilcoxon Signed Rank tests. The effect of treatment on the number of trichomes per leaf was determined by ANOVAs and paired t-tests. To determine the effect of treatment on the number of leaves per plant we ran Kruskal-Wallis, ANOVA, and paired t-tests.

Effect of Increased Atmospheric Nitrogen Deposition and Elevated CO₂ on the Investment in Carnivory of D. intermedia

Eighty *D. intermedia* obtained from Meadowview Biological Research Station were

planted in 4x4x4-inch pots in the UMBS greenhouse on July 3. Pots were filled almost to the top with a moist soil mixture of 50% sand and 50% *Sphagnum* peat moss, and placed in individual plastic cups containing standing water. Plants were grouped into sets of four based primarily on similar coloration and secondarily on number of leaves. The four size-matched plants in each set were randomly assigned to the four treatments: ambient CO₂ and no nitrogen addition, ambient CO₂ and high (A1FI) nitrogen addition (0.000871g of NH₄NO₃/week), elevated CO₂ (750ppm) and no nitrogen addition, and elevated CO₂ and high nitrogen addition. The nitrogen addition corresponding to an A1FI future was calculated as described above, and scaled for a 4X4-inch area.

Two days after transplanting, plants were moved to one of 40 open-topped CO₂ chambers, 20 of which were maintained at ambient CO₂ (hereafter “ambient chambers”) and 20 of which were maintained at elevated (approximately 750ppm) CO₂ (hereafter “elevated chambers”) (Karowe and Grubb 2011). From each set of four plants, two plants, one receiving nitrogen addition and the other not, were moved into an ambient chamber and the other two plants, one receiving nitrogen addition and the other not, were moved into an adjacent elevated chamber. The two chambers therefore constituted a block and, in principle, experienced similar position effects within the array.

For plants receiving nitrogen addition, NH₄NO₃ was dissolved in water such that 10mL of solution contained 0.000871g. This amount was delivered to plants once a week using a handheld spray bottle.

To determine the separate and combined effects of increased carbon and nitrogen availability, all plants were photographed once a week. Photos were taken between 9AM and

1PM using a shade tent to reduce glare on the leaves. Adobe Photoshop was used to analyze the coloration of the plant and the number of trichomes per leaf using the methods described above.

To determine the effects of treatments on leaf stickiness, the weight of the sticky dew on each plant was measured after 5 weeks of growth in the chambers. On August 12 between 4PM and 9PM, dew was collected onto a pre-weighted filter paper disc by brushing the disc repeatedly along all open and half open leaves on the plant. The disc was immediately reweighed, and the difference indicated the weight of dew collected. The total dew weight was divided by the number of leaves sampled to calculate the weight of dew per leaf. The filter paper disc was then placed into a microfuge tube to allow for future analysis of the sugar content of the dew.

We determined the effect of treatment on the coloration using a mixture of Kruskal-Wallis, ANOVA, paired t-test, and Wilcoxon Signed Rank tests. The effect of treatment on the number of trichomes was determined by running Kruskal-Wallis, ANOVA, paired t-test, and Wilcoxon Signed Rank tests. To determine the effect of treatment on the number of leaves per plant we ran Kruskal-Wallis, ANOVA, and paired t-tests. We determined the effect of treatment on dew per plant by running a Kruskal-Wallis test. Post hoc pairwise comparisons were done using Mann-Whitney U tests.

Results

Effect of Increased Atmospheric Nitrogen Deposition on D. rotundifolia Investment in Carnivory

Prior to the application of treatments, sundew plants assigned to the control, B2 (low nitrogen addition), and A1FI (high nitrogen addition) treatment groups did not differ in percent redness ($X^2 = 0.68$, $df = 2$, $p = 0.71$), trichome number ($F = 1.33$, $df = 2$, $p = 0.27$), or leaf number

($X^2 = 0.49$, $df = 2$, $p = 0.78$), (Table 1). At the end of the experiment, this was again true for percent redness ($F = 1.47$, $df = 2$, $p = 0.24$), trichome number ($F = 0.01$, $df = 2$, $p = 0.99$), and leaf number ($F = 0.82$, $df = 2$, $p = 0.45$) (Table 1). Consistent with these observations, ANOVA did not detect any difference among treatment groups in change in percent redness ($F = 0.34$, $df = 2$, $p = 0.17$), trichome number ($F = 0.99$, $df = 2$, $p = 0.79$), or leaf number ($F = 0.72$, $df = 2$, $p = 0.49$) over the course of the experiment.

We also used paired tests within each treatment to ask whether prey-capture traits responded similarly to all treatments. Paired tests indicated that plants receiving the control and A1FI treatments showed no significant change in percent red tissue (Wilcoxon $Z = -1.41$, $p = 0.16$; paired $t = -0.52$, $df = 22$, $p = 0.61$, respectively). In contrast, percent redness decreased significantly for plants receiving the B2 treatment (paired $t = 2.10$, $df = 23$, $p = 0.047$; indicated by an asterisk in Table 1).

Paired tests also revealed treatment-specific responses in the number of trichomes per leaf. Trichome number decreased significantly for plants that received the A1FI and B2 treatments (paired $t = 2.48$, $df = 22$, $p = 0.021$; paired $t = 2.11$, $df = 22$, $p = 0.047$, respectively). In contrast, plants that received the control treatment showed no significant difference between initial and final number of trichomes (paired $t = 1.42$, $df = 24$, $p = 0.17$) (Table 1).

Paired tests also revealed that the number of leaves decreased significantly for plants that received the control and A1FI treatments (paired $t = 2.70$, $df = 15$, $p = 0.017$; paired $t = 2.73$, $df = 15$, $p = 0.015$, respectively). In contrast, plants that received the B2 treatment showed no significant change in number of leaves, though the trend was in the same direction (paired $t = 1.78$, $df = 15$, $p = 0.096$) (Table 1).

Table 1. Summary of effect of treatment on *D. rotundifolia* prey-capture traits. Asterisks indicate significant differences between initial and final values for plants within a given treatment ($^+ 0.10 < p < 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	Control	B2	A1FI	Among Treatment p-value
Initial % Redness	52.6 ± 3.2	57.0 ± 4.7	52.0 ± 4.5	0.71
Final % Redness	43.2 ± 2.2	45.2 ± 1.8*	51.8 ± 2.0	0.24
Initial Trichome Number	59.0 ± 4.5	67.9 ± 4.0	68.7 ± 5.5	0.27
Final Trichome Number	53.3 ± 11.1	53.3 ± 15.1*	53.7 ± 11.6*	0.99
Initial Leaf Number	5.8 ± 1.1	5.6 ± 0.9	5.8 ± 1.0	0.78
Final Leaf Number	5.1 ± 1.5*	5.0 ± 1.2 ⁺	4.7 ± 1.5*	0.45

Effect of Increased Atmospheric Nitrogen Deposition and Elevated CO₂ on D. intermedia

Investment in Carnivory

Prior to application of treatments, sundew plants assigned to the 4 treatments (ambient or elevated CO₂ with or without simulated A1FI nitrogen deposition) did not differ in percent redness ($X^2 = 1.41$, $df = 3$, $p = 0.70$), trichome number ($X^2 = 3.59$, $df = 3$, $p = 0.31$), or leaf number ($F = 0.13$, $df = 76$, $p = 0.94$) (Table 2). At the end of the experiment, this was again true for percent redness ($F = 0.90$, $df = 67$, $p = 0.45$), trichome number ($F = 0.37$, $df = 67$, $p = 0.77$), and leaf number ($F = 0.81$, $df = 76$, $p = 0.49$) (Table 2). Consistent with these observations, ANOVA did not detect any difference among treatment groups in change in percent redness ($F = 0.37$, $df = 3$, $p = 0.77$), trichome number ($F = 0.67$, $df = 3$, $p = 0.57$), or leaf number ($X^2 = 4.86$, $df = 3$, $p = 0.18$) over the course of the experiment.

We again used paired tests within each treatment to ask whether prey-capture traits

responded similarly to all treatments. Paired tests indicated that percent redness of *D. intermedia* increased significantly in response to all treatments (ambient CO₂ without nitrogen addition paired $t = 3.77$, $df = 16$, $p = 0.002$; ambient CO₂ with nitrogen addition paired $t = 2.23$, $df = 15$, $p = 0.041$; elevated CO₂ without nitrogen addition paired $t = 3.83$, $df = 17$, $p = 0.001$; elevated CO₂ with nitrogen addition Wilcoxon $Z = 2.91$, $p = 0.004$, indicated by asterisks in Table 2). The increase in redness was greatest for plants receiving elevated CO₂ and no nitrogen addition, and least for plants receiving ambient CO₂ and nitrogen addition (Table 2).

Paired tests also revealed similar responses of trichome number for plants within each of the four treatments. The number of trichomes per leaf increased in response to each combination of CO₂ and nitrogen addition (ambient CO₂ without nitrogen addition Wilcoxon $Z = -2.86$, $p = 0.004$; ambient CO₂ with nitrogen addition paired $t = -3.88$, $df = 15$, $p = 0.001$; elevated CO₂ without nitrogen addition paired $t = -3.87$, $df = 16$, $p = 0.001$; elevated CO₂ with nitrogen addition paired $t = -2.62$, $df = 16$, $p = 0.019$, indicated by asterisks in Table 2). The increase in trichome number was greatest for plants receiving ambient CO₂ and nitrogen addition, and least for plants receiving elevated CO₂ and nitrogen addition (Table 2).

Paired tests also revealed treatment-specific responses in the number of leaves per plant. Leaf number did not change significantly for plants experiencing ambient CO₂ without and with nitrogen addition, or elevated CO₂ without nitrogen addition (paired $t = 1.22$, $df = 19$, $p = 0.24$; paired $t = 0.53$, $df = 19$, $p = 0.61$; paired $t = 1.08$, $df = 19$, $p = 0.30$, respectively) In contrast, plants experiencing elevated CO₂ with nitrogen addition showed a significant decrease in number of leaves per plant (paired $t = 2.82$, $df = 19$, $p = 0.01$, indicated by an asterisk in Table 2).

After five weeks in the chambers, the weight of sticky dew per leaf did not differ among

D. intermedia plants in the four treatments, though there was some suggestion that they might if exposed to the treatments for a longer time ($X^2 = 6.16$, $df = 3$, $p = 0.10$) (Table 2). In fact, post hoc pairwise comparisons revealed several differences between treatments. Plants experiencing elevated CO₂ without nitrogen addition produced significantly more dew than those experiencing ambient CO₂ without nitrogen (Mann-Whitney U = 63.0, $n = 31$, $p = 0.024$), and nearly significantly more dew than those experiencing elevated CO₂ with nitrogen addition (Mann-Whitney U = 70.5, $n = 30$, $p = 0.081$).

Table 2. Summary of effect of treatment on *D. intermedia* prey-capture traits when exposed to ambient or elevated CO₂ with or without simulated increased atmospheric nitrogen deposition. Initial and final values for % redness were measured on July 7 and August 11, for trichome number on July 7 and August 11, and for leaf number on July 7 and August 11. Stickiness was only measured once, at the end of the study, on August 12. Stickiness was measured as mg of dew per leaf. Asterisks indicate significant differences between initial and final values for plants within a given treatment (* p < 0.05, ** p < 0.01, *** p < 0.001).

CO ₂ Level Nitrogen Addition	Ambient No	Ambient Yes	Elevated No	Elevated Yes	Among Treatment p-value
Initial % Redness	34.7 ± 22.1	40.9 ± 18.8	41.7 ± 25.4	37.0 ± 21.7	0.70
Final % Redness	56.8 ± 19.0**	59.6 ± 18.0*	66.2 ± 15.7***	61.8 ± 17.8**	0.45
Initial Trichome Number	31.6 ± 12.6	26.7 ± 8.6	26.1 ± 9.3	30.7 ± 9.3	0.31
Final Trichome Number	43.1 ± 8.1**	41.9 ± 11.4***	40.6 ± 10.2***	39.6 ± 11.0*	0.77
Initial Leaf Number	10.7 ± 3.6	11.2 ± 3.1	11.2 ± 3.2	11.3 ± 3.0	0.94
Final Leaf Number	9.3 ± 5.7	10.4 ± 6.7	9.8 ± 5.6	7.7 ± 5.3*	0.49
Stickiness (mg dew)	0.7 ± 0.2	1.0 ± 0.6	1.2 ± 0.7	0.8 ± 0.7	0.10

Discussion

Human activities are increasing the levels of both carbon and nitrogen available to plants. Since their leaves serve the dual functions of carbon and nitrogen capture, it will be very important for carnivorous plants to adjust to the changing availability of both in the future. The results of this study suggest that the carnivorous sundews, *Drosera rotundifolia* and *D. intermedia*, are to some extent able to alter their morphology and physiology in ways that are likely to be advantageous in the future.

We expected that, when exposed to simulated increases in atmospheric nitrogen deposition, *D. rotundifolia* would become less red (more green), since red tissue enhances nitrogen acquisition via prey capture (Schaefer and Ruxton 2008; Newell and Nastase 1998). Consistent with this expectation, *D. rotundifolia* plants in the B2 (low nitrogen addition) treatment became significantly (approximately 12%) greener, and plants in the control treatment did not display a significant change in color. However, contrary to expectation, plants in the A1FI (high nitrogen addition) treatment also did not display a significant change in color. It is possible that high nitrogen addition allowed the A1FI plants to retain their leaves longer, and that older leaves are redder. Carbon not used to make new leaves may have been invested in root growth, but we cannot evaluate this hypothesis.

Consistent with expectation, the number of trichomes per leaf decreased significantly for *D. rotundifolia* in the B2 and A1FI treatments (by 14.6 and 15.0, respectively), but not for plants in the control treatment. This suggests that with increasing nitrogen deposition, sundews will invest less in trichomes, leading to an overall reduction in stickiness and, thus, prey capture.

Overall, sundews experiencing the B2 (low nitrogen addition) treatment became greener

with fewer trichomes. Sundews experiencing the A1FI (high nitrogen addition) did not change color, but produced fewer trichomes. It appears that A1FI plants invested more in carbon capture since nitrogen was not as limiting a nutrient as it was for B2 plants. Together, these observations suggest that *D. rotundifolia* prey capture traits are phenotypically plastic in response to altered nitrogen availability, though this plasticity may be more effective as a response to a moderate rather than a large increase in nitrogen availability.

When plants have little nitrogen, older leaves must be shed and replaced by younger, stickier leaves. This may have been the case with the plants that received the control and B2 treatments. However, under the A1FI (high) nitrogen deposition, sundews do not need younger, stickier leaves because plants would rely less on prey-derived nitrogen and more on root-derived nitrogen (Millet, et al., 2012). This would allow plants in the A1FI treatment to retain their older leaves for longer without needing to replenish lost trichomes. Therefore, nitrogen addition may have led to slower leaf turnover and hence redder leaves with fewer trichomes.

However, changes in leaf number do not support this hypothesis. Leaf number decreased significantly for plants in the control and A1FI treatments (by 1.1 leaves), but not for plants in the B2 treatment. One variable that was not included in this study was leaf turnover rate. The number of leaves per plant was only measured at the start and end of the study. It is possible that leaf turnover rates were slower in the A1FI treatments than the other treatments. Alternatively, many *Drosera* species lose a considerable amount of the total N when leaves senesce (Adamec 1997). If this is the case, plants receiving the A1FI treatment could have afforded higher leaf turnover rates, whereas plants receiving the control and B2 treatments could not. If this study were to be repeated, we would measure the number of leaves weekly to better understand how nitrogen deposition directly affects leaf turnover and, therefore, indirectly affects percent red

tissue and number of trichomes per leaf.

When exposed to increased atmospheric CO₂ and nitrogen deposition, *D. intermedia* plants in all four treatments became significantly redder, and ANOVA detected no effect of treatment on this response. However, it is interesting that plants experiencing elevated CO₂ without or with nitrogen addition displayed a larger increase in redness (24.5% and 24.8%) than plants experiencing ambient CO₂ with or without nitrogen addition (18.7 and 22.1%), suggesting that future analysis using a split plot two-way ANOVA may reveal a significant CO₂ effect. This is consistent with our expectation that, under elevated CO₂, plants would invest more in nitrogen capture, as it is an even more limiting nutrient. Moreover, plants experiencing ambient CO₂ and nitrogen addition displayed the smallest increase in redness. It appears that these plants were receiving enough nitrogen from their roots that reliance on prey-derived nitrogen could be reduced. Therefore, the leaves could enhance carbon capture by slowing the transition of green tissue to red tissue that happens with age.

For plants in all treatments, the number of trichomes per leaf increased significantly, indicating that *D. intermedia* invested more in this prey capture trait over time. However, in contrast to redness, there was no obvious pattern of differences among treatments. The largest increases were observed for plants receiving ambient CO₂ with added nitrogen and elevated CO₂ without added nitrogen (15.2 and 14.5), and the smallest increases were observed for plants receiving ambient CO₂ without added nitrogen and elevated CO₂ with added nitrogen (11.5 and 8.9), suggesting that this prey capture trait will not respond in a simple manner to elevated CO₂ and increased atmospheric nitrogen deposition. It is interesting that plants experiencing elevated CO₂ and nitrogen addition showed the smallest increase in trichome number, since it was not obvious how sundews would react under simultaneous increases in both carbon and nitrogen

availability. It appears that elevated CO₂, which by our reasoning should increase trichomes, and nitrogen addition, which should decrease trichomes, partially cancel each other out when simultaneously acting on *D. intermedia*, resulting in a minor increase in the number of trichomes per leaf.

Across all treatments the number of leaves per plant decreased, but the change was significant only for plants receiving elevated CO₂ with nitrogen addition (by 3.6 leaves). It is not clear why these plants decreased their amount of surface area dedicated to carbon and nitrogen capture.

ANOVA did not detect a significant overall treatment effect on the weight of dew per leaf, our measure of stickiness of *D. intermedia* plants. However, pairwise comparisons revealed that plants that experienced elevated CO₂ alone were significantly stickier than those that experienced ambient CO₂ alone. This aligns with our expectation that increased carbon availability would result in increased investment in prey-capture traits, both because nitrogen is more limiting and more carbon is available to produce carbon-rich dew (Michalko et al., 2013). This would allow sundews to convert excess carbon into captured nitrogen. It is worth noting that, among plants experiencing elevated CO₂, those receiving no nitrogen addition were nearly significantly stickier than those receiving nitrogen addition. This suggests that *D. intermedia* also adjusts investment in prey-capture traits in response to nitrogen availability. Interestingly, there was very little difference in dew production between plants experiencing no added carbon or nitrogen and plants receiving both added carbon and nitrogen, suggesting that simultaneous future increases in carbon and nitrogen may largely cancel each other out with respect to dew production.

Our results suggest that high (A1FI) nitrogen addition affects the two sundew species in dissimilar ways. Percent redness did not change for *D. rotundifolia*, but increased for *D. intermedia*. Trichome number decreased for *D. rotundifolia* but increased for *D. intermedia*. This suggests that responses of carnivorous plants to future increases in atmospheric nitrogen deposition may be species-specific. Alternatively, the difference in response may be due to the fact that *D. rotundifolia* were in their natural habitat while *D. intermedia* were in more artificial conditions (in pots in chambers).

Our results may also have been affected by the choice of leaves for color and trichome analysis. Leaves directly facing the lens were the most in focus, and were selected for color analysis. However, this selection method does not guarantee that the analyzed leaf was the most representative leaf on the plant, or even that the same leaf was selected for analysis from week to week. Nonetheless, we suspect that this selection method introduced “noise” into our analyses and therefore is more likely to have obscured than created differences among treatments.

Unfortunately, our *D. intermedia* sample size was reduced because all of the leaves of 19 chamber plants had been eaten by herbivores (probably grasshoppers). Fortunately, eaten plants were distributed fairly equally among treatments (four in ambient CO₂ without nitrogen addition, five each in ambient CO₂ with nitrogen addition and elevated CO₂ without nitrogen, and six in elevated CO₂ with nitrogen addition).

If this study were to be repeated, we would increase our sample sizes in order to avoid a large influence of herbivory. Additionally, we would attempt to analyze the change in stickiness for both species. A future study would also benefit from a longer duration, ideally including multiple growing seasons.

In summary, the carnivorous sundews *D. rotundifolia* and *D. intermedia* appear capable of adjusting investment in prey-capture traits in response to increasing levels of atmospheric carbon and nitrogen. However, their ability to adaptively adjust investment in red coloration, trichomes, and sticky dew is far from complete, and will likely be species-specific. Additional research is needed to understand how sundews and other carnivorous plants will respond to anthropogenic alteration of Earth's atmosphere.

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Appendix

Total nitrogen deposition

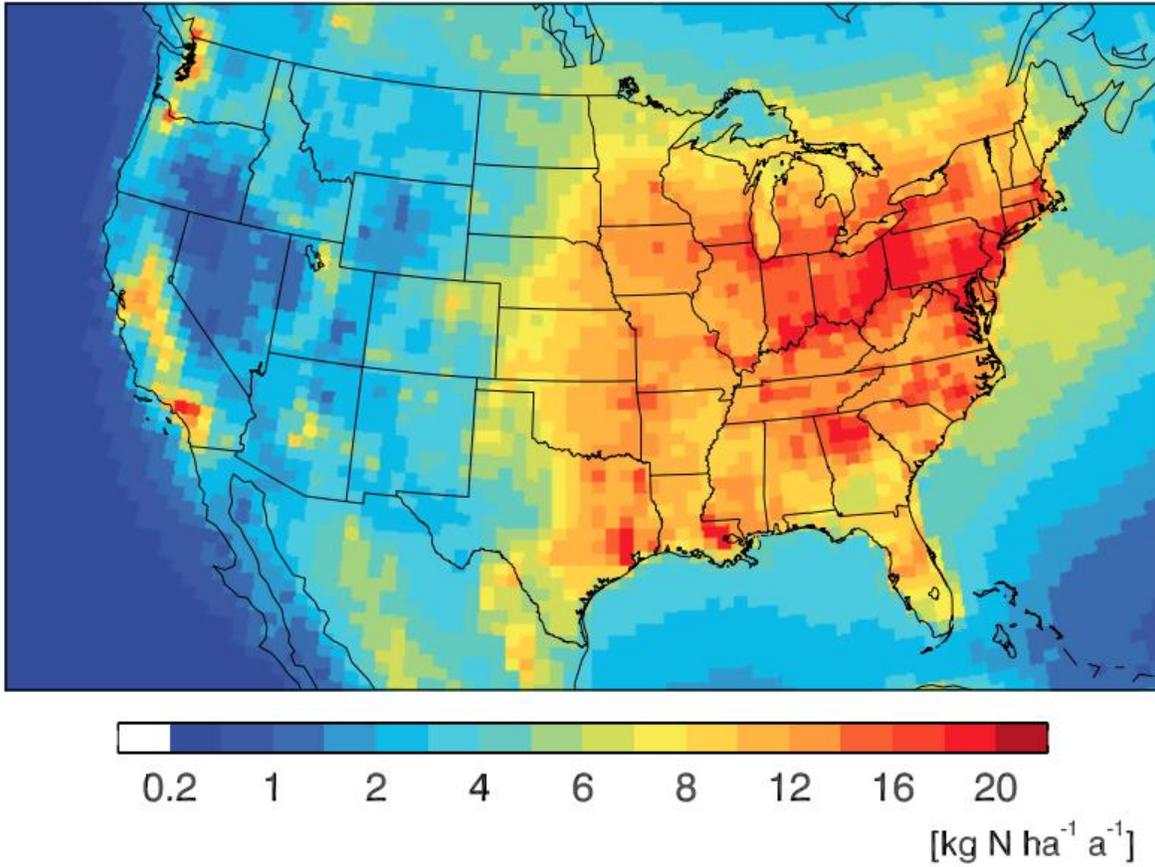


Figure 1. Annual total nitrogen deposition fluxes. Values are GEOS-Chem averages for 2006-2008. (Zhang et al. 2012)

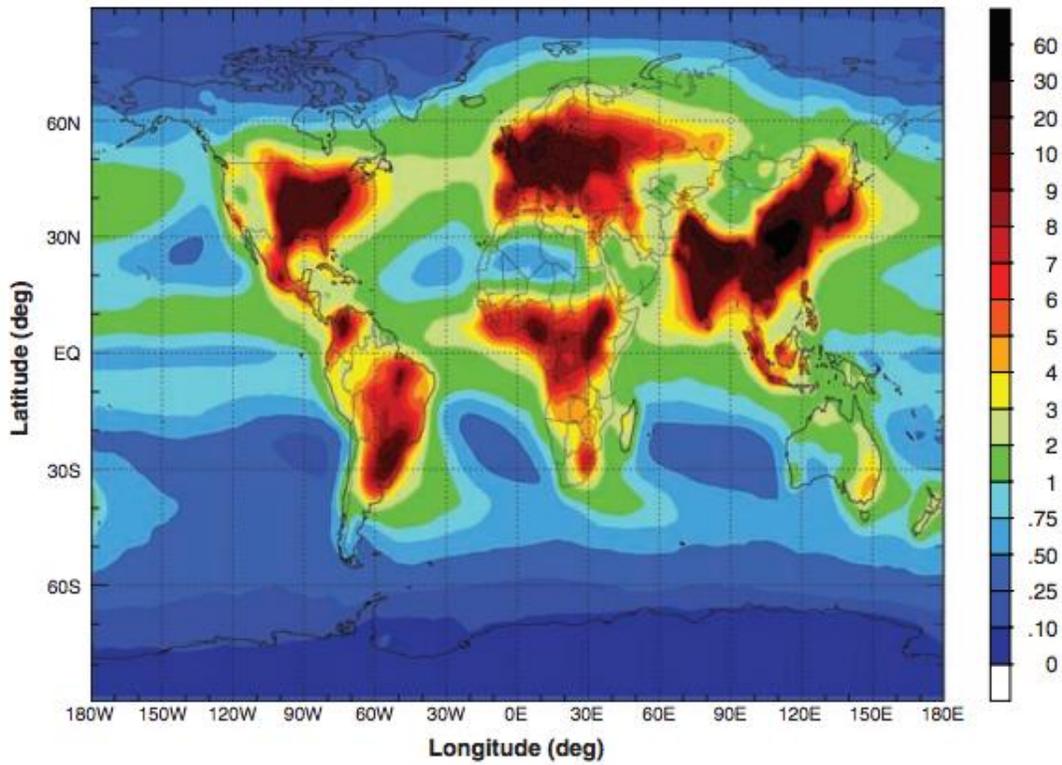


Figure 2. Estimated N deposition from global total N (NO_y and NH_x) emissions, totaling 105 Tg N y^{-1} . The unit scale is $\text{kg N ha}^{-1} \text{ y}^{-1}$, modified from the original units ($\text{mg m}^{-2} \text{ y}^{-1}$) (Galloway, et al., 2008).

Year	B2	A1FI
2020	43	50
2050	55	95
2100	61	110

Figure 3. NO_x emissions in MtN/yr under two IPCC SRES scenarios (IPCC, 2000).



Figure 4. *D. rotundifolia* in Mud Lake Bog has green and red leaf tissue (White, 2014).

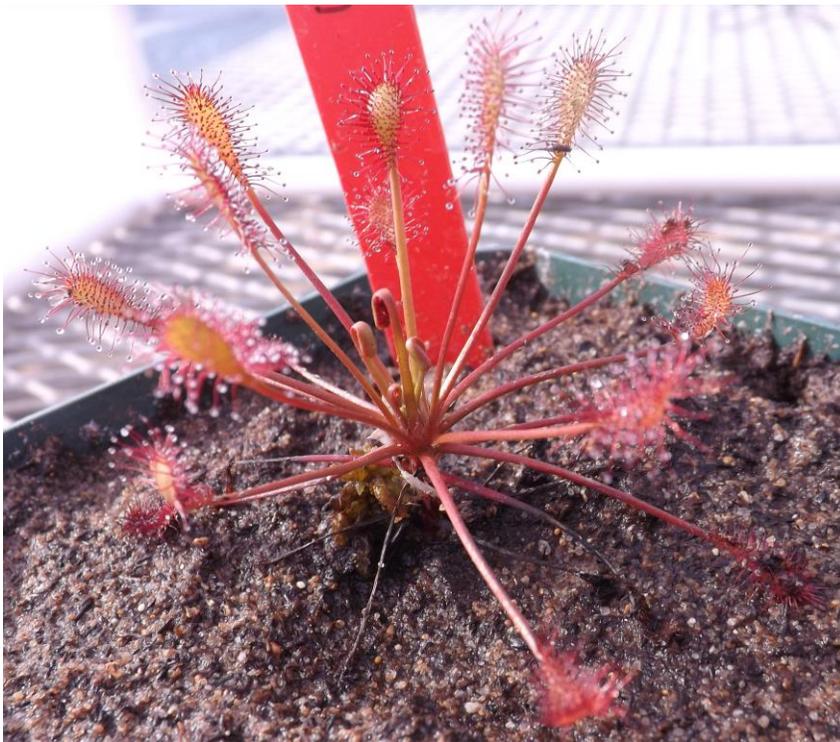


Figure 5. *D. intermedia* has green and red leaf tissue (White, 2014).

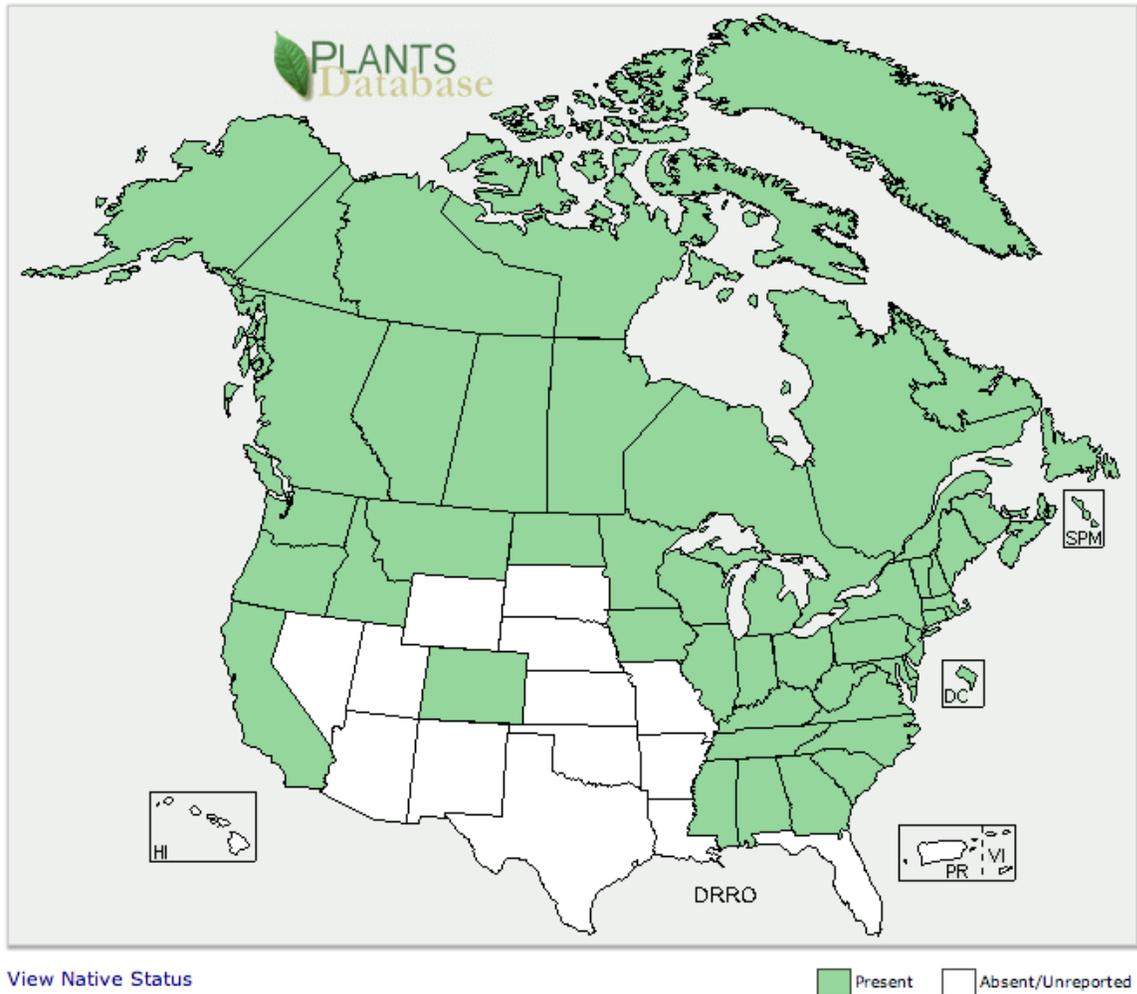


Figure 6. Distribution of *Drosera rotundifolia* (United States Department of Agriculture, n.d.)

