

Effect of environmental factors on prey-derived nitrogen in sundews (Drosera rotundifolia) in a northern Michigan bog



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Abstract

In this study, $\delta^{15}\text{N}$ values of insectivorous plants, their prey, and non-carnivorous reference plants were used to determine the relative contributions of nitrogen originating from prey and soil to the nutrition of *Drosera rotundifolia* plants in a northern Michigan bog. To determine which environmental factors that most strongly affect the relative contributions of plant- and insect-derived N within a single population of carnivorous plants, soil pH, temperature, dissolved inorganic nitrogen (as NH_4^+), and insect availability adjacent to 26 sundew plants were measured. Stable nitrogen isotope analysis was used to determine which variable was most strongly correlated with the proportion of prey-derived nitrogen across plants. This study provided a test of the hypothesis that carnivory evolved in acidic, low-nutrient habitats largely because low pH inhibits decomposition, thereby reducing the availability of plant-derived nitrogen and creating strong selection for modification of leaves into structures capable of capturing insect nitrogen. The population of *Drosera rotundifolia* was found to derive an average of 44% of its nitrogen from prey. However, the variation among individuals and thus the relative importance of carnivory varied across individuals by almost 4-fold. Neither pH nor temperature, nor insect availability as a whole were significantly correlated with the proportion of prey-derived nitrogen. The commonly held belief that pH slows decomposition which decreases ammonium availability did not appear to be upheld in this bog. The major driver of the relative importance of carnivory in Mud Lake Bog may be the abundance of small, weak flying prey that are likely to be attracted to the red glistening leaves of sundews such as midges.

Introduction

Carnivory has evolved independently among angiosperms several times, and *ca.* 630 species of carnivorous plants have been described (Porembski and Barthlott 2006). In general, carnivorous plants are sun-loving, weak rooted perennials that live in acidic, low nutrient habitats. Carnivorous plants are able to tolerate low-nutrient soils in large part because they have evolved an array of mechanisms for luring, trapping, digesting, and absorbing the digestive products from animal prey (Schnell 2002).

The common sundew, *Drosera rotundifolia*, uses adhesive traps to capture prey. *Drosera rotundifolia* is a perennial herb with fibrous roots, short stems, prominent petioles, and leaves covered in mucilaginous stalked and sessile glands. Prey, usually small insects, either land on or crawl over the leaf blade surface and become stuck in the mucilage. The long, glandular tentacles attached to the prey bend inward while the glands produce digestive juices with proteases, esterases, and acid phosphatases, and the insect is digested on the leaf surface. *Drosera rotundifolia* is a rosette found commonly in *Sphagnum* bogs and can grow vigorously in areas that are too harsh for other carnivorous plant species (Schnell 2002).

Bogs are an ecosystem abundant in the glaciated circumboreal landscapes of the Northern hemisphere region (Larsden 1982). Bogs develop when kettle holes are invaded by *Sphagnum* moss which acidifies the water by cation exchange, resulting in a low pH usually in the range of 3.3-4.5 (Clymo 1964). Such acidic conditions slow the rates of decomposition and nutrient cycling, and thereby substantially decrease the availability of dissolved inorganic nitrogen (DIN) to plants. Availability of ammonium in particular is lower in areas of low pH (Bragazza and Gerdol 2002). Thus, despite the presence of much undecomposed organic matter, bogs are a nutrient-poor environment.

Insect capture is assumed to be an adaptation to a low nutrient environment. Givnish et al. (1984) propose that carnivorous plants evolved in sunny, wet areas with low nitrogen availability, and offer a cost-benefit model that postulates a trade-off between the photosynthetic costs of carnivorous structures and the photosynthetic benefits accrued through additional nutrient acquisition. In their interpretation, carnivory should evolve if the energetic benefits outweigh the costs of expensive investments in carnivorous adaptations because plants with these special structures should gain a competitive edge in sunny, wet, nutrient poor habitats (Givnish et al. 1984). However, their position that carnivory may increase a plant's total rate of photosynthesis as a result of increased mineral absorption remains unsupported by current research. In fact, the photosynthetic nutrient use efficiency (PNUE = $\mu\text{mol CO}_2$ fixed per mol nutrient per unit time) of carnivorous plants may be as low as 20-50% of their non-carnivorous counterparts (Ellison 2006).

Since the benefits of carnivory are mainly tied to nutritional gain, benefits should decrease as soil nutrient availability decreases. Indeed, a study conducted in Finland found that *D. rotundifolia* individuals that received added nutrients decreased their investment in carnivory which was quantified in terms of leaf stickiness (Thoren et al. 2003). This study predicts an increasing investment in insect-capturing structures as nutrient availability decreases. Since the nutrient gradient should vary with the pH in a bog, one would expect to see plants with higher percentages of prey-derived nitrogen content in areas of low pH and presumably lower nutrient availability. As bogs are nutrient depleted, waterlogged, sunny habitats in which carnivorous plants can be found in abundance, they represent a good habitat in which to study in ecological time the factors that presumably have driven the evolution of carnivory.

Natural abundance stable isotope measurement is an advanced method of directly determining the contribution of two nitrogen (N) sources to a single N sink (Peterson & Fry 1987). Because $\delta^{15}\text{N}$, the ratio of the two stable isotopes of nitrogen (^{14}N and the less common ^{15}N), becomes enriched in ^{15}N by 3-4% at each increased trophic level (Post 2002), stable nitrogen isotope analysis can be used to determine the relative contributions of N from soil (lower $\delta^{15}\text{N}$) and from insect prey (higher $\delta^{15}\text{N}$) for a natural population of carnivorous plants. Differences $\delta^{15}\text{N}$ are caused because enzyme mediated-reactions commonly discriminate against the heavier ^{15}N (Dawson et al. 2002).

Several studies have used stable nitrogen isotopes to determine the percent of nitrogen carnivorous plants derive from prey (Schulze et al. 1991, Moran et al. 2001, Millet et al. 2003). For instance, Millet et al. (2003) determined that about 50% of the total N in *D. rotundifolia* is of insect origin. However, the relative contribution of insect-derived N varied among individual sundews from 30 to 65 %. Similarly, Schulze et al. (1991) reported a 50% difference within a population of Australian sundews. Though individual plants within a population clearly vary in the proportion of nitrogen derived from insects, to our knowledge no study has addressed the underlying cause(s) of this variation. In principle, many factors, such as soil pH, temperature, availability of insects, the composition of the microbial community, and competition from other plants could vary over small spatial scales in a manner that would produce variation in the relative importance of plant- and insect-derived N to individual carnivorous plants. For instance, lower pH and lower temperature might inhibit microbial decomposition, thereby reducing local availability of plant-derived N and, presumably, increasing the relative contribution of insect-derived N to carnivorous plants. Higher local insect abundance and stronger local competition from other plants could produce the same effect.

In this study, $\delta^{15}\text{N}$ values of insectivorous plants, their prey, and non-carnivorous reference plants are used to determine the relative contributions of nitrogen originating from prey and soil to the nutrition of *Drosera rotundifolia* plants in a northern Michigan bog. The main goal of this study is to determine the environmental factors that most strongly affect the relative contributions of plant- and insect-derived N within a single population of carnivorous plants. To do so, soil pH, temperature, dissolved inorganic

nitrogen (as NH_4^+), and insect availability adjacent to 26 sundew plants will be measured. Stable nitrogen isotope analysis will be used to determine which of these variables is most strongly correlated with the percent of insect-derived nitrogen across plants. In addition, this study provides a test of the hypothesis that carnivory evolved in acidic, low-nutrient habitats largely because low pH inhibits decomposition, thereby reducing the availability of plant-derived nitrogen and creating strong selection for modification of leaves into structures capable of capturing insect nitrogen. If this scenario is true, we would expect pH to be positively correlated with NH_4^+ and, therefore, negatively correlated with the proportion of sundew N derived from insects.

Materials and Methods

Plants were collected from Mud Lake Bog (45° 36' N, 84°35' W) located six miles NE of the University of Michigan Biological Station in Pellston, Michigan. Samples were collected in July and August 2007. Mud Lake Bog is *Sphagnum* dominated and is inhabited by three species of carnivorous plants: *Sarracenia purpurea* (purple pitcher plant), *Drosera intermedia* (spatulate-leaved sundew), and *Drosera rotundifolia* (round-leaved sundew). *Drosera rotundifolia* was chosen for its local abundance and occurrence across a wide range of pH. Twenty six sample sites, each containing at least one sundew plant, were chosen throughout the bog lawn and closer to the lake on the *Sphagnum* floating mat. All sample sites were mapped with a GPS device (Figure 1).

At each sample site, one sundew plant was chosen and pH and temperature of the soil next to the plant were measured several times throughout the summer. pH was determined by inserting into the substrate a 6" PVC collar with holes drilled in the sides, allowing the surrounding water to enter (hereafter "well water"), and measuring pH with a pH meter. At the same time, temperature of the well water was measured with an alcohol thermometer and pH readings were corrected for differences in temperature (Table 1). pH was measured five times throughout the summer. In addition, soil temperature was measured three times with a thermocouple immediately under and also 10 cm below the sundew rosette; these depths were chosen to characterize the local temperature most relevant to decomposers. It is unlikely that *Drosera* root hairs penetrate deeper than 10 cm (Adlassnig et al. 2005). To determine the local availability of dissolved inorganic nitrogen, 60 mL of well water was collected twice at each site and analyzed for NH_4^+ with an AutoAnalyzer 3 Digital Colorimeter. At five sites, well water was also analyzed for NO_3^- to verify that nitrate levels were negligible.

Three to four leaves (every other leaf so as not to kill the plant) were collected from a single *D. rotundifolia* plant at each sample site. Each leaf was cleaned of any insect parts with a forceps under a dissecting scope. Since it was impossible for us to directly analyze the dissolved inorganic nitrogen collected from the well water for $\delta^{15}\text{N}$, we reasoned that *Sphagnum* spp. would be the most accurate proxy because they also directly absorb the NH_4^+ available to the very shallow *Drosera* roots, and therefore should have a $\delta^{15}\text{N}$ signature very similar to that absorbed by *Drosera* roots (Millett et al. 2003). In addition, *Sphagnum* is likely the major contributor to soil nitrogen in Mud Lake Bog. However, since individual sample sites were surrounded by various mixtures of non-carnivorous plants, if the $\delta^{15}\text{N}$ signature varied among non-carnivorous plants, then it seemed likely that the $\delta^{15}\text{N}$ of the *Sphagnum* would also differ among sample sites. Indeed, $\delta^{15}\text{N}$ signatures of the 13 most common non-carnivorous species (*Andromeda polifolia*, *Alnus rugosa*, *Chamaedaphne calyculata*, *Carex lasiocarpa*, *Dulichium arundinaceum*, *Kalmia polifolia*, *Larix laricina*, *Picea mariana*, *Potentilla palustris*, *Rhynchospora alba*, *Sphagnum* spp., *Scheuchzeria palustris*, *Vaccinium oxycoccos*) varied from -1.0 to -11.5 (Table 2). Therefore, we collected a separate sample of undecayed *Sphagnum* spp. from the surface and a sample of partially decomposed peat from directly under the surface at each sample site.

All samples were frozen at -80°C for several hours, lyophilized for 24-48 hours, ground to a fine powder with a mortar and pestle, and analyzed for stable nitrogen isotope ratio and C:N ratio using an Elemental Analysis Combustion 2010 System and Thermo Finnigan Plus XP mass spectrometer. C:N ratio was measured as an indicator of N stress and thus as a proxy for plant health.

To estimate the abundance of insects at each site, Aphid Whitefly Sticky traps made of nitrogen free petroleum wax (ARBICO-organics, Tucson, AZ) were placed at each site for 65 h from July 27 to July 30. The contents of the sticky traps were counted and identified. Using a dissecting microscope, the number of individuals smaller than approximately 10 mm (the maximum size assumed to be caught by sundews) were counted and classified into the following categories: homopterans, acalypterate muscoid flies, dolichopodid flies, midges, wasps, thrips, and springtails. Occasionally, traps caught small beetles, mites, spiders, sciomyzid flies, and moths, but they were too rare to be included in our analyses. To estimate the average biomass of each insect category, insects were collected on August 2 and August 4 using a sweep net and aspirator. Four to fifteen individuals from each category were dried at 60°C for approximately 12 h and weighed collectively to provide an average weight. After weighing, individuals from each category were ground with a mortar and pestle, and analyzed for $\delta^{15}\text{N}$ signature.

Whether an insect is a carnivore or herbivore and whether the nymphs are terrestrial or aquatic should presumably affect its $\delta^{15}\text{N}$. To account for this difference, a weighted average $\delta^{15}\text{N}$ value for each individual site was created using the biomass and abundance data collected through the sticky traps and sweep net samples. Insects taken directly from leaves were not used as they were unidentifiable, partially digested, and covered in digestive juices that might alter their correct $\delta^{15}\text{N}$ values.

Mixing Model

The relative contribution of insect nitrogen to the total nitrogen content of *D. rotundifolia* plants was calculated by using a single isotope two source weighted mixing model, using the $\delta^{15}\text{N}$ of the *D. rotundifolia* individuals ($\delta^{15}\text{N}_D$), the $\delta^{15}\text{N}$ of insect prey ($\delta^{15}\text{N}_I$), and the $\delta^{15}\text{N}$ of the *Sphagnum* moss ($\delta^{15}\text{N}_S$) (Schulze et al. 1991).

$$\%N \text{ from insect prey} = (\delta^{15}\text{N}_D - \delta^{15}\text{N}_S) / (\delta^{15}\text{N}_I - \delta^{15}\text{N}_S)$$

The $\delta^{15}\text{N}_I$ values were calculated individually for each sample site using a weighted average of the insects caught at that site's sticky trap:

$$\delta^{15}\text{N}_I = \sum_i (\text{Biomass}_i * \delta^{15}\text{N}_i) / \text{Total Insect Biomass}$$

where Biomass_i = the biomass of insect category *i* (e.g. midges) and $\delta^{15}\text{N}_i$ = the $\delta^{15}\text{N}$ signature of insect category *i*. This weighted average was calculated both using all categories for which we could collect sufficient numbers to determine an average weight (homopterans, acalypterate muscoid flies, dolichopodid flies, midges, and wasps) and for flies only (acalypterate muscoid flies, dolichopodid flies, and midges), which are most likely to be attracted to the glistening surface of sundew plants (Brian Scholtens, personal communication).

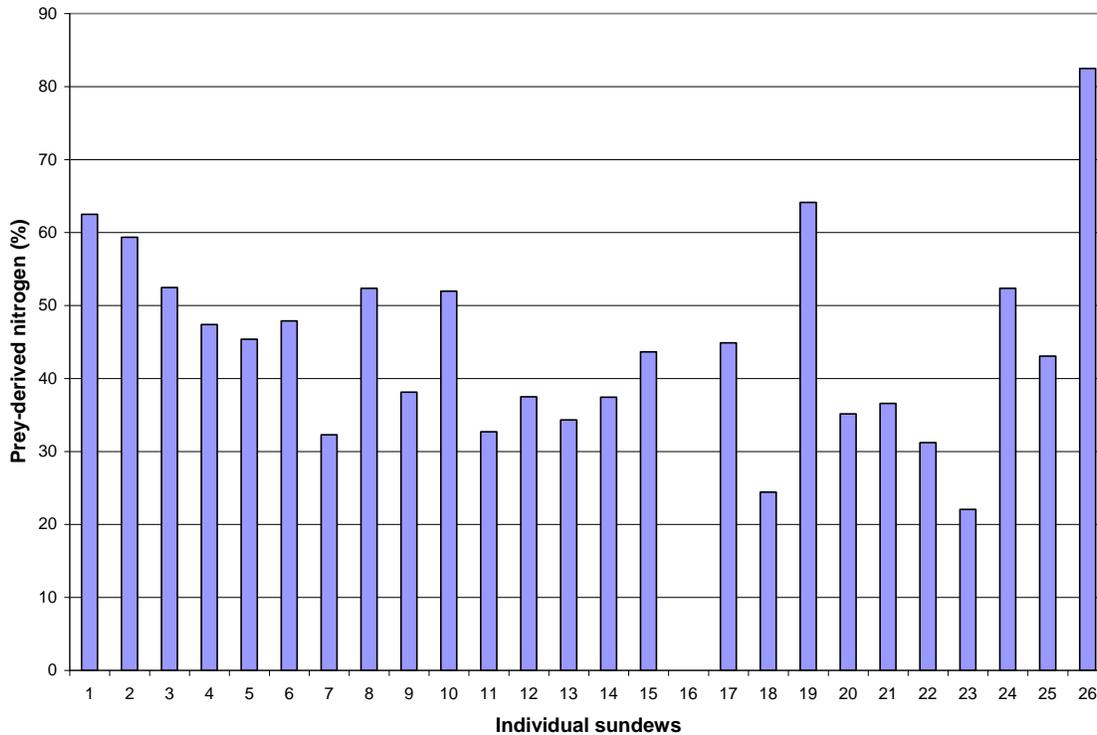
Data Analysis

Linear regression/correlation tests were performed (using SPSS 15.0 for Windows) to determine if pH or temperature was correlated with ammonium, whether the percent of N derived from insect prey was correlated with ammonium, temperature, pH, and/or local availability of insects, and the C:N ratio of sundew leaves.

Results

On average, *D. rotundifolia* at Mud Lake Bog obtained 44% of their N from insect prey. However, the relative importance of insect-derived N varied greatly among individual sundews; plants obtained as little as 22.1 % or as much as 82.5% of their N from insects (Figure 2).

Figure 2. Percentage of prey-derived N for 25 sundew plants in Mud Lake Bog collected in July of 2007 in northern Michigan.



Environmental Factors that Affect Relative Importance of Carnivory

Given that the percentage of prey-derived N, and therefore the relative importance of carnivory, varied almost 4-fold among individual sundew plants, it was of interest to determine which environmental factor(s) may be responsible for this variation.

Across the 26 sample sites, the ammonium levels ranged from 32-1099 $\mu\text{gN/l}$. Across the 26 sample sites, pH ranged from 3.29-5.17 (see Figure 1 for spatial distribution of pH). Contrary to our expectation of a positive correlation between ammonium and pH, ammonium and pH were nearly significantly negatively correlated (d.f. = 25, $R = -0.336$, $p = 0.093$; Figure 3).

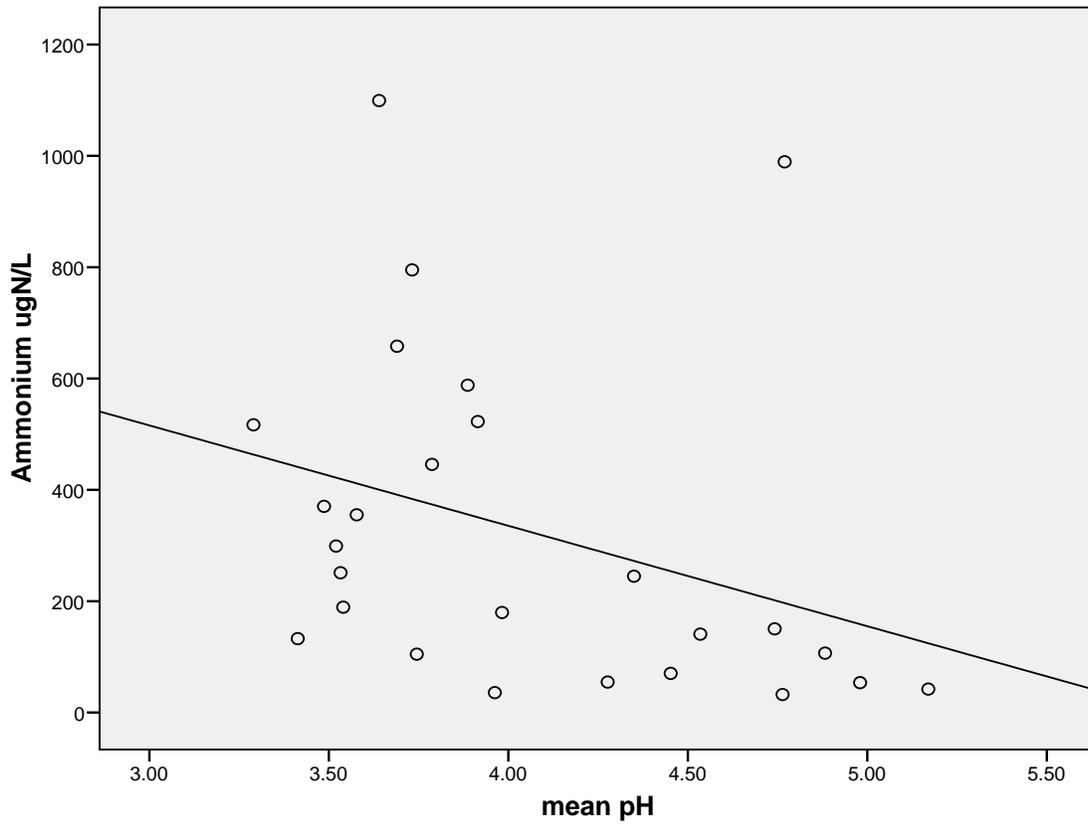


Figure 3. Correlation between mean pH and ammonium levels in well water. Each point represents conditions adjacent to an individual sundew plant.

Surprisingly, the percentage of insect-derived N was not correlated with variation in pH among sites (d.f. = 24, $R = 0.156$, $p = 0.457$; Figure 4).

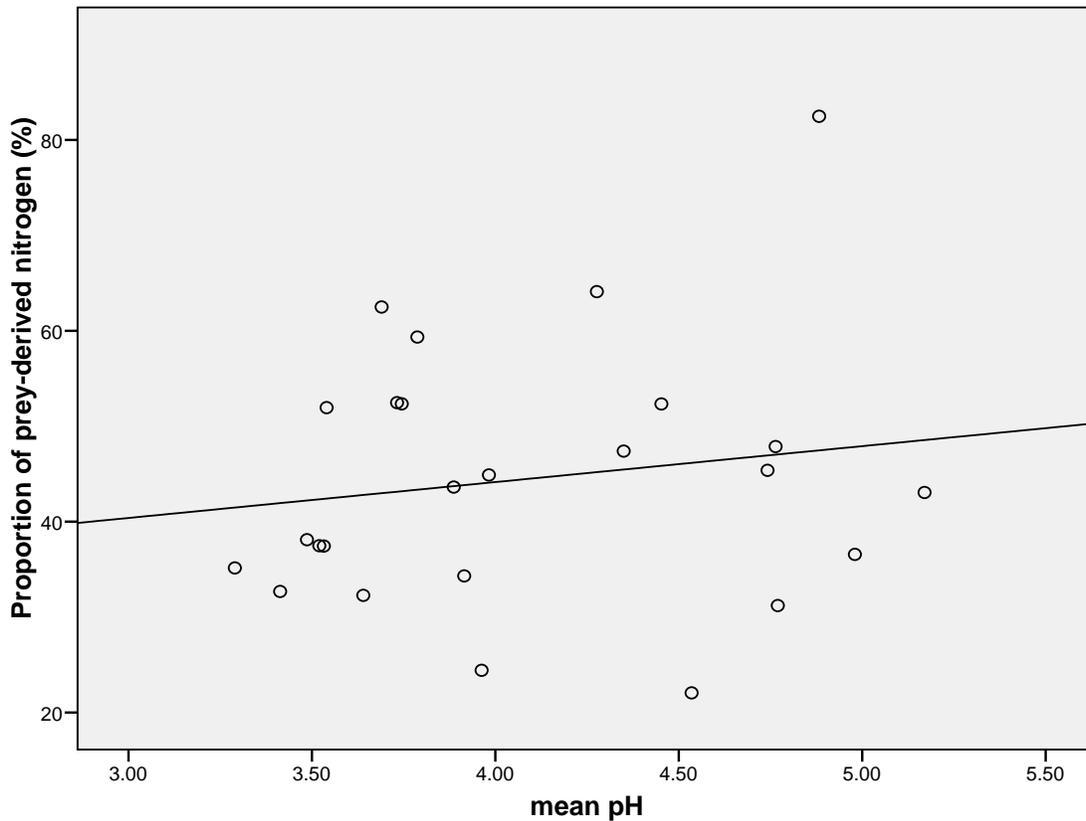


Figure 4. Lack of correlation between prey-derived N of each individual sundew and mean pH adjacent to each sundew (N = 25).

Average surface temperature across three dates was 28.4 °C, and average temperature 10 cm below the surface was 19.4 °C. However, at individual sites average surface temperature ranged from 23.5 to 32.3 °C, and average temperature at 10 cm ranged from 17.2 to 21.9. Among sites, average surface and 10 cm temperatures were positively correlated (d.f. = 25, $R = 0.686$, $p < 0.0005$; data not shown). The percent of prey-derived N was not correlated with average surface temperature or average 10 cm temperature (d.f. = 24, $R = -0.290$, $p = 0.175$ and d.f. = 24, $R = 0.109$, $p = 0.605$, respectively; Figure 5 and 6).

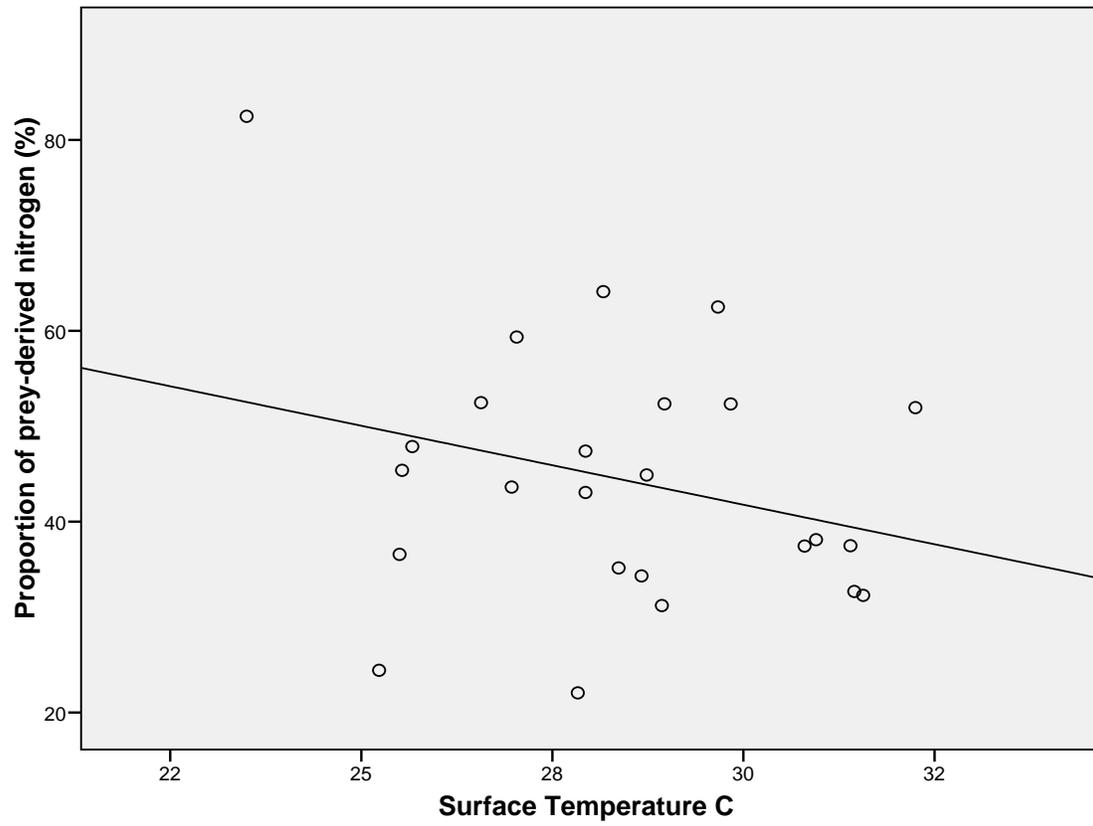


Figure 5. Lack of correlation between prey-derived nitrogen of each individual sundew and average surface temperature (°C) (N = 25).

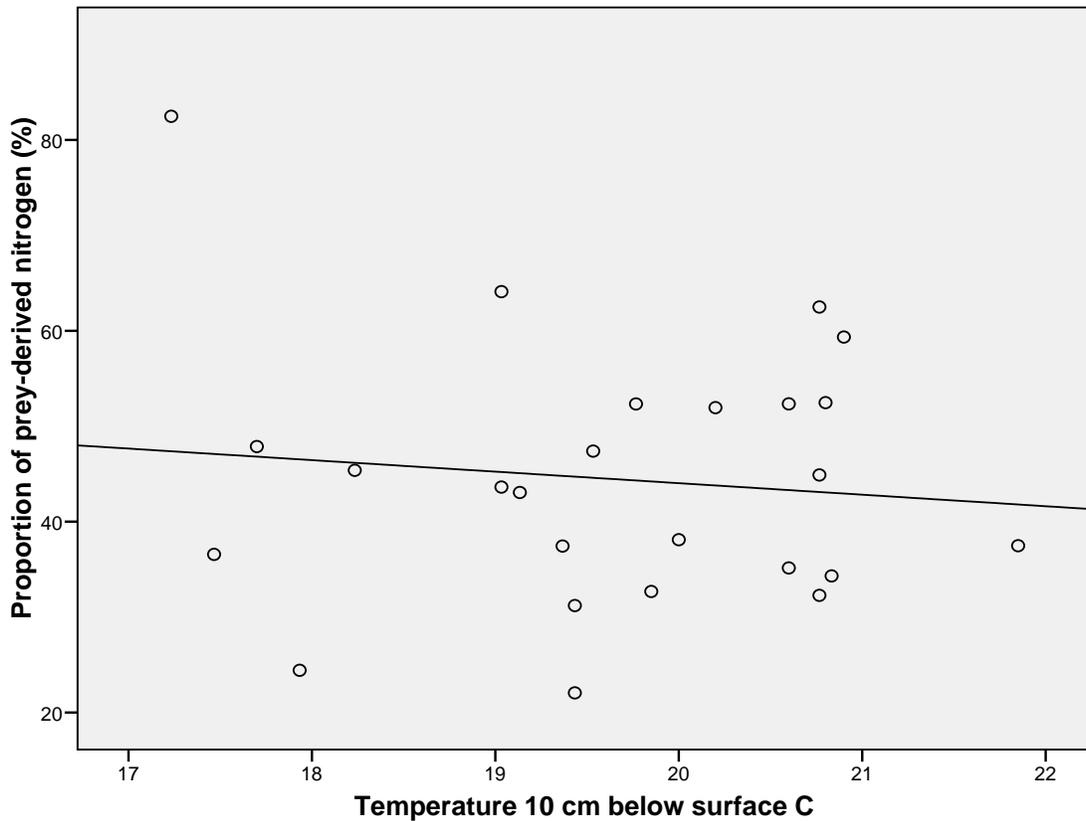


Figure 6. Lack of correlation between percent of prey-derived nitrogen for each individual sundew and the average temperature at 10 cm below the surface ($^{\circ}\text{C}$) ($N = 25$).

Average surface temperature was not correlated with ammonium levels across the 26 sites (d.f. = 25, $R = 0.267$, $p = 0.187$; Figure 7). However, average temperature 10 cm below the surface was significantly positively correlated with ammonium availability (d.f. = 25, $R = 0.479$, $p = 0.013$; Figure 8).

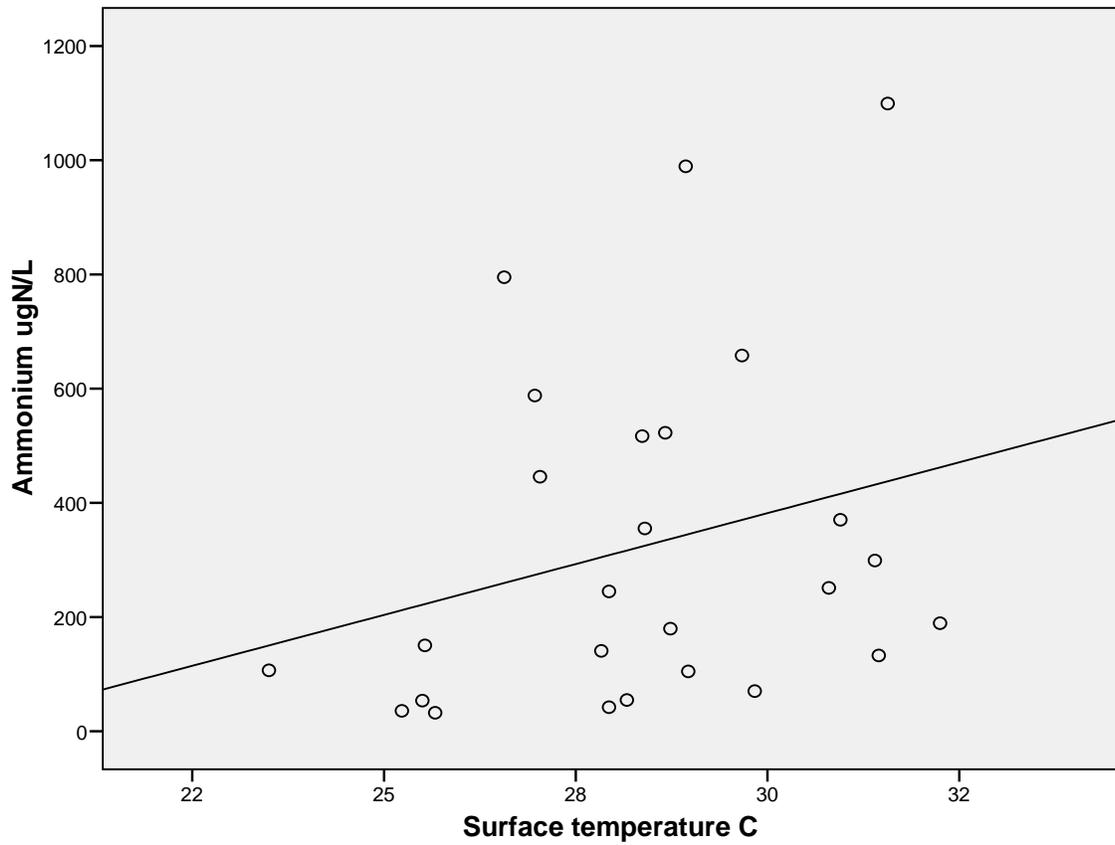


Figure 7. Lack of correlation between average ammonium levels of well water and average surface temperature ($^{\circ}\text{C}$) at each sundew ($N = 26$).

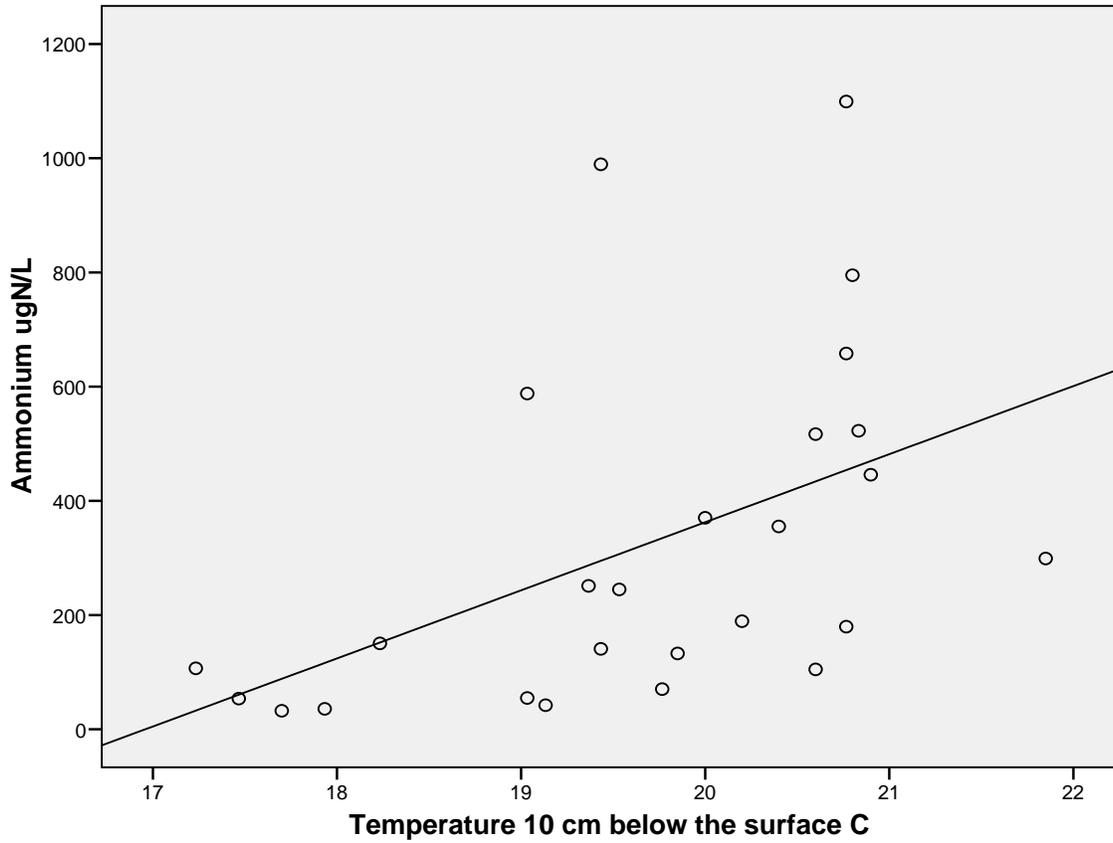


Figure 8. Correlation between average ammonium levels in well water and average temperature ($^{\circ}\text{C}$) 10 cm below the surface of each sundew plant ($N = 26$). Each point represents the conditions adjacent to each individual sundew.

The average fly biomass accessible to each individual sundew was 5.52 mg, but fly biomass ranged from 2.6 to 12.3 mg across sites. The average insect biomass at each site was 10.82 mg, but access to insects was quite variable and ranged from 4.2 to 17.3 mg. The proportion of prey-derived N was not correlated with total prey biomass (d.f. = 24, $R = 0.043$, $p = 0.840$; Figure 9) or with fly biomass (d.f. = 24, $R = 0.091$, $p = 0.666$; Figure 10).

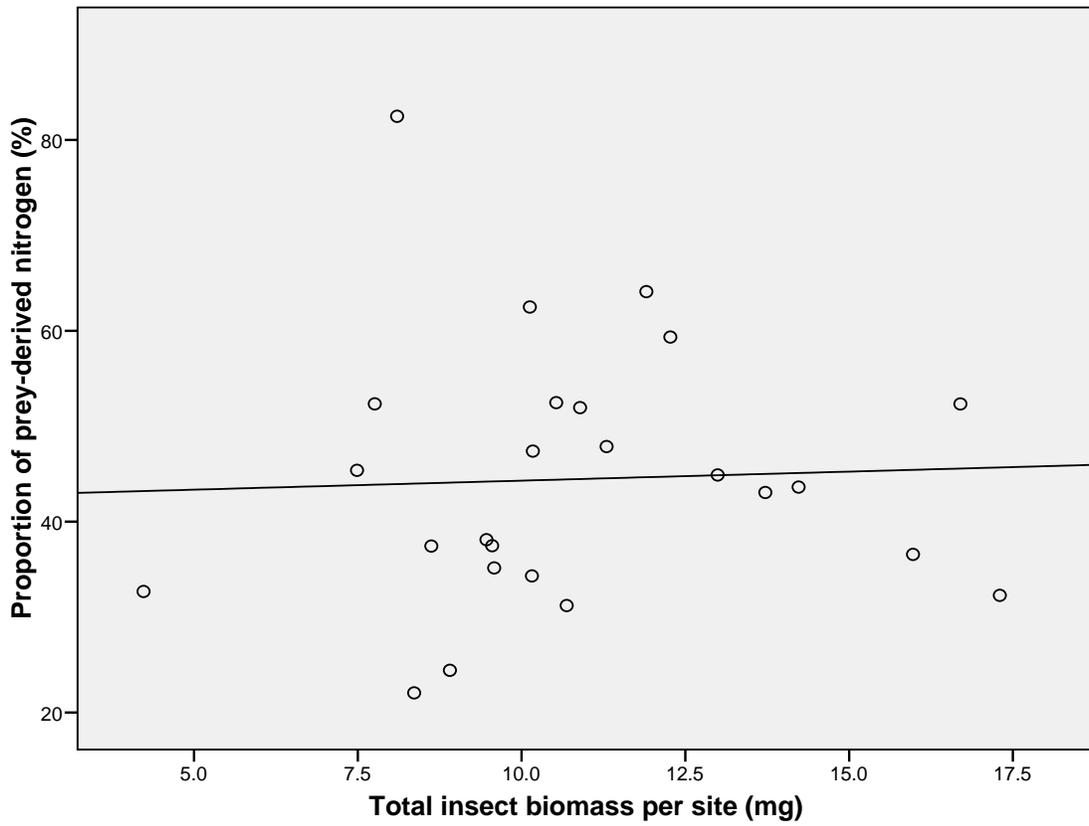


Figure 9. Lack of correlation between prey-derived nitrogen per sundew and total insect biomass in mg available to each sundew ($N = 25$).

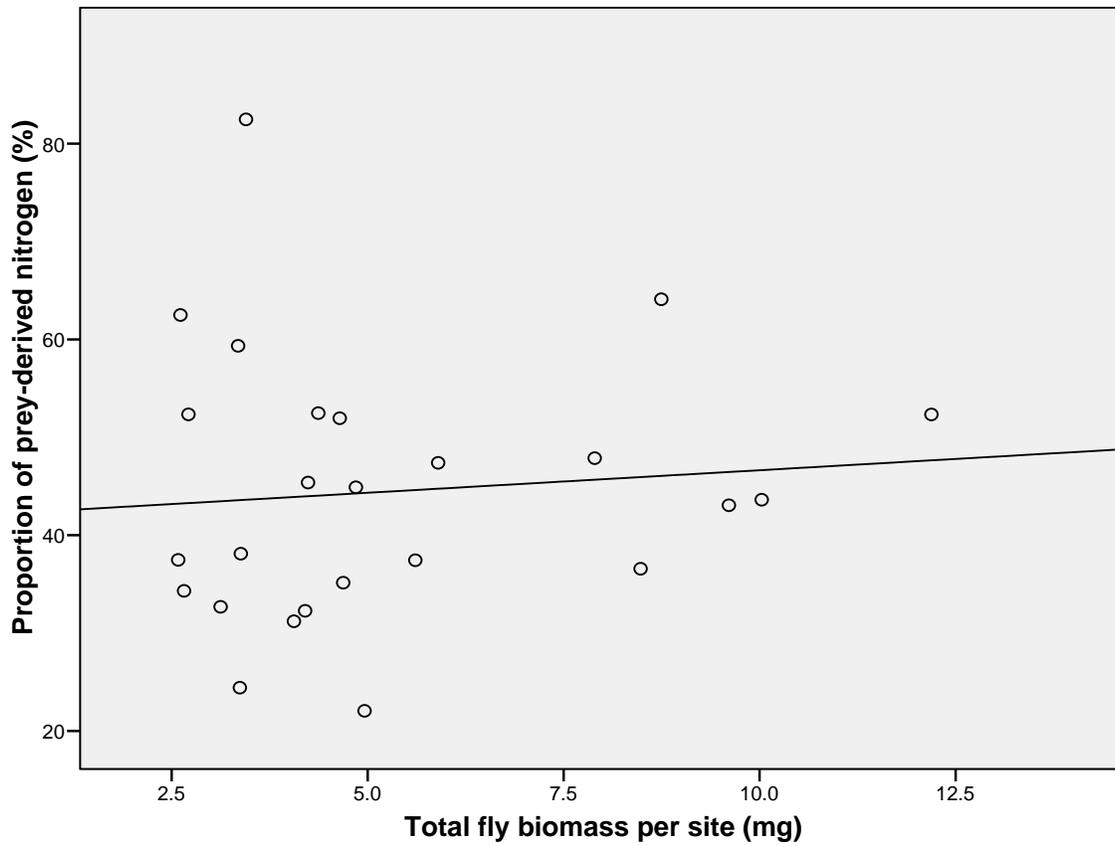


Figure 10.

Lack of correlation between prey-derived nitrogen per sundew and total fly biomass available at each site ($N = 25$). Each point represents the amount of available fly biomass in mg to each individual sundew.

In contrast, when comparing the percent of prey-derived nitrogen with the biomass of a single insect group, midge biomass is nearly significantly positively correlated with percent of prey-derived nitrogen ($d.f. = 24$, $R = 0.381$, $p = 0.06$; Figure 11).

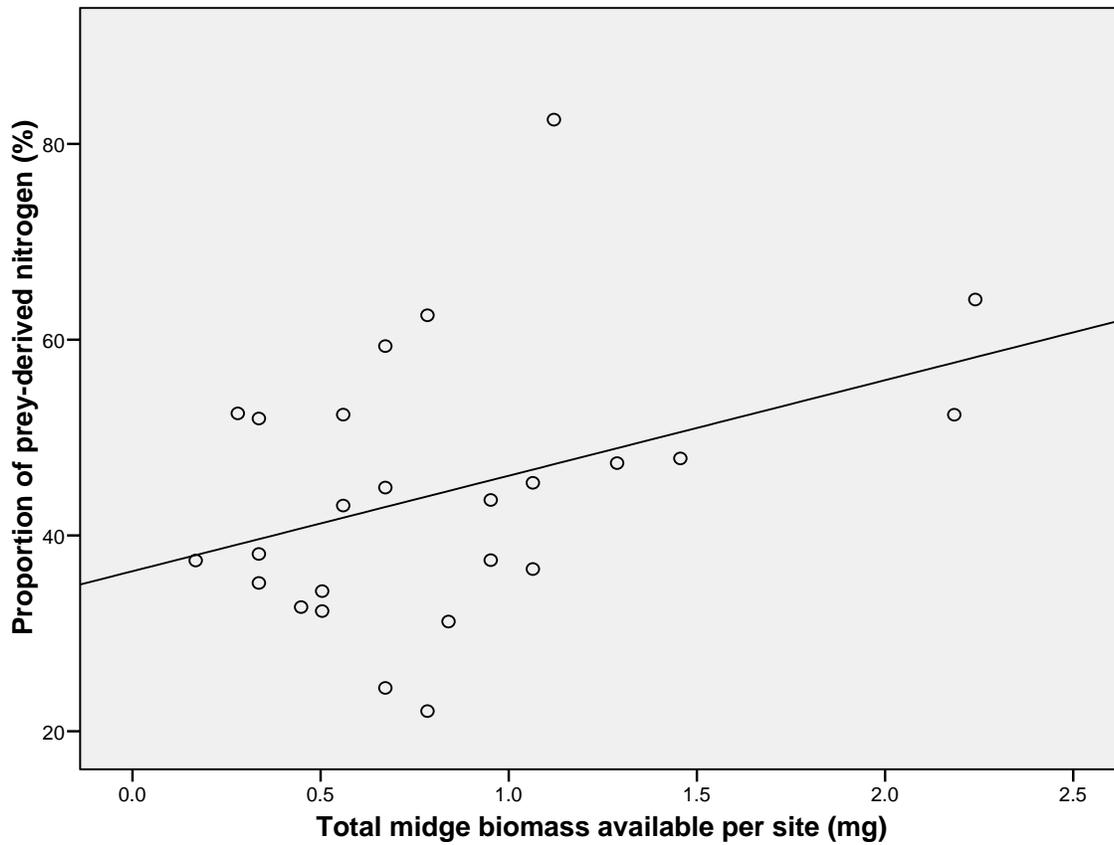


Figure 11. Correlation between prey-derived nitrogen per sundew and total midge biomass in mg available to each sundew (N = 25).

Additionally, the percent of prey-derived nitrogen was not correlated with the number of flies available at each site (d.f. = 24, $R = 0.174$, $p = 0.404$; Figure 12) or the number of total insects (d.f. = 24, $R = 0.135$, $p = 0.519$; Figure 13).

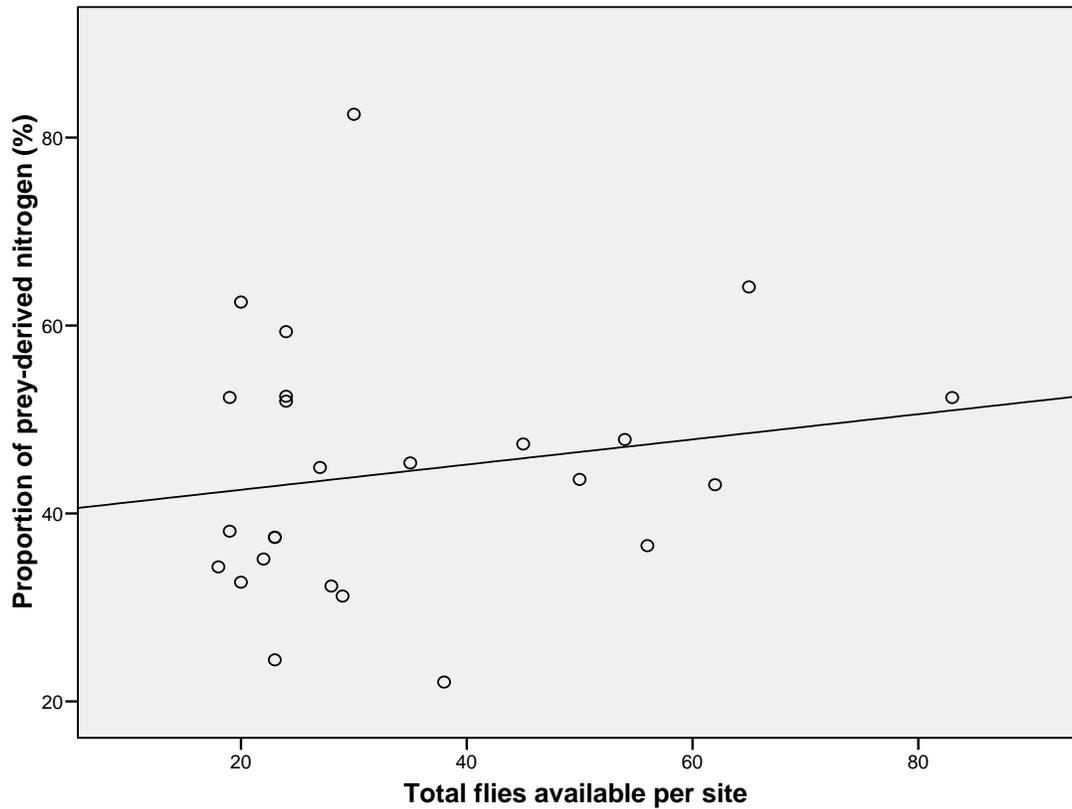


Figure 12. Lack of correlation between the percent of prey-derived nitrogen for each sundew and total number of flies at each site ($N = 25$). Each point represents the number of flies available to each sundew.

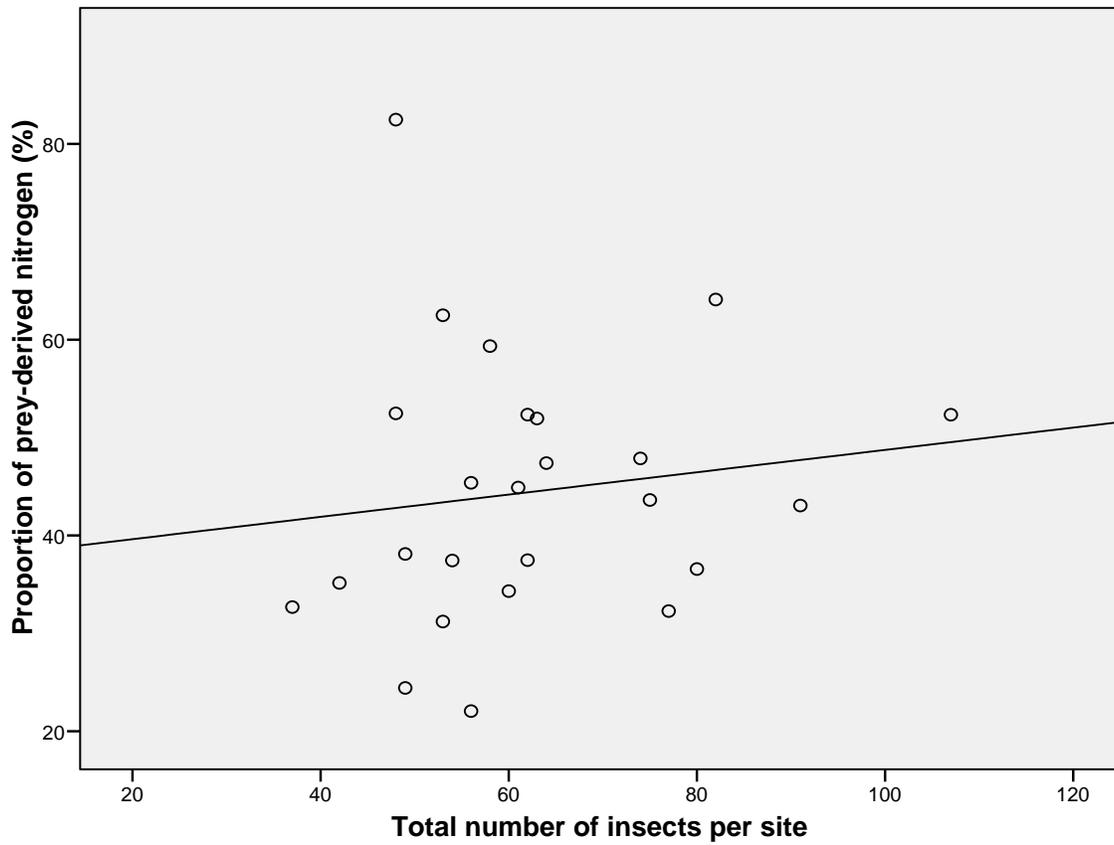


Figure 13. Lack of correlation between the percent of prey-derived nitrogen and total number of insects per site ($N = 25$). Each point represents the number of insects available to each individual sundew.

*Importance of prey-derived N for plant “health”
% of prey-derived N vs. C:N*

Finally, although individual sundews varied greatly in the proportion of N derived from insects, this variation was not reflected in the nutritional composition of their leaves. The average leaf C:N ratio was 31.54, with a range of 19 to 44. The average percentage of nitrogen reflected in the leaves was 1.69%, and ranged from 1.07-2.59%. Leaf C:N ratio was not correlated with the percentage of prey-derived nitrogen (d.f. = 23, $R = 0.017$, $p = 0.937$; Figure 14).

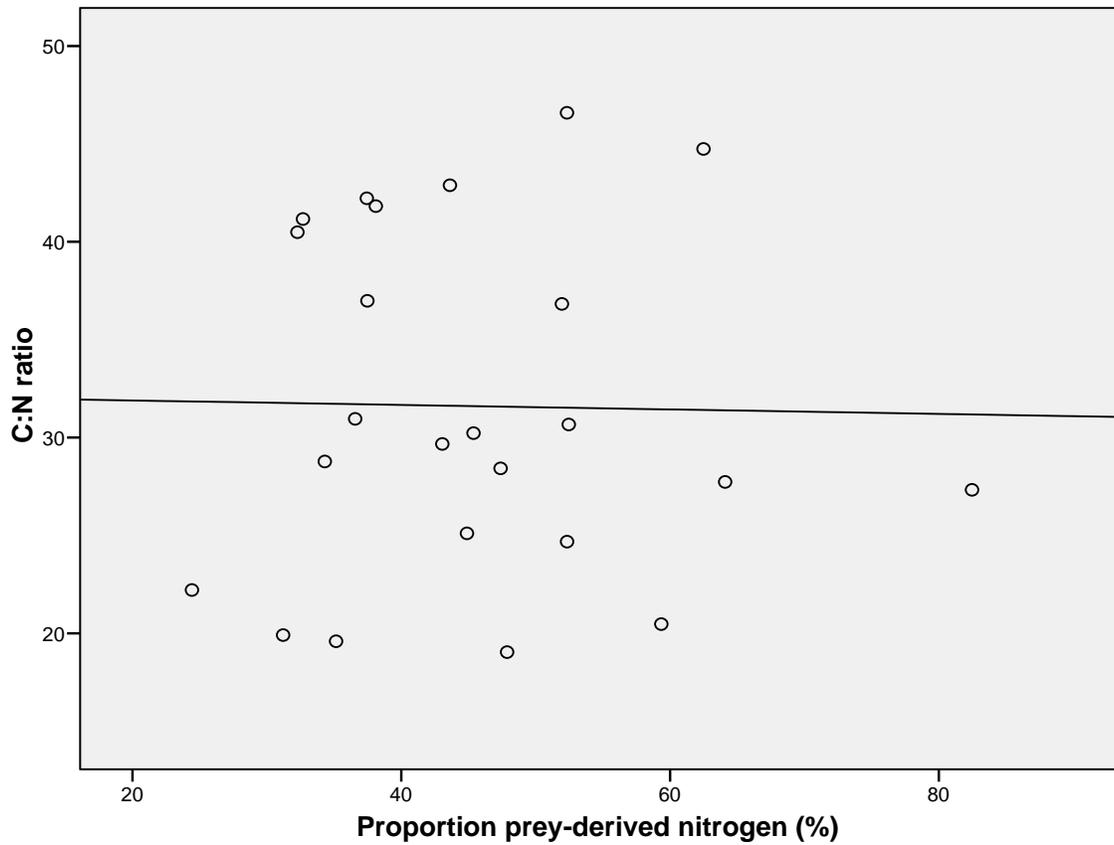


Figure 14. Correlation between the percent of prey-derived nitrogen and C:N ratio of each sundew. C:N ratio and percent of prey-derived nitrogen were calculated individually for each sundew.

Discussion

We found that on average, *D. rotundifolia* individuals in Mud Lake Bog obtain 44% of their nitrogen from insects. However, a large amount of individual variation occurs across a pH of 3.29-5.17: percent of prey-derived nitrogen ranged from 22.1-82.5%. Our results suggest that the proportion of prey-derived nitrogen varies significantly across individuals. As it appears that the relative importance of carnivory varies across individuals by almost 4-fold, we feel it is necessary to investigate the underlying drivers of this variation.

Given that carnivorous plants mainly occur in acidic habitats, and acidity decreases decomposition rates, which would make fewer nutrients available, it is reasonable to assume that pH is a driver for adaptations for plants to capture nitrogen from other sources. Therefore, we would expect the relative importance of carnivory to increase in more acidic areas that would presumably have lower decomposition rates and thus less available nutrients. Should pH not be the main driver of decomposition rates, we expected that temperature may be a factor affecting decomposition and thus nitrogen availability. We expected that areas with higher temperatures would have more microbial activity and thus more nitrogen availability, leading plants to have a lower percentages of prey-derived nitrogen. Another factor that we reasoned may affect the amount of prey-derived nitrogen is insect availability. Plants with more access to insects should have a more nitrogen derived from prey. However, we did not find this to be the case.

Virtually all of our results failed to support the hypotheses that pH, temperature, or insect availability are major drivers of the variation among individuals in the relative importance of carnivory. We found no evidence that microhabitat pH level affects the relative contribution of insect nitrogen to sundews. Not only are the percentage of prey-derived nitrogen and pH not correlated, but pH and ammonium were nearly significantly negatively correlated. A Russian study on the bacteria *Burkholderia*, a typical component of the microbial community of *Sphagnum* bogs, found the bacteria develop optimally at pH 5-7, but can develop in pH 3.5-7.4 (Belova et al. 2006). Given that we sampled over a pH range of 3.29-5.17, it is particularly surprising that pH and ammonium availability were not positively correlated.

The hypothesis that the response to prey decreases as soil nutrient availability increases was not supported in a study done on *D. rotundifolia* and other carnivorous plants in a subarctic environment (Karlsson et al. 1991). In one case, the response to prey in terms of winter bud weight was higher for plants supplied with soil nutrients. Thus, plants in areas with more ammonium availability may not necessarily seek less nitrogen from insects. Perhaps plants with more access to ammonium are able to invest more in insect trapping structures and are thus more successful in luring and obtaining insect nitrogen. If this were the case, we would not see a negative correlation between ammonium and percent of prey-derived nitrogen.

That ammonium and pH may be negatively correlated is certainly surprising, but there may be some reasons why ammonium would artificially appear to be lower in areas of high pH. Competition may be higher in the areas of higher pH. The plant community on the floating mat, which typically has a higher pH than the bog lawn, is different than that of the bog lawn. Perhaps the plants on the floating mat, which have deeper roots than the weak-rooted sundews, are better competitors and sequester the extra ammonium at a level deeper than we measured. Thus, even though pH may in fact lead to a wider availability of ammonium, other plants with deeper roots may have faster access to the ammonium and use it before the sundews can get to it. Vestigial root systems occur in species of *Drosera*, and the roots can be short lived, reduced, frail, and thin (Adlassnig et al. 2005). If competition is a major factor, increased ammonium availability would not affect percent of prey-derived nitrogen because other plants would get to the ammonium first and sundews would still have to seek nitrogen from aerial sources.

Mycorrhizal colonization has been demonstrated at a low level in *Drosera intermedia*, a species closely related to *D. rotundifolia* (Fuchs and Haselwandter 2004). AMF colonization intensity can be slightly higher in alkaline versus acid soils (Clark and Zeto 1996). Thus, pH may affect AMF colonization. It is possible that *D. rotundifolia* or the other bog plants are more heavily colonized by mycorrhizae in the areas of higher pH, and are thus more capable of absorbing ammonium, so the standing levels of ammonium appear lower. If *D. rotundifolia* are mycorrhizal and acidity inhibits colonization, then they would be less capable of collecting the ammonium in areas of lower pH and the ammonium may sit longer before being absorbed. Even if sundews were not mycorrhizal but the surrounding vascular plants were, the same effect may be seen.

We cannot ignore the inherent and inescapable limitations of taking a point measurement to characterize a factor that may vary over a larger time scale over the life of a long lived perennial such as *D. rotundifolia*. Point sampling may not be as reliable as we would like. The ammonium levels measured on the first day were not correlated with the ammonium levels measured on the second day (d.f. = 24, R = 0.284 p = 0.170). Bragazza and Gerdol (2002) found ammonium levels of 13-54 $\mu\text{gN/l}$ in *Sphagnum*-dominated peatlands in the Italian Alps, and Millet et al. (2003) found ammonium levels of 848-1345 $\mu\text{gN/l}$ in *Sphagnum*-dominated bogs in the UK. As we found ammonium levels of 32-1099 $\mu\text{gN/l}$, it is evident that ammonium levels are highly variable across space and time. Thus, it may be difficult to correlate a time integrated factor such as percent of prey-derived nitrogen with a character that is

ephemerally measured such as ammonium. Sampling over a short time interval may not accurately represent the pH and ammonium levels the sundews experience over their lifetimes.

Prey-derived nitrogen was not only uncorrelated with pH, but it was also not correlated with temperature. Percent of prey-derived nitrogen was not correlated with either surface temperature or temperature 10 cm down. Ammonium was also not correlated with surface temperature, but it was correlated with temperature 10 cm below the surface. It was not possible to collect enough water at the surface where most *Drosera* roots exist to do ammonium analysis to determine how much ammonium is at the surface. Thus, the ammonium levels are closer estimates to the ammonium levels at 10 cm down than at the surface. It is likely that the major microbial drivers of decomposition are not directly at the surface but are in fact closer to 10 cm below the surface. If more microbes are present at 10 cm below the surface, it would make sense that ammonium and temperature are positively correlated, because bacteria should be more active at higher temperatures. However, although temperature at 10 cm and ammonium are positively correlated, percent of prey-derived nitrogen and temperature at 10 cm are not correlated. Since *Drosera* roots occur mainly at the surface, any ammonium that is produced from microbial activity below would have to diffuse vertically in order to reach the *Drosera* roots. Perhaps other plants that root deeper than *D. rotundifolia* are able to intercept and take advantage of the ammonium that is available further below the surface. A study done in a bog in subarctic Sweden concludes that *Sphagnum fuscum* has a competitive advantage over *D. rotundifolia* as it has first access to mineral nutrients (Svensson 1995). As ammonium may be intercepted as it diffuses vertically to the sundew roots, variation in ammonium levels at 10 cm may not be reflective of variation near *Drosera* roots. In this case, the proportion of N derived from prey would not be correlated with temperature at 10 cm.

The percent of prey-derived nitrogen was not correlated with total fly or insect biomass or with the total amount of flies or insects available to each sundew. It is possible that *D. rotundifolia* do in fact take advantage of the other insects besides flies, especially the ones for which we were unable to quantify biomass and $\delta^{15}\text{N}$ such as springtails and thrips. The same factors that made them difficult to quantify such as their small size could also make them more readily incorporated into a sundew diet. Additionally, perhaps what landed on our trap over a 65 hour period is not representative of what has landed on the *Drosera* over years of its life. Given this, a correlation does indeed seem to appear when prey-derived nitrogen is compared with just a single group of insects that is likely to be eaten by a sundew because of its small size and attraction to glistening surfaces: the midge. In Mud Lake Bog, midges may be a primary source of insect N in *D. rotundifolia*. Thus, the major determinant of local variation in relative importance of carnivory for sundews in Mud Lake bog may not be pH or temperature but the availability of midges or other small, weak flying prey.

Finally, the percent of prey-derived nitrogen was also not correlated with C:N ratio. Sundews have low average % N in their leaves and high C:N ratios when compared to a typical C_3 plant. It is not likely that they are satiated in terms of nitrogen. However, our results did not corroborate with findings that C:N ratio decreased with increasing prey-derived N (Millet 2003). Our results do not support the idea that plants deriving more of their N from prey are less nitrogen stressed than plants deriving nitrogen from soil.

Carnivory has evolved multiple times independently in diverse plant families (Ellison and Gotelli 2001), suggesting that it is an adaptive trait. Bogs are harsh habitats and not many plants are capable of thriving there. Sundews have managed to flourish in such a nitrogen poor habitat by evolving the capacity for capturing and digesting nitrogen rich insect prey. While our results do not support the hypotheses of pH or temperature driving variation in the relative importance of carnivory to sundews, it appears as though availability of small insect prey such as midges is a viable determinant of prey-derived nitrogen.

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Figure 1. Distribution of pH conditions across microhabitats of Mud Lake Bog measured July-August 2007. Shrub/Scrub Wetland represents the bog lawn, and Aquatic Bed Wetland represents the bog floating mat.

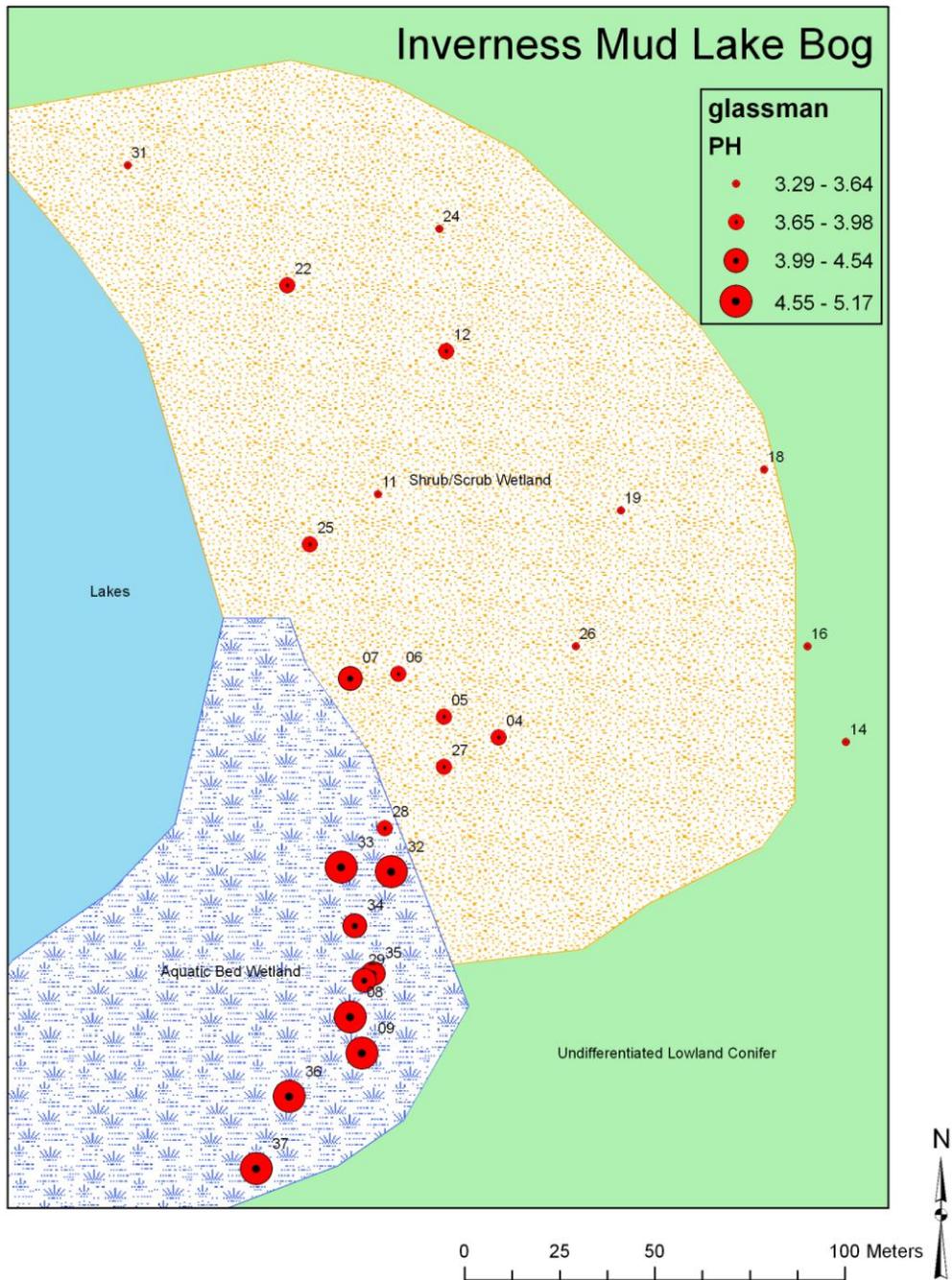


Table 1. pH Temperature Correction Factors at Varying Temperatures from Standard (25°C)
(<http://h2o.enr.state.nc.us/lau/documents/TemperatureCorrection.doc>).

Temp°C	Correction Factor
40	Plus 0.45
39	Plus 0.42
38	Plus 0.39
37	Plus 0.36
36	Plus 0.33
35	Plus 0.30
34	Plus 0.27
33	Plus 0.24
32	Plus 0.21
31	Plus 0.18
30	Plus 0.15
29	Plus 0.12
28	Plus 0.09
27	Plus 0.06
26	Plus 0.03
25	0.00
24	Minus 0.03
23	Minus 0.06
22	Minus 0.09
21	Minus 0.12
20	Minus 0.15
19	Minus 0.18
18	Minus 0.21
17	Minus 0.24
16	Minus 0.27
15	Minus 0.30
14	Minus 0.33
13	Minus 0.36
12	Minus 0.39
11	Minus 0.42
10	Minus 0.45

Table 2. The $\delta^{15}\text{N}$ values of the thirteen most common non carnivorous plant species in Mud Lake Bog.

Species	mean $\delta^{15}\text{N}$ vs. Air	Standard Dev.
<i>Andromeda polifolia</i>	-10.6	0.709
<i>Alnus rugosa</i>	-2.2	0.014
<i>Chamaedaphne calyculata</i>	-9.7	3.045
<i>Carex lasiocarpa</i>	-1	0.978
<i>Dulichium arundinaceum</i>	-1.3	0.421
<i>Kalmia polifolia</i>	-9.7	1.007
<i>Larix laricina</i>	-7.3	0.276
<i>Picea mariana</i>	-10.2	1.769
<i>Potentilla palustris</i>	-1.7	0.047
<i>Rhynchospora alba</i>	-1.2	0.876
<i>Sphagnum spp.</i>	-2.7	0.123
<i>Scheuchzeria palustris</i>	-2	0.039
<i>Vaccinium oxycoccos</i>	-11.5	0.000