The tradeoff between polyuronic acid content and photosynthesis rates in Sphagnum

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Abstract

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Sphagnum mosses often dominate peatland ecosystems. Because of the low availability of nutrients in these environments, *Sphagnum* mosses have developed a cation exchange system within their cell walls to take up nutrients using polyuronic acids (PUA). Increasing amounts of PUA may come at a physiological tradeoff and cause decreasing photosynthetic rates. The objectives of this study were 1) to assess variation in polyuronic acid content and structure by analyzing component monomers from three different *Sphagnum* species representative of different microhabitats, 2) to investigate how this variation affects the cation exchange capacity of the peat moss species, and 3) to evaluate whether uronic acid concentrations show a negative association with maximum photosynthetic rates. Maximum photosynthetic rates were lowest among species that grew in nutrient poor environments. Cation exchange capacity was measured, with the species that grew in less nutrient poor having the lowest capacity. Cell walls were isolated, hydrolyzed, and an evaluation of monomer concentrations was done. The data from this experiment was inconclusive. While the protocol was shown to be an effective method to quantify the uronic acid content of the species, the analytical techniques may have been too simplistic causing the results to be skewed.

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Introduction

Boreal ecosystems are integral to the world's carbon cycle, as they store 180 to 455 petagrams, or about one-third of the world's soil carbon pool (Smith et. al., 2004), much of which is stored in peatlands. These peatland systems are primarily located in the northern hemisphere in the USSR, USA, Canada and Fenno-scandian countries (Gorham, 1991), and have a potential major impact on global climate change (Bridgham, 1995). Peatlands are characterized by having severely limited or no nutrient input from runoff. Instead they must obtain their nutrient supply from wet deposition from dissolved nutrients in rainwater or dry deposition from the atmosphere. A second defining characteristic is that the peatlands are usually acidic. This serves to preserve plant matter by limiting the growth of microbes, which leads to the buildup of the peat deposits, and also limits the species of plants that can survive in the habitat.

Sphagnum mosses are a fundamental species of peatland ecosystems and they play an important ecological role, as they thrive in the nutrient poor conditions and influence the succession (Soudzilvskaia, 2010) and patterning of peatlands (Eppinga, 2009). Species of *Sphagnum* have several physiological traits that allow them to survive. *Sphagnum* mosses, unlike vascular plants, do not have roots to scavenge for water. Instead their leaves are made up of two different types of cells: large hyaline cells surrounded by chlorophyllose cells that are photosynthetically active. Hyaline cells function to store water for the plant. They are dead and have pores to allow for water movement into and out of the cell. To salvage enough resources in the nutrient poor environment, *Sphagnum* mosses have also developed a cation exchange system within their cell walls to take up nutrients using polyuronic acids (PUA). These compounds,

made up of monomers such as galacturonic acid and glucuronic acid, are found in the plants' cell walls and are able to give off a proton and in exchange take up a cation from the environment that the plant can use, such as NH_4^+, Mg^{2+}, Ca^{2+}, and K^+ (Spearing, 1972; van Breeman, 1995).

These PUAs are important keystone compounds in the bog ecosystem. They allow for nutrient uptake, account for as much as 25% of the dry weight of Sphagnum (Clymo, 1964), and acidify the bog as they release more H^+ ions into the environment. PUA have been shown to exhibit an ability to preserve tissue, a common characteristic of bogs. Their ability to bind nitrogen slows the growth of microbes that would otherwise decompose tissue and allows the peat mosses to thrive (Borsheim, Christensen, & Painter, 2001). The PUA compounds may be used in the transport of nutrients around the *Sphagnum* moss (Rydin, & Clymo, 1989) and are a factor in the characteristic patterning of peatlands (Eppinga, et. al., 2009). The acidification of the environment by *Sphagnum* results in an environment that is difficult for many vascular plants, which cannot take up the necessary nutrients for growth in such an environment. The PUA and acidifying properties of *Sphagnum* are important in shaping the environment for other plants (Eppinga, et. al., 2009).

Within the bog itself there are several different microhabitats. The "hollow" habitat consists of the low levels that are closer to the water table. They have relatively more nutrient content when compared to other areas of the bog and are inhabited by species such as *S. fallax.* The "hummock" areas of the bog are raised areas, or mounds in the bog. Because they are higher above the water table they more nutrient poor as they receive less nutrients from decay, and rely solely on atmospheric deposition. Only

Sphagnum species that are able to survive in really low nutrient availability, such as *S. fuscum,* are found in these areas. Species of *Sphagnum* have developed different adaptations and physiology to thrive in the microhabitat they are found in. One example is water holding capacity. Titus and Wagner (1984) looked at two species, *S. fallax* and *S. nemoreum,* and their water holding capacity. *S. nemoreum,* a hummock species has developed physiological adaptations to hold a higher capacity of water and lose it more slowly, allowing it to dominant hummock areas, were water availability was low. *S fallax*, which dominates hollow microhabitats did not have the same physiological traits and was not able to move into low water environments (Titus and Wagner, 1984). The hyaline cells that are used by *Sphagnum* to store water vary in their size among different species. Characteristics such as pore size and number also vary by species. Another example of *Sphagnum* physiological adaptation is that as distance above the water table increases (i.e. hollow microhabitat to a hummock microhabitat) the amount of galacturonic acid contained in the organism and its CEC also tend to increase (Spearing, 1972). As the level above the water table increases, conditions become more nutrient poor, therefore species that have adapted and have a physiology that will thrive in the environment (high CEC to bind free nutrients), are dominant.

These differences in physiology can be used to show important trade-offs in *Sphagnum.* Species that have the highest photosynthetic rates do not dominate universally. They tend to dominate in areas closer to the water table where other factors such as nutrients and water availability are not limiting. Similarly, species that have high CEC do not dominate universally either. They tend to dominate areas where nutrients are limiting. In these areas species with high photosynthetic rates are not able to outcompete

them. The differences in the species present in these hollow and hummock areas clearly demonstrate these important functional trade-offs in these mosses. For example there is an inverse relationship between the water holding capacity of the mosses and their maximum rate of photosynthesis (Rice et. al., 2008). Species such as *S.fallax*, which have relatively small hyaline cells, can allocate a greater percentage of their leaves towards photosynthetic cells, but by doing so, sacrifice water storage ability. Species such as *S. fuscum*, which have large hyaline cells, devote a greater percentage of their leaf volume towards water storage, but sacrifice photosynthetic ability. Therefore in environments like hollows, where water is less limiting, smaller hyaline celled species are able to dominant other species by outcompeting them photosynthetically. In environments such as hummocks, where water is more limiting, species that have larger hyaline cells are able to dominate, even though they have a lower maximum photosynthetic rate, because they can hold more water and resist desiccation during drought.

Other studies have looked at the physiological differences among species as the water table level increases. Differences in galacturonic acid content (Spearing, 1972), cation exchange capacity, (Spearing, 1972), and photosynthesis rates (Titus, 1982) have been shown to vary with level above the water table. However no studies have demonstrated a link between an increase in the peat moss' CEC, PUA content, and a decrease in photosynthetic rates. By looking at only one physiological trait, studies have shown adaptations of species to their environment but they have not shown trade-offs between the traits, or more fundamentally the cost of investing heavily in one trait. Studies such as Spearing (1972) only show the benefit to having high galacturonic acid

(GalA) content. She fails to investigate the cost to synthesizing galacturonic acid. Because high galacturonic acid containing species only dominate high above the water table and not everywhere, there must be a cost to synthesizing GalA that allows other low GalA species to outcompete them closer to the water table. Our study seeks to further explore the costs of having higher CEC and PUA content, in terms of a trade-off with maximum photosynthetic rates.

We hypothesize that a similar relationship is true between PUA content and maximum photosynthetic rates of *Sphagnum*. In our overall study we will measure the concentration of different PUA monomers in species of *Sphagnum*, the cation exchange capacity (CEC), and the maximum photosynthetic rate of three mosses species that occupy different microhabitat. We hypothesize that mosses found in low nutrient environments will have higher CEC and PUA content than high nutrient species because they will need to scavenge the environment for more resources. There are two main ways that species could achieve this higher level of PUA. The first way would be for the *Sphagnum* moss to keep the concentration of PUA in the cell wall the same, but just have more cell walls. The second would be to have the same amount of cell walls but have a higher percentage of the cell walls devoted to PUA. Some combination of these two methods would have to account for higher PUA. As a trade-off, low nutrient environment mosses will have lower maximum rates of photosynthesis, as they will have devoted more resources to building PUA and less towards building chloroplasts, photosynthetic enzymes and other materials for photosynthesis.

The objectives of this study are to use a comparison approach with three *Sphagnum* species from different microhabitats 1) to assess variation in polyuronic acid

content and structure by analyzing component monomers and their linkages from different *Sphagnum* species, 2) to investigate how this variation affects the cation exchange capacity of the peat moss species, and 3) to evaluate whether polyuronic acid concentrations, as well as specific monomer concentrations, affect maximum photosynthetic rates.

Methods

Maximum Photosynthetic Rate

Samples of *S. fallax, S. magellanicum, and S. fuscum* were collected in rings of 3.5cm PVC pipe from Twila's bog in Queensbury, NY. Efforts were made during collection to keep moss canopies intact. Samples were maintained in a controlled environment with 16 hours of light per day for 2 weeks and kept between 16°C and 18°C. Before analysis, excess water was removed by blotting dry with paper towels from the top and bottom of the sample tubes for approximately 15 minutes. Light curves were established for each of the three species to determine saturating light levels. Six samples of each species were analyzed at 95% saturating light levels (at 550 μmol photons $m^{-2}s^{-2}$) for maximum photosynthesis rates using a LICOR 6400 photosynthesis system (LiCor, Lincoln, NB). Measurements were taken at temperatures ranging from 25°C to 28°C and humidities ranging between 75% and 85%. Following photosynthetic analysis, the samples were dried at 65 °C for 72 hours to remove excess water and dry weights for each sample where taken. Photosynthesis was then calculated on per area and per dry weight bases. Statistical significance was measured using ANOVA implemented in SPSS. (Somers, NY).

Cell Wall Extraction

Samples of *S. fallax (*3 samples*), S. magellanicum (*3 samples*)* and *S. fuscum (*2 samples*)* were collected from Twila's bog. Branches from the top 1 cm below the stem and capitulum were removed and stored in a freezer at -70°C until needed. The procedure for cell wall extraction followed Zablackis et. al. (1995). Samples of each species were ground in liquid nitrogen to a powder to help break up the tissue. Approximately 0.3g of powder were put into each sample tube. Then the sample tubes were extracted with 1.5% SDS followed by two extractions with 0.5% SDS to break open the cell membranes, with each extraction being followed by a 5 minute centrifuge at 3000 rpm. Then the sample was extracted 0.5M KPO_4 and 1.5M KPO_4 and centrifuged again. After a wash with water, the sample was extracted with a phenol:acetic acid:water (2:1:1) solution and dried with acetone twice. Samples were air dried for 2 weeks, then dried at 50 C for 24 hours, and then analyzed. Cell wall content was calculated as sample dry weight after extraction divided by sample dry weight before extraction. Statistical significance was measured using Vassar Statistics (ANOVA).

Cation Exchange Capacity

The cation exchange capacity (CEC) of the three species was determined using the procedure outlined by Soudzilovskaia et. al. (2010). Six 100 mg samples from each of the three species were placed into mesh bags. Dry tissue was used as there is no significant difference in CEC between wet and dry tissue (Soudzilovskaia et. al., 2010). The sample bags were then placed in a beaker with 20 ml of 0.02M hydrochloric acid for

1 hour to remove all ions that the PUA were holding. After the hour long treatment, the sample bags were placed into 500 mL of milliQ water for 20 minutes to remove the acid from the tissue. The sample bags were then put into a treatment of 20 ml of 0.02M sodium chloride, which saturated all cation exchange sites on the plant tissue with sodium ions. The sample bags were left in this solution for 2 hours in total (two 1 hours treatments). After the two changes of sodium chloride, the sample bags were again washed in 500 mL of milliQ water to remove all sodium ions that were not bound to cation exchange sites. The sample bags were then placed in a final treatment of 0.02M hydrochloric acid to unbind all sodium ion from the cation exchange sites and release them into solution. The resulting solution was then be analyzed for sodium content to determine CEC, using inductively coupled plasma mass spectrometry (ICP-MS).

Polyuronic Acid Content

The content of polyuronic acid content was determined using a protocol outlined by Foster et. al. (2010). First the cell walls were broken down into monomers. Two mg of dry cell wall material (obtained from cell wall isolation procedure) was taken from the six sample of each species and placed into a glass screw cap tube. Two addition sample \ tubes containing two mg of galacturonic acid and glucuronic acid respectively, were taken through the treatment to use as eternal standards. To make sure that all the material was attached to the side of the tube, 250 μL of acetone was used to wash the sides of the tube and collect all the material at the bottom. The acetone was then evaporated off. The

cell walls were then hydrolyzed by adding 250 μ of 2 M trifluoroacetic acid, capping the tubes and autoclaving the samples at 121° for a 90 minute cycle. Following the incubation period, the resulting solution was then centrifuged for 10 minutes at 10,000 rpm to separate the solid crystalline cellulose from the dissolved matrix monosaccharides, which contain monomers of the polyuronic acids. Once the samples had been centrifuged, 75 μL of the supernatant was pipette off and placed into test tubes. The trifluoroacetic acid was then evaporated off leaving a film of non-cellulose cell wall monomers. To remove all traces of acid, 300 μL of 2-propanol was added to each sample and evaporated off three times. Ten mL of water was added to each sample as a solvent. The samples were then analyzed for uronic acid monomers using a triple quadrupole liquid chromatography- mass spectrometer.

Results

Maximum Photosynthetic Rate

The maximum photosynthetic rate on a per area basis differed significantly between *S. fallax, S. magellanicum, and S. fuscum (*$p<0.01$ among all species, ANOVA). *S. fallax* had the highest maximum photosynthetic rate/area and averaged 4.28 μmol $CO_2/m^2/s$. *S. magellanicum* averaged 2.73 μmol $CO_2/m^2/s$. *S. fuscum* had the lowest photosynthetic rate/area and averaged 1.66 μmol $CO_2/m^2/s$ (figure 1).

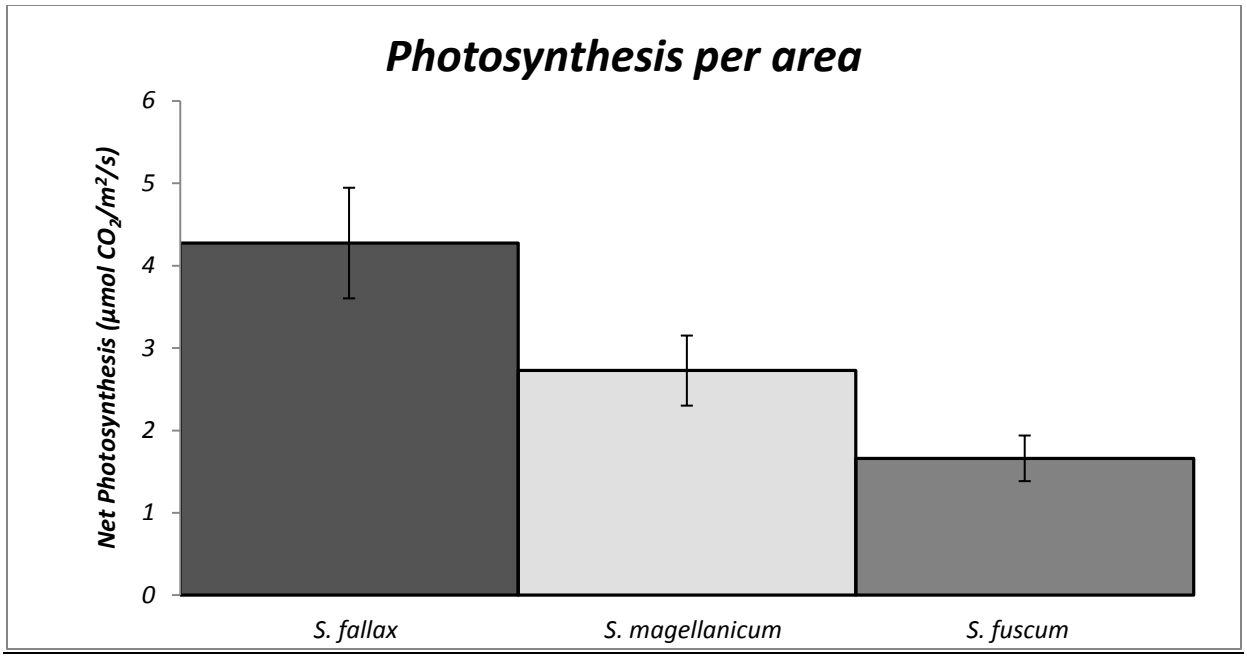

Figure 1: Maximum photosynthetic rate on a per area basis differed significantly between *S. fallax, S. magellanicum, and S. fuscum (*$p<0.01$ between all species, ANOVA). *S. fallax* averaged 4.275 μmol $CO_2/m^2/s$. *S. magellanicum* averaged 2.727 μmol $CO_2/m^2/s$. *S. fuscum* averaged 1.662 μmol $CO_2/m^2/s$.

The maximum photosynthetic rate on a per dry weight basis also differed significantly between *S. fallax, S. magellanicum, and S. fuscum (*$p<0.01$ between all species, ANOVA). *S. fallax* had the highest photosynthetic rate/dry weight and averaged 0.0149 μmol $CO_2/g/s$. *S. magellanicum* averaged 0.0079 μmol $CO_2/g/s$. *S. fuscum* had the lowest photosynthetic rate/ dry weight and averaged 0.0012 μmol $CO_2/g/s$ (figure 2).

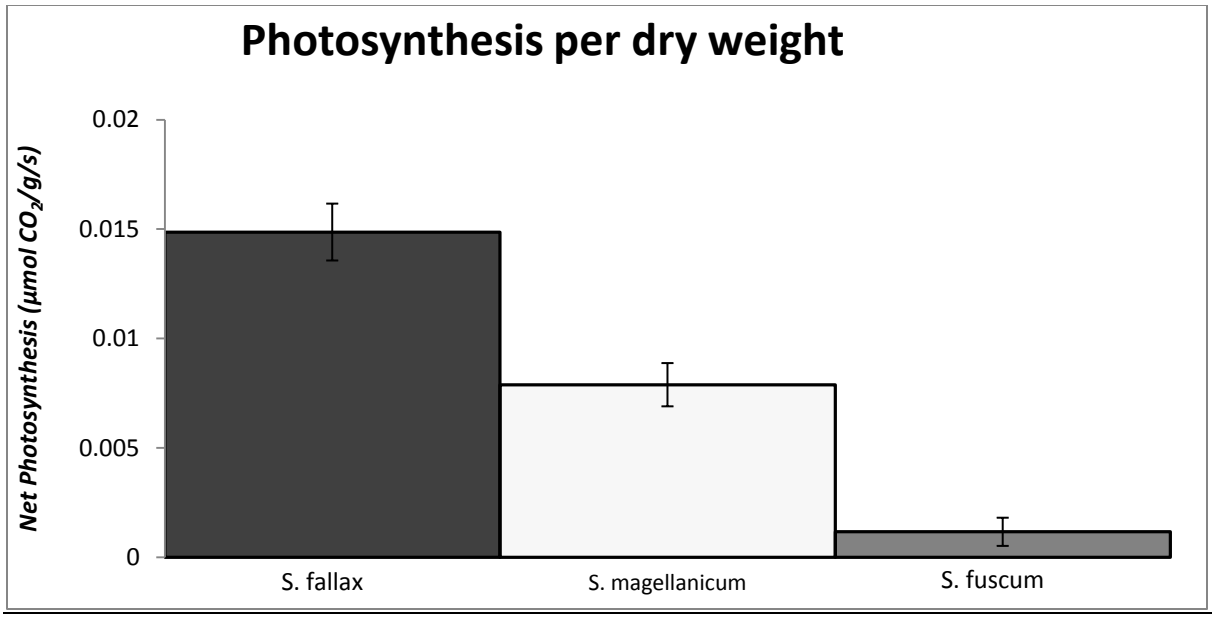

Figure 2: The maximum photosynthetic rate on a per dry weight basis differed significantly between *S. fallax, S. magellanicum, and S. fuscum (*$p<0.01$ between all species, ANOVA). *S. fallax* averaged 0.0149 µmol CO_2/g/s. *S. magellanicum* averaged 0.0079 µmol CO_2/g/s. *S. fuscum* averaged 0.0012 µmol CO_2/g/s.

Cell Wall Analysis

For all three species the cell wall accounted for over 70% of dry weight. *S. fallax* averaged 71.42% cell wall/dry mass, *S. magellanicum* averaged 77.60% cell wall/ dry mass, and *S. fuscum* averaged 79.48% cell wall/dry mass (Figure 3). None of these differences were significant.

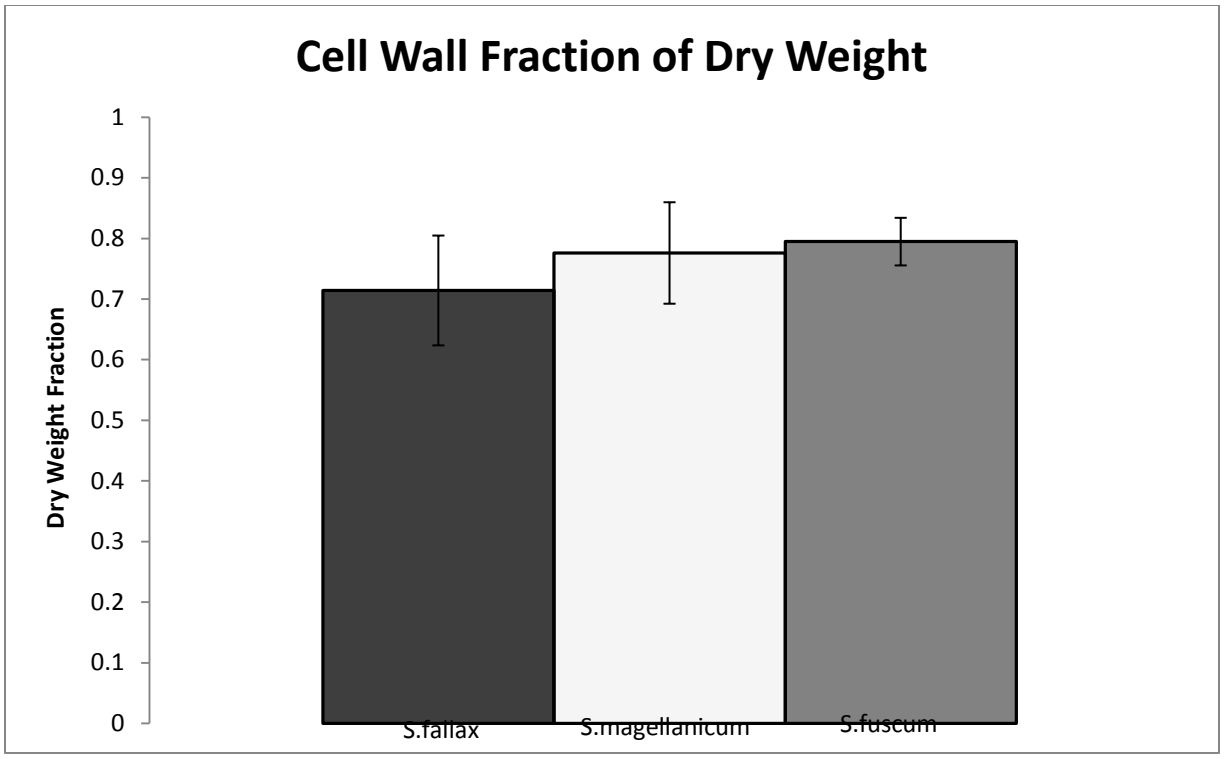

Figure 3: The fraction of dry weight made up of cell wall. *S. fuscum* had a cell wall/ dry weight ratio of 0.794. *S. fallax* had a cell wall / dry weight ratio of 0.714. *S. magellanicum* had a cell wall / dry weight ratio of 0.776. The differences were not significant.

Cation Exchange Capacity

The ion exchange capacity, or the ability of the plant to exchange hydrogen ions from nutrient cations in the environment was measured in parts per million (ppm) of sodium ion (Figure 4). *S. fallax* had the lowest cation exchange capacity (15.00 ppm). *S. magellanicum* and *S. fuscum* had higher ion exchange capacities or 60.07 ppm and 50.09 ppm respectively. The standard deviation for all species, especially *S. magellanicum* and *S. fallax* were all high. *S. magellanicum* was significantly different from *S. fallax.* All other pair-wise comparisons were not significant.

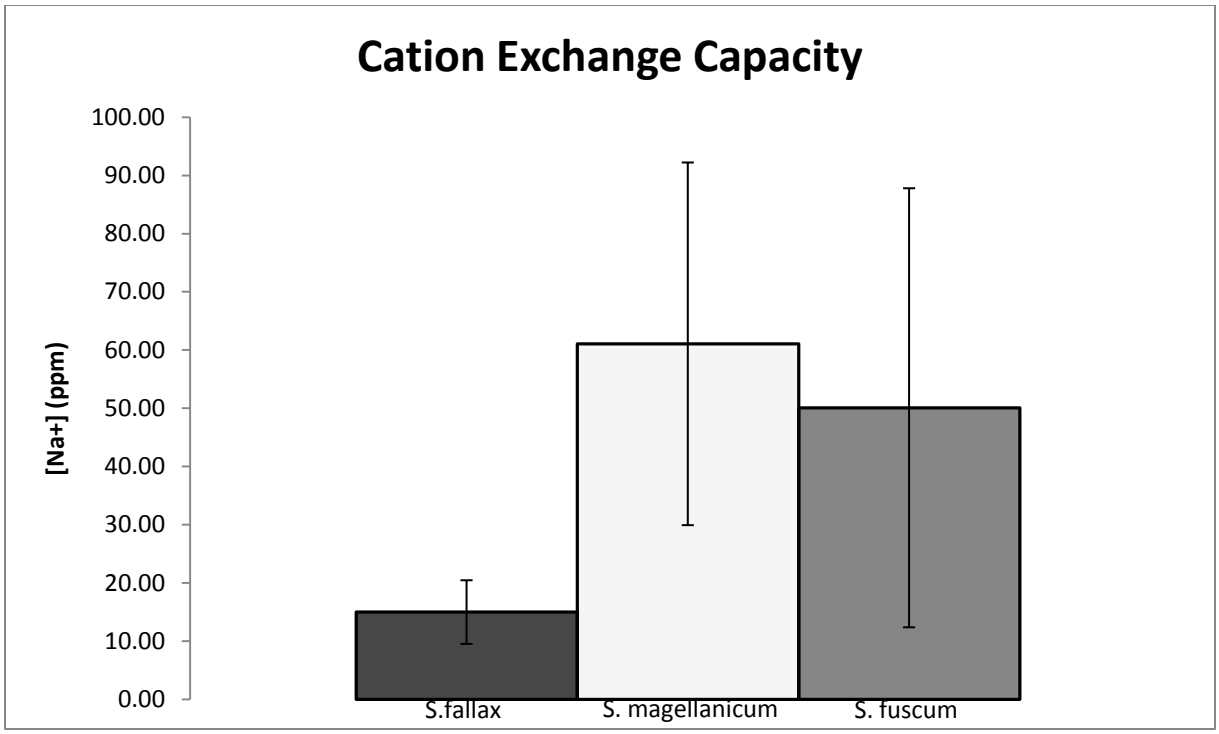

Figure 4: The cation exchange capacity (CEC) measured in parts per million of sodium ions. *S. fallax* had the lowest CEC of 15.00ppm. *S. magellanicum* and *S. fuscum* had higher CEC of 60.07 ppm and 50.09 ppm respectively. *S. fallax* was significantly different from *S. magellanicum* ($p<0.01$).

Polyuronic Acid Content

We found that the *S. fallax* and *S. magellanicum* devoted 9.56% and 9.14% respectively of their cell walls towards polyuronic acids (Figure 5). *S. fuscum* devoted a lower percentage of 6.21% of their cell walls towards polyuronic acids. The two former species differed significantly from the latter species ($p<0.01$ for both *S. fallax* vs. *S. fuscum* and *S. magellanicum* and *S. fuscum). S. fallax* and *S. magellanicum* were not statistically different from each other though.

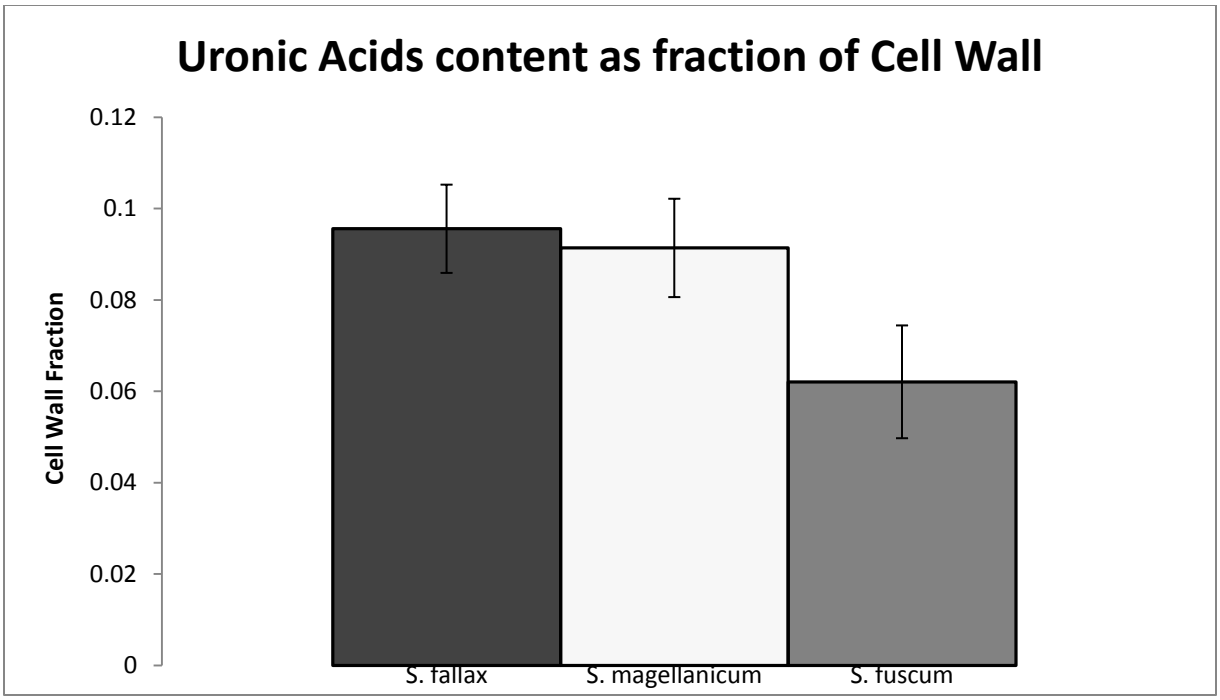

Figure 5: The fraction of cell wall devoted to uronic acids. The fraction was highest in *S. fallax* and *S. magellanicum (*0.956 and 0.914 respectively). These two species differed significantly from *S. fuscum*, which devoted only 6.21% of its cell wall toward uronic acids ($p<0.01$ for both comparisions).

When uronic acid content was examined on the basis of dry weight instead of cell wall, *S. magellanicum* had the highest percentage of uronic acids (7.09% of dry weight, Figure 6). *S. fallax* had a slightly lower percentage of dry weight devotes towards uronic acids (6.88% of dry weight). *S. fuscum* again had the lowest percentage with only 4.60% of dry weight. There was not a significant difference between *S. fallax* and *S. magellanicum* or between *S. fallax* and *S. fuscum.* There was a significant difference between *S. magellanicum* and *S. fuscum* though ($p<0.01$).

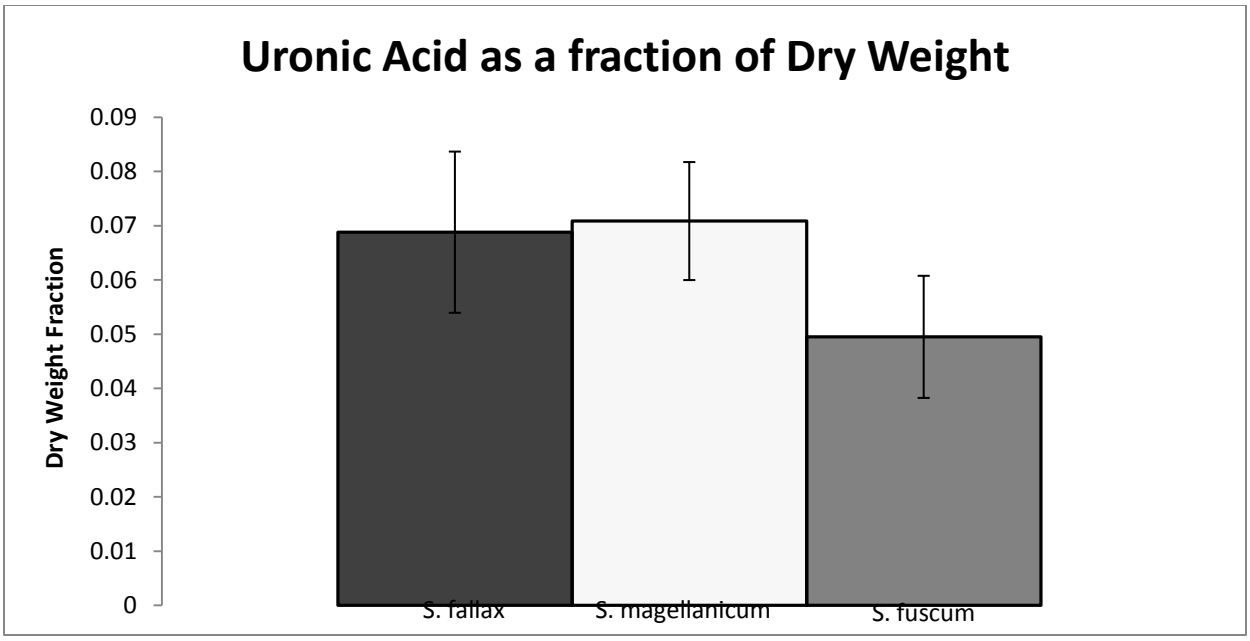

Figure 6: The fraction of dry weight devoted toward uronic acids. *S. magellanicum* and *S. fallax* had the highest fraction of dry weight devoted toward uronic acids (0.709 and 0.688 respectively). *S. fuscum* devoted only 4.60% of dry weight to uronic acids. *S. magellanicum* and *S. fuscum* differed significantly ($p<0.01$).

Discussion

The results from the analysis of photosynthesis expressed on area ($PS_{area)}$ or mass (PS_{dw}) bases agreed strongly with each other. In both cases, *S. fallax* had the highest maximum photosynthetic rate, *S. fuscum* had the lowest photosynthetic rates, and *S. magellanicum* was in the middle. It is important that these two agreed, because either measurement on its own would not completely quantify the trait. There are a few factors that cause this. First, the different species grow at very different densities. *S. fuscum* grows in very dense canopies. The individual plants are very intertwined with each other leaving almost no gaps. *S. fallax*, on the other hand, grows in much less dense canopies

and is not intertwined. These differences in canopy structure could skew the photosynthetic measure on a per area basis. A dense canopy will have more photosynthetically active biomass per unit area. If only PS_{area} was measured, this difference in canopy density would be largely ignored, and a major morphological difference between *S. fuscum* and *S. fallax* with not be accounted for. Secondly, the species may not conduct photosynthesis equally at the same depths into their canopy. For example *S. fallax* allows light to penetrate much deeper into its canopy then *S. magellanicum* or *S. fuscum (*Aclander, 2006). This difference could cause us to include tissue that is effectively photosynthetically active in our measure of dry weight for *S. magellanicum or S. fuscum,* skewing our measurement of PS_{dw}. The measurement of PS_{area} accounts for any differences in photosynthetically active tissue depth between species. By measuring both PS_{area} and PS_{dw} we can take both of these factors into account. Because our analysis yields results for both of these that not only were strongly suggestive of a trend by themselves, but also agreed strongly with each other, it lends even more support to *S. fallax* having the highest photosynthetic rate among the three species, *S. fuscum* having the lowest among the three, and *S. magellanicum* being in between.

In terms of our initial hypothesis, this result was expected. *S. fallax*, the species that to grows in a hollow environment that is less nutrient poor had the highest photosynthetic rates. We had hypothesized that hollow species, such as *S. fallax,* would not have to devote large amounts of resources towards building up nutrient scavenging compounds, allowing it to put more of its resources toward photosynthetic machinery, thus yielding a higher photosynthetic rate. We had hypothesized that the lowest

photosynthetic rate would be for a hummock species, such as S. *fuscum.* Because the environment that it grew in was more nutrient poor, the hummock species would have to devote relatively more resources towards nutrient scavenging compounds. This allocation would leave relatively less resources to put towards photosynthetic machinery, yielding a lower photosynthetic rate. This result also agreed with Titus et. al. (1983), which looked at photosynthetic rates for hummock and hollow species (*S. nemoreum* and *S. fallax* respectively) in a similar way that we did. They also found that the hollow species was able to photosynthesize at a higher rate than the hummock species.

In hollow microhabitats, photosynthesis seems to be the driving force of competition. The species that has the highest photosynthetic rate (*S. fallax*) is able to assimilate more carbon, grow faster, and outcompete species with lesser photosynthetic rates. *S. fallax* though, gets outcompeted by *S. fuscum* in hummock environments even though the former has a higher photosynthetic rate. There must therefore be other physiological characteristics that favor *S. fuscum* in a hummock microhabitat and allow it to outcompete *S. fallax*, which should be able to outcompete it photosynthetically. We had hypothesized that one such factor was the ability of species to scavenge the nutrient poor environment for nutrient cations.

Our results for the comparison of cation exchange capacity, or the ability of the moss to exchange a hydrogen ion for a nutrient cation in the environment, somewhat agreed with our hypothesis. *S. fallax* had the lowest cation exchange capacity. We would have expected this result because *S. fallax* grows in a hollow environment. This microhabitat is not as nutrient poor, therefore the plant does not need to put as many resources in increasing its cation exchange capacity. There are already enough nutrients

in the environment that are freely available to the moss without having to put energy it nutrient gathering. *S. magellanicum* and *S. fuscum*, which thrive on the side of hummocks and on the top of hummocks respectively, had higher cation exchange capacities. The environment that they live in has fewer nutrients available and a higher cation exchange capacity allows them to scavenge for nutrients and survive.

The cation exchange capacity data that was obtained showed high variation. The results, especially among *S. fuscum* and *S. magellanicum*, varied by extreme amounts. In the case of *S. fuscum* the highest and lowest sample differed by a factor of 6. This made it hard to find any statistical significance in the data. Soudzilovskaia et. al. (2010), who utilized a similar method of measuring cation exchange capacity, did not have the same variation. In fact their samples hardly varied at all. The excessive variation in our samples may be due to our protocol. One possible source of intra-species variation would be the washes after the sodium chloride treatments. This step in the protocol is meant to remove all of the sodium ions in the solution that are not bound to cation exchange site on the plant tissue. If the wash is not thorough enough, sodium ions that are not bound will remain on the plant tissue and cause artificial inflation of the sodium ion concentration in the final solution. The wash becomes even more important because of the sample bags that we had the moss contained in. These mesh bags were used to keep the tissue collected and allowed for easy transfers between solutions. The down side to using the bags was that it allowed for an addition site for sodium ions to get trapped in. A thorough wash after the sodium treatment was therefore all the more important to remove all unbound ions from both the tissue and the bag.

Our wash protocol may not have been adequate. We left the sample bags in 500 mL of milliQ water for 20 minutes. To increase the effectiveness of the water and possibly reduce the intra-species variation, the samples should be left in the water for a longer period. An hour may be more appropriate than 20 minutes as the other treatments of acid and sodium chloride each were an hour long. Also the wash may be more effective if the beakers containing the wash are shaken for the hour-long wash instead of just left stationary.

By measuring cation exchange capacity we can get a better idea of the relative physiological differences between the species' ability to scavenge for nutrients. But this ability to exchange ions may be directly correlated to the amount of polyuronic acid compounds in the moss' cell wall. By investigating the nature of uronic acids in the cell walls of the plant we may be better able to characterize the plant's nutrient scavenging abilities. Because the cell walls contain the uronic acid compounds, the more dry weight that a plant allocates towards cell wall, the more uronic acids the moss will contain, assuming uronic acid concentration in the cell wall is constant between species. We did not see this in the data. There was not a significant difference between any of the three species in terms of their cell wall concentration.

Like the cation exchange capacity results, the cell wall analysis did not yield may statistically significant results due to excessive intra-species variation (although less variation than the CEC). Also like the cation exchange capacity experiment, the methods could be improved to attempt to decrease this variation. The protocol to isolate cell walls consisted of a series of washes to break up cell membranes and wash away all internal components of the cells, leaving behind only cell wall. In order for the solutions to be

effective at breaking up the membrane, it must first be able to penetrate the cell wall that surrounds the cell membrane. There was no step in the procedure to attempt to break up the cell wall by chemical means in order to allow the other solutions access to the cell membrane. Instead the cell walls were broken up manually by grinding the tissue with a mortar and pestle in liquid nitrogen. Even though the samples were ground thoroughly, it was difficult to ensure that every cell had its cell wall manually broken up allowing the following solutions access to the membrane. The result was that after all the solution treatments there were two layers of tissue. One layer contained the pure cell wall resulting from the adequately ground plant tissue. The second layer contained the result of the inadequately ground plant tissue and was a mix of cell wall and other cell components. This method therefore yielded enough pure cell wall to use for uronic acid analysis, but may have not accurately showed how much dry weight the mosses devoted to cell wall. Because the second layer contained other cell components, the measurement of the fraction of cell wall may have been overestimated with this protocol.

In our analysis of the implications of cell wall content on nutrient uptake ability we assumed that polyuronic acid concentration in the cell wall was constant between species. Our results from the uronic acid experiment showed that this assumption may not be valid. *S. fuscum* had a significantly lower concentration of uronic acids in their cell walls compared with the other two species. This result means that in order to fully quantify the amount of nutrient uptake machinery it is not sufficient to use cell wall content as a proxy for uronic acid content. The cell walls of different species contain different concentrations of polyuronic acids and therefore a full analysis of uronic acid monomer content must be done to understand the cation exchange compounds.

The results from the uronic acid experiment also showed that the novel method of acid hydrolysis of the cell walls to obtain uronic acid monomers and the analysis of the resulting molecules by LC-MS/MS was a valid way to measure uronic acid concentration. The results showed that uronic acids made up between made up between 3.7% and 8.8% of the dry mass of the moss. Clymo (1963) looked at species where uronic acids made up 12-25 percent of the dry mass of the plant, so our results were comparable. Also the method allowed for reliable and repeatable results and yielded low intra-species variation.

In terms of our hypothesis, the fraction of dry weight that each species devoted to uronic acids was not what we originally expected. We had hypothesized *S. fuscum* would have the highest amount of uronic acids per dry weight because it grew in a nutrient poor environment that would require the moss to devote a lot of resources towards building nutrient gathering compounds. Instead what we saw was that *S. fuscum* had the lowest amount of uronic acids per dry weight. There are a few possible reasons for this discrepancy. For example there may be other compounds that are involved in nutrient uptake that we did not measure. There are many acidic compounds in the cell wall of *Sphagnum* species, such as phenol compounds, that may be able to donate a proton to take up nutrient cations. Perhaps *S. fuscum* utilizes other molecules in addition to uronic acids to fulfill its nutrient needs.

Another possible factor is the way that the uronic acid monomers are linked into polyuronic chains may vary between species. Differences in monomer linkages of the cell wall have been shown in other plant species (Zablakis et. al., 1995). In *Sphagnum* species, these differences in the linkages may results in more or less cation exchange sites

being exposed to the environment and available to nutrient uptake. *S. fuscum* may have a linkage conformation that is more favorable to cation exchange and is therefore able to utilize fewer uronic acid monomers to uptake nutrients more efficiently. The problem with this linkage hypothesis is that forming favorable linkage group does not necessarily come at a cost to the moss, whereas building addition polyuronic acid polymers to increase nutrient uptake comes at an obvious metabolic cost to the moss, and would result in less resources being available for photosynthetic machinery. Linking the monomer together in a certain way does not imply the same energetic costs that would be detrimental to photosynthesis, therefore linkage effects on the tradeoff between nutrient uptake and photosynthesis may be negligible.

A third factor that may have led to *S. fuscum* having the lowest uronic acid fraction of dry weight is that different uronic acids may not be present in equal concentrations among different species. There are many uronic acids that are found in the plant cell walls. The two main uronic acids in polyuronic chains are galacturonic acid, which is the major monomer, and glucuronic acid, which is present to a lesser extent. The two uronic acids are isomers with the same molecular weight, meaning that we could not easily distinguish between the two in the LC-MS/MS analysis of the samples and determine their relative concentrations. Because we did not therefore know the relative concentrations of the different uronic acids, we averaged the peaks obtained from the galacturonic acid standard and the glucuronic acid standard in order to estimate the amount of uronic acid in the samples. This makes the assumption that the two uronic acids are present in equal concentrations in the sample, which is likely untrue. Because the peak obtained from galacturonic acid standard resulted in a peak on the LC-MS/MS

that was half the magnitude of a glucuronic acid standard of identical concentration, this assumption would severely underestimate the uronic acid content of species high in galacturonic acid and severely overestimate the uronic acid content of species high in glucuronic acid. Therefore the assumption that was necessary for calculation could have skewed the results and underestimated *S. fuscum*'s uronic acid content if the species is high in galacturonic acid monomers. Another consequence of this assumption was that the uronic acid content of all three species in general was underestimated. Instead of 3.7 to 8.8 percent of dry weight, the actual fraction may be higher and more towards the Clymo (1963) estimate of 12 to 25 percent of dry weight.

In order to avoid making this invalid assumption in the future, the samples may need to be acetylated prior to LC-MS/S anlaysis. By removing hydrogens from all of the hydroxyl groups on the uronic acids and replacing them with acetyl groups, differences between the monomers are accentuated. This may allow the different monomers to be separated in the LC column by retention time and allow the individual monomer concentrations to be measured, resulting in more accurate measurements of uronic acid content differences between species. Accounting for individual monomer concentrations rather than lumping all uronic acids together may be even more essential if the different uronic acids have different affinities for nutrient cations. If galacturonic acid is better able to scavenge for nutrients than glucuronic acid, it would be even more important to determine individual monomer concentrations separately.

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