^{13}C Composition in Bryophyte Primary Sugars as an Indicator of Water Availability

By

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ABSTRACT

WILLIAMSON, OLIVIA ^{13}C Composition in Bryophyte Primary Sugars as an Indicator of Water Availability

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Bryophytes (mosses and their relatives) are a major carbon sink, and their productivity, is expected to be affected by climate change. Changes in plant productivity caused by changes in the climate can be tracked through stable carbon isotopes. This research aims to find a connection between stable carbon isotope signatures and water availability in bryophytes by examining the composition of ^{13}C in soluble sugars and bulk tissue. Similar to trees, which leave rings of growth every year, mosses build up peat deposits, which can be used to gain information about the weather and water availability of a region. Information on weather can be determined by the stable carbon isotope composition of bryophytes. Atmospheric carbon exists in two isotopic forms: ^{12}C (approximately 99% of the total CO_2 in the atmosphere) and ^{13}C (1%) and rubisco (the enzyme responsible for carbon fixation in photosynthesis) prefers the lighter isotope. Carbon dioxide diffuses 10,000 times slower in water than in air; consequently, when a water film forms on a plant leaf (particularly on moss leaves, which lack stomata) the plant will assimilate more ^{13}C, as rubisco becomes less selective when CO_2 becomes limiting. Differences in $\delta^{13}C$ in plant tissues are caused by such discrimination processes. Studies have shown a link between carbon isotope signatures in bulk tissues and moisture over time periods of several weeks, but there is little correlation on shorter time scales. Soluble sugars are primarily the result of new photosynthesis and sugars that have yet to be

integrated into the bulk tissue of the bryophyte. The goal of this experiment is to explore the timing of carbon isotope discrimination in bryophytes and determine if a short-term link can be made between carbon isotope composition and moisture levels by examining $\delta^{13}C$ at soluble sugars. Samples of *Sphagnum papillosum* were maintained in growth champers under two water conditions (dry and moist) over a time period of four weeks. Each week, a sample of plants (n=5) was collected and soluble sugars were extracted from their tissues. The carbon isotope composition of their bulk tissues was determined and a significant effect of both treatment and week was found. Over time, wet plants were less discriminating against ^{13}C where drier plants maintained the same carbon isotope composition throughout the experiment. In addition, a control experiment was preformed to find the carbon isotope composition of the soluble sugars.

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I would like to thank Professor Steven Rice for advising me through this project and introducing my to *Sphagnum.* Additionally, I would also like to thank professors David Gillikin and Anouk Verheyden-Gillikin for conducting $\delta^{13}C$ analyses and to acknowledge the NSF MRI grant (#1229258 to D. Gillikin): Acquisition of a Stable Isotope Ratio Mass Spectrometer for Interdisciplinary Research and Undergraduate Research Training. A Union College Student Research Grant provided addition funding for this research.

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INTRODUCTION

Faced with the growing concern of global climate change, it is important to understand the function and productivity of major carbon reservoirs, like bogs and fens. Plant productivities contributes massively to the carbon cycle and variation in water availability, which is amplified by climate change, affects this productivity. Plants become more or less productive depending on temperature, sun light availability, and water availability and scientists often turn to plants to learn about a regions recent meteorological history, through tree rings and branch growth scars (Loader *et. al,* 2003). Similar to trees, which leave rings of growth every year, some bryophytes (mosses and their relatives) build up peat deposits, which can be used to gain information about the seasonal climate of an area (Glime 2006). Understanding the mechanisms of plant productivity and water availability allows studies to be done on past environments by relating what we know about present day water use to samples from ice, tree, and bog cores. Understanding the timing and magnitude of plant productivity could potentially be used to monitor water availability in different habitats.

The moss studied in this experiment, *Sphagnum papillosum,* grow in and are an integral part of bog and fen type ecosystems. These nonvascular plants grow from the shoot apex forming thick mats, sometime meters above the water table. These mats have very low nutrient levels, getting most of their nitrogen and minerals from rainwater and run off (in the case of fens). As the moss grows, deeper sections are shaded out and die (depending on canopy development of the species); since there are such low nutrient levels and limited oxygen in bogs and fens, very few bacteria grow here to decompose the moss (Glime 2006). This builds up what are called peat deposits and leaves a record of the

environmental conditions at the time of growth (Loisel *et al.* 2009) analogous to tree rings. This growth record is in the form of carbon isotope composition at various depths of peat. When studying past environments, the peat record has some advantages over other available data. A prominent part of peat, *Sphagnum* mosses are a more direct reflection of environmental conditions compared to their vascular counterparts. Vascular plants have a higher concentration of lignin to maintain their complex vascular structures. Lignin breaks down more slowly than cellulose (the primary structural component of moss) and could then skew information of carbon assimilation (McCarroll & Loader, 2004). *Sphagnum* mosses are primarily water and cell wall, and amongst their non-structural components, soluble sugars, like sucrose are most prevalent (Marschall 2010).

Bryophytes follow the C3 pathway of carbon fixation so the plants are limited by the rate of carbon dioxide diffusion to the leaf. To assimilate carbon for photosynthesis bryophytes, like all C3 land plants, depend on the enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco). Carbon dioxide (CO_2) exists in two non-radioactive isotopic forms: $^{12}CO_2$ (99% of atmospheric CO_2) and $^{13}CO_2$ (1% of atmospheric CO_2). Rubisco preferentially fixes ^{12}C over ^{13}C, causing a fractionation and ultimately making bulk tissue of plants significantly more negative than the air. $^{12}CO_2$ is more plentiful in the atmosphere, and it diffuses slightly faster than $^{13}CO_2$ through water. However, in moist conditions, rubisco (which naturally prefers $^{12}CO_2$) becomes less selective and begins to assimilate stable carbon isotopes $^{13}CO_2$ at a higher rate (Rice & Giles 1996). The ratio of ^{13}C:^{12}C, also known as the $\delta^{13}C$, can be measured using a mass spectrometer.

As non vascular plants, bryophytes have developed unique water storing and transport systems that do not rely on conductive tissues. Amongst bryophytes, *Sphagnum*

sp. have a unique system for water transport. *Sphagnum* leaves contain a large network of dead hyaline cells, with spiral thickenings, which are used to store water. During development each hyaline cell forms a pore, (similar to stomata in vascular plant leaves, but without the desiccation control of guard cells), which allows the dead cell to fill with water, making it available to photosynthetic cells. The branching pattern of *Sphagnum* mosses also contributes to the water dynamics of the plant. *Sphagnum* shoots consist of several bundles of branches, each with two or three spreading branches, and two or three hanging. The hanging branches allow for water to be conducted up the stem via capillary action, while the spreading branches are primarily responsible for photosynthesis. *Sphagnum* mosses form thick mats in which each stalk contributes to the overall water dynamics of the canopy as a whole (Glime, 2006).

Sphagnum plants fractionate carbon at a rate similar to other terrestrial plants though they do not have guard cells flanking pores for water and gas exchange. Each component of the moss expresses a different isotopic composition but consistently in the same relative positions with the stem showing the highest fractionation followed by spreading branches and hanging branches showing the least discrimination (Loader *et al.* 2007). This data demonstrates the importance photosynthetic rate, vulnerability to drying play on the $\delta^{13}C$ composition of the plant. The stem is less actively photosynthetic and thus more discriminating against heavier carbon isotopes. Hanging branches help conduct water up the stem of the plant, and thus are more likely to develop a thick water film and be less discriminating. Spreading branches are the most photosynthetically active meaning they require more carbon (which could lead to less fractionation) but they are more likely

to dry out and loose their water film than hanging branches. This study will focus on the capitulum of the plant.

Sphagnum sp. have leaves of a single cell layer, and they rely solely on diffusion of carbon dioxide to undergo photosynthesis. Since bryophytes lack stomata in their ecologically dominant form, they have no control of their carbon dioxide and water uptake, beyond the holding power of hyaline cells. Consequently, water film development is a very important factor in the plant's overall productivity (Rice 2000). Photosynthetic cells are arranged in different characteristic patterns between species of *Sphagnum*, sometimes wedged between hyaline cells, so they have direct contact with the surface of the leaf, and sometimes they fall completely surrounded by hyaline cells, though this does not seem to dramatically affect resistance to diffusion of CO_2 (Rice and Giles 1996). Instead, diffusional resistance to CO_2 in *Sphagnum* and water film development accounts for a much higher limitation on the total photosynthetic. Rice and Giles (1996) found that in plants with free surface water, water film thickness controls carbon assimilation rates in situations where light is non-limiting.

To understand water film development as a limiting factor of growth rate, the mosses can be considered under two extreme conditions, those that are dry on the surface, and those that are submerged in water. Under high moisture conditions a water film can form on the leaves of the plants, and since the rate of carbon dioxide diffusion is 10,000 times slower through water then air, there is less carbon dioxide available to the plant. However, in completely dry situations the moss is unable to preform the biochemical functions it needs to produce new growth, meaning there is a threshold of moisture to achieve maximum photosynthesis. The presence or absence of external water films are

more significant to carbon isotope discrimination than the anatomy of individual leaves, though leaf structure can contribute to the development of these films (Rice & Giles 1996).

Moisture levels not only affect the carbon isotope signature of *Sphagnum sp.* but also the rate of carbon assimilation of the plant. A study done by Robroek *et al.* (2009) looked at *S. cuspidatum, S. magellanicum* and *S. rubellum* (three co-occurring species) to assess the interaction of water table height and moisture levels (precipitation) on carbon assimilation. The moisture manipulations were created by artificially maintaining a water table of either 10 cm or 1 cm below the capitulum of the *Sphagnum.* These samples were kept outdoors where they were naturally exposed to rainwater for 23 days. The assimilation of carbon was higher when the plants were grown in higher water tables. A lack of precipitation had a larger effect on carbon uptake than water table height and this association varied interspecifically.

There tends to be a high degree of variation when sampling bulk tissue directly from the field (Loader *et al.* 2007). A study by Rice (2000) investigated the impact of genetic variation, season, and microsite in both field and greenhouse common gardens using three species of *Sphagnum.* This study aimed to gain an understanding of the many factors that may contribute to the observed variation in carbon isotope discrimination amongst peat mosses. Three *Sphagnum* species were studied, all native to hollow, hummock, or lawn-type microtopographies. The study found that hollow species were the most discriminating against heavy carbon isotopes because the structure of their canopy is less conducive to water film formation. Wet plants show less discrimination to ^{13}C and as the plant dries it becomes more fractionated as rubisco always prefers lighter carbon isotopes as they become more available (Bramley-Alves *et al.* 2015). Rice (2000) found seasonal

variation in the carbon isotope discrimination in each of the three species of *Sphagnum.* In the spring when the plants experience little evaporation, water films develop, but when temperatures are ideal for photosynthesis, lawn and hollow species will grow rapidly and show a high demand for carbon and thus low discrimination. However, slow growing hummock species will not need as much carbon because they often display less photosynthetic activity, leading to higher discrimination as more carbon dioxide of both forms is available. In the summer, with higher rates of evaporation, boundary level resistance becomes more relevant as species (particularly lawn species) are subject to desiccation and increase in carbon isotope discrimination as photosynthesis slows. This theory applies on a long-term scale to the bulk tissue of the mosses, but because bulk tissue integrates carbon acquired the previous 1-3 months (depending on the rate of growth of the plant) it may not correlate well with water availability over shorter periods of time. This study was done testing only the bulk tissue for carbon isotope and saw these changes over several weeks. It is possible that targeting specific carbon based molecules could be valuable in determining more rapid responses to water availability.

In Antarctic bryophytes, Bramley-Alves *et al* (2015) investigated the effectiveness of using carbon isotope signatures of soluble sugars and other components of mosses as a measure of short-term water availability. This study found that over an Antarctic growing season (of about 5 weeks), the $\delta^{13}C_{sugar}$ showed variation in response to moisture levels. Bramley-Alves *et al* (2015) also looked at the carbon isotope composition of cellulose in the moss and bulk tissue. The study found that it was not until 22 weeks of the experiment that all three parameters (sugar, cellulose, and bulk tissue) correlated. Though $\delta^{13}C_{celluose}$ values were significantly less negative in wet environments, these values were consistently

more negative than $\delta^{13}C_{sugar}$, and closer to those of $\delta^{13}C_{bulk}$. This suggests that the mosses being studied quickly sequester new carbon in the form of soluble sugars but are less efficient when forming new structural tissue (as the carbon used to make new cellulose is developed from the soluble sugars). All three parameters of this study did not respond the same which means that $\delta^{13}C_{cellulose}$ and $\delta^{13}C_{bulk}$ may be more reasonable proxies for interseasonal changes. This study focused on artic mosses that experience a short growing season and fluctuations outside of ideal growing temperatures. According to photosynthetic temperature response curves, colder than ideal temperatures slow both carbon assimilation and new tissue development in mosses. *Sphagnum papillosum* is accustomed to a much longer growing season with higher rates of net photosynthesis, compared to their Antarctic counterparts, which may allow sugars to be developed into bulk tissues more quickly. Rates of carbon assimilation are also faster in *Sphagnum* than in the Antartic bryophytes leading to a more sensitive reaction to difference in moisture.

This experiment aims to determine the timing of carbon discrimination in bulk tissue and soluble sugars of *Sphagnum* mosses. Expanding on the work of Bramley-Alves *et al* (2015), this project will investigate carbon isotope discrimination of wet versus dry *Sphagnum papillosum* both in terms of bulk tissue and soluble sugar. I expect to find if plants are grown in moister environments, their bulk tissue and soluble sugars will have a heavier carbon isotope signature than their drier counterparts. Furthermore, soluble sugars will show a faster response than bulk tissues. Soluble sugars are expected to be a more accurate reflection of moisture level than bulk tissue over the four-week course of this experiment.

METHODOLOGY

To determine the timing of carbon isotope discrimination in *Sphagnum* mosses as it relates to moisture levels, a four-week long experiment was run using *Sphagnum papillosum.* Plants were exposed to either wet or dry conditions and samples were harvested each week. Soluble sugars were extracted from the moss and their carbon isotope composition was determined and compared to bulk tissue. In addition, a control study was conducted to determine if nutrient spray affected $\delta^{13}C$ in plants.

Plant Collection:

Sphagnum papillosum plants were collected from a fen in the Woodlawn Reserve, Schenectady NY. Plants were collected on October 14th, 2015 from a variety of locations across the fen. On location, capitulum were counted in areas equal to the size of the experimental cups to determine an average density of approximately 15 capitula per cup, which is equivalent to about 7,600m^{-2}. In the lab, plants were randomly placed into cups, leading to a mixture of locations being represented in each of the 50 6.5 cm tall cups, because location across the fen has been shown to affect carbon isotope signature of the plants.

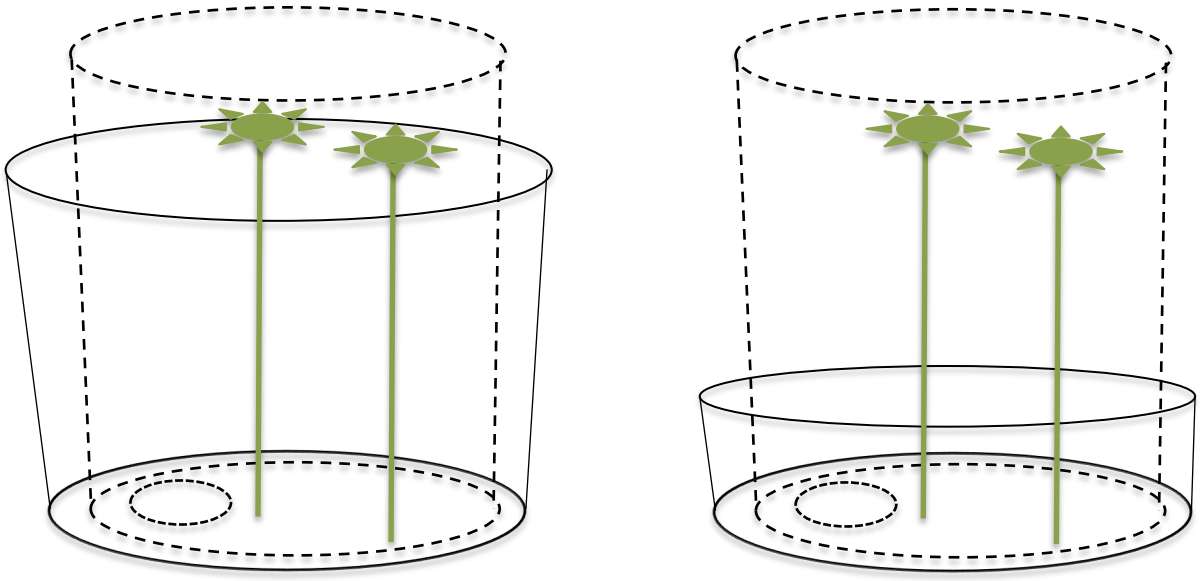

Figure 1. Experimental cup apparatus representing moist conditions (left) and dry (right). The outer cups are represented by solid lines. The inner cups are dashed. Water from the inner cup drains through the bottom hole into the outer cup and the outer cups are filled to the brim with deionized water. This keeps the water at either high or low levels similar to the water table experiments used by Robroek *et al.* (2009). The capitula are represented by the green figures, and 15 plants were assigned to each cup. Plants were watered 5.5cm up their stem in the wet condition, leaving approximately 1cm above the open cup, and 1cm up their stem in dry conditions, leaving 5.5cm of exposed plant.

Moisture Manipulation:

This experiment called for two manipulations to moisture levels: wet and dry (Figure 1). The wet plants were saturated 5.5cm up their stem, just to the bottom of the capitulum, and the dry plants were only watered 1.0cm from the bottom, about 5 cm from the bottom of their capitulum. Holes were drilled at the bottom of each of the 50 experimental cups using a 0.64 cm drill bit. The experimental cups were then placed inside one of 2 possible outer cups. These cups were cut to either a high or low height (Figure 1). The experimental cups were each filled with 15 capitulum. Upon the first fill, water was poured directly on to plants, all subsequent waterings were made by adding water to the

outer cup. The experiment ran for 4 weeks each week was assigned 10 samples, 5 for each of the two conditions (including a set of initials, for a total of 50 cups each with 15 individual plants).

The cups were randomized and placed under a grow light in a cold room. The average light intensity under the grow light was 230 (μmol photons m^{-2} s^{-1} of photosynthetically active radiation). The room was kept at high relative humidity and humidity and temperature levels were monitored throughout the experiment. Temperature and relative humidity was monitored with probes. The average temperature for the duration of the experiment was 19.0^{o}C and the average humidity was 92.1%.

Every week, 10 cups were harvested (5 wet and 5 dry). The handling was done wearing gloves to prevent oils from the skin to contaminate the moss. After removing that week's moss, the remaining plants were lightly sprayed with 1/10th Gamborgs B-5 Basal Medium (with minimal organics) diluted to 0.32g/liter of miliQ water. Spraying was done in a grid type formation, 9 sprays covering the entire moss surface in the early weeks of the experiment, and fewer sprays once less samples remained. The carbon isotope signature of this dilution of the nutrient spray was determined.

After harvesting for weeks 2 and 4 of the experiment, the capitula were weighed before and after drying to determine water content. Quantum Yield (Fv/Fm), an indicator of photosystem II functionality was measured using a Walz Mini PAM Chlorophyll Flouorometer; this measurement is also an indication of water content. One stalk from each repetition was placed in darkness for 10 minutes before the chlorophyll fluorescence was measured.

Following weight the wet plants, the capitulum were removed from the stem and dried in an oven at 60^{o}C for two days. Dried plants were placed in a desiccator to prevent any additional moisture from being absorbed before mosses were pulverized. Pulverization was done using a Wig-L-Bug and each vessel was cleaned of moss residue between samples using pressurized air.

Nutrient Spray Test:

The 1/10th Gamborgs B-5 Basal Medium may have an affect on the carbon isotope signature of the plants. To test this a separate experiment was preformed where plants were arbitrarily split into three groups: one that served as an initial read of carbon signature, one that was sprayed, and one that was not. 15 plants were put in each of the 15 cups (n=5), following the procedure outlined above and watered in the wet treatment (wet plants were chosen as they are usually more photosynthetically active and thus more likely to assimilate carbon from this source). The plants were harvested from the Woodlawn Reserve in the winter and given 10 days in the cold room under the grow light to become photosynthetically active once more. The chlorophyll fluorescence was measured and within normal range of a regular summer-time plant. The initial plants were harvested then both experimental groups the following week. These tissues were pulverized and processed comparably to the moisture manipulation tests.

Sugar extraction:

Sugar extraction was adapted from the procedure outlined by Brugnoli *et al* (1988) To extract sugars from the moss, 0.1 mL of pulverized moss was weighed into an eppendorf

tube. After several failed attempts using only miliQ water, 1.5mL of a 4:1 methanol dilution was added to the sample and heated to 100^{o}C for 30 minutes. Samples were allowed to cool for 15 minutes, vortexed, then spun at 10,000 rpm for 30 minutes. Next, 400µL of supernatant was placed in a syringe pushed through two columns: the Dionex OnGuard II H Ion Exchange Cartridge, and Dionex OnGuard II A Ion Exchange Cartridge (in that order). The columns were washed with 2mL of miliQ water. The remaining liquid should only contain soluble sugars.

Isotope Analysis:

The isotope values were analyzed using a Thermo Delta Advantage mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer via a ConFlo III at Union College. Bulk tissue samples were made by weighing 0.05mg of pulverized tissue into 8x5mm tin cups and carefully folding the cups into neat spheres. Soluble sugar samples were made by pipetting the soluble sugar solution into tin cups in three 50µL repetitions, allowing the solution to dry under the hood between each repetition, then the cups were folded. The isotopic value of the nutrient spray was quantified by pipetting 50 or 100µL of the diluted spray into tin cups. Samples were left in a desiccation chamber until being processed by the Union College Geology Department.

Analysis:

All analysis was done using JMP 8 software. Statistical tools used include two way analysis of variance, Student T-Test, least square mean of differences Tukey honest

significant difference, and LSMeans contrast. Figures were created on Excel using results from JMP analysis.

RESULTS

The results presented below do not include data from soluble sugar extracts of the water manipulations described above, but instead they indicate just reason to continue with extraction of these tissues.

Water manipulation

Though the initial water content was not measured, it can be assumed that all the plants started with roughly the same water content when they were collected from the field. Particularly wet and particularly dry areas were avoided and the *S. papillosum* were collected primarily from lawn type microtopography. The water content was not recorded until week 2 of the experiment. Figure 2 below shows the final water content in grams. The average water content was taken from each of the two groups and plotted with standard error. After the 4 week period, moss under the dry condition held significantly less water in their capitulum than plants in under wet conditions (p-value >0.001).

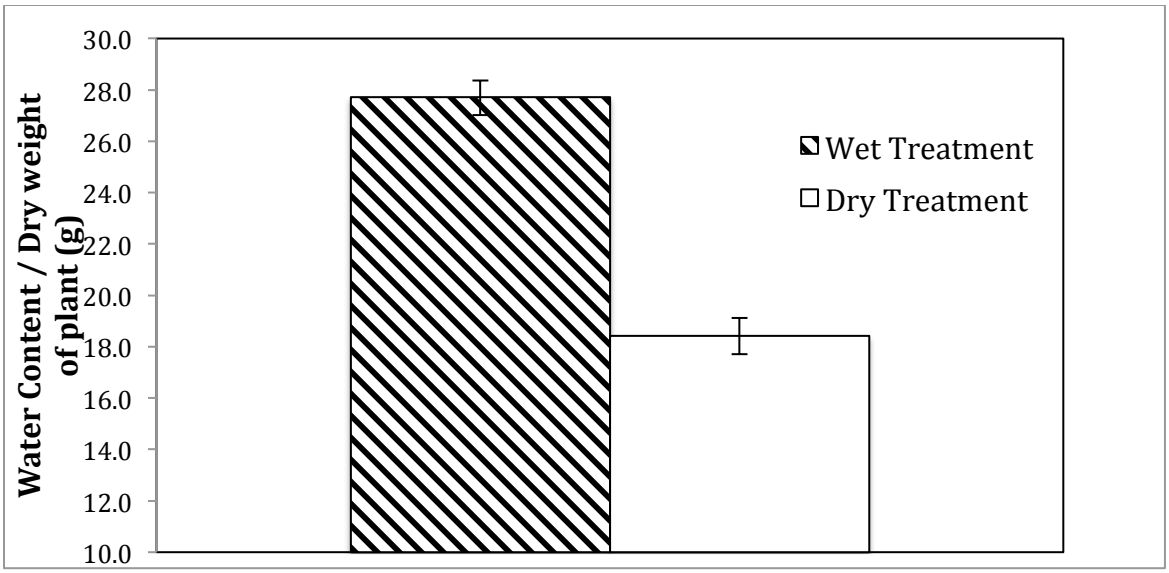

Figure 2. Water content per gram of dry tissue, week 4. Values shown are the average of 5 samples for either manipulation. P-value>0.0001. The average mass of water per gram of dry moss in the capitulum of the wet treatment was 27.7g and the dry treatment was 18.4g.

There was no significant affect on chlorophyll fluorescence between wet and dry plants within the same week (t=0.17). Chlorophyll fluorescence was not affected by week either (t=0.97), though this was the expected result. There was an interactive effect of the two independent variables (t=0.03) meaning the wet and dry plants changed differently over time.

Bulk Tissue Carbon Signature

There was no significant difference in the carbon isotope signature of bulk from the initial sample of plants (W0A-E and D0A-E) (p-value=0.9833). After week 0, there were significant differences between wet and dry bulk tissue each week (p-value (week 1) =0.03, p-value (week 2)=0.008, p-value (week 3)<0.0001, p-value (week 4)<0.00001). There was also an obvious affect of both week and treatment separately for the entire experiment (p-

value<0.0001). There was a strong positive correlation in the wet data (dashed line, y = 0.38x - 29.28 r^2=0.726). The slope of the trend line for the dry samples (solid line) does not vary significantly from 0 the r^2 value for this line was not reported because it is not significant.

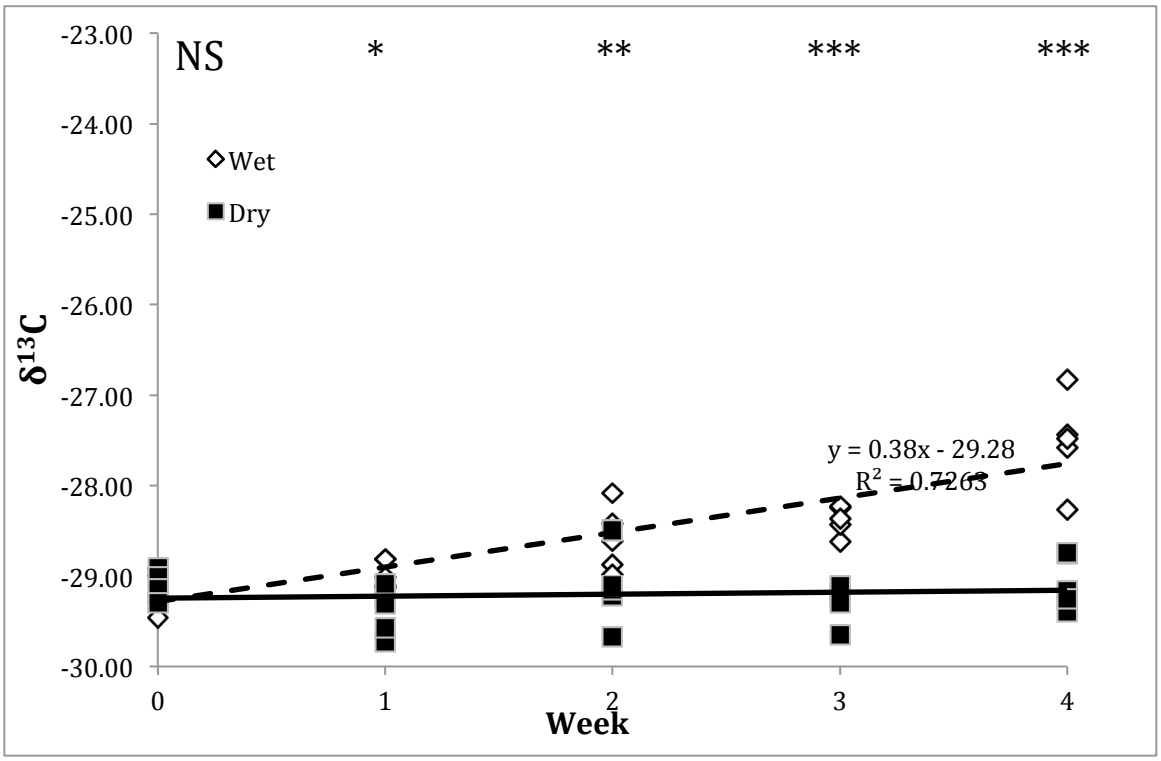

Figure 3. $\delta^{13}C$ signature of bulk tissues in *S. papillosum* wet and dry treatments by time. Empty diamonds and the dashed line represent the wet and filled squares and solid lines represent the dry treatments. There is a positive increase in carbon 13-isotope assimilation in wet treatment plants while there is no difference in the dry treatment. Significant differences between wet and dry samples per week are indicated by *. NS not significant; single * 0.05>p>0.01; double ** 0.01>p>0.001; triple *** p>0.001

Soluble Sugar Carbon Signature

Like the bulk tissue, there was no significant difference in carbon isotope signature of the soluble sugars at the start of the experiment. The wet treatment shows an

immediate increase in ^{13}C signature, which is not reflected in the dry tissue until week 2. Following the increase, both wet and dry saw a decrease in ^{13}C in the subsequent week, 2 and 3, respectively. The wet treatment then experienced a steady increase in ^{13}C while the dry treatment had no more change.

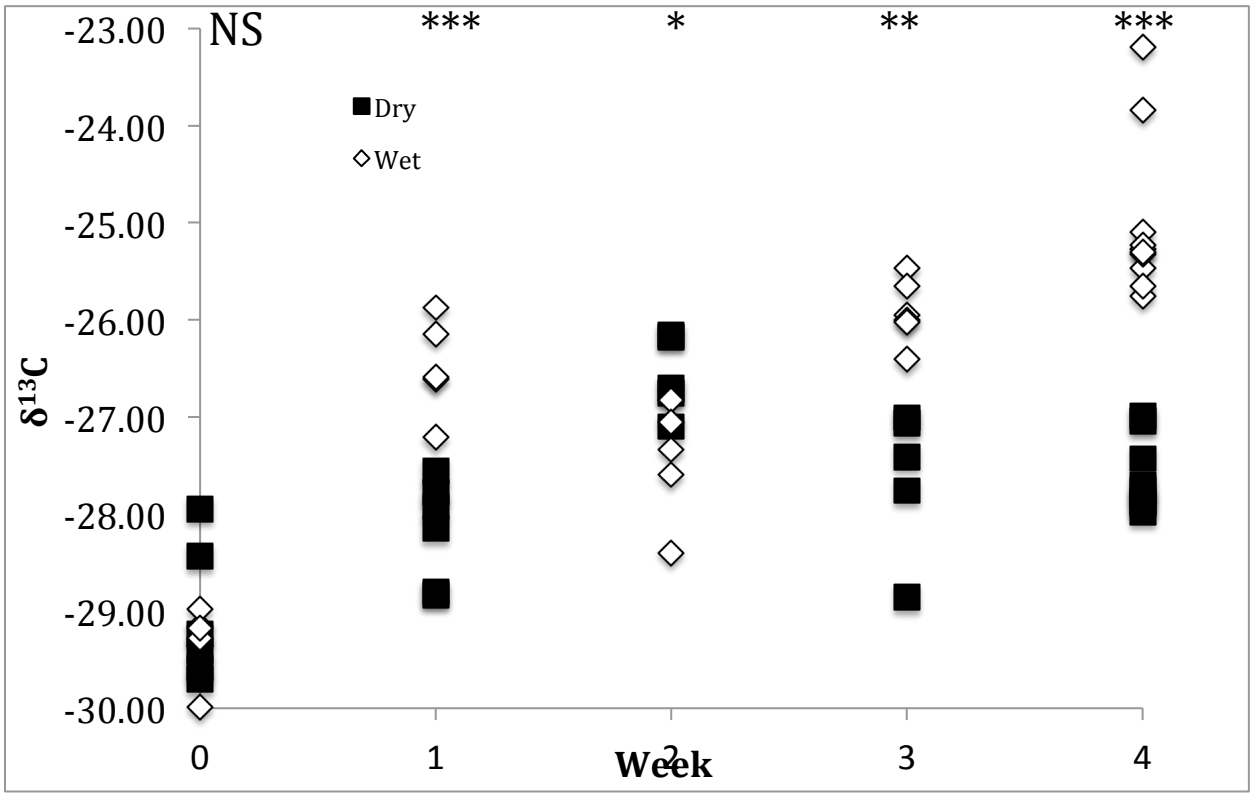

Figure 4. $\delta^{13}C$ signature of soluble sugars in *S. papillosum* wet and dry treatments by time. Empty diamonds represent the wet and filled squares represent the dry treatments. There is an initial increase in of ^{13}C in both treatments followed by a decrease. The dry treatment then stays at the same level for the final two weeks of the experiments, while the wet treatments increase in signature. Significant differences between wet and dry samples per week are indicated by *. NS not significant; single * 0.05>p>0.01; double ** 0.01>p>0.001; triple *** p>0.001

Nutrient Spray Analysis

The plants were sprayed with a potentially confounding nutrient spray. An experiment to test the effect of the spray was preformed. The chlorophyll fluorescence averaged 681 µmol photons m^{-2} s^{-1}. Soluble sugars were extracted from the plants and their carbon isotope signatures were measured. There was a significant difference between the initial carbon isotope values and the two experimental groups. Figure 4 below graphs the means of these values with standard error. At one week there was not a discernable difference between the sprayed vs. non-sprayed groups.

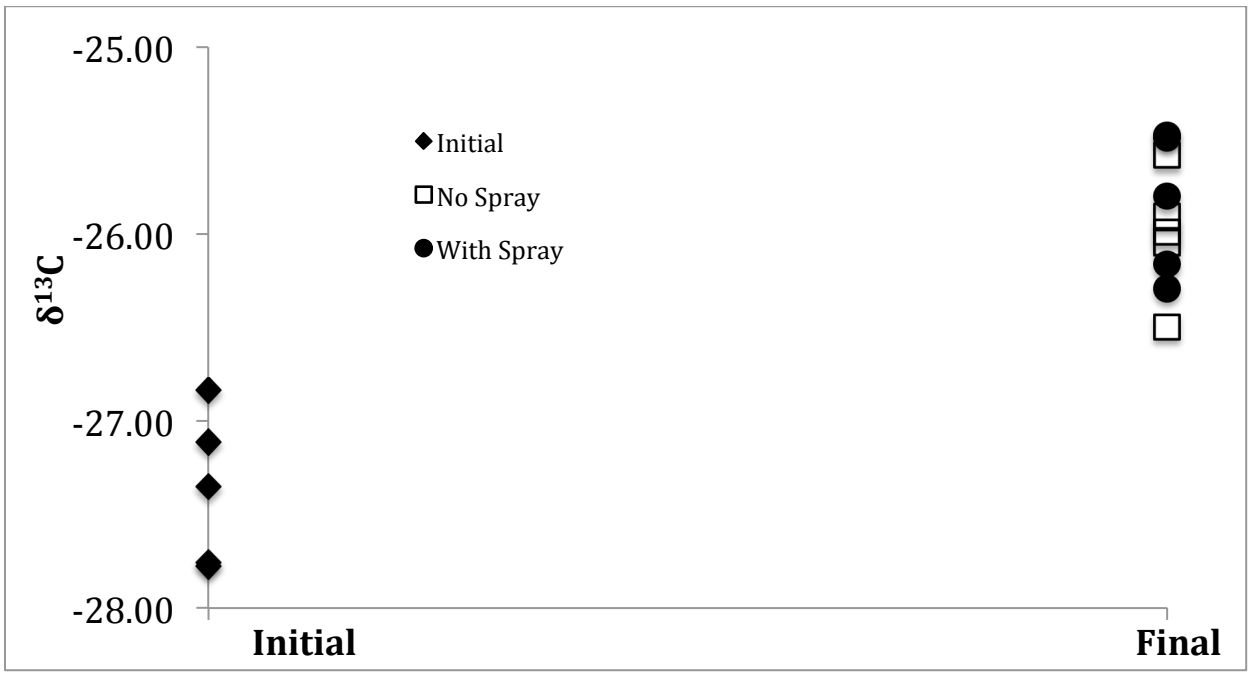

Figure 5. Carbon isotope values of soluble sugars in nutrient spray manipulations. Closed diamonds represent the initial group of plants (n=5), this value is likely representative of each of the groups at the beginning of this experiment (week 0). The open squares represent samples at week 1 that were not sprayed with nutrients. The closed circles were sprayed. There is no significant difference between these two groups but they are both significantly different from the initial group.

DISCUSSION

The manipulation of the water table was successful and the plants had significantly different water content by the end of the experiment. Productivity of the plants increases in higher water tables according to Robroek *et al.* (2009), which had a very similar wet condition (1 cm below the capitulum). This study did have presumably drier dry manipulations meaning the difference of carbon assimilation may have been more dramatic if our plants were drier.

The initial group of test plants (W0A-E and D0A-E) did not differ in their carbon isotope signature ($p=0.9833$). This implies that the samples were sufficiently randomized and it can be assumed that each plant started with roughly similar carbon isotope signatures at the beginning of the experiment. Loader *et al.* (2007) did find a high degree of variation in carbon isotope signature of plants taken directly from the field. This may be because they sampled from various locations through the bog, where this study focused on collecting from lawn type microtopography. Since there is a link between moisture and bulk tissue carbon assimilation (Rice & Giles 1996, Loader *et al,* 2007, Bramley-Alves *et al.* 2015) by collecting samples from similar areas through the fen and randomizing the plants prior to the experiment, we minimized this potential for error.

There was a significant difference between wet and dry groups after just the first week of water manipulation, which is consistent to the timing of plants in Robroek *et al.* (2009), but quicker than what was predicted in Bramley-Alves *et al* 2015. The drier plants did not vary much in carbon isotope signature throughout the experiment compared to the wet plants. There was no difference in the $\delta^{13}C$ in the dry plants, as seen in figure 3 the solid line, which represents a best-fit line for carbon isotope signature in the dry plants

does not differ from 0. There is however, a significant change in this trend as the average $\delta^{13}C$ value is initially -29.13 and ultimately increases to -29.06. These plants were kept moist only 1 cm from the bottom of their stalk, leaving about 5.5cm exposed, which is much less exposed stem than the manipulation by Robroek *et al.* (2009), so perhaps if longer plant samples were collected and more stem as left unexposed the plants would have showed the most carbon fractionation. As it is now, enough of a water film must have formed for carbon to be slightly limiting to plant growth as rubisco assimilated more ^{13}C than it did in the field.

The wet manipulation was significantly different not only from the dry treatment but also from the initial after just one week. There is a dramatic increase in $\delta^{13}C$ assimilation amongst the wet plants, which increases linearly throughout the course of this experiment (figure 3). These plants have formed a water film that remains consistent throughout the experiment and as the plants grow, a greater percent of the capitulum is made of newly assimilated material. As the carbon that existed in the plant at harvesting develops into stems and branches, more of the carbon in the plant is the result of new photosynthesis under wet conditions. If the experiment were to run longer, one would expect the carbon isotope signature not to continue on the same trajectory but to stabilize at a level reflective of consistently limited carbon.

The soluble sugar results were less obvious than the bulk tissue. The wet treatments more quickly reacted to the changing conditions (moving from a fen into a laboratory experiment) and as expected, the more metabolically slow dry plants reacted more slowly. The increase in $\delta^{13}C$ that both groups experienced was most likely a reflection of mobilization of stored starch to compensate for new conditions. After this

point, the dry group dipped closer to the original $\delta^{13}C$ signature and remained there, showing there was no pressure for the plant to assimilate ^{13}C as ^{12}C was not limiting. On the other hand, the wet treatment showed a steady increase in ^{13}C assimilation, which was reflected in the bulk tissue.

It is possible that this difference was in part due to the nutrient spray with minimal organics that was sprayed on all of the samples. Though the wet and dry plants were equally sprayed, the wet plants were more metabolically active. *Sphagnum* could possibly absorb and assimilate sugars on their leaves, so the more metabolically active plants may have been assimilating these organics more quickly than the drier plants.

Consequently, second experiment was done to see if the nutrient spray really does affect the carbon isotope signature of the sugars in the plants. For this test the plants were kept under wet conditions to increase metabolic activity and the chances carbon is limiting and the plants would take up the new source or carbon. The soluble sugars were tested instead of merely testing the bulk tissue because the soluble sugars should be more sensitive to changes in metabolic activity as it comes to carbon composition. There was a significant change in metabolic activity between the initial group and the two experimental groups (figure 4), but the experimental groups themselves were not significantly different. Furthermore when testing the carbon isotope signature of just the diluted nutrient spray, concentration of carbon was two small for the mass spec to measure. This implies that it was in fact the wet and dry conditions that cause the difference in bulk tissue seen in figure 3 and the nutrient spray did not have an overwhelming affect even when testing samples as low in carbon as the sugar extract.

CONCLUSION

This experiment successfully manipulated moisture levels, leading to bulk tissue samples that displayed significant individual and interactive affects. Soluble sugars were also extracted from the mosses and concentrated with little degree of error. The carbon isotope signatures of the soluble sugars changed more rapidly and to a larger degree than the bulk tissues in both the wet and dry conditions. Both the bulk tissue samples and the soluble sugar samples for the wet treatment showed an overall increase in ^{13}C assimilation, while the dry treatments did not experience this change. There does not appear to have been an affect of the nutrient spray that was added to the plants, so the next step in this process would be to analyze the soluble sugar from the water manipulation experiment. Further experimentation could involve a longer time period so one could see the ultimate carbon isotope signature of the bulk tissue compared to the sugar samples. This would allow one to determine how quickly carbon goes from being assimilated into the plant to becoming an actual structural component.

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