Carbon and Nitrogen Abundance, Isotope Fractionation, and Aquatic vegetation decay rates in Patuxent Freshwater Wetlands

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Abstract

To maintain elevation equilibrium, sediment accretion in tidal marsh channels must equal local sea-level rise. Sediment accretion consists of mineral and organic matter accumulation, and an important aspect of organic matter accumulation is decomposition, which has not been extensively examined in freshwater tidal marsh inlets. The role of decomposition in organic matter net accretion was the focus of this study. I examined organic matter decomposition rates using the litter-bag method and sediment cores studies. Three genera of marsh channel vegetation – *Zizania*, *Nuphar*, and *Hydrilla* – were collected, dried, weighed, and buried in the marsh upper sediment, then measured for weight loss and isotopic composition at one, two, four, and twelve week intervals. Initial isotope ratios of the plant material indicated significant differences among plant species. However, after 2-4 weeks of decomposition there was significant overlap in the range of $\delta^{13}C$ and $\delta^{15}N$ compositions of the two emergent plants *Zizania* and *Nuphar*, whereas both were significantly different than *Hydrilla*. Decay rates indicated that *Zizania* decomposed significantly slower than either *Nuphar* or *Hydrilla*. Sediment core data from the upper 50 cm of channel sediment indicate significant organic content in the upper five to ten centimeters of sediment, with significant decreases in organic content with depth. Organic-rich layers were analyzed for isotopic composition of identified plant fragments and for weight proportion of organic matter by bulk sediment mass loss on ignition. The organic matter found in the top ten centimeters of the marsh cores had similar isotopic compositions to the end-member litter bag samples. Isotopic changes occurred as a function of depth in the sediment cores and were different for nitrogen and carbon isotopes.

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Background

The survivability of coastal wetlands is a major concern due to their importance in protecting coastlines from storm surges associated with large storm events, such as Hurricane Sandy. Types of coastal tidal wetlands include freshwater tidal marshes, salt tidal marshes, and brackish tidal marshes. Salt marshes have been most extensively studied (e.g. Neubauer et al., 2002) due to their role as buffers against coastal erosion and storm surge events. Tidal salt marshes are found along shallow coastlines which allow frequent flooding by tidal seawater, whereas freshwater marshes are found in the transition between terrestrial rivers and shallow marine systems (e.g. Mitsch and Gosselink, 2000). Freshwater tidal marshes are composed of both marsh flats and adjacent tidal channels; all require a supply of fresh water and a tidally varying flow (e.g. Mitsch and Gosselink, 2000). Coastal wetlands perform important ecological functions; they are sites for sediment deposition and nutrient retention, including denitrification, and thus protect coastal water from eutrophication (e.g. Kim Lan et al., 2006; Seldomridge and Prestegaard, 2012; 2013).

An increase in relative sea level, which is the elevation of sea level in relation to a benchmark elevation, can result from sea level rise and/or decreased local subsidence. In the Chesapeake Bay region, sea level is rising at 3-4 mm a year (e.g. Glick et al., 2008). If this increase in sea-level is not offset by local deposition or other responses, then the volume of water in tidal channels will increase, which can affect tidal velocities and geomorphic responses. Marsh tidal channels can respond to these changes through land-ward channel extension, channel bank erosion, incision of the main channel, or increased flooding over the marsh platform (e.g. Mitsch and Gosselink, 2000). Freshwater tidal marsh areas may expand, if channel erosion does not undermine the stability of the marsh platform.

Marsh platform and channel elevation is maintained if sediment accumulation rates are equal to sea level rise rates. Local accretion includes both mineral and organic sediment. Most studies of tidal channel morphology have focused on mineral accumulation and erosion (e.g. Cloern et al., 2002), although organic matter accumulation and decay is the focus of most marsh platform studies (e.g. Walker et al., 2005). In tidal channels with extensive emergent vegetation, organic accumulation may be a significant factor in elevation maintenance.

The tidal freshwater wetlands in the Chesapeake Bay region have several common species of emergent and submerged channel vegetation. Emergent species include *Zizania* and *Nuphar* (fig. 1a, 1b). *Zizania* is, unusually, a C-3 grass; most grasses utilize the C-4 pathway for carbon fixation, which involves transporting atmospheric carbon from mesophyll cells to bundle

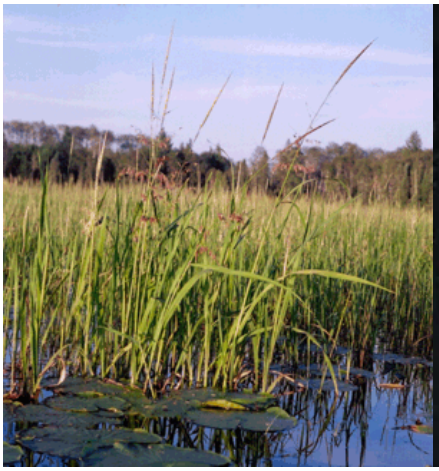

Figure 1a: *Zizania*

Figure 1b: *Nuphar* stem and leaf

Figure 1c: *Hydrilla*

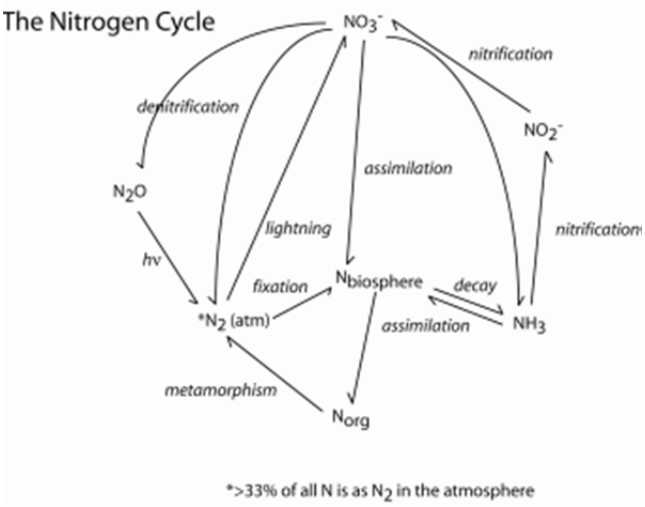

Figure 2: The nitrogen cycle

sheath cells for use in the Calvin Cycle (fig 3). *Nuphar* is also a C-3 plant, meaning both emergent genera fix carbon through open stomata for photosynthesis. *Nuphar* is perennial and grows rapidly from the rhizome in the spring, while *Zizania* is an annual plant that grows from seeds (e.g. Walker et al., 2005).

Hydrilla (fig. 1c) is an invasive submerged aquatic plant that grows at depths between high and low tide. *Hydrilla* is capable of switching to alternative carbon fixation pathways when under stress (e.g. Rao et al., 2002). Unlike the other plants being studied, which get nitrogen from ammonia, *Hydrilla* acquires nitrogen through the uptake of nitrate (Kaufman, personal comm.).

How a plant takes in carbon and nitrogen is important because it can affect isotope fractionation within the plant tissues. Utilizing the same carbon fixation pathways can mean the plants have similar isotope abundances within their tissues. For example, carbon fixation processes prefer ^{12}C, which means plants that fixate carbon from the atmosphere directly will take in less carbon 13. On the other hand, C-4 plants tend to undergo less carbon isotope fractionation than C-3 plants, because all isotopes of carbon are brought into bundle sheath cells before carbon fixation occurs, which results in all isotopes of carbon being used (e.g. Rao et al., 2002). Nitrogen isotopes are also favored by the different reactions of the nitrogen cycle (fig. 2), resulting in isotope fractionation during fixation, decay, nitrification, and denitrification. How a plant takes in nitrogen thus affects nitrogen isotope abundance in plant tissue.

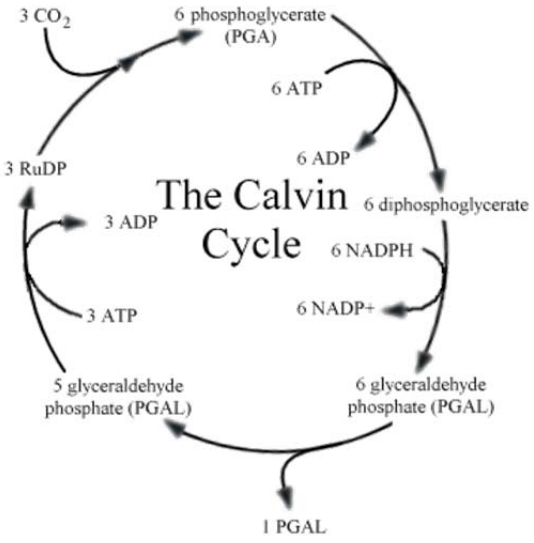

Figure 3: The Calvin Cycle

These plants have a significant impact on channel morphology. *Zizania* contributes a large amount of organic material after the winter die back – the plant is fibrous and leaves significant identifiable fragments that remain in the sediment for an extensive period of time. This sediment builds up the banks of the channel and eventually provides nutrients for plants in the spring (e.g. Walker et al. 2005). Most plant material left by *Zizania* remains on the surface of the marsh platform when first deposited; the shoots topple over, lying on top of the sediment as it decomposes (e.g. Lan et al., 2006). Both *Zizania* and *Nuphar* modify the shape of tidal channels by slowing the flow of water, catching and accumulating sediment where they grow (Stakieweisz pers. comm.). When *Nuphar* dies back in the fall and winter, the shape of the channel cross section changes as sediment previously accumulated by the plants is eroded away and the main channel incision is filled, giving the channel a more

uniformly concave shape (Stakieweisz pers. comm.). Although not native to the Chesapeake region, Hydrilla is observed in the summer months to capture sediment on its stem and leaves. It propagates by drifting portions of its stem into new sites and is largely absent from channel beds by late fall.

Changes in carbon isotope, nitrogen isotope, and carbon to nitrogen ratios in organic material from freshwater tidal channels were analyzed through vegetation and sediment core analysis. Samples were obtained from areas of the marsh dominated by *Nuphar*, *Zizania*, and *Hydrilla*, located in Anne Arundel County off of the Patuxent River. Three types of material were collected by kayaking into the wetlands: fresh vegetation samples of the three genera of plants (obtained in the fall), decomposing vegetation experiments in the form of nylon mesh bags of plant material (referred to as the litter bag method) buried in the channel sediment, and sediment cores from sections of the marsh dominated either by *Nuphar* or *Zizania*. These samples were analyzed via mass spectrometry to identify δ13C and δ15N and C:N ratios; these values were calculated through the following equations (e.g. Cloern et al. 2002):

$$\delta 13C = [(^{13}C/^{12}Cplant)/(^{13}C/^{12}Cstandard)-1] *1000$$

$$\delta 15N = [(^{15}N/^{14}Nplant)/(^{15}N/^{14}Nstandard)-1] *1000$$

Where $^{13}C/^{12}Cplant$ is the abundance of carbon 13 to carbon 12 in the plant material, $^{13}C/^{12}Cstandard$ is the abundance of carbon isotopes in a standard substance of VPDB, $^{15}N/^{14}Nplant$ is the nitrogen isotope abundances in the plant material, and $^{15}N/^{14}Nstandard$ is the nitrogen isotope abundance of the atmosphere (e.g. Troxler et al. 2009). Carbon to nitrogen ratios are calculated as a weight percentage of total carbon to nitrogen, determined by mass spectrometry.

Decomposition is often measured using the litterbag method. Plant matter is sown into permeable bags with small enough openings to prevent loss of material, but large enough to allow small insects that facilitate decomposition. These bags are then placed in the field for a period of time to decompose (e.g. Cloern et al. 2007). The three species of plants are made of different types of tissues, use different sources of nitrogen, and may decompose at different rates due to organic matter of different composition.

Hypotheses

The goals of this study were to test the following hypotheses:

1. The initial $\delta^{13}C$ values of the three plants will be similar, as all use the C-3 photosynthetic pathway and should thus undergo similar C isotope fractionation.
2. The C:N ratios and $\delta^{15}N$ of the plants will be distinct due to differences in N sources among the plants; *Hydrilla* utilizes nitrate instead of ammonia and should have a different N isotopic composition than *Nuphar* and *Zizania*.
3. The $\delta^{13}C$ values in the residual plant matter will increase overtime with decomposition. Plant components such as lignin that are ^{13}C-enriched decay slower than other plant components (e.g. Benner et al. 1987).

4. *Nuphar* will decompose the fastest, giving a higher $\delta^{13}C$ value than *Hydrilla* or *Zizania*, the latter of which is expected to have the lowest $\delta^{13}C$ change due to decomposition. N isotopic composition will separate Hydrilla from the two other species.
5. The three plant species will each have unique isotopic compositions in core samples. Organic matter from sediment cores will also have similar isotopic compositions to the end members of the decomposition experiments.

Study site and Methods

Choice of Study site

The plant samples, decomposition experiments, and core samples were obtained from tidal inlets that are currently being studied by Stakiewiscz and Prestegaard (pers. Comm.). They have been measuring vegetation density, shear stress distributions, and accretion in tidal channel inlets. They found that each plant species occupies a different, but overlapping, section of the marsh channel – in a 29.0 meter channel, Zizania is found at the shallowest depths (0.1 to 0.45 m), Nuphar is found between 0.25 and 0.7 meters, and Hydrilla is found between 0.4 and 1.2 meters (Prestegaard and Stakieweiz, personal comm.). Although the emergent species Nuphar and Zizania grow in different sections of the marsh channel, each is the dominant species in different tidal inlets; rarely do they grow side by side. The plant and sediment core samples were obtained from these same tidal inlets (fig. 4).

Sample Collection

Fresh samples of *Nuphar Zizania* and *Hydrilla* were collected with the help of Dr. Prestegaard and graduate student Anna Statkiewicz, from a tidal channel of the Patuxent River in Anne Arundel County (fig. 4). The vegetation samples were collected for both litter bag experiments and for isotopic analysis. Collection of the plant material involved kayaking into the marsh, clipping the standing vegetation, and storing them temporarily in plastic bags. The

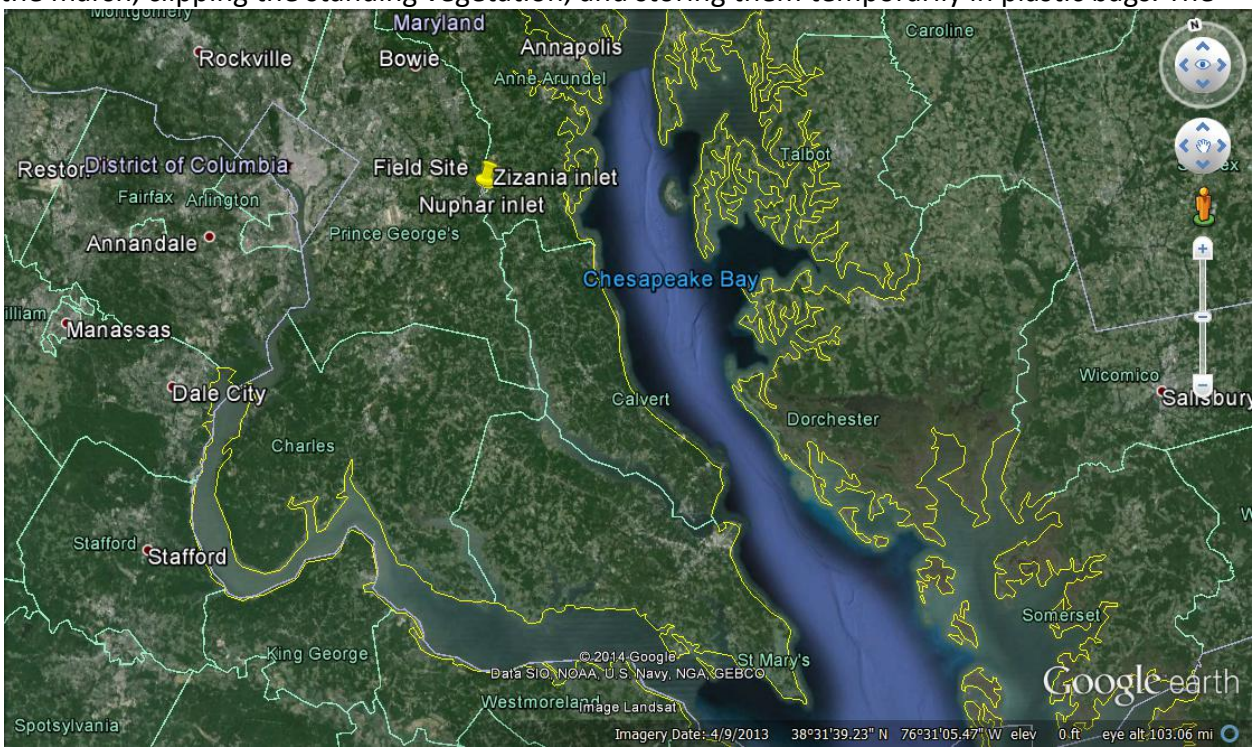

Figure 4: Google Earth location of field site relative to the Chesapeake Bay

vegetation was washed and dried at ~60 ° Celsius for two to three days in an oven located in Dr. Prestegaard's lab. The dried material was then either stored in the freezer for isotopic analysis or used as material in litter bag experiments.

Litter Bag Experiments

Litter bag experiments are a common experimental procedure in ecological studies. Live or standing dead leaves and stems are collected, dried, weighed into aliquots, and sewn into bags. Multiple bags are placed at the field sites and are retrieved at periodic intervals. The material in the bags is dried and reweighed to determine the amount of mass lost. Organic matter in the litter bags are exposed to the normal fluctuations in temperature and moisture experienced at the site. The mesh allows small insects and microorganisms, such as bacteria and fungi, access to the material.

For this study, dry vegetation was weighed into allotments of the same mass, 11.0 grams for *Zizania* and *Hydrilla* and 8.3 grams for *Nuphar*, placed in individual mesh bags made of two millimeter nylon webbing, and sewed shut on all sides with nylon thread (fig. 5). The remaining plant material was kept frozen, to prevent decomposition, until ready for isotopic analysis.

Figure 5: A nylon mesh bags, cell phone to scale

The vegetation decomposition experiments required periodic field excursions for sample collection. Dried vegetation had been weighed and sewn into individual two millimeter nylon mesh bags with nylon string sown into the side. Nylon fabric is essential for the experiment, as the material is inert, unlike other materials such as cotton which would decompose with the

Figure 6: the PVC pipe posts created, with holes drilled into the top, allowing strings to be tied

Figure 7: Posts in the tidal channels. Sediment samples tend to float, and must be buried

plant material. PVC pipe was cut into posts using a hacksaw with holes drilled in the top so the nylon string could be tied (fig 6). These PVC pipe sections were then taken into the field by kayak, with the help of Dr. Prestegaard, and stuck into the shallow organic sediment of two channel inlets, where the mesh bags were buried to prevent floating (fig 7). The posts were placed in a *Nuphar* dominated inlet towards the south (fig 8) and a *Zizania* dominated inlet towards the north of the marsh (fig 9). The vegetation in the mesh bags at each location correspond to the dominate type of vegetation found there; the northern inlet had twelve samples of *Zizania* and eight samples of *Hydrilla*, and the southern inlet had twelve samples of *Nuphar*.

Due to the potential inaccessibility of the northern marsh in hazardous winter weather conditions (it can only be accessed by kayakingboat), samples of *Zizania* were also placed in the southern inlet (fig 8). The southern marsh is closer to the road, and can be accessed without kayaks, allowing for samples of *Zizania* to be retrieved if the northern marsh was inaccessible. However, because microbes responsible for decomposition of specific plants may vary by location, these results may be less accurate. All litter bags were successfully located in the appropriate marshes by the conclusion of study.

Samples were collected at approximately one, two, four, and eight week intervals, dried, weighed, and frozen to preserve the organic matter, before being prepared for isotopic analysis using the previously outlined crushing and microbalance measuring procedures. Although freezing, thawing, drying, re-wetting, then drying of the vegetation may impact the decomposition process, freezing and thawing were common processes at the field during the study period, from September 2013 to April 2014.

After each time interval, three mesh bags of *Zizania*, *Nuphar*, and two of *Hydrilla* were collected, resulting in four different intervals to determine decomposition rate and calculate variance. The time intervals were staggered, so samples of *Zizania* and *Hydrilla* were collected on different dates then *Hydrilla*. The mesh bags were cut open over aluminum trays, to catch any organic material that may fall out of the bags. As much plant material within the litter bags as possible was carefully removed from the mesh by hand, which were washed between sample handling to prevent contamination. However, algae living on the plants, especially *Nuphar*, is difficult to remove, and affects the measured isotopic signature. For the purposes of this study, no attempts were made to scrap off algae from the plant material, as algae

Figure 8a: The area of the southern marsh where samples of *Hydrilla* and *Zizania* were placed; below in map view, with location marked by white arrow.

contribute to the decomposition process and organic sediment accumulation of the plants being studied.

Figure 8b: The southern marsh in map view

Figure 9: The northern marsh location where samples of *Hydrilla* and *Zizania* were placed, in map view

<u>Elemental Analysis</u>

All plant material collected wad stored in plastic bags for data analysis and record keeping. Emptied bags were stored in zip lock bags labeled with the plant material, date of collection, and number of days decomposing, as a record of the amount of material left in the bags. The aluminum trays containing individual samples were labeled with the sample type, number, and date of collection, and are dried in an oven at 60° Celsius in Dr. Prestegaard's lab for two to three days, then weighed on a mass balance to measure the mass lost during decomposition. The samples were stored in plastic bags before an aliquot of each sample (*Nuphar* stems, *Nuphar* leaves, *Zizania*, and *Hydrilla*) were prepared in Dr. Kaufman's lab.

An aliquot of each plant sample was taken, including *Nuphar* stems, *Nuphar* leaves, *Zizania*, and *Hydrilla*, frozen with liquid nitrogen, and crushed with a mortar and pestle into powder, performed using Dr. Kaufman's lab and equipment. The goal was to create a completely homogenized powder of plant material; however, some plant material proved more resilient to the crushing process. *Zizania* in particular is tough to obtain a homogenized powder, due to its higher lignin composition (Kim Lan et al. 2006), making homogenization relatively difficult. *Nuphar* stems and *Nuphar* leaves were separated to determine whether there is a difference in isotopic signature between different plant components. Between each sample crushing, the mortar and pestle was cleaned with ethanol solution and kimwipes, and then used to grind baked sand. This was done to remove traces of previous plant material, preventing the contamination of future samples.

Tin capsules were used for mass spectrometry. A microgram balance was zeroed to the weight of each capsule, at which point 100 µg of crushed plant material was placed in each capsule using a microscoop and tweezers. Two tin capsules containing material of each sample were used (two for *Nuphar* stems, two for *Zizania*, etc.), for a total of eight plant samples for the initial carbon isotope analysis. Standards of urea were weighed with the plant sample capsules to establish the instrumental precision. Standards of urea are used because the carbon and nitrogen abundance and isotope ratios are well known, so deviations in measurements of urea standards can identify the accuracy of results obtained for the plant samples. A separate batch of samples was prepared for analyzing the δ15N and C:N ratios of the plant material, as the amount of nitrogen present in the initial 100 µg was not enough to yield interpretable results. Eight additional samples were prepared, containing 2000 µg of plant material. Capsules were sealed and crushed into spheres with tweezers, then placed in labeled tray slots so that they could be run through the mass spectrometer with the help of Lab Manager Rebecca Plummer.

The amount of organic matter remaining at each point in time was plotted as a function of time and a first order exponential function was fit to the data. Decay rates, k, were calculated using the fitted equation:

$m_t = m_0 * e^{-k*t}$

(e.g. Kim Lan et al. 2005; Longhi et al. 2008), where m_t is the mass of a plant sample after t days, m_0 is the initial mass of the plant material, and k is the decay constant.

	Weight of Dried Plant Material	Number of Samples placed	Date Placed
Zizania (Upper Marsh)	11.0 g	12	10/16/2013
Zizania (Lower Marsh)	11.0 g	4	10/20/2013
Hydrilla (Upper Marsh)	11.0 g	8	10/16/2013
Nuphar 1 (Lower Marsh)	11.5 g	3	10/20/2013
Nuphar 2 (Lower Marsh)	8.3 g	9	10/26/2013

Table I: the amount of material placed in litter bags at each site

Sediment Core Collection and Analysis

Sediment cores were collected from two different marsh locations – the upper *Zizania* dominated marsh and the lower *Nuphar* dominated marsh. A peat sampler was driven into the ground with the blade pointed out and then closed. This left a half meter, half cylinder column of sediment (fig 10). The cores were separated into two centimeter intervals (for the top 10 cm of the core) and five centimeter intervals (for the lower 40 cm). Sediment from these interevals were placed into individual labeled plastic bags (fig 10) while in the field. These sections were kept frozen until February, at which point the sections were cut in half, dried, and examined for visible plant fragments. Half of the five sections from each sediment core that had the most visible plant matter were identified, taken to Dr. Kaufman's lab, and broken apart. The organic material was then crushed via mortar and pestle, isotopically analyzed, and graphed with depth of sediment core acting as a proxy for time.

The remaining half of the sediment core sections were kept frozen until bulk density and total organic content could be assessed. These sections were dried in March, at which point they were crushed into powder and weighed using a microbalance. Then, the powder was placed in crucibles in an oven set for 450 degrees Celsius in order to ignite the organic matter.

After eight hours, at which point all organic matter had been ignited, the sediment was allowed to cool for two to three hours before measuring the mass on a microbalance to determine the difference from the initial mass, which represents the organic sediments that had been ignited.

Figure 10: Sectioning the core and dividing them into labeled plastic bags. The bags are labeled with the sediment core number, the marsh location, and the depth range of that section.

Results

Litter Bag Experiments: Decomposition Rates

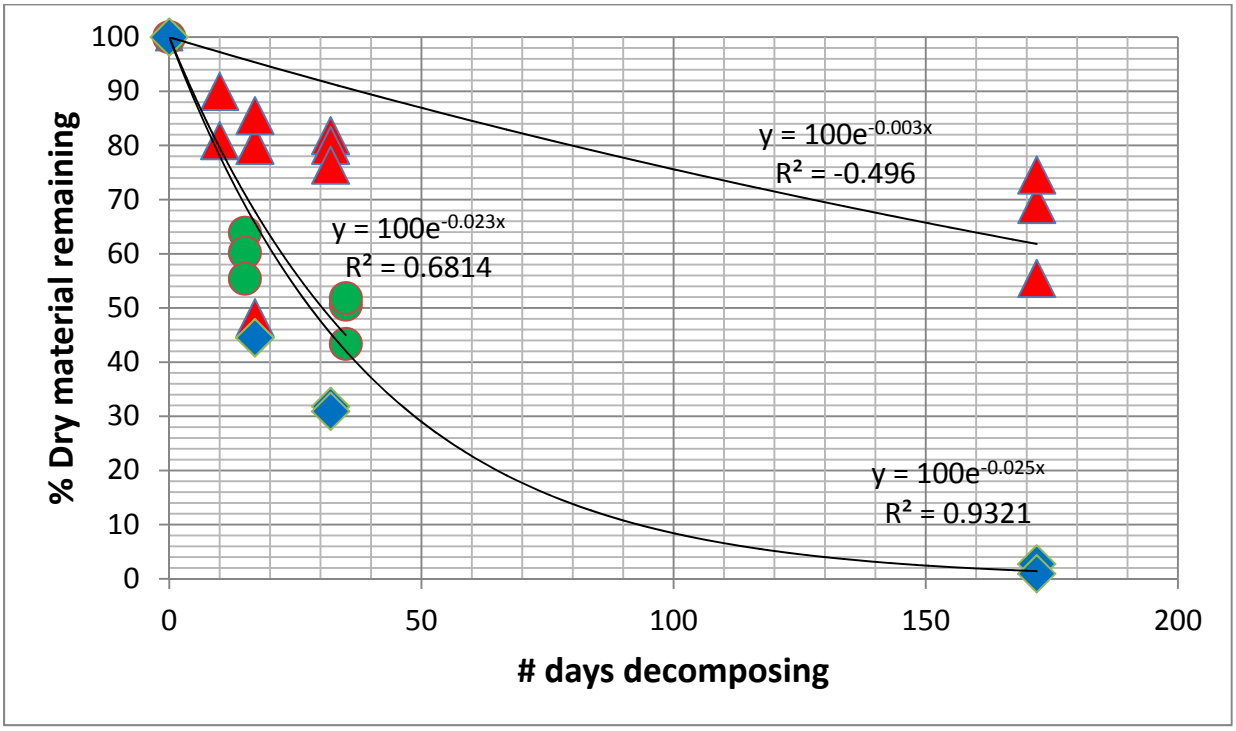

Table II: The remaining plant material from litter bag experiments at each time interval.

The decomposition rate of *Zizania* was distinct from those of *Nuphar* and *Hydrilla*. *Hydrilla* decomposed the fastest, with less than 5% of the organic material remaining after 170 days of decomposition. *Nuphar* also lost mass quickly, averaging around 45% of its mass remaining after 35 days. The last samples of *Nuphar* were unobtainable from the field; however, *Hydrilla* had at the same time interval about 10% less mass. The decay rates for both were very similar, with .023 for *Nuphar* and .025 for *Hydrilla*. *Zizania* averaged more than 60% of its mass remaining after 172 days, but had more outliers and a wider range of mass lost recorded, making it difficult to fit an accurate decay rate equation. However, it is unmistakable that the resilience of *Zizania* tissues is higher than that of *Nuphar* or *Hydrilla*, with a decay constant of .003.

Litter Bag Experiments: Isotopic Compositions

Fresh *Hydrilla* plant material is isotopically distinguishable from both *Nuphar* and *Zizania* due to significantly higher $\delta^{15}N$ values and lower $\delta^{13}C$ values obtained than either of the other plants (Appendix I, Table III). Samples of *Zizania* and *Nuphar* are also distinguishable from each other by $\delta^{15}N$ per mil, as *Zizania* has a significantly higher concentration of ^{15}N than *Nuphar*. However, they are not distinguishable based on ^{13}C concentration, with *Zizania* and *Nuphar* having a large degree of overlap in $\delta^{13}C$ values. *Nuphar* stems and leaves were separated to determine the isotopic distinction between different plant tissues within a single plant could be; in terms of $\delta^{13}C$ and $\delta^{15}N$ there are no significant differences in concentrations for *Nuphar*.

The elemental composition of carbon and nitrogen in fresh plant material is similar between all three plants studied, falling within one to two percent of the each other (Appendix I, Table IV). Nitrogen displays a similar degree of variation, with all samples falling under four percent. There is a slight difference between *Nuphar* stems and leaves, as *Nuphar* leaves have a little over a two percent higher concentration of nitrogen than stems do. However, one *Zizania* sample contained a significantly higher percentage of carbon than the rest of the plant samples, indicating a high degree of variability between plant samples.

Over time, the isotopic signature distinction in the litter bag experiments between *Zizania* and *Nuphar* decreased, while *Hydrilla* remained distinguishable (Appendix I, Tables V, VI, VII, VIII). After two weeks, $\delta^{13}C$ values of *Nuphar* and *Zizania* retained a high degree of overlap, while both plants converged between 6.5 and 8 $\delta^{15}N$ per mil (Table V). The carbon compositions of both also overlap to a significant degree (Table VII). *Nuphar* stems and leaves saw an increase in the proportion of $\delta^{15}N$ and nitrogen in overall composition, while *Zizania* lost a relatively high amount of ^{15}N and nitrogen percent. The range of values obtained from *Nuphar* stems and leaves also increases as decomposition proceeds. These trends continue through the litter bag analyses from the four week samples, where the $\delta^{13}C$ and $\delta^{15}N$ values of these two plants are completely indistinguishable from each other (Table VI). However, after up four weeks of decomposition, *Hydrilla* remains isotopically distinct. Samples of *Zizania* and *Nuphar* never reach 10 $\delta^{15}N$ per mil, while *Hydrilla* always remains between ten and twelve per mil (Table V and VI). *Hydrilla* also decreases in $\delta^{13}C$ concentration quicker than the other plants, increasing the difference in ^{13}C over time. However, the difference in carbon composition of *Hydrilla* and *Zizania/Nuphar* decreases throughout decomposition, while the nitrogen composition of *Hydrilla* remains indistinct from either (Table VII and VIII).

There were few consistent trends in $\delta^{13}C$ over the course of several time intervals in the litter bag experiments (Table IX). Both *Nuphar* stems and leaves had an increased spread of values over time, and the average at each time interval does not indicate a trend of increasing or decreasing concentration. The same is true for *Zizania*. An ANOVA analysis was performed to determine whether or not the results obtained were significantly distinct to identify them as from separate populations. The total variance calculated was less than one between *Nuphar* and *Zizania* samples, indicating a very low chance of a statistically significant distinction between the two plants. *Hydrilla* was found to be statistically relevant, being significantly lower in concentration and displaying a limited within group variability. The means $\delta^{13}C$ at each interval demonstrates a consistent decreasing concentration trend.

The same process was applied to the nitrogen isotopic data as well (Table X). *Hydrilla* is less distinct from *Zizania* and *Nuphar* in ^{15}N concentration than ^{13}C, and decreases in ^{15}N over time. The overall statistical significance of the nitrogen results was less than that of the carbon, due to less distinction between *Hydrilla* and the other two types of plants and a greater overlap in data ranges for *Zizania* and *Nuphar*.

Sediment Core Analysis

Four sediment cores were analyzed – two from a *Zizania* dominated northern marsh inlet and two from a *Nuphar* dominated southern marsh inlet. The five most organic rich sections from each core were selected for organic matter content and isotopic composition (Appendix II, Table XI and XII). All four sediment cores have an organic rich upper layer from the surface to 10 cm deep, with each sediment core varying in subsequent organic layer depths.

Organic matter from the top 10 cm have similar $\delta^{13}C$ and $\delta^{15}N$ values as the four week litter bag experiments. With depth along the sediment core, $\delta^{13}C$ values become higher in *Nuphar* cores before flat lining around 20 to 30 cm deep, while in *Zizania* cores $\delta^{13}C$ shows a more consistent increase with depth throughout. There's a high degree of overlap between *Nuphar* and *Zizania* $\delta^{13}C$ values obtained, as in the litter bag experiments. All four sediment cores show a similar pattern in $\delta^{15}N$ depletion. *Zizania* and *Nuphar* cores have similar starting $\delta^{15}N$ values, and decrease in concentration at similar rates further down the sediment core. The similarities in values make plant identification based on isotopic analysis of the cores impossible.

Percent organic matter was observed to consistently decrease with depth in all four sediment cores (Table XIII). Starting from 20 to 30% mass lost on ignition in the top 5 cm of the sediment core, by 30 cm depth the sections were less than 10% organic content. Despite observable bands of dark organic matter throughout the sediment core, the percentage of sediment that is organic decreases with time. *Zizania* cores were not found to have significantly higher organic matter content, nor were *Nuphar* cores.

Discussion

Hypotheses

The data from the isotopic analysis of the fresh plant material confirms the first hypothesis. Isotope analysis of the fresh plant material showed that initially the three marsh plants had similar $\delta^{13}C$ values, except *Hydrilla*, but could be distinguished from each other

based on $\delta^{15}N$ concentration, which was unique to each species. However, the C:N ratios of the initial plant material for each species were not distinguishable, with C and N compositions varying within plant species as much as they varied between the plant species. The second hypothesis, that C:N ratios and $\delta^{15}N$ concentrations for each plant type would be unique, is partially confirmed.

The third hypothesis was that $\delta^{13}C$ values for each plant would increase with decomposition, due to higher concentrations of $\delta^{13}C$ in more resilient plant tissues (e.g. Benner et al. 1987). No consistent trend was found in *Zizania* or *Nuphar* $\delta^{13}C$ concentrations, where as *Hydrilla* decreased in $\delta^{13}C$ over time. This indicates that there are more variables in isotopic fractionation of decomposing organic matter than the isotope abundances of resilient tissue. During the process of denitrification, CO_2 is released alongside nitrogen; bacterial preferences for certain carbon isotopes could impact isotope fractionation.

The fourth hypothesis of this study was that due to greater resilience of certain plant tissues that tend to be $\delta^{13}C$ enriched, the $\delta^{13}C$ values seen in decomposed plant material would increase over time, and *Zizania* would decompose the least, yielding the lowest change in isotope abundance. *Nuphar* was expected to decompose the fastest, corresponding with the greatest increase in $\delta^{13}C$. While *Zizania* did decompose the slowest, *Hydrilla* was found to decompose faster than *Nuphar*, and no consistent trend of isotope fractionation could be identified in *Zizania* or *Nuphar*. *Hydrilla* demonstrated a consistent decrease in $\delta^{13}C$ with decomposition, and had the lowest $\delta^{13}C$ values at each time interval with the greatest degree of isotope fractionation. This indicates no correlation between $\delta^{13}C$ concentration and decay rates in Patuxent marsh vegetation. However, *Nuphar* stems and leaves yielded unique isotope signatures initially, confirming that there is a difference in isotope abundances among different plant tissues. There are a couple explanations for this – first, while a literature review did indicate a pattern in the isotope fractionation in certain plants' various tissues, these plants were not the same marsh vegetation this study examined, and thus may not hold the same pattern of fractionation. Additionally, there are environmental differences between the Patuxent River and other study areas such as the San Francisco Estuary.

The final hypothesis links the litter bag experiments with the sediment core analysis, predicting that the organic rich top layer of the sediment cores will isotopically match the end member fractionation results from the litter bag experiments. *Nuphar* sediment cores were predicted to have higher $\delta^{13}C$ values initially due to the falsified idea that higher decomposition rates would result in higher $\delta^{13}C$ values. The sediment cores' organic matter was also proposed to be distinguishable isotopically, and *Zizania* was predicted to have higher organic matter content due to a smaller amount of mass lost from decomposition. Both types of sediment cores, *Zizania* and *Nuphar*, match the isotopic signature of the end member litter bag experiments in both $\delta^{13}C$ and $\delta^{15}N$, confirming the first part. This supports the idea that the organic sediment contributions consist mainly of plant matter falling into the sediment in situ, and not being washed farther downstream, which is what was observed in the field. *Zizania* was observed to topple over in situ, and *Nuphar* tended to shrivel and decay in place. However, the lack of significantly distinct isotope fractionations between different plants, both in the litter bags and sediment cores, indicates that the plant material comes from similar plants, but not necessarily the dominant vegetation in that specific area. Sediment from a *Nuphar* marsh being

deposited in a *Zizania* marsh would not be detectable, as they do not contain significantly higher concentrations of ^{13}C, as was suggested, or significantly distinct concentrations of ^{15}N.

Implications

As shown in the litter bag experiment, *Zizania* and *Nuphar* values became less distinct as decomposition proceeded. This makes identification of *Zizania* and *Nuphar* from each other based on isotopic analysis of deceased organic matter impossible, which is confirmed by sediment core data, disproving the second part of the fifth hypothesis. Isotopic analysis of the substrate is thus not adequate in determining what type of marsh vegetation was living in an area at the time of sediment deposition. However, identification of plant fragments found in the sediment cores was possible, especially for *Zizania*, which retains characteristic stalks of plant material due to its resilience. *Nuphar* also had several fragments that were easily identified, although more ambiguity exists due to further decomposition. It is therefore plausible that the type of marsh vegetation living at the time of deposition can be determined through sediment core analysis. This, however, was only observed in the top 50 cm of sediment; identifiable plant fragments may not exist at increasing depths.

Based on sediment core analysis, it appears that other factors have a strong influence on isotopic composition besides plant material. While there was not a clear trend of increasing or decreasing $\delta^{13}C$ values in the litter bags, the *Nuphar* sediment cores display an increase in $\delta^{13}C$ before flat lining, and the *Zizania* cores demonstrate a consistent increase in $\delta^{13}C$ per mil. This flat lining could indicate the end of the denitrification process, which releases carbon from the plant material, as organic sediment stops decomposing due to sediment burial, preserving the remaining plant material. However, within the same time frame as the $\delta^{13}C$ flat line, $\delta^{15}N$ continues to decrease in concentration, indicating some microbial process could still be at work. Quick burial of organic matter by mineral sediments could preserve the organic fragments, preventing further decomposition. Assuming that $\delta^{13}C$ decreases with decomposition, as is the case with *Hydrilla*, the only plant that demonstrated any consistent pattern in isotope fractionation, than the trend exhibited in Table X could also indicate that burial of the organic matter occurred earlier in the season than it does now.

The percentage of organic material in the different sediment cores did not indicate a significant difference between *Zizania* marsh percent organic mass and *Nuphar* marsh percent organic mass. This indicates it is unlikely that *Zizania* contributes more sediment long term than *Nuphar*, as both have a similar degree of organic matter preservation. In addition, organic content within the sediment cores decreased consistently with depth, and therefore time. Despite identifiable plant fragments existing at depths up to 40 cm, it does not seem to matter whether organic material is buried by mineral sediments rapidly or not. Organic mass is still being lost after burial, possibly through a combination of erosion and decomposition. Continued decreases in $\delta^{15}N$ and consistent decreases in percent organic matter indicates decomposition does not likely end with burial. However, between 25 and 40cm the decrease in percent organic matter appears to flat line. This could indicate the depth at which decomposition processes stop and organic material becomes a more permanent part of the substrate.

Initial organic contribution to sediment accumulation ranges from 20 to 35% of total sediment mass. This indicates that organic material contributes significantly to elevation

equilibrium initially. However, decomposition removes the majority of this mass long term, resulting in about 3 to 8% of long term sediment being organic material. The importance of organic sediment accumulation therefore rests in maintaining elevation equilibrium through yearly diebacks that maintain the elevation of the marsh through large quick inputs of sediment. These bursts of sedimentation account for a considerable contribution to local elevation, as 30% of the top 10cm of sediment is organic material, or about 3 cm. This sediment undergoes significant decomposition and erosion every year, and so local elevation equilibrium must rely on consistent yearly contributions of organic sediment to match rising sea level. Any decrease in vegetation biomass will result in less short term organic sediment accumulation, which could allow an increase in water volume in tidal channels, potentially harming the future of key wetlands.

Future Work

There are still aspects of elevation equilibrium and sediment accumulation to be studied in the Chesapeake Bay watershed region. While this study focused on the impact of organic sediment decomposition in the Patuxent River wetlands, organic and mineral sediment accumulation has not been sufficiently examined. An analysis of deeper sediment cores would be helpful in determining the extent of decomposition processes within buried sediment. Examining other plant species in the wetlands will help determine if any significant isotope fractionation occurs between plant species in the same tidal channels. Analyzing total plant biomass accumulation during the spring and summer growing season could give insight into how much organic sediment is provided to the wetlands over the course of a year. Studying the effects of storm events, flooding, or year to year mineral accumulation rates could also help determine how much total accumulation will occur in a given year.

Additionally, the impact of rising relative sea level on the region should be looked into further. Measuring channel width, main incision depth, and inland extension of the channels over several years would give insight into how the Patuxent responds to the increase in water volume. The Potomac and Susquehanna rivers, as well as other tributaries to the Chesapeake Bay, should also be examined for a complete understanding of Chesapeake Bay elevation equilibrium and the responses to increasing sea level rise rates.

Acknowledgements

I'd like to thank my advisors Dr. Karen Prestegaard and Dr. A. J. Kaufman, for their instruction and assistance in the field and lab, and for allowing access to their equipment day in and day out. Dr. Prestegaard was of great help in the field, taking the time to instruct me on field safety and procedures and invaluable in helping me collect the samples needed to conduct this research. Dr. Kaufman was generous both in allowing the use of his laboratory equipment and instructing me on the procedures needed to analyze the acquired samples for the necessary data, without which this research would also not be possible. Both were immeasurably helpful in discussing the background knowledge, research progress, and results obtained throughout the project. I thank Dr. Candela for running an excellent thesis program, his constant assistance in the progression of multiple projects, and his writing expertise that has

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Appendix I – Litter Bag Data

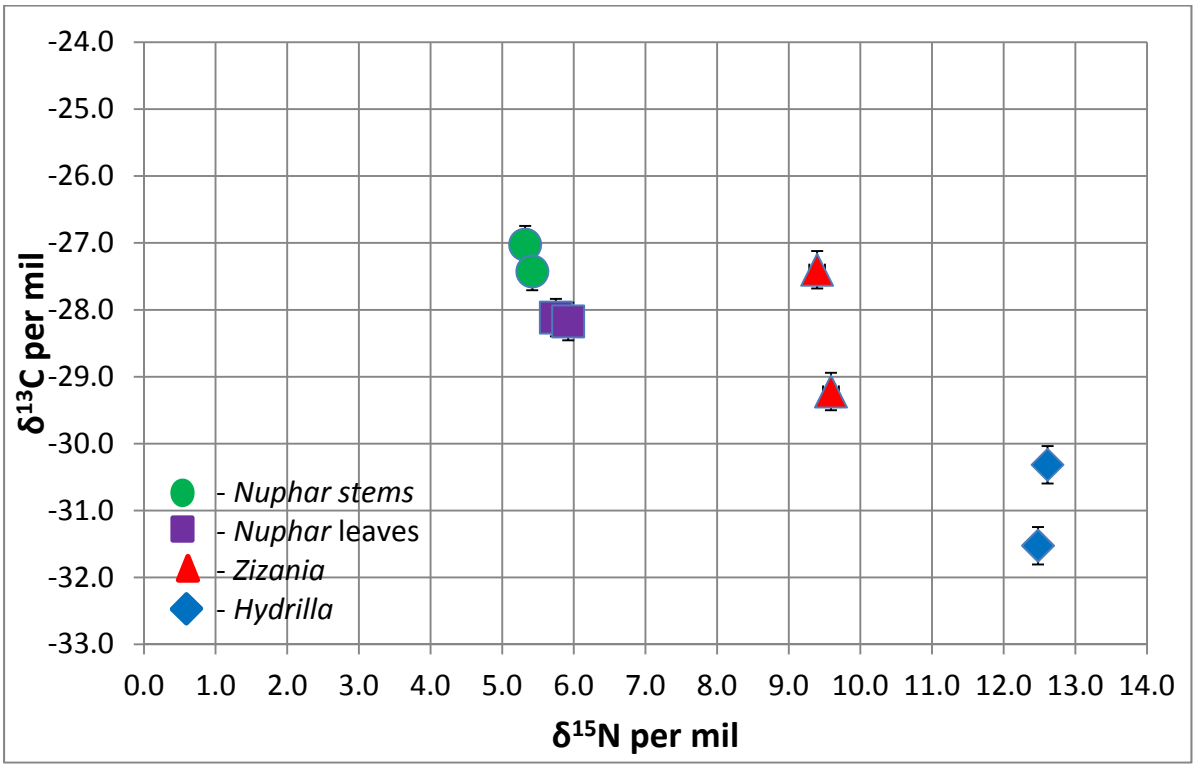

Table III: $\delta^{13}C$: $\delta^{15}N$ ratio of initial plant material. Initial results indicate plant sediments may be distinguishable based on isotopic composition

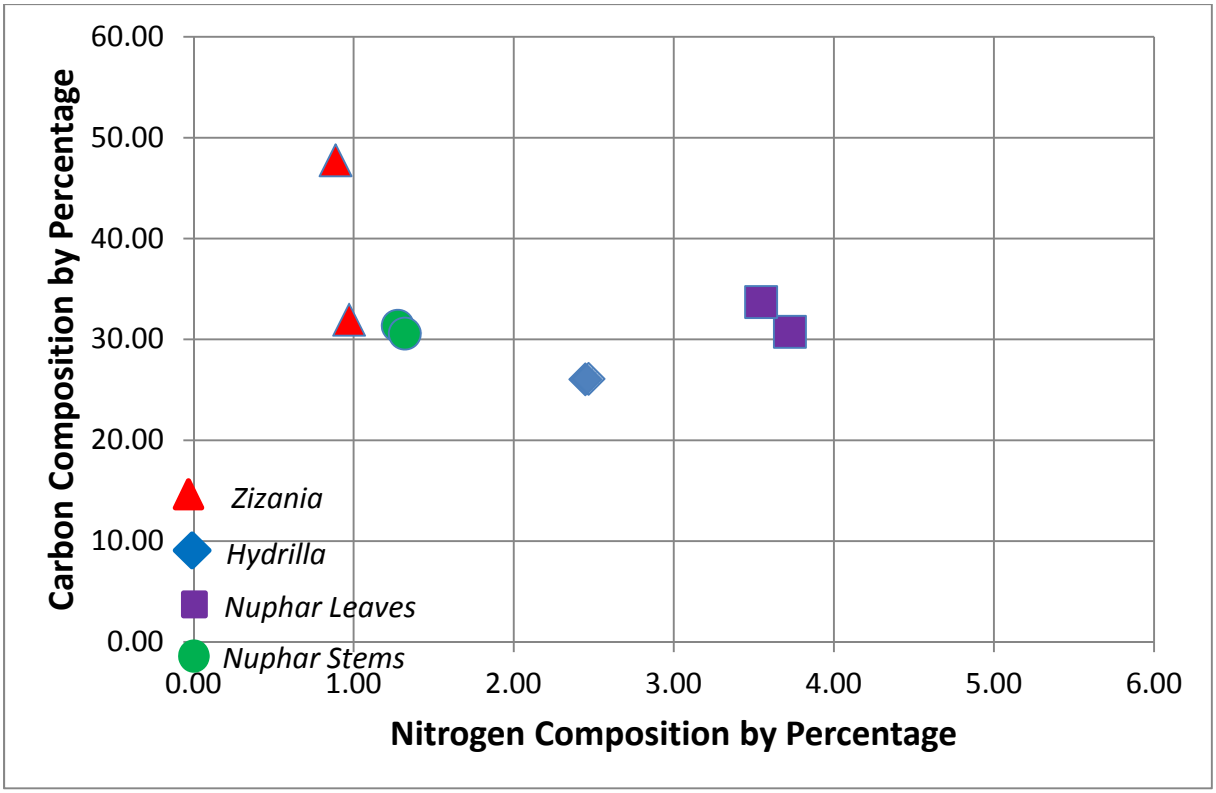

Table IV: C:N ratio of initial plant material. Elemental composition does not vary significantly between plants

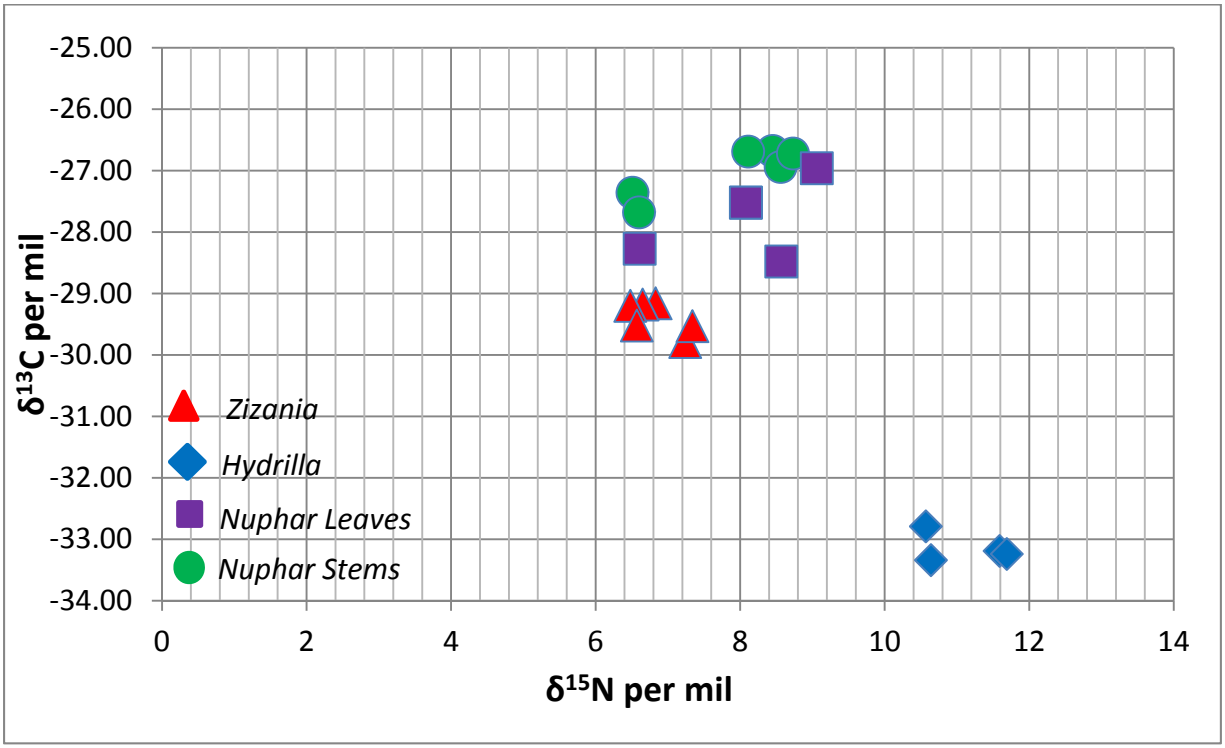

Table V: $\delta^{13}C$:$\delta^{15}N$ ratio of plant material after 2 weeks of decomposition. Note how *Zizania* and *Nuphar* have significant overlap in both isotope compositions.

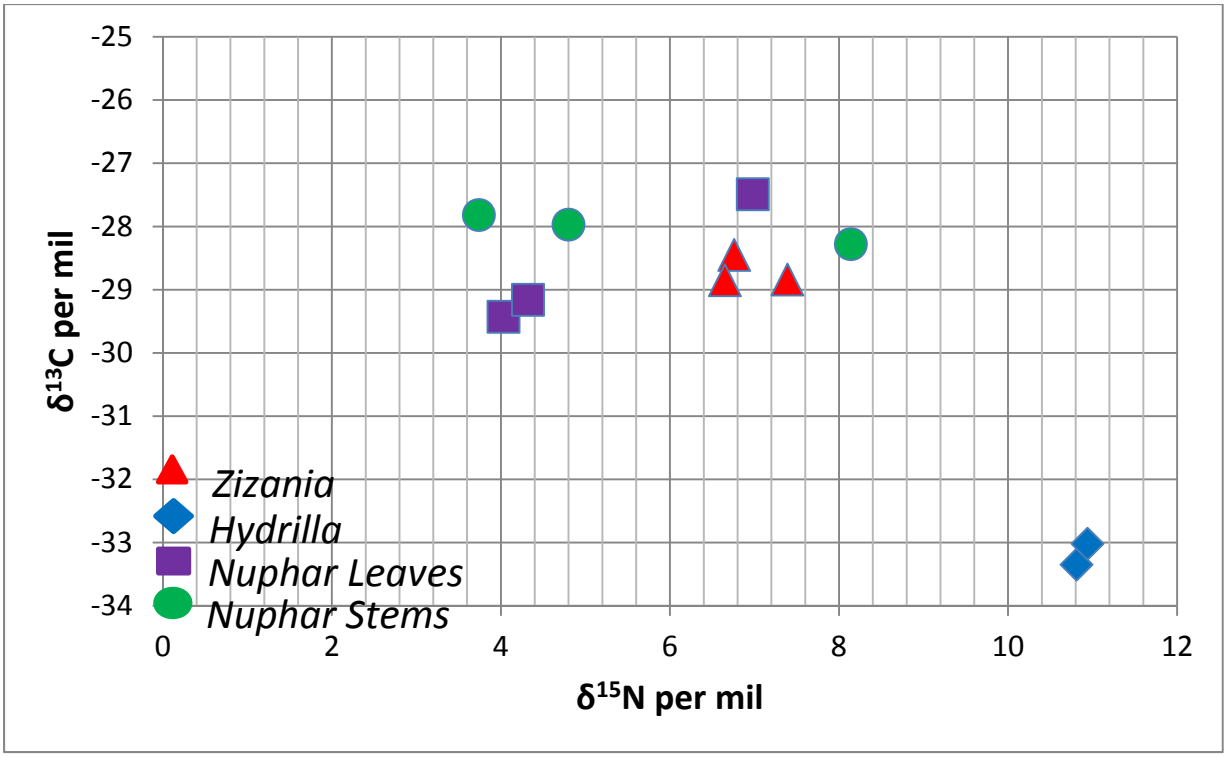

Table VI: $\delta^{13}C$: $\delta^{15}N$ ratio of plant material after 4 weeks of decomposition. *Hydrilla* is the only plant type distinguishable from the others.

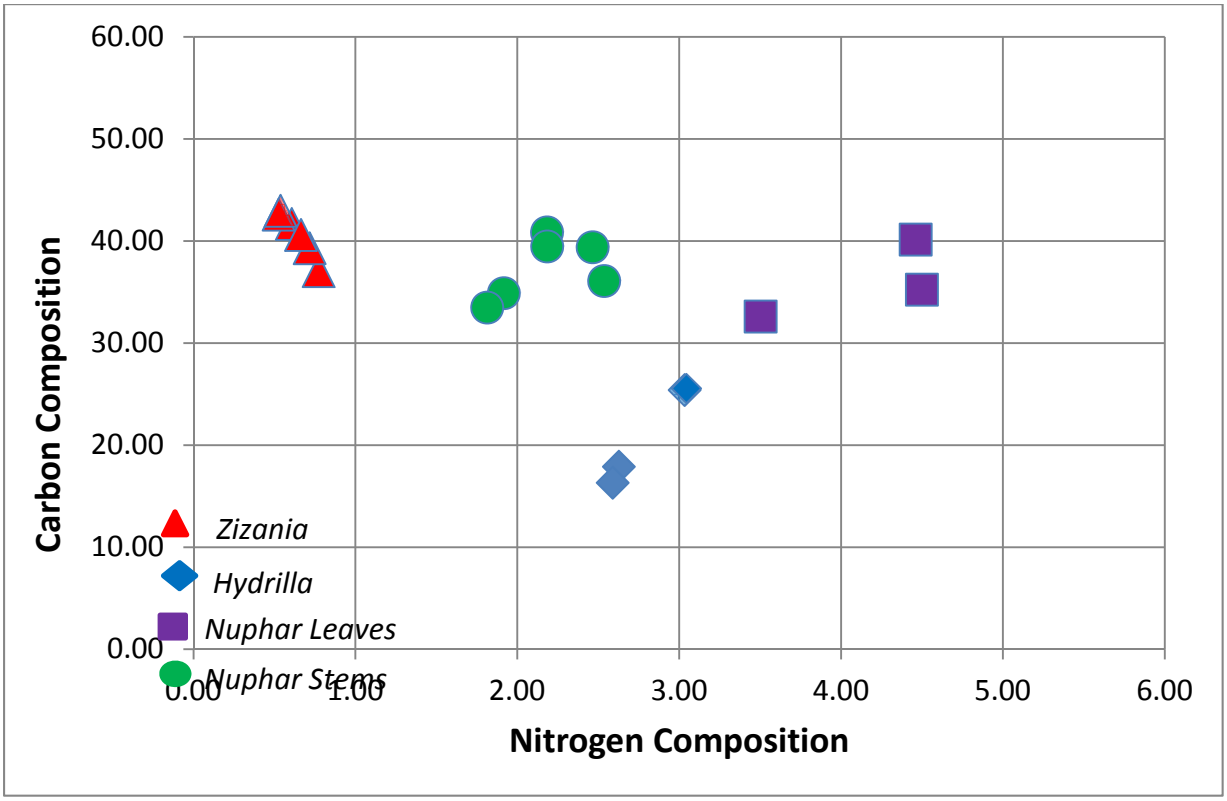

Table VII: C:N ratio of plant material after 2 weeks of decomposition. There is no significant difference between *Zizania* and *Nuphar* compositions. *Hydrilla* and *Zizania* carbon compositions are distinguishable however.

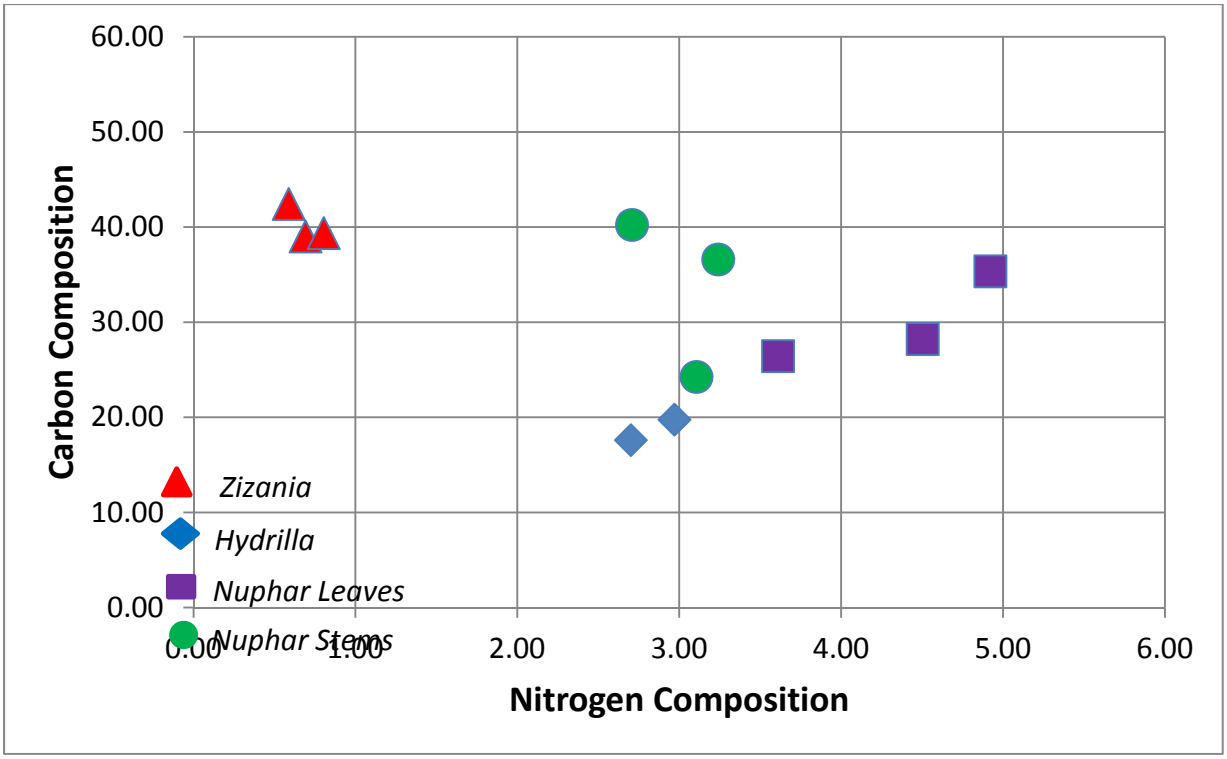

Table VIII: C:N ratio of plant material after 4 weeks of decomposition. There is no significant change in composition from 2 weeks of decomposition.

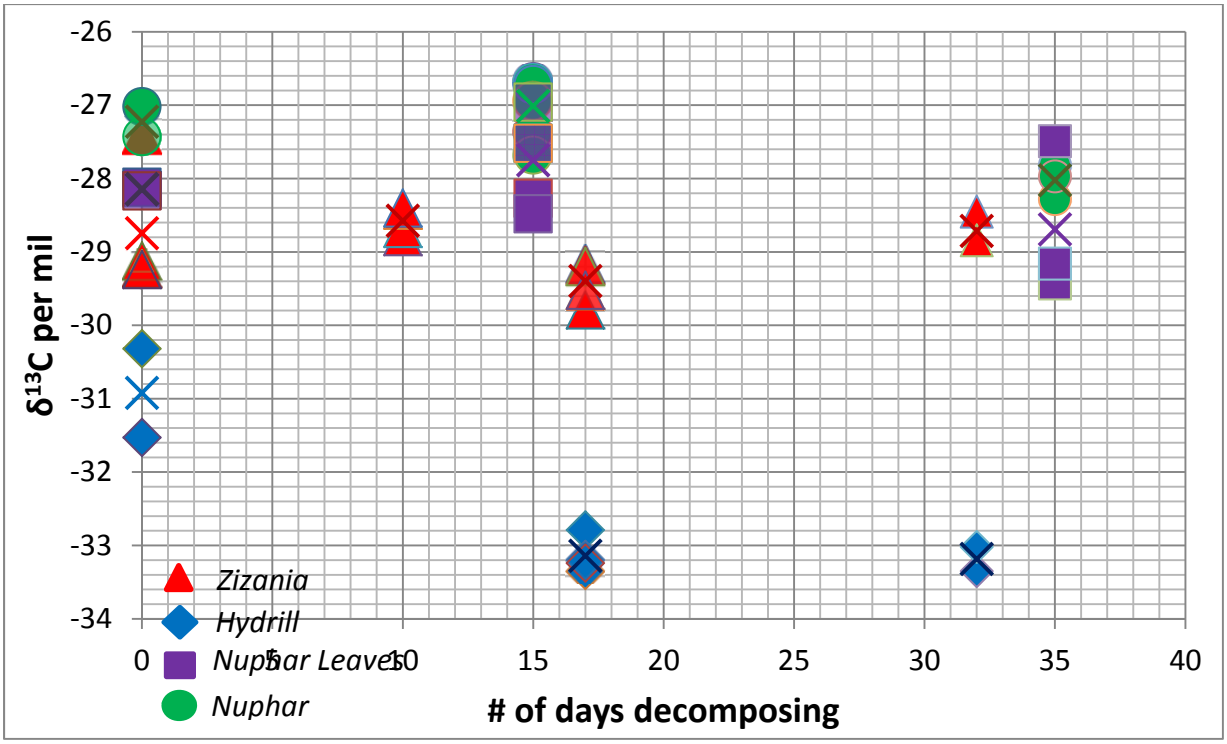

Table IX: $\delta^{13}C$ per mil over time. *Zizania* and *Nuphar* demonstrate no clear trends. *Hydrilla* however shows a clear decrease in $\delta^{13}C$ with decomposition. Colored X's indicate averages for the appropriate plant material at that time interval.

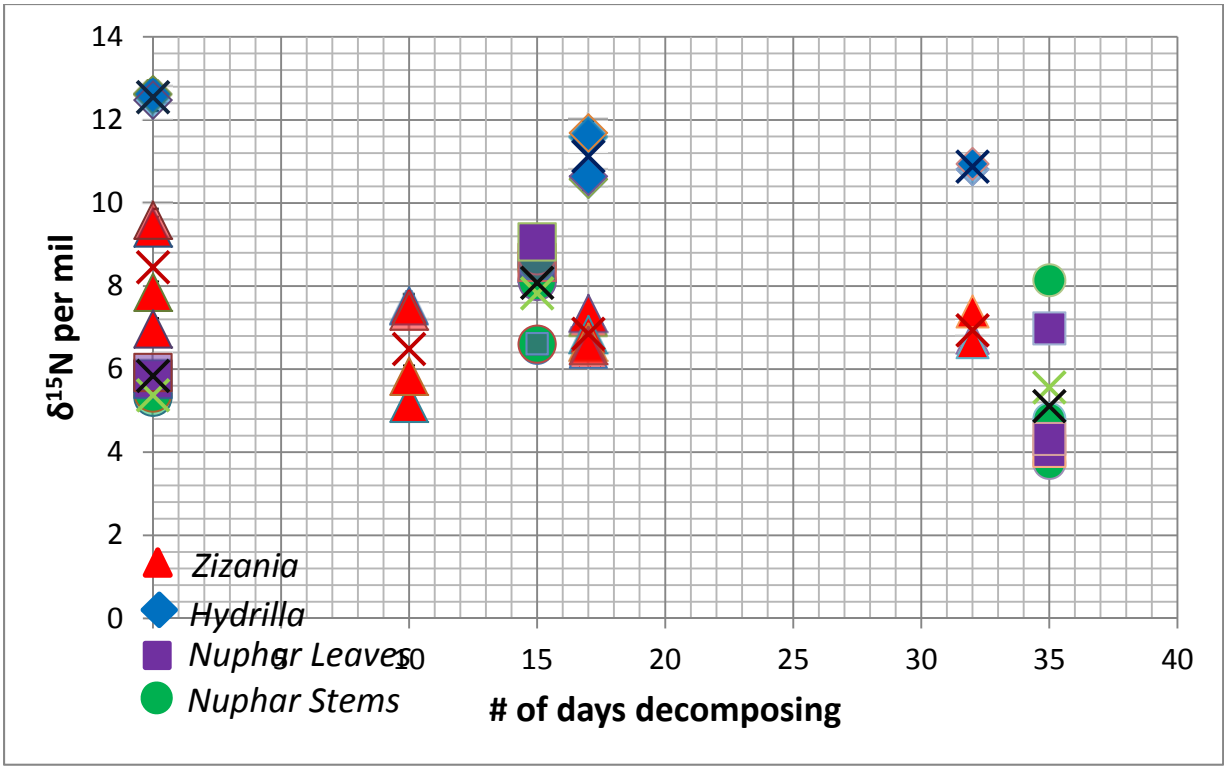

Table X: $\delta^{15}N$ per mil over time. *Zizania* and *Nuphar* demonstrate no clear trends and are indistinguishable from each other. *Hydrilla* demonstrates a slight decrease in $\delta^{15}N$ with decomposition. Colored X's indicate averages for the appropriate plant material at that time

Appendix II – Sediment Core Data

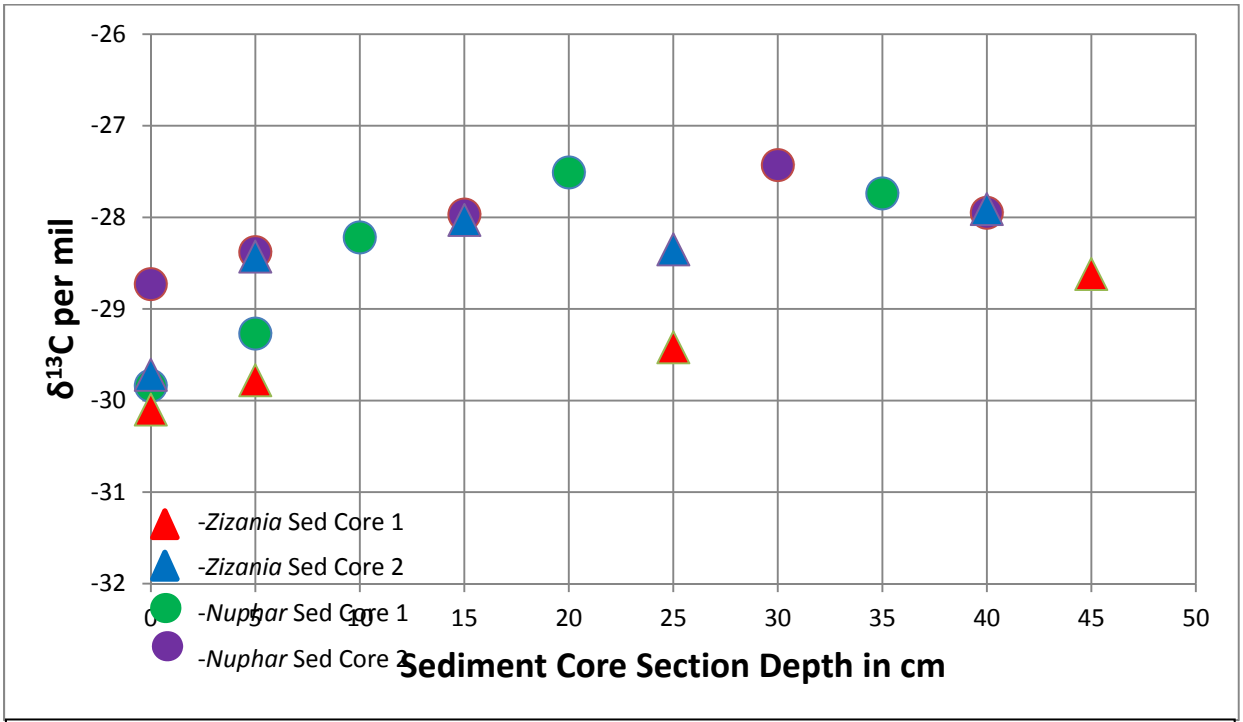

Table XI: $\delta^{13}C$ per mil of organic material with depth in four individual sediment cores. At the surface, the organic material has similar $\delta^{13}C$ values as the end members of the litter bag experiments. *Zizania* and *Nuphar* cores are not distinguishable based on isotopic data. Deeper sections of the core seem to indicate decreasing $\delta^{13}C$ content after flat lining near the middle.

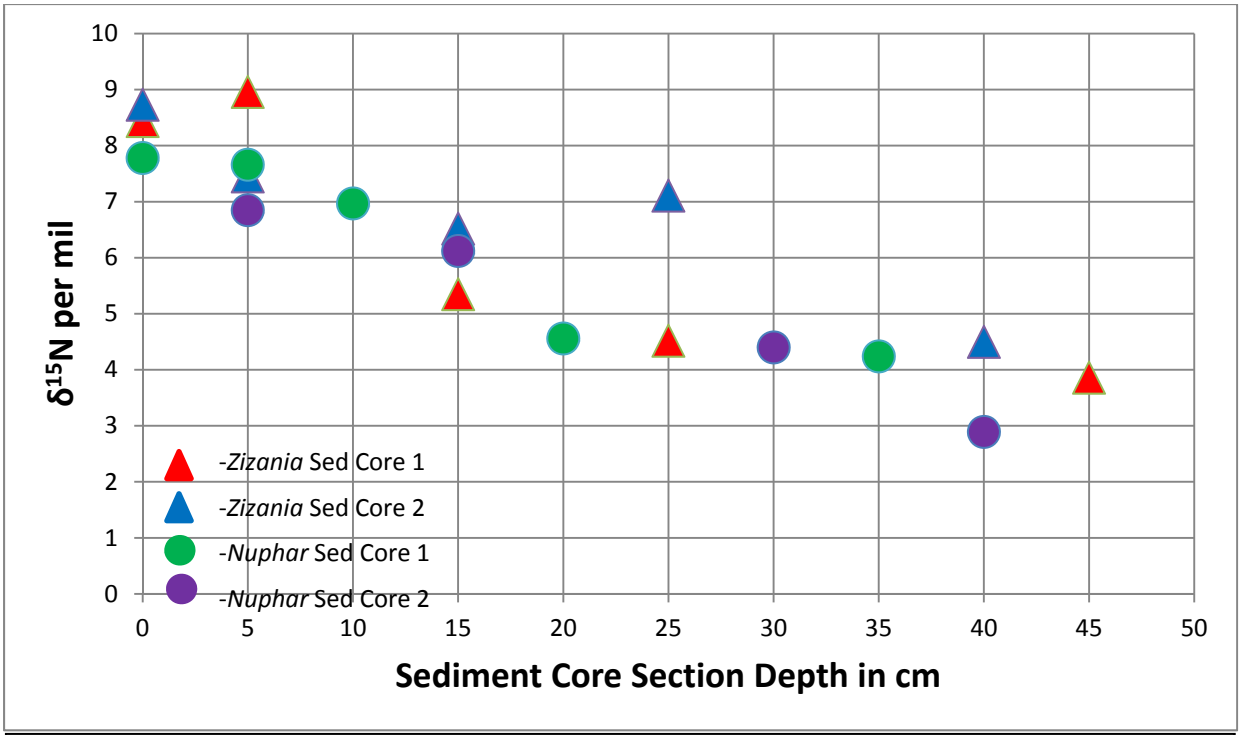

Table XII: $\delta^{15}N$ per mil of organic material with depth in four individual sediment cores. At the surface, organic matter has similar $\delta^{15}N$ values as the end members of the litter bag experiments. *Zizania* and *Nuphar* cores are not distinguishable based on isotopic data, and demonstrate consistent decreases in $\delta^{15}N$ values with depth.

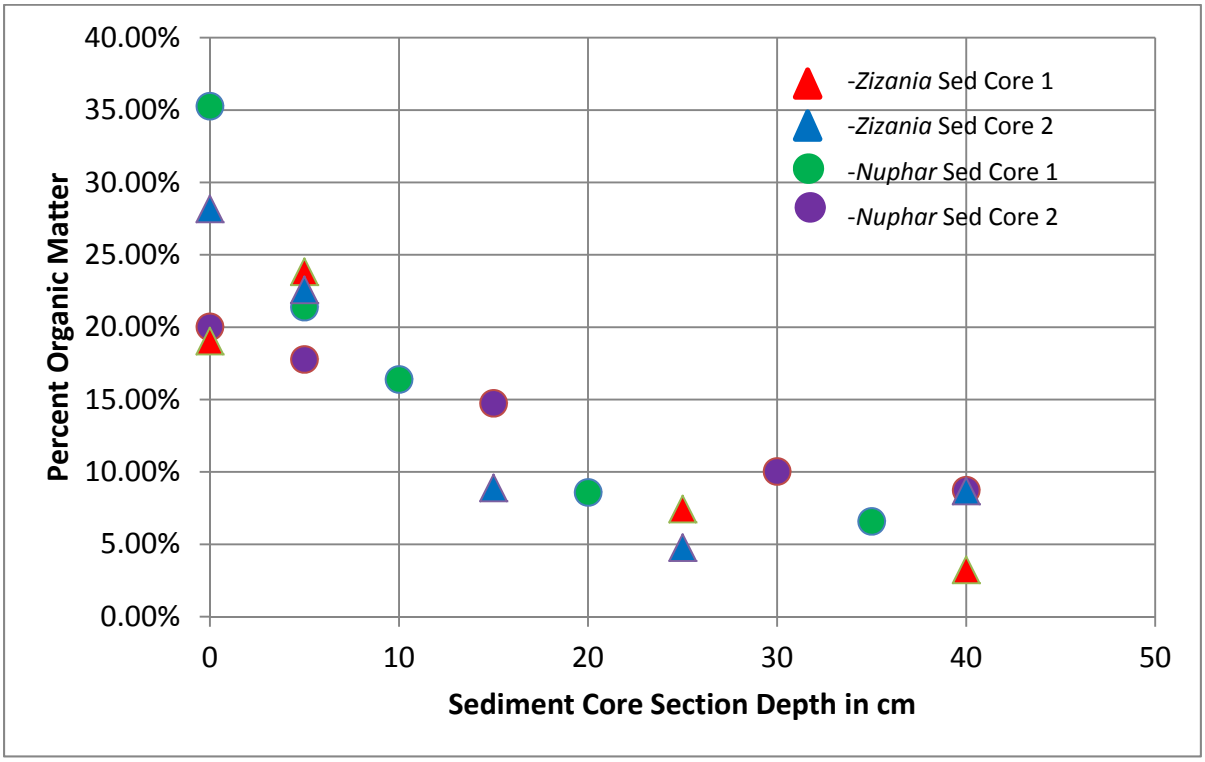

Table XIII: Percent organic matter of four unique sediment cores. All show a consistent trend of decreasing organic content with depth.

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Office of the Minnesota Secretary of State, http://www.sos.state.mn.us/index.aspx?page=164 – *Zizania* photo

USFWS BayScapes Conservation Landscaping Program, http://www.nps.gov/plants/pubs/chesapeake/plant/776.htm - *Nuphar* photo

Aquatic Nuisance Species Program, http://www.dnr.sc.gov/invasiveweeds/*Hydrilla*.html - *Hydrilla* photo