The use of senescent plant biomass to investigate relationships between potential particulate and dissolved organic matter in a wetland ecosystem

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Abstract

The purpose of this research was to (1) determine if different species of wetland vegetation produced characteristically different dissolved organic matter (DOM) based upon both chemical and physical characteristics and (2) determine if any relationships exist between characteristics of DOM derived from freshly senescent tissues of different wetland plant species common to the Florida Everglades and characteristics of the senescent plant tissue itself. Senescent plant tissues were used to represent potential particulate organic matter (POM) and leachates derived from them through cold water extraction were used to simulate abiotically produced labile DOM. Leachate DOM was characterized by total phosphorus (TP), nitrogen (TN), and carbon (TC), total carbohydrate content (TCC), total phenolic content (TPC), E4/E6 ratios, stable isotopes (δ13C, δ15N) and molecular mass fractionation (MMF). Senescent plant tissue (POM) was characterized by TP, TN, TC, E4/E6 ratio, stable isotopes (δ13C, δ15N), and fiber fractionation analysis (soluble content, hemicellulose, cellulose, and lignin).

Comparisons of DOM mean values for MMF, TCC, and TPC among species revealed significant differences, which was further supported by observed separation of species in principal components analysis. Regression analysis between POM and DOM characteristics suggests that POM N:P ratios are useful predictors of DOM N:P ratios ($r^2 = 0.83$, $P < 0.001$) and that POM levels of soluble constituents and hemicellulose can be a significant predictors of DOM TC ($r^2 = 0.82$, $P < 0.001$). Comparisons of E4/E6 ratios and stable isotopes (δ13C, δ15N) of DOM and POM, however, did not reveal significant relationships. The results of this study suggest that plant community structure may be a significant modulator of DOM quality and quantity through species specific contributions of characteristically different DOM and that plant tissue concentrations of nutrients and structural components can significantly influence chemical characteristics of DOM derived from them.

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1. Introduction

Dissolved organic matter (DOM) represents a majority of the organic carbon exported from terrestrial ecosystems into aquatic habitats (Wetzel, 1992). This DOM forms the basis for heterotrophic production and thus is very important in the transfer of energy in aquatic food webs (Moran and Hodson, 1990; Tranvik, 1992). It also has been found to be a significant source and sink of essential nutrients such as nitrogen and phosphorus (Lewis, 2002; Qualls and Richardson, 2003). The chemical characteristics of the DOM pool can control the availability and lability of metals and toxic organics in the aquatic ecosystem as well as affect water chemistry and light availability (McKnight et al., 1985; Strober et al., 1995).

A recent review of the literature finds hundreds of studies that use a variety of techniques, both qualitative and quantitative, to characterize the DOM fraction (Findlay and Sinsibaugh, 2003). There exists a great range in values of these characteristics for both lentic and lotic waters globally. Further, it is often unclear as to the origin of the DOM, whether it is autochthonous or allochthonous, terrestrial or aquatic in origin. While it is commonly asserted that most DOM in freshwater wetlands originates from terrestrial or aquatic macrophytic or woody species, little attention has been given to determination of the direct sources of this material, i.e. what plant species are responsible for the wide range of characteristics found in different wetland systems. This is likely because DOM in
aquatic systems undergoes continual biotic and abiotic decomposition via alteration by microbial communities and UV photolysis (Wetzel et al., 1995; Amon et al., 2001). Therefore, identifying a single source of DOM can be extremely challenging, especially in the presence of many plant or algal sources and an unknown age of the material. Thus, the role of different plant communities in the production of DOM is relatively unknown. Further, it has yet to be shown that different species of macrophytes contribute characteristically different portions to the DOM pool. Understanding the linkage between chemical characteristics of senescent macrophyte tissue (a dominant source of POM in the Everglades) and the chemical characteristics of the resulting DOM produced can be a powerful tool in determining the role of vegetation type in DOM dynamics and the associated effects that changes to dominant vegetation patterns may have to the ecosystem with respect to DOM dynamics.

In an effort to better understand the role of individual plant species potential contributions to DOM dynamics of the Florida Everglades, we conducted experiments to determine: (1) if different species of wetland vegetation produce significantly different forms of DOM and (2) if relationships exist between the chemical composition of parent plant materials and the nature of DOM produced from these materials. The hypothesis for this study was that wetland vegetation types will produce characteristically different DOM. Further, the chemical characteristics of the parent material, or POM, will influence the chemical and physical characteristics of the DOM that originates from this material.

2. Methods

2.1. Plant species and sample locations

To investigate the relationships between plant derived POM and DOM in wetlands, selected species of dominant wetland vegetation were chosen and the standing dead biomass collected from sites within the Florida Everglades. Specific vegetation types chosen for this study were common to the greater Everglades wetland ecosystem and included Typha domingensis Pers., Cladium jamaicense Crantz, Panicum hemitomon Schult., Spartina bakerii Merr., Eleocharis interstincta (Vahl) R. and S., Thalia geniculata L., Taxodium disticum (Vahl) R. and S., Charis interstincta (Typ); Nymphaea odorata (Pan); P. hemitomon (Pan); T. disticum (Tax). Collection of samples occurred during the months of November–December 2002 and in 2003 during months of natural senescence. A broad range of collection sites was employed to ensure representation of vegetation across the spectrum of moderately impacted to pristine areas of the Greater Everglades Ecosystem. Plant tissue collection consisted of harvest of recently senesced above ground biomass. For each species, a minimum of 10 samples were collected, returned to the laboratory and immediately dried at 55 °C. The temperature of 55 °C was chosen to avoid artificial lignification of the tissues observed with higher drying temperatures (Roberts and Rowland, 1998). Tissue samples were subsequently ground in a large Wiley mill to <1 mm² and thoroughly mixed to produce three aggregate samples. Sub samples of the ground tissue were ball milled as necessary for nutrient analysis.

2.2. Particulate organic matter nutrient analysis

Analysis of plant material total nitrogen (TN) and total carbon (TC) was conducted on a Carlo-Erba CN analyzer, total phosphorus (TP) was analyzed by ashing, acid digestion, and colorimetric analysis on an auto analyzer (Bran Leubbe Auto Analyzer 3 with digital colorimeter), δ¹³C and δ¹⁵N signatures were analyzed using a Costech model 4010 Elemental Analyzer (Costech Analytical Industries, Valencia, CA) coupled to a Finnigan Mat Delta Plus XL Isotope Ratio Mass Spectrometer (Thermo Finnigan, San Jose, CA) via a Finnigan Conflo II interface.

2.3. Particulate organic matter fiber analysis

Soluble tissue constituents (lipids, proteins, cellular constituents, etc.), hemicellulose, cellulose, and lignin fractions were quantified by a modified sequential fiber extraction method (Ankom Technology, Fairport, NY) modified from the feed and forage analysis by Van Soest (1970). This method has
been semi-automated by employing an Ankom Total Fiber Analyzer 200 and has been shown to be repeatable and consistent for a given plant type (Roberts and Rowland, 1998).

2.4. Particulate organic matter E4/E6

To obtain E4/E6 measurements on plant detrital material, 3 g ground sample material was shaken overnight with 25 mL of 0.1 M NaOH. The extracts were then diluted to standard concentration of 25 mg L⁻¹ C and analyzed for absorbance at 465 and 665 nm on a Shimadzu UV-160 UV–vis spectrophotometer (Dilling and Kaiser, 2002). Ratios of absorbance at 465/665 nm (E4/E6) above 5 are considered to indicate a dominance of fulvic acid type materials while values below 5 are indicative of humic acid materials. In the case of plant extracts, E4/E6 ratios are indicative the presence of humic or fulvic acid precursors in the soluble portions of plant tissues.

2.5. Dissolved organic matter production

To determine the potential for detrital material to produce DOM, ground senescent plant tissue (5 g) was extracted in room temperature distilled deionized water (300 mL) for 3 h with continuous stirring. Extracts were sequentially filtered through pre-combusted Whatman 934-AH 90 mm glass fiber filters, Whatman GF/F (0.7 μm) 47 mm glass fiber filters, and pre-leached Pall Supor-450 (0.45 μm) 47 mm membrane filters to remove particulate material. A 24 h extraction experiment was performed and it was determined that 3 h was sufficient to maximize release of DOM from the ground material. Potential extractable DOM was quantified and used as a descriptor for POM as well. DOC concentrations in each extract were standardized based upon carbon content requirements for each chemical analysis. DOC concentration was determined by high temperature oxidation of DOM to CO₂ coupled with IR detection of CO₂ in a Shimadzu TOC-5050 (Columbia, Maryland). Bulk DOM for all other analysis was created by leaching excessive amounts (approximately 200 g) of ground plant material in 1 L of distilled deionized water to achieve very high concentrations of DOC. These extracts were used as stock solutions for making DOM of various concentrations for separate analyses.

2.6. Ultra filtration

Molecular mass fractions (MMF) of leachates were determined by ultra filtration techniques utilizing Amicon 8500 continuously stirred ultra filtration cells. Millipore regenerated cellulose filter membranes (76 mm diameter) of nominal molecular mass limit 1000 (YM1), 3000 (YM3), and 10,000 (YM10) Da were used under 55 psi of ultra pure nitrogen gas in continually stirred cells (Tadanier et al., 2000).

2.7. Dissolved organic matter nutrient analysis

Total carbon (TC) of DOM was determined by analyzing for DOC on a Shimadzu TOC-5050. Total nitrogen (TN) analysis was performed following methods for total Kjeldahl nitrogen (TKN) which entailed acid digestion and autoclaving of samples to digest organic matrix and release nitrogen for colorimetric analysis (EPA method 351.2). Total phosphorus (TP) analysis also employed a TKN digestion and colorimetric analysis for P (EPA method 351.2). Nitrate content was determined on diluted samples by Alpkem Rapid Flow Analyzer with coupled cadmium reduction column (EPA method 353.2) and ammonium by colorimetric analysis on an Alpkem 300 Series auto analyzer (EPA method 351.2). Soluble reactive phosphorus (SRP) was determined on diluted samples colorimetrically using a Bran Leubbe AA3 with digital colorimeter (EPA method 365.1).

2.8. Dissolved organic matter total carbohydrate and total phenolic content

To determine total carbohydrate content in the leachates, a modified version of the phenol–sulfuric acid method was used (Liu et al., 1973). Total phenolics content, content of both mono and poly phenolics compounds, was determined following the colorimetric method of Price and Butler (1977).

2.9. Dissolved organic matter specific absorbance and E4/E6

Specific UV absorbance (SUVA) was obtained by measuring absorbance of pure sample at 254 nm on a Shimadzu UV-160 UV–vis spectrophotometer and dividing by carbon content. This ratio is used to estimate the aromatic content of the DOM sample (Dilling and Kaiser, 2002). Similarly, pure samples of leachates standardized to 25 mg C L⁻¹ were measured for absorbance at 465 and 665 nm to determine E4/E6 ratios. This ratio is used to estimate the level of humic like substances in the leachates. Again, ratios above 5 are considered to indicate a dominance of fulvic acid type materials while values below 5 are indicative of humic acid materials.

2.10. Statistical analyses

Significant differences between DOM derived from different vegetation types were evaluated by comparison of means via ANOVA with a Duncan’s Multiple Range post hoc. To determine if any characteristics of the POM of senescent plant tissues influenced the characteristics of the leachate DOM, regression analyses were performed on the results of the characterization data of POM versus DOM for all characteristics. Finally, to further investigate distinct separation of species DOM characteristics, principal component analysis (PCA) was used to plot all species DOM characteristics in two dimensional space. Statistical analyses were performed using NCSS software package (Number Cruncher Statistical System, East Kaysville, Utah).
Table 2
Nutrient analysis for all species bulk tissue samples

<table>
<thead>
<tr>
<th>Species</th>
<th>TC (g kg(^{-1}))</th>
<th>TN (g kg(^{-1}))</th>
<th>TP (g kg(^{-1}))</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eleo</td>
<td>428</td>
<td>10.1</td>
<td>0.382</td>
<td>42.4</td>
<td>1121</td>
<td>26.4</td>
</tr>
<tr>
<td>Typ</td>
<td>459</td>
<td>7.7</td>
<td>0.342</td>
<td>59.6</td>
<td>1342</td>
<td>22.5</td>
</tr>
<tr>
<td>Clad</td>
<td>442</td>
<td>7.6</td>
<td>0.358</td>
<td>58.2</td>
<td>1236</td>
<td>21.2</td>
</tr>
<tr>
<td>Spar</td>
<td>462</td>
<td>8.9</td>
<td>0.736</td>
<td>51.9</td>
<td>628</td>
<td>12.1</td>
</tr>
<tr>
<td>Thal</td>
<td>416</td>
<td>9.5</td>
<td>0.703</td>
<td>43.8</td>
<td>592</td>
<td>13.5</td>
</tr>
<tr>
<td>Nuph</td>
<td>440</td>
<td>19</td>
<td>1.310</td>
<td>23.2</td>
<td>337</td>
<td>14.6</td>
</tr>
<tr>
<td>Nym</td>
<td>438</td>
<td>15.9</td>
<td>1.120</td>
<td>27.6</td>
<td>391</td>
<td>14.2</td>
</tr>
<tr>
<td>Pan</td>
<td>437</td>
<td>12.7</td>
<td>1.148</td>
<td>34.4</td>
<td>380</td>
<td>11.0</td>
</tr>
<tr>
<td>Tax</td>
<td>478</td>
<td>17.4</td>
<td>1.808</td>
<td>27.5</td>
<td>264</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Species names are abbreviated as follows: E. interstincta (Eleo); T. domingensis (Typ); C. jamaicense (Clad); S. bakerii (Spar); T. geniculata (Thal); N. odorata (Nym); P. hemitomon (Pan); T. disticum (Tax). Total carbon, total nitrogen, and total phosphorus are represented by TC, TN, and TP, respectively. Mass ratios of carbon to nitrogen, carbon to phosphorus, and nitrogen to phosphorus are indicated by C:N, C:P, and N:P.

3. Results

3.1. Particulate and dissolved organic matter nutrient analysis

Analysis of plant detrital material revealed that TC values ranged from 417 to 479 g kg\(^{-1}\) (T. geniculata and T. disticum, respectively) with a mean value of 445 g kg\(^{-1}\) or 45% of the bulk material (Table 2). Total nitrogen (TN) values ranged from 7.6 to 19 g kg\(^{-1}\) (C. jamaicense and N. luteum, respectively) with a mean value of 12 g kg\(^{-1}\) or 1.2% of the bulk material. Total phosphorus ranged from 0.342 to 1.81 g kg\(^{-1}\) (T. domingensis and T. disticum, respectively) with a mean value of 0.841 g kg\(^{-1}\) or 0.84% of the bulk material. Ratios of C to N ranged from 264.8 (T. disticum) to a high of 14.8 (E. interstincta). Soluble reactive phosphorus (SRP) exhibited quite a range with T. domingensis being the low value of 0.014 g kg\(^{-1}\) and T. disticum the high value of 0.99 g kg\(^{-1}\), a difference of almost two orders of magnitude. While nearly half the species did not produce measurable ammonium, there was a two order of magnitude difference between the five species that did produce ammonium. N. luteum produced the lowest measured value of 0.02 g kg\(^{-1}\) and S. bakerii the highest with 1.38 g kg\(^{-1}\). Unlike ammonium, nitrate was detected in all leachates with C. jamaicense producing the least nitrate (0.006 g kg\(^{-1}\)) and N. luteum producing the most (0.161 g kg\(^{-1}\)).

Regression analysis of TC, TN, and TP content of POM versus DOM did not reveal any significant relationship, however, POM TN did exhibit a weak positive correlation with DOM TC ($r^2 = 0.49$, $P < 0.001$). Evidence that nutrient ratios remain intact during leaching was suggested by mass ratios of N and P for POM and DOM, which did show a significant correlation ($r^2 = 0.83$, $P < 0.001$). Of note, DOM TN did exhibit a weak negative correlation with DOM TP ($r^2 = 0.34$, $P < 0.001$) (Fig. 1a).

3.2. Particulate organic matter fiber content

Analysis of the senescent plant tissue for fiber fractions revealed a wide range of values for the soluble fiber fraction (Table 4). S. bakerii represented the lowest value of soluble tissue content at 11% while N. luteum represented the highest at 56%. N. odorata (51%) and T. disticum (46%) were also very high in relation to the other species. Reciprocally,
**Table 4**

Results of fiber analysis for all species

<table>
<thead>
<tr>
<th>Species</th>
<th>Soluble (%)</th>
<th>Hemicellulose (%)</th>
<th>Cellulose (%)</th>
<th>Lignin (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eleo</td>
<td>24.6 (1.0)</td>
<td>28.6 (1.0)</td>
<td>40.0 (1.2)</td>
<td>6.2 (0.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>Typ</td>
<td>27.2 (1.6)</td>
<td>23.4 (2.4)</td>
<td>39.7 (1.6)</td>
<td>9.4 (1.1)</td>
<td>1.1</td>
</tr>
<tr>
<td>Clad</td>
<td>19.9 (0.7)</td>
<td>32.7 (1.3)</td>
<td>37.2 (1.5)</td>
<td>9.8 (0.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Spar</td>
<td>10.5 (1.9)</td>
<td>40.0 (2.0)</td>
<td>38.7 (0.5)</td>
<td>10 (0.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>Thal</td>
<td>32.2 (2.0)</td>
<td>21.9 (1.9)</td>
<td>38.3 (2.0)</td>
<td>6.9 (0.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>Nuph</td>
<td>56.2 (3.5)</td>
<td>17.6 (0.9)</td>
<td>18.4 (1.4)</td>
<td>7.4 (0.6)</td>
<td>0.6</td>
</tr>
<tr>
<td>Nym</td>
<td>50.9 (4.1)</td>
<td>16.4 (1.9)</td>
<td>23.6 (0.9)</td>
<td>7.8 (0.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Pan</td>
<td>22.0 (2.3)</td>
<td>33.6 (3.0)</td>
<td>37.1 (2.6)</td>
<td>5.9 (0.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>Tax</td>
<td>46.2 (2.0)</td>
<td>13.4 (1.8)</td>
<td>23.1 (1.8)</td>
<td>17 (1.4)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Data presented as percent (%) of total mass of senescent plant material. Species are abbreviated as follows: *E. interstincta* (Eleo); *T. domingensis* (Typ); *C. jamaicense* (Clad); *S. bakerii* (Spar); *T. geniculata* (Thal); *N. luteum* (Nuph); *N. odorata* (Nym); *P. hemitomon* (Pan); *T. disticum* (Tax). Values are means with ±1 S.E.

Values ranged from 6% (*P. hemitomon*) to 17% (*T. disticum*), with most species containing 6–10% lignin in their tissues.

Regression analysis of POM fiber fractions with DOM characteristics resulted in a positive correlation between POM soluble and hemicellulose fractions and DOM TC ($r^2 = 0.82$, $P < 0.001$) (Fig. 1b) and a slightly less significant positive correlation between POM soluble content and DOM nitrate concentration ($r^2 = 0.73$, $P < 0.01$). Regression analysis found no other significant correlations between POM fiber fractions and DOM characteristics.

### 3.3. Particulate and dissolved organic matter E4/E6

Extraction of POM with weak NaOH resulted in a wide range E4/E6 ratio values. *E. interstincta* had the highest value (11.5) and *N. odorata* the lowest (3.2). Ratios above 5 are considered to indicate a dominance of fulvic acid type materials while values below 5 are indicative of humic acid materials. Investigation of E4/E6 ratios for leachates found a similar range of values as those of E4/E6 ratios of the tissue NaOH extraction. As in the analysis of POM, *N. odorata* had the lowest observed value, 1.9. Unlike the tissue analysis, *T. domingensis* had the highest value of 11.1. In five of the nine species, DOM E4/E6 ratio increased in relation to the POM E4/E6, in two species, the value decreased in the DOM, and two species E4/E6 ratios did not change significantly.

### 3.4. Isotopic analysis

Analysis of POM concentrations of $\delta^{15}N$ and $\delta^{13}C$ revealed a broad range of values for $\delta^{15}N$ with the lowest value being *S. bakerii* (0.11%) and the highest, *T. geniculata* (4.00%). In the analysis of $\delta^{13}C$, less variable results were observed. With the exception of the C4 grass *S. bakerii* (−15.24%), the other species exhibited values in the expected range of −25.44 to −29.96%, *N. luteum* and *E. interstincta*, respectively.

Results of the $\delta^{15}N$ and $\delta^{13}C$ analysis revealed some unexpected changes for the DOM samples. The values for $\delta^{15}N$ decreased in all but two species (*S. bakerii* + 1.1% and *T. geniculata* + 3.4%). Decreases in per mil concentration of $\delta^{15}N$ ranged from 0.5 to 5%. Changes in per mil concentration of $\delta^{13}C$ were less dramatic with three species decreasing (*T. domingensis*, *S. bakerii*, and *T. geniculata*), two increasing (*Eleocharis* and *P. hemitomon*), and four not changing significantly (*C. jamaicense*, *N. luteum*, *N. odorata*, *T. disticum*). Magnitude of change in per mil concentrations of $\delta^{13}C$ in leachates ranged from −1.5 to + 2.0%.

### 3.5. Dissolved organic matter molecular weight fractionation

Fractionation of DOM by molecular mass suggested three groupings in the smallest molecular mass cut-off of 1 kDa (Table 5). *E. interstincta* and *N. luteum* partitioned 4.3 and 6.5% of their carbon content, respectively, in this fraction. *S. bakerii* and *T. geniculata* contained 29 and 32%, respectively, in this size fraction, representing the highest content of this fraction,
Table 5
Molecular mass fractionations of DOM fractions of <1, 1–3, 3–10, and >10 kDa

<table>
<thead>
<tr>
<th>Species</th>
<th>&lt;1 kDa</th>
<th>1–3 kDa</th>
<th>3–10 kDa</th>
<th>&gt;10 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eleo</td>
<td>4.3</td>
<td>15.8</td>
<td>28.2</td>
<td>51.6</td>
</tr>
<tr>
<td>Typ</td>
<td>15.4</td>
<td>12.0</td>
<td>12.3</td>
<td>60.2</td>
</tr>
<tr>
<td>Clad</td>
<td>20.4</td>
<td>2.9</td>
<td>13.7</td>
<td>62.9</td>
</tr>
<tr>
<td>Spar</td>
<td>29.4</td>
<td>2.8</td>
<td>10.0</td>
<td>57.7</td>
</tr>
<tr>
<td>Thal</td>
<td>32.2</td>
<td>2.0</td>
<td>2.1</td>
<td>63.6</td>
</tr>
<tr>
<td>Nuph</td>
<td>6.5</td>
<td>30.3</td>
<td>12.3</td>
<td>50.8</td>
</tr>
<tr>
<td>Nym</td>
<td>16.1</td>
<td>8.4</td>
<td>18.0</td>
<td>57.3</td>
</tr>
<tr>
<td>Pan</td>
<td>15.1</td>
<td>15.0</td>
<td>8.9</td>
<td>60.9</td>
</tr>
<tr>
<td>Tax</td>
<td>19.2</td>
<td>13.8</td>
<td>7.4</td>
<td>59.6</td>
</tr>
</tbody>
</table>

Values are presented as % of total DOC in each fraction. Species are abbreviated as follows: E. interstincta (Eleo); T. domingensis (Typ); C. jamaicense (Clad); S. bakerii (Spar); T. geniculata (Thal); N. luteum (Nuph); N. odorata (Nym); P. hemitomon (Pan); T. disticum (Tax). Values are means ± (2%) error.

while T. domingensis, C. jamaicense, N. odorata, P. hemitomon and T. disticum were in the range of 15–20%. These three groupings were found to be significantly different from each other by ANOVA with Duncan’s Multiple Range test (n = 4, α = 0.05, P < 0.01). The 1–3 kDa fraction exhibited similar separation of distinct groupings with C. jamaicense, S. bakerii, and T. geniculata representing the low values in the range of 2–3%. N. odorata was significantly different from the others at 8%. Similarly, T. domingensis was significantly different from the other species at 12%. T. disticum, E. interstincta, and P. hemitomon partitioned between 14 and 16% of their carbon into this fraction and N. luteum was significantly higher than all other species at 30%. The 3–10 kDa fraction exhibited less variation in that T. geniculata alone represented the lowest values at 2% of its total carbon being partitioned into this fraction. T. disticum, P. hemitomon, and S. bakerii made up the next separate group with values ranging from 7 to 10%. N. luteum, T. domingensis, and C. jamaicense were grouped into the range of 12–14%. N. odorata was alone at 18% at the higher end of the partition values, and E. interstincta contained the highest amount of carbon in this size class at 28%. The least variable size fraction analyzed was the high molecular mass partition >10 kDa, which ranged from 51% (N. luteum) to 64% (T. geniculata). All species exhibited a majority of carbon content in this size fraction. N. luteum and E. interstincta represented the low end grouping of the range (51–52%) and all other species fell into the high end grouping of 57–64%. No significant correlations were found between the MMF of DOM and POM characteristics.

3.6. Dissolved organic matter total carbohydrate and phenolic content

Analysis of DOM total carbohydrate content is presented as percent of total DOM carbon in the form of carbohydrate (Fig. 2a). C. jamaicense, S. bakerii, and N. luteum represent the significantly lower grouping with carbohydrate contents ranging from 29 to 32% (ANOVA α = 0.05, P < 0.01) with Duncan’s Multiple Range test, E. interstincta, N. odorata, and P. hemitomon represent another significantly different grouping with median values of 36–40%. T. geniculata and T. disticum were not significantly different from each other with values of 43 and 45% carbohydrate content, respectively, but were different from the two lower groups. T. geniculata was not found to be significantly different from T. domingensis, which had the highest carbohydrate content at 49%. No significant correlations were observed in the regression analysis between DOM TCC and any POM characteristics.

The results of the total phenolics content analysis revealed similar results as the total carbohydrate analysis as there were four distinct groups of values, yet the species composition of those groups was not the same (Fig. 2b). E. interstincta, S. bakerii, P. hemitomon, and T. disticum made up the group with the lowest values (4.7–7.5%). The next significantly different group included C. jamaicense and T. geniculata with a range of 11–12%. N. odorata and N. luteum were similar with values of 27 and 30%, respectively, and T. domingensis and N. luteum represented the grouping with the highest values at 37 and 30%, respectively. Groupings were determined as before with ANOVA (n = 5, α = 0.05, P < 0.01) and Duncan’s Multiple Range test. Regression analysis between DOM TPC and POM characteristics did not reveal any significant relationships.
3.7. Principal components analysis

Although no two leachates were ever grouped in to the same statistically similar groups for all of the analysis conducted, a principal components analysis (PCA) was used to further investigate if the chemical characteristics of the leachates were truly different (Fig. 3). The total variance explained by component 1 and 2 was 76% of the total variance in all of the DOM characteristics data. Plotting vectors of analytical data along the axis of component 1 and 2 of the PCA results in a two dimensional representation of relationships among the data, with positive values of PC axis 1 being influenced by nutrient content and negative values being influenced by high molecular mass fractions (10 kDa) and E4/E6 ratios. Positive values on the axis of PC 2 were influenced by nutrient mass ratios and TPC, while negative values were influenced by TCC and low MMF (1 kDa). Projection of all DOM data groups into principal component dimensional space indicates no overlap of species data clusters.

4. Discussion

4.1. POM and DOM nutrients

Tissue levels of TC, TN, and TP were found to be quite variable and the dominate species such as *C. jamaicense*, *E. interstincta*, and *T. domingensis* contained levels of nutrients close to those observed in Everglades soils (DeBusk and Reddy, 1998, 2003). Similar senescent tissue values are found in the literature for *T. domingensis* and *C. jamaicense* (Davis, 1991).

The high levels of N and P found in some of the other species upon senescence are interesting in that these plants may be significant sources of nutrients to the system during decomposition. Nutrient concentrations of the resulting DOM produced from these tissues was not found to be predictable based upon tissue concentration of nutrients and was also highly variable. Similar values were found in an Everglades study of *T. domingensis* and *C. jamaicense* leachates (Qualls and Richardson, 2003). These findings suggest that different species allocate N and P differently in their structural tissues and therefore DOM from these species can be quite different in respect to nutrient content (Davis and Van der Valk, 1983). At the broad level of tissue fractionation and nutrient analysis employed, the relationships of storage could not be determined. It is also important to note that different species of aquatic plants will reallocate nutrients to rhizomes and other structures upon senescence, therefore adding another level of variability to the DOM produced from these materials (Twilley et al., 1977). To better understand this process, more in-depth research into the structural allocation of N and P would be required. The results of these experiments suggest that leachate levels of N and P are significantly different and therefore species can play a role in determination of DOM N and P content. Furthermore, because no relationship was found between tissue and leachate concentrations of N and P, there exists no strong relationship between species tissues and the resulting leachates with respect to N or P. However, a significant relationship between N:P ratios of DOM and POM was observed suggesting similar allocation strategy within soluble fiber among the different species with respect to these nutrients (Fig. 1a). The tissue levels of TN did show a moderate correlation to the TC of the leachates ($r^2 = 0.49$, $P < 0.001$), likely due to growing conditions of these plants and the amount of N allocated to intercellular uses.

The correlation between the soluble fiber plus hemicellulose fractions and the leachate TC was significant ($r^2 = 0.82$, $P < 0.001$). This is likely due to the water solubility of these two tissue fractions. It does provide a useful tool in predicting the amount of leachable carbon with respect to plant species (Fig. 1b). This finding also suggests that the bulk quantity of DOM derived from a given plant community in a given time period may be altered if the species composition were to change. Therefore, the relationship of species specific DOM quantity produced for a given plant type could exert significant influence over the dynamics of DOM.

The relationship observed between soluble fiber content in the POM and DOM nitrate concentration ($r^2 = 0.73$, $P < 0.01$) suggests similar allocation of nitrate by the plants. Due to the manner in which nitrate is used by plants, it is necessary for it to be located in the plant cell. Because the leaching process extracts soluble cellular constituents, the level of soluble material in the tissues should correlate to the amount of nitrate and other N forms, as well as, SRP. In the case of ammonium and SRP, no relationship was found. This disparity suggests that the species used in this experiment differentially reallocate SRP and possibly free ammonium upon senescence. Although no relationship was found between tissue nutrient content of structural components and concentrations of SRP or ammonium in DOM, the data do suggest that different species are capable of producing DOM with different levels of these two constituents. Again, this suggests the potential for species specific influence on DOM cycling in the Everglades, especially in relation to microbially mediated decomposition and cycling of limiting nutrients such as N and P.
4.2. POM and DOM chemical characteristics

The E4/E6 spectrophotometric analysis revealed little about the POM or DOM and proved to be unsatisfactory as a predictor of leachate characteristics. Some species POM values were higher than leachates and vice versa. This suggests that some other factors outside of the ones measured in this study are controlling this parameter. Often employed to characterize DOM in bulk DOM studies, the E4/E6 ratio, in this case, could only be used to further the argument that the characteristics of different species leachates are measurably different and that the POM content of humic and fulvic acid precursors does not accurately reflect the nature of the DOM formed from leaching these materials.

As was the case for the E4/E6 analyses, stable isotope analysis did not reveal any notable relationships between DOM and its POM precursor. POM levels of δ¹³C and δ¹⁵N were not reflected in the resulting DOM which raised the question as to how the individual species of plants allocate C and N to their tissues. In the case of δ¹³C, unidirectional changes in depletion are expected (Dawson et al., 2002), but not all species exhibited this. This result suggests that there is some differential allocation to δ¹³C in tissues that would require analysis of fiber fractions to elucidate the relationships. It also suggests that not all species do this in the same way hence the differences in leachates observed with respect to δ¹³C (Kracht and Gleixner, 2000). This phenomenon was observed even more drastically in the δ¹⁵N fraction of POM and DOM. There were large changes in the per mil concentration of δ¹⁵N within some species and not others. These findings suggest that there is even more preferential allocation of δ¹⁵N within plant tissues. The literature supports this in that various N pools (TN, amino acids, NH₄⁺) in soil organic matter can have drastically different values for δ¹⁵N (Griffiths, 1998) when the plant source is the same.

Values of δ¹³C measured for POM were within the range of those reported for plants in the northern Everglades by (Wang et al., 2002), and while there was no significant predictability between the POM values and the resulting DOM values for either δ¹³C or δ¹⁵N, the data do suggest that different species produce different DOM isotopic signatures, especially with respect to δ¹⁵N.

The great diversity in molecular mass fractions present in different plant species was expected to relate to the bulk tissue fiber and nutrient status; however, no relationship was found. Studies of bulk DOM from freshwaters and marine ecosystems report higher values for the smaller size fractions. Burdige and Gardner (1998) report 60–90% of the DOM in an estuarine study to be in the fraction less than 3 kDa and 73% of lake bulk DOM was found to be less than 1 kDa by Waiser and Robarts (2000). In the case of this study, the largest fraction of DOM was found in the >10 kDa fraction, suggesting that this fraction will undergo significant biotic and abiotic decomposition before approaching the values reported for the Northern Everglades by Wang et al. (2002) who found that on average, 50% of the bulk DOM was in the <1 kDa fraction. While molecular fractionation schemes vary by investigator, the use of four fractions was believed to be adequate to reveal any relationships present. While no correlations to tissue parameters were observed, the fractionation scheme did provide ample information to support the hypothesis that there is significant differences present in the physical character of DOM produced by different species of plants. This finding further suggests that individual species of plants may contribute different qualities of DOM, based on differential lability of these fractions, and thus influence DOM dynamics at the scale of microbial interaction.

It was also expected that soluble fiber and hemicellulose would correlate with leachate carbohydrate content based upon the simplicity of the hemicellulose structure (as opposed to cellulose and lignin), but no relationship was observed. Likely, the tissue levels of simple sugars are the parameters that would reveal better any relationship between the two. Similar values have been reported for the species T. disticum (29% versus 42% reported here) by Opsahl and Benner (1999) and other studies have shown that similar levels of carbohydrate in the bulk DOM pool can be found in lakes and streams as well (Volk et al., 1997; Dai et al., 2001). The analysis of total carbohydrate in the leachate DOM did, however, reveal that there is a large amount of variability among DOM samples which support the hypothesis that different species produce characteristically different leachable DOM. Because carbohydrates are often highly labile portions of the DOM pool, these results suggest that species specific contributions of DOM could influence DOM cycling at the microbial level.

Similar to our expectations of tissue fiber fractions correlating to carbohydrate content, it was expected that factors such as cellulose content of the POM would influence the total phenolic content of DOM to a large degree due to the aromatic nature of this complex structural material, but no relationship was found with regression analysis. It is likely that different species of plants have adapted different chemical defense strategies (secondary plant metabolites), which contain various levels of phenolic compounds. These compounds are likely contained mostly in the leaves and used to defend against herbivory. This is well documented for terrestrial plants (Waterman and Mole, 1994). T. domingensis, the species with the highest phenolic content has been shown to have allelopathic interactions with other plants through production and exudation of phenolic and volatile fatty acid exudates (Ervin and Wetzel, 2003). Richardson et al. (1999) reported that phenolic content of C. jamaicense decreased with increased P availability and argued that under conditions of elevated limiting nutrients, C. jamaicense and other plants would allocate more energy into growth and less into anti-herbivory compounds. In this study, comparisons of tissue TN and TP found no relationship with total phenolic content, however, the plant collection methodology employed in this study intentionally diluted the effects of elevated nutrient status in plants growing in nutrient impacted areas of the Everglades. Comparisons of TPC measurements with all tissue parameters did not expose any valuable relationships. The analyses for total phenolic content did, however, suggest another level of separation of species specific leachate characteristics and thus further supports the hypothesis that these leachates are significantly different.
The use of PCA was not intended to demonstrate clustering of species specific DOM characteristics, rather the separation of individual species based upon the inclusion of all measured DOM chemical and physical characteristics. The results of the PCA indicate no species groups overlap each other, suggesting species separation is distinct. Because no two species projections occupy the same dimensional space with respect to the principal components 1 and 2, species specific DOM produced from these plants is significantly different based upon the parameters measured in this study.

The results of this study demonstrate that individual species of wetland vegetation commonly found in the Everglades ecosystem produce characteristically different DOM products based upon major nutrient content (TC, TN, TP), total carbohydrate and phenolic content, and molecular mass fractionation. Results of significant groupings in these analyses, and the separation observed among species in the principal component analysis, support this conclusion. The limited correlations observed between POM and DOM suggests that some attributes of senescent plant material, such as soluble constituents, hemicellulose content, and major nutrient content can influence the characteristics of DOM derived from these materials. Because hydrologic and nutrient impacts to wetlands often result in changes to dominant vegetation patterns, as is the case in the Everglades, these findings suggest a need to better understand the role of individual plant species as contributors to the DOM pool and possible modulators of DOM cycling.

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Reference