

Sources and composition of high-molecular-weight dissolved organic carbon in a southern Louisiana tidal stream (Bayou Trepagnier)

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Abstract

The composition and cycling of high-molecular-weight dissolved organic carbon (HMW-DOC) were examined in a tidal stream (Bayou Trepagnier) with seasonally high DOC concentrations (1.0–5.6 mM). ¹³C nuclear magnetic resonance (¹³C NMR) was used to examine the bulk chemical composition of natural HMW-DOC from two field sites over 1.5 yr. The HMW-DOC at both sites was dominated by aliphatic (41%), carbohydrate (33%), and carboxyl (16%) carbon, with relatively low aromatic carbon abundance (10%). A comparison of ¹³C NMR signatures of bayou HMW-DOC and leachate HMW-DOC collected from leaf litter and soils revealed that plant litter leachate appears to be a more important source of HMW-DOC than soil. Dominant sources of HMW-DOC were likely allochthonous inputs of terrestrial plant litter with periodic inputs of soil organic matter during flooding events. The low aromaticity of bayou HMW-DOC may reflect the influence of low-oxygen conditions, which inhibit the decomposition of particulate aromatic macromolecules such as lignin and humic material to HMW-DOC. Lignin-phenol biomarker concentrations (Λ_6 , in mg/100 mg OC) were much lower in HMW-DOC (1.2) than in plant (5.2) and soil (6.8) organic matter, indicating that a significant fraction of this highly aromatic material was not degraded to HMW-DOC. Finally, this study demonstrated that lignin and other compounds from terrestrially derived organic matter in sediments and adjacent soils are not a significant source of more soluble moieties that enter the HMW-DOC pool of the bayou.

In both freshwater and marine systems, a significant fraction of total dissolved organic carbon (DOC) is composed of high-molecular-weight DOC (HMW-DOC) or colloidal organic carbon. The composition of HMW-DOC is important to nutrient cycling and aquatic chemistry, because this fraction contains substantial amounts of potential bacterial substrates and influences physicochemical characteristics through light attenuation, pH buffering, and metal complexation (McKnight et al. 1994; Battin 1998). Despite the abundance of HMW-DOC in inland waters, most studies advancing our understanding of HMW-DOC cycling have been conducted in the open ocean or large estuaries. In those systems, HMW-DOC appears to be derived mainly from direct exudation by phytoplankton and is rich in polysaccharides, pigments, lipids, and nucleic acids (Bianchi et al. 1995; Bidanda and Benner 1997). In freshwater systems, humic substances derived from diagenesis of structural materials from soils and higher plants are also important contributors to HMW-DOC (Frimmel and Christman 1988; Gustafsson and Gschwend 1997).

Relatively little is known about HMW-DOC cycling in coastal wetlands, although wetlands have high DOC concentrations and could be important HMW-DOC sources to es-

tuaries. Since Orem and Hatcher (1987) compared pore-water HMW-DOC composition in several wetlands, a few studies have examined the abundance and properties of bulk HMW-DOC in freshwater wetlands (Mann and Wetzel 1995; Chin et al. 1998). Yet to our knowledge, there has been no seasonal study focusing on composition and sources of HMW-DOC in a wetland or tidal stream.

Molecular biomarker analyses (i.e., fatty acids, lipids, carbohydrates, lignin-phenols, etc.) have provided valuable information about the composition of HMW-DOC (Benner et al. 1990b; Bianchi et al. 1995; Guo and Santschi 1997). However, many single-biomarker studies follow the production or fate of a small suite of compounds that may represent only a small percentage of bulk particulate or dissolved organic carbon. Therefore, much recent work has focused on the use of multiple biomarkers or spectroscopic analyses that are more likely to reveal the composition of bulk dissolved organic matter (DOM), including ¹³C nuclear magnetic resonance (NMR) spectroscopy. The NMR method allows non-destructive measurement of all major functional groups in complex material as a percentage of the total carbon in a sample (Schnitzer and Preston 1986; Benner et al. 1992; Hedges et al. 1992). In addition, certain peaks in NMR spectra can be used to indicate the presence of specific molecules or classes of compounds, such as lignin (Hatcher 1987; Benner et al. 1990a). Although a number of studies have used ¹³C NMR to study humic substances isolated from the natural environment by use of XAD resin extraction methods, which chemically alter DOM (Schnitzer and Preston 1986; Malcolm 1990; Filip et al. 1991; Lobartini et al. 1991; Hedges et al. 1992), few have addressed the spectroscopic characteristics of bulk DOM and its potential source materials (Orem and Hatcher 1987; Benner et al. 1992; Knicker and Ludemann 1995).

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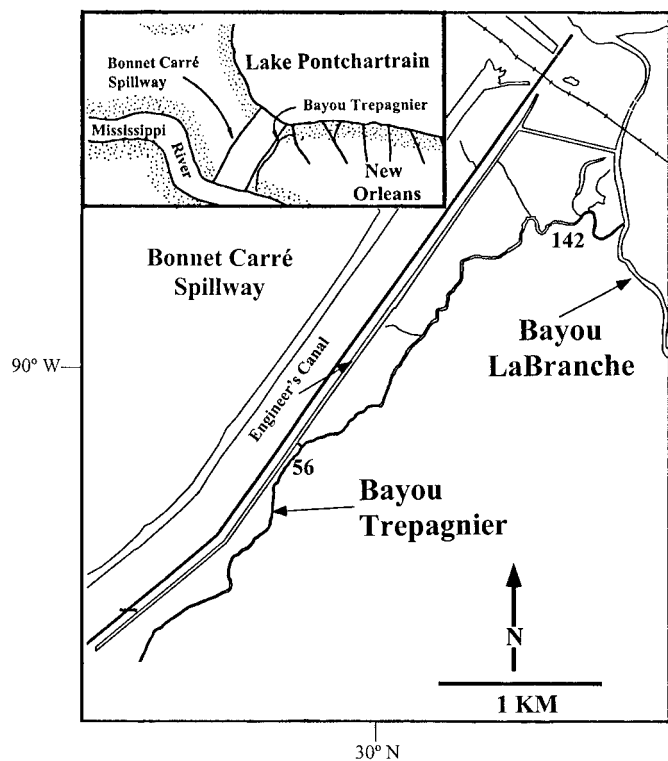


Fig. 1. Map of Bayou Trepagnier, Louisiana, and the surrounding Lake Pontchartrain estuary.

This study examines HMW-DOC concentrations, sources, and composition in a forested tidal stream. The close coupling of littoral and pelagic zones and a long hydraulic residence time (weeks–months) make this small system an excellent field mesocosm for examining the pathway of HMW-DOC production and the relative contributions of potential organic matter sources.

Materials and methods

Study site—Bayou Trepagnier is a 4.9-km forested tidal stream ranging from 0.5 to 2 m in depth. The bayou is located west of New Orleans, Louisiana, and connects with Lake Pontchartrain via Bayou La Branche at its northern end (Fig. 1). As a result of tidal and wind-driven exchange with Lake Pontchartrain, the bayou is slightly brackish (generally $<2.0\text{‰}$). During periods of minimal tidal movement, flow rates are very low ($0\text{--}0.03\text{ m s}^{-1}$; Flowers et al. 1998). The bayou drains cypress swamps, freshwater and brackish marshes, and deciduous bottomland hardwood forests of predominantly red maple (*Acer rubrum*) and hackberry (*Celtis laevigata*).

Bayou Trepagnier was used as a site for industrial dumping of petrochemical by-products, including heavy metals and polycyclic aromatic hydrocarbons, until the mid-1970s (Flowers et al. 1998). Site markers from previous studies were used for this work and were located every 30.5 m (100 ft.), beginning with marker 0 at the southern end of the bayou and ending with marker 160 at the mouth of the bayou, adjoining Bayou La Branche (Fig. 1). Two field sites with

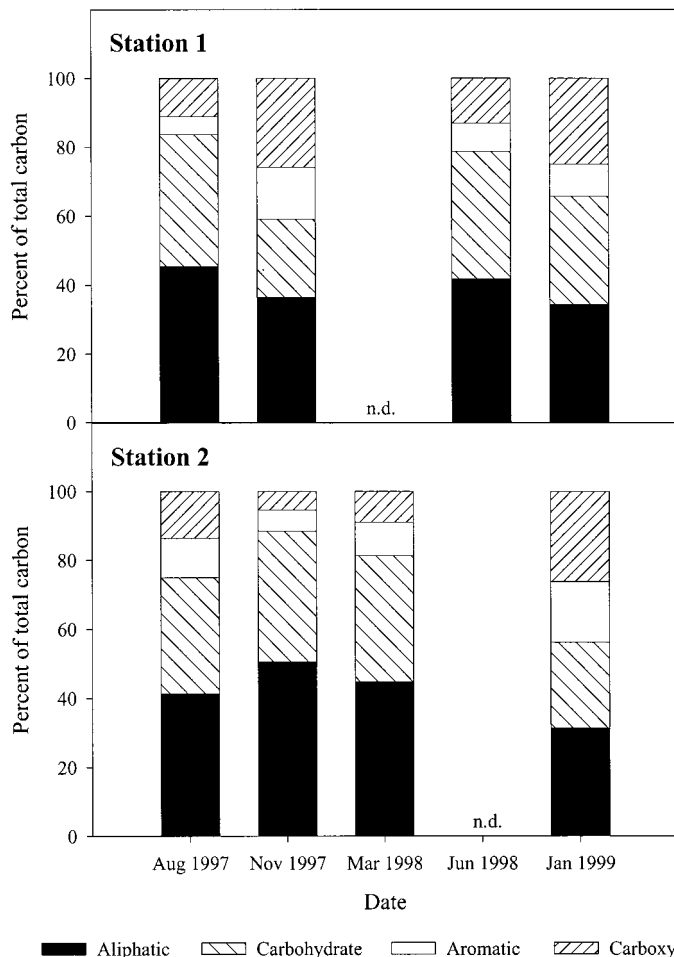


Fig. 2. Functional group composition of HMW-DOC collected from August 1997 to January 1999 (n.d., no data).

similar DOC and HMW-DOC concentrations and physicochemical characteristics were chosen in Bayou Trepagnier. The sites have different canopy cover densities (Engelhaupt et al. unpubl. data). Sta. 1 is in the upper bayou in an area closely coupled to surrounding wetlands (marker 56), and Sta. 2 is in the lower bayou, closer to the Lake Pontchartrain estuary (marker 142) (Fig. 1).

Field sampling—Seasonal water-column sampling was conducted in Bayou Trepagnier from August 1997 to January 1999. Temperature, salinity, water column pH, alkalinity, dissolved oxygen, and total light flux were measured in the field.

Water samples (20–30 liter) were pumped through 25-cm Nuclepore 0.2- μm polycarbonate in-line prefilter cartridges into acid-cleaned 20-liter LDPE containers. Samples were fractionated in the laboratory by use of tangential cross-flow ultrafiltration, as described by Guo et al. (1994). The prefiltered water was pumped through an Amicon Proflux Tangential System Model M12 (80% pump speed and 40 psi outlet pressure) consisting of two Amicon spiral ultrafiltration polysulfone cartridges with nominal pore sizes of 1 kDa aligned in series. Concentrations of DOC in each fraction

(<0.2 μm , <1 kDa, and 0.2 μm > x > 1 kDa) were measured during ultrafiltration to calculate a carbon mass balance. Samples of DOC and HMW-DOC were collected and lyophilized for ^{13}C NMR, lignin, and elemental analysis (CHN). Liquid subsamples were retained for ultraviolet (UV) spectrophotometry.

Laboratory—To better understand the relative importance of different terrestrial carbon sources in the bayou, soil and predominant plant material entering the bayou were characterized by use of solid-state ^{13}C NMR, lignin-phenol analysis, and elemental analysis (C, H, and N). Tree leaves as well as floating and emergent aquatic vegetation are major components of organic matter entering aquatic systems, and soil organic matter can contribute to DOM via groundwater or runoff (Wetzel 1983; Hinton et al. 1998). Therefore, plant leaves and soil were collected from both field sites in Bayou Trepagnier and were leached to collect dissolved organic matter. In August 1997, *Lemna minor* plant material was collected near Sta. 1 in Bayou Trepagnier and allowed to decompose anaerobically for 18 d. The resulting ultrafiltered leachate HMW-DOC was lyophilized and characterized by use of ^{13}C NMR.

In November 1998, leaves were collected at Sta. 1 from red swamp maple (*A. rubrum*), hackberry (*C. laevigata*), and wax myrtle (*Myrica cerifera*). These species made up ~75% of the tree cover near the two field sites (Whitbeck unpubl. data). At Sta. 2, leaves were collected from these species as well as from bulrushes (*Scirpus* sp.), which grow along one bank of the bayou at that site. Soil was collected 1 m from the edge of the bayou at 0–2 cm depth below the litter layer at the upper A horizon. Leaves were beginning to senesce when collection was performed.

Subsamples of plant and soil material were lyophilized, ground in a Tecator sample mill, and analyzed by use of ^{13}C NMR. The remaining material was weighed, sorted into duplicates of four treatments, and placed in 55-liter acid-leached plastic containers with 10 liters of Nanopure filtered water (adjusted to pH 6.5 to simulate local rainwater). Different proportions of the various plant species were used to approximate the observed species composition of the two field sites. The mesocosm treatments were as follows: (1) Sta. 1 leaves, 70% maple, 25% hackberry, and 5% wax myrtle by mass; (2) Sta. 2 leaves, 35% maple, 25% hackberry, 5% wax myrtle, and 35% bulrush; (3) Sta. 1 soil; and (4) Sta. 2 soil. A control container contained only filtered water. Soil treatments contained 1.5 kg (wet weight) of soil. Plant materials were not rinsed, to maintain the natural microbial community. All containers were loosely covered and allowed to leach for 5 weeks at ambient temperature. The pH, [DOC], and $[\text{O}_2]$ of the water were measured at the beginning and end of the experiment. After 5 weeks, plant material was removed with use of sieves. Leachate samples were prefiltered to remove large particles, filtered by use of a 0.2- μm Nuclepore filter cartridge, and then ultrafiltered to recover HMW-DOC and lyophilized for ^{13}C NMR and lignin analyses.

Analysis—All DOC measurements were made by the high temperature catalytic oxidation method (HTCO) with use of

a Shimadzu TOC 5000 Analyzer. Samples were acidified with 40 μl of 2N HCl and sparged for 4 min with ultrapure oxygen (150 ml min^{-1}) to remove inorganic carbon prior to measurement. The mean of five to six injections was reported for each sample. UV-irradiated Nanopure water was used as a sample blank in each sample set. Reagent-grade potassium biphthalate ($\text{KHC}_8\text{H}_4\text{O}_4$, Kanto Chemical) was used as a standard. The precision of the HTCO method, expressed as a coefficient of variation, was <2%.

^{13}C NMR spectroscopy was performed according to methods described by Benner et al. (1990). Cross polarization/magic angle spinning (MAS) ^{13}C spectra of lyophilized HMW-DOC were measured on a 300-MHz Bruker NMR spectrometer with either a 4- or 7-mm diameter probe. General parameters of the standard pulse program included a spinning rate of 7 kHz, contact time of 1 ms, and a pulse delay of 700 ms. Between 25,000 and 100,000 scans were collected for each sample. Selected samples, including a humic acid standard (Nittany Ridge humic acids), were cross-calibrated with a 100 MHz Chemagnetics CMX spectrometer with a 9-mm diameter probe (P. Hatcher, The Ohio State University). Although cross-polarization dynamics and Hartman-Hahn matching conditions of 100-MHz spectrometers differ from those of the 300 MHz used in this study, comparison of spectra obtained from the same standard provided useful information regarding the overall characteristics of spectra. Spectra collected on the two instruments by use of the same sample were compared and found to have nearly identical peak areas. Chemical shifts were referenced to a secondary standard, adamantane (methyl carbon at 17.35 ppm) and Nittany Ridge humic acids. Peak areas were reported without correction for spinning side bands, since the high MAS frequency suppressed sideband signals to levels near background noise. The low level of sideband intensity was confirmed by comparing peak areas for the same sample run at different spin rates (7 and 14 kHz) and verifying that the same peak areas were obtained. Finally, since the length of the pulse delay can be important at long run times, the effects of using different numbers of scans for a given sample were examined to confirm that the delay time was adequate to allow even relaxation of all carbon bond types. Relative peak areas of a humic acid standard (Nittany Ridge) run for 2,000, 4,000, and 10,000 scans were compared and found to be similar ($\pm 1\%$).

Percentages of the total carbon represented by each of four functional groups were estimated by integrating total peak area in each region with use of Bruker software (Xwinplot version 2.2.0). The four functional groups were identified based on their position (shift) in each spectrum as aliphatic (0–60 ppm), oxygenated aliphatic (also referred to as carbohydrate; 60–110 ppm), aromatic (110–165 ppm), and carboxyl/carbonyl (165–220 ppm).

Total carbon and nitrogen percentages were determined by use of a Carlo Erba CHNS Elemental Analyzer. Soil samples were acidified with 12 N HCl fumes.

Lignin-phenols of plant material, soils, and HMW-DOC were extracted by use of the CuO oxidation procedure of Hedges and Ertel (1982) and analyzed by gas chromatography–mass spectrometry. A Hewlett-Packard 6890 Series gas chromatographic mass spectrometer was used in selec-

Table 1. DOC and HMW-DOC concentrations, % HWM-DOC, and % carbon recovery for ultrafiltration of plant and soil leachates (two replicates) and in the water column (Sta. 1 and 2) in Bayou Trepagnier, Louisiana.

Date	Sta.	Sample type	DOC (mM)	HMW-DOC (mM)	% HMW-DOC	% carbon recovery
Aug 1997	2	Water column	10.0	2.9	28.9	93.1
	1	Water column	13.4	3.6	27.0	87.2
Nov 1997	2	Water column	4.1	2.5	61.8	138.2
	1	Water column	4.3	2.3	52.9	112.0
Feb 1998	2	Water column	2.0	0.4	30.3	106.4
Mar 1998	2	Water column	2.2	1.1	50.6	101.1
	1	Water column	3.2	1.1	37.1	89.5
Jun 1998	2	Water column	2.1	0.2	11.7	86.5
	1	Water column	1.0	0.3	28.3	93.8
Oct 1998	2	Water column	4.0	2.3	58.4	91.4
	1	Water column	4.6	3.0	65.2	108.3
Jan 1999	2	Water column	1.8	0.9	51.4	116.7
	1	Water column	2.3	0.6	22.4	74.4
Nov 1998	2	Plant Leachate	8.5	4.3	50.0	90.9
	2	Plant Leachate	9.6	4.7	48.9	85.4
	1	Plant Leachate	33.7	11.9	35.4	89.1
	1	Plant Leachate	33.3	12.8	38.5	90.4
	2	Soil Leachate	4.1	2.8	68.0	95.3
	2	Soil Leachate	3.1	2.2	71.1	96.7
	1	Soil Leachate	3.0	2.2	71.9	99.1
	1	Soil Leachate	2.6	2.1	79.8	96.8

tive ion monitoring mode with a 30 m × 0.25 mm i.d. × 1 μm film thickness DB-5MS capillary column (J&W Scientific). Peak areas and calculated response factors for phenolic standards (Sigma Chemical) were used to determine phenol concentration on the basis of recovery of the internal standard ethyl vanillin. Lignin-phenols were expressed relative to organic carbon by use of the lambda index, Λ_6 , calculated as the sum of the six syringyl and vanillyl lignin-phenols in mg/100 mg organic carbon.

Paired *t*-tests were conducted in SigmaPlot 4.0 to test for differences in physicochemical parameters and carbon functional groups between sites. 95% confidence intervals (CI) are reported as ±CI. Precipitation data were obtained from the National Climatic Data Center for New Orleans International Airport.

Results

Carbon and nitrogen—Concentrations of DOC in Bayou Trepagnier were high relative to most brackish or freshwater systems, averaging 2.8 mM, with a range of 1.0–4.6 mM. Concentrations were generally higher at Sta. 1, although this difference was not significant ($P > 0.05$). Occasional large freshwater inputs to the bayou (from storm events or diversion of Mississippi River floodwaters) resulted in exceptionally high DOC concentrations (>4.0 mM), likely because of surface runoff and groundwater inputs from flooding of local wetlands. The highest DOC concentrations occurred in October 1998 after storm events (tropical storm Frances on 11 September and Hurricane Georges on 28 September). Total rainfall during the 3 weeks prior to sampling gave the optimal correlation between precipitation and DOC concentrations, explaining 59% of variability in DOC concentrations at Sta. 1 and 30% at Sta. 2.

Concentrations of HMW-DOC in the water column ranged from 0.2 to 3.0 mM (average 1.3 mM). HMW-DOC contributed significantly to the DOC pool in Bayou Trepagnier, representing an average of 39% of total DOC. The percentage of HMW-DOC in the total DOC was highly variable, ranging from 12% to 65%, with highest values occurring in November 1997 and October 1998. The fraction of HMW-DOC/total DOC versus rainfall followed the same pattern as DOC, with rainfall predicting 62% of the variability in HMW-DOC/total DOC at Sta. 1 and 29% at Sta. 2. Percent recovery values for ultrafiltered carbon averaged 97%, with most values between 86% and 102%, indicating little net loss or contamination of DOC during ultrafiltration (Table 1). Bayou Trepagnier high-molecular-weight dissolved organic matter (HMW-DOM) contained 5%–16% carbon and <1% nitrogen with C:N ratios from 16 to 24.

¹³C NMR-field HMW-DOC—Aliphatic carbon made up the largest percentage of HMW-DOC in Bayou Trepagnier, averaging 41% of total carbon in all field samples (Tables 2, 3; Fig. 2). Peaks were distributed relatively evenly across the aliphatic region (0–60 ppm), indicating equal contributions from aliphatic carbons with and without adjacent electron-withdrawing groups (such as COO).

Carbohydrate carbon (60–110 ppm) constituted 33% of total carbon, with a peak centered around 72 ppm, whereas aromatic/olefinic carbon (110–165 ppm) constituted 10%. The carboxyl/carbonyl region (165–220 ppm) contributed 16% of total area. Within this region, carbonyl carbon (180–220 ppm) contributions were minor (<5% of area). Peak area in the carboxyl/carbonyl region resulted almost entirely from a broad peak centered around 175 ppm, which has been confirmed as carboxyl carbon using dipolar dephasing/MAS

Table 2. Relative abundance of functional groups in ^{13}C NMR spectra of all samples of Bayou Trepagnier HMW-DOC, plants, and soil collected during the sampling period of August 1997 through January 1999, as percentages of total carbon in each sample.

Category, date	Sta. No./substrate	% aliphatic	% carbohydrate	% aromatic	% carboxyl
Field					
Aug 1997	1	46	38	5.2	11
	2	41	34	11	14
HMW-DOC					
Nov 1997	1	37	23	15	26
	2	51	38	6.1	5.3
Mar 1998	1	ND	ND	ND	ND
	2	45	37	9.7	8.9
Jun 1998	1	42	37	8.3	13
	2	ND	ND	ND	ND
Jan 1999	1	34	31	9.3	25
	2	31	25	18	26
Lab experiments					
Plant leaves	<i>L. minor</i>	12	66	8.0	14
	<i>C. laevigata</i>	28	46	11	15
	<i>M. cerifera</i>	28	46	17	8
	<i>A. rubrum</i>	28	49	17	6.8
	<i>Scirpus</i> sp.	27	50	14	9.6
Plant leachate	1	34	36	11	18
	1	34	26	17	23
	2	36	35	9.7	19
	2	38	32	12	19
Soil leachate	1	30	24	19	27
	2	26	24	17	33
Nittany Ridge	Humic acid (May 98)	60	10	18	12
	Humic acid (May 99)	61	11	17	11

ND, no data.

to discriminate between protonated and nonprotonated or freely rotating (e.g., methyl) carbons (Wilson 1987).

Characterization of organic matter sources: leachate experiments—The HMW-DOC collected from *L. minor* leachate was composed primarily of oxygenated aliphatic carbon, which contributed 66% of the total ^{13}C NMR signal (Table 2). The major peak occurred at 73 ppm, indicating C-O bonds from carbohydrates (Orem and Hatcher 1987). Aromatic carbon made up only 8% of the signal. There was also no notable peak at 55 ppm to indicate the presence of lignin in *L. minor* (Benner et al. 1990). This result was confirmed with lignin-phenol analysis, which revealed a Λ_6 value of <0.02. This value is very low in comparison with values from most terrestrial plants, which contain more woody, lignified structural material (Hedges and Ertel 1982). *L. minor*

plant material contained 35.3% C and 4.8% N, with a C:N value of 7.3.

In undecomposed leaves collected from four common plant species at the field sites, an average of 48% of the total carbon was composed of carbohydrate carbon (Table 3); the major peak occurred at 72–73 ppm for all species. Aliphatic and aromatic carbon comprised an average of 28% and 15% of the total carbon, whereas carboxyl carbon accounted for an average of 10%. Leaves from plant species used in the leachate experiment contained an average of 43% C and 1.8% N and had an average C:N ratio of 26. Leachates obtained from these leaves contained an average of 29% C and 1.6% N, with a C:N ratio averaging 23.

The distribution of carbon functional groups in HMW-DOC leachates collected after 5 weeks of decomposition had changed substantially and was similar to HMW-DOC col-

Table 3. Average relative abundance of functional groups in ^{13}C NMR spectra of HMW-DOC, plant leaves and leachate, and soil and soil leachate collected from August 1997 to January 1999 in Bayou Trepagnier, Louisiana, as percentages of total carbon in each sample. Standard errors are shown in parentheses.

Sample	% aliphatic	% carbohydrate	% aromatic	% carboxyl
Sta. 1 HMW-DOC	40 (2.5)	32 (3.6)	9.5 (2.1)	19 (3.9)
Sta. 2 HMW-DOC	42 (4.0)	33 (2.9)	11 (2.4)	14 (4.5)
All field samples HMW-DOC	41 (2.3)	33 (2.2)	10 (1.5)	16 (2.9)
Plant material	28 (0.3)	48 (1.0)	15 (1.5)	9.9 (1.7)
Plant leachate	36 (0.9)	32 (2.2)	12 (1.5)	20 (1.2)
Soil leachate	28 (2.2)	24 (0.1)	18 (0.8)	30 (3.1)

Table 4. Lignin oxidation products of HMW-DOC collected in Bayou Trepagnier in August and November 1997, soil and plant leaves collected in November 1998, and HMW-DOC fraction of plant leachate. Units are mg 100 mg OC⁻¹.

	V	S	C	Λ_6	S/V	C/V	[Ad/Al] _v	[Ad/Al] _s
Sta. 1 soil	3.92	4.35	1.43	8.27	1.11	0.36	0.74	1.59
Sta. 2 soil	33.3	2.04	0.01	5.37	0.61	NQ	NQ	0.01
<i>Acer rubrum</i>	4.03	3.50	0.47	7.53	0.87	0.12	0.26	1.28
<i>Celtis laevigata</i>	1.78	1.09	0.00	2.87	0.61	0.00	0.01	0.01
<i>Lemna minor</i>	0.003	0.011	0.003	0.014	3.55	0.83	1.1	0.41
HMW-DOC								
Sta. 1, 6 Aug 97	0.21	0.24	0.01	0.45	1.15	0.05	0.04	NQ
Sta. 1, 19 Nov 97	0.60	0.72	0.59	1.32	1.20	0.98	2.49	4.65
Sta. 2, 19 Nov 97	0.91	1.03	0.61	1.94	1.13	0.68	3.14	5.07
Plant leachate								
Sta. 1	0.90	0.53	0.02	1.43	0.59	0.02	0.06	0.07
Sta. 2	0.58	0.52	NQ	1.10	0.91	0.00	NQ	0.01
Leached leaves								
Sta. 1	1.99	0.85	0.01	2.83	0.43	0.01	0.01	NQ
Sta. 2	1.46	0.29	0.00	1.76	0.20	NQ	0.02	0.06

V, vanillyl phenols; S, syringyl phenols; C, cinnamyl phenols; Λ_6 , S + V; [Ad/Al]_v, vanillic acid/vanillin; [Ad/Al]_s, syringic acid/syringaldehyde; NQ, not quantifiable.

lected from the bayou (Table 2). Carbohydrates and aromatic carbon had decreased, constituting an average of 32% and 12% of the total carbon atoms, respectively. Meanwhile, the relative contribution from aliphatic and carboxyl carbon increased, contributing an average of 35% and 20% of total carbon, respectively. Of total DOC in plant leachates, 35%–50% was in the HMW-DOC fraction.

The signature of soil organic matter was spread relatively equally across the four major functional groups (Table 2).

Leachates from these soils had a similar composition, averaging 28% aliphatic, 24% carbohydrate, 18% aromatic, and 30% carboxyl. The aliphatic and carboxylic carbon were higher in soil leachates than in Bayou Trepagnier HMW-DOC or plant leachates. Soils in Bayou Trepagnier contained, on average, 9% C and 0.6% N, with a C:N ratio averaging 14. Leachates from these soils averaged 19% C, 0.9% N, and had a C:N ratio averaging 21. Soil leachate DOC contained 68%–80% HMW-DOC.

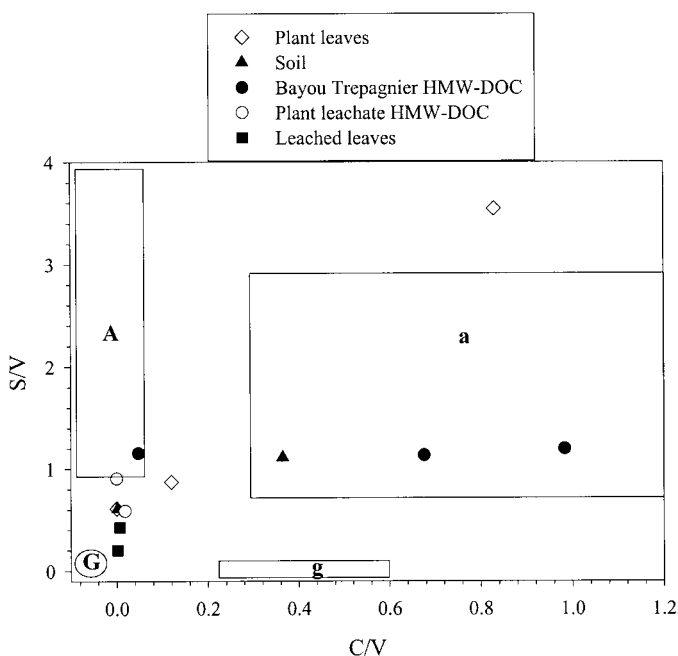


Fig. 3. Plot of C:V versus S:V ratios for HMW-DOC, plant leaves and leachates, and soil from Bayou Trepagnier. Boxes indicate compositional ranges of major vascular plant tissues (Goni et al. 1998 and references therein). A, angiosperm wood; a, angiosperm leaves and grasses; G, gymnosperm wood; and g, gymnosperm needles.

Lignin-phenol analyses—In leaves from *A. rubrum* and *C. laevigata*, Λ_6 values (in mg/100 mg organic carbon) were 7.5 and 2.9, respectively. Soils from Bayou Trepagnier had slightly higher Λ_6 values of 8.3 and 5.4, respectively. The average Λ_6 for Bayou Trepagnier HMW-DOC was much lower (1.2; Table 4). Sources of vascular plant material in soil and HMW-DOC were examined by use of ratios of specific phenols (Fig. 3). Ratios of syringyl to vanillyl (S:V) phenols provide information on the origin of lignin (i.e., angiosperm vs. gymnosperm); ratios in all samples were appreciably greater than 0, indicating the presence of angiosperm tissue. S:V ratios averaged 1.2 in Bayou Trepagnier HMW-DOC and were 0.9 and 0.6 in fresh leaves of *A. rubrum* and *C. laevigata*, respectively, within the range found for fresh angiosperm tissues in previous studies (Hedges and Mann 1979). In Bayou Trepagnier soils, S:V ratios were similar to those of leaves (0.6–1.1). Cinnamyl:vanillyl (C:V) ratios indicate whether source tissues are woody or non-woody, with cinnamyl phenols present in herbaceous tissues only (Goni et al. 1998). C:V ratios for leaf material were lower than expected, with no cinnamyls detected in *C. laevigata* and a ratio of only 0.1 in *A. rubrum*. Soils from Sta. 2 had an average C:V of 0.2. HMW-DOC from Sta. 2 had a C:V value of 0.6. Acid:aldehyde ratios of syringyl and vanillyl phenols are presented in Table 4. The ratios obtained for *L. minor* plant material must be viewed with some caution because of the propagation of error associated with dividing small numbers.

Discussion

DOC and HMW-DOC cycling—DOC and HMW-DOC concentrations in Bayou Trepagnier varied seasonally, with higher concentrations in fall and lower concentrations in spring and early summer. Although DOC concentrations might be expected to increase in summer, when biological productivity is high, this trend most likely resulted from allochthonous inputs of terrestrial material during fall. DOC concentrations were generally lower at Sta. 2, likely resulting from dilution with low-[DOC] water from Lake Pontchartrain. DOC concentrations (Argyrou et al. 1997) were also influenced by flushing of organic matter into the bayou during rain events. Variability in rainfall accounted for 59% of the variability in DOC concentrations at Sta. 1. Previous work in streams has shown that storm runoff exports much of the DOC in riparian soils (Hinton et al. 1998). This work also showed that in streams adjacent to wetlands, DOC produced by decomposition and leaching of wetland organic matter can represent as much as 32%–46% of the total DOC export to the stream. Earlier work demonstrated that stream channels in wetlands are the primary source of stream DOC (Hemond 1990). It has also been suggested that DOC inputs from groundwaters to streams (Brunke and Gonser 1997) may either provide a significant source or have a dilution effect on surface-water DOC concentrations. Few studies have attempted to correlate stream DOC dynamics with groundwater inputs (Easthouse et al. 1992). Thus, during episodic flooding in Bayou Trepagnier, both surface runoff and increased groundwater flow may contribute to the amounts of accumulated particulate organic carbon and DOC in the bayou.

Plant and soil leachates were enriched in HMW-DOC relative to total DOC (35%–80%) and were likely major sources of HMW-DOC in the bayou. The DOC pool in Bayou Trepagnier contained as much as 65% HMW-DOC. Contributions of HMW-DOC to the bulk DOC pool are much lower in the Lake Pontchartrain estuary adjoining Bayou Trepagnier, with only 4%–11% of the bulk DOC in the HMW-DOC fraction (Argyrou et al. 1997). The percentage of HMW-DOC in Bayou Trepagnier was highly variable, ranging from 12% to 65%, with highest values occurring in November 1997 and October 1998 (Table 1). This variability could result from the temporal variability of HMW-DOC entering the bayou during flooding of local wetlands.

Dominant sources of HMW-DOC— ^{13}C NMR spectra of Bayou Trepagnier HMW-DOC were dominated by aliphatic and carbohydrate carbon, consistent with the HMW-DOC composition found in reducing environments (Orem and Hatcher 1987). The ratio of aromatic to carbohydrate carbon in Bayou Trepagnier HMW-DOC, averaging 0.35, was within the range found in pore waters from reducing environments in that study. Orem and Hatcher suggested that anaerobic conditions in sediments of a sapropel lake resulted in an accumulation of carbohydrate and aliphatic (also called paraffinic) carbon and low aromaticity in pore-water HMW-DOC because of minimal lignin degradation (which releases aromatic moieties from the lignin macromolecule). The low aromaticity in Bayou Trepagnier HMW-DOC could likewise

be related to low oxygen conditions in the sediments and water column. Although dissolved O_2 concentrations measured in this study were above values considered hypoxic (2.0 ppm), more frequent monitoring of the bayou has shown that dissolved O_2 has a mean concentration of ~ 2.0 – 2.5 ppm (Flowers et al. 1998); dissolved oxygen concentrations < 1 ppm have been measured in the water column (Flowers unpubl. data). The sediments, where much allochthonous carbon is deposited, are thus likely to be anaerobic.

The periodically low oxygen environment in Bayou Trepagnier could contribute to high DOC concentrations and long DOC residence times because of incomplete mineralization of DOM by anaerobic microbes, especially in pore waters, where oxygen concentrations likely remain low (Orem and Hatcher 1987). The low oxygen values measured in this study on 2 October 1998 were likely due to impacts of tropical storm Frances and Hurricane Georges. Such storms are noted for transporting significant quantities of detritus into aquatic systems; this detritus decomposes over time, leading to low oxygen conditions.

Concentrations of lignin-phenols were very low in HMW-DOC relative to the surrounding terrestrial plant sources and riparian soils (Table 4), in agreement with the ^{13}C NMR data indicating low aromaticity in Bayou Trepagnier HMW-DOC. Dividing the Λ_6 values (carbon-normalized lignin abundance) of HMW-DOC by the average Λ_6 value of dominant plant and soil materials (*A. rubrum*, *C. laevigata*, and soil from each site; average $\Lambda_6 = 6.0$) in the bayou's watershed provided an estimate of the vascular plant-derived fraction in the HMW-DOC pool. It was estimated that only 7%–32% of the lignin in Bayou Trepagnier leaves and soil was incorporated into HMW-DOC (21%, using the average Λ_6 of HMW-DOC; Table 4). This estimate is conservative in that the calculation assumes that the average Λ_6 of leaves and soil represents all lignin sources to HMW-DOC—the percentage could be lower. These percentages are considerably lower than what might be expected on the basis of the lignin concentrations in estuarine and coastal water HMW-DOC (Bianchi et al. 1997; Mitra et al. 2000). Thus, it appears that much of the lignin in plant and soil materials is not being degraded into smaller, more soluble moieties and entering into the HMW-DOC pool.

The dominant sources of HMW-DOC in Bayou Trepagnier were likely allochthonous inputs from both terrestrial plants and the floating duckweed *L. minor*. ^{13}C NMR spectra of HMW-DOC in Bayou Trepagnier bore a strong resemblance to HMW-DOC from plant leachates, dominated by aliphatic and carbohydrate carbon with lower aromaticity than soil organic matter (Fig. 4, Table 3). Also, lignin phenols in Bayou Trepagnier HMW-DOC had S:V and C:V ratios indicative of angiosperm tissues, with significant inputs from angiosperm leaves and grasses (Fig. 3). S:V and C:V ratios of HMW-DOC from Bayou Trepagnier generally fell within the ranges found for HMW-DOC from leaf leachates, particularly those of the tree species. Other studies have shown that wetland plants produce (via excretion of photosynthate and leaching of plant materials) a significant fraction of the total DOC in freshwater systems (Wetzel and Manny 1972; Findlay et al. 1986). C:N ratios of Bayou Trepagnier HMW-DOC revealed that this material was of moderate lability (C:N ~ 15 –

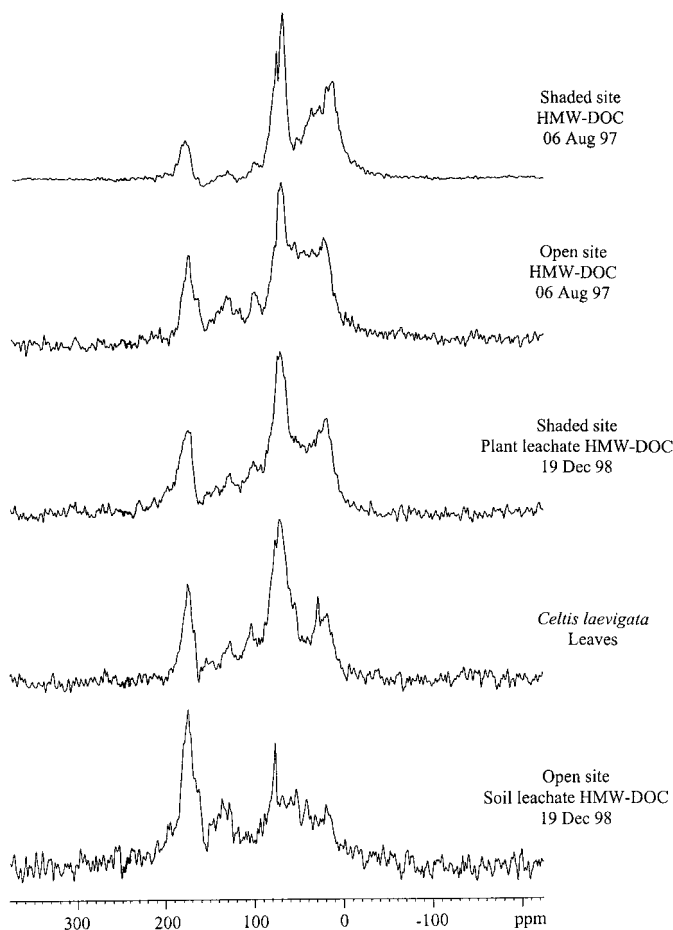


Fig. 4. ^{13}C NMR spectra of HMW-DOC collected from Sta. 1 and 2 in Bayou Trepagnier, Louisiana, *Celtis* leaves, and plant and soil leachates.

20). C:N ratios in this range generally indicate the presence of N-poor terrestrial components such as humic substances (Frimmel and Christman 1988; Valiela 1995). A significant fraction of HMW-DOM was likely derived from older plant leachates in the bayou that had been decomposing over time, due to the longer residence time of the more recalcitrant fraction. For example, it has been shown that the labile component of DOC leaf leachate (hickory and maple) has a half-life of ~ 1.92 d; this value is 80 d for the more recalcitrant fraction. The ratio of acid to aldehyde vanillyl and syringyl CuO oxidation products is also used as an estimate of the diagenetic state of lignin, with higher ratios indicating more extensive degradation (Hedges et al. 1988). This pattern has been demonstrated during subaqueous decomposition of herbaceous vascular plant material. In this study, $(\text{Ad:Al})_s$ and $(\text{Ad:Al})_v$ ratios were higher in HMW-DOC (3.2 ± 2.3 and 1.9 ± 1.3 , respectively) than in soils or fresh plant material (0.72 ± 0.72 and 0.25 ± 0.30). Other researchers have noted increases in Ad:Al ratios only after 2–4 yr of subaqueous decomposition (Opsahl and Benner 1995). The increased ratios in Bayou Trepagnier HMW-DOC may have resulted from processes occurring on this timescale or longer.

During the 5-week decomposition and leaching experiment, a fraction of the carbohydrate and aromatic carbon in

plant leaves did not enter the dissolved pool. In particular, it appeared that carbohydrate material was lost from plants during initial decomposition, as reported elsewhere (Benner et al. 1990a; Knicker and Ludemann 1995). Carbohydrates from plant material may be completely degraded by microbes and respired as CO_2 or partially degraded and released into the DOM pool (Orem et al. 1987). In this case, DOM leachate from decomposing plants contained a smaller percentage of carbohydrate carbon than starting material contained. Even so, a component of the carbohydrate pool continued to contribute significantly to the bulk signature of plant leachates, with the leachate composition after 5 weeks being quite similar to HMW-DOC in the bayou's water column. This remaining pool of carbohydrates may be more resistant to microbial degradation, especially in low-oxygen environments. HMW-DOC in leachate from decomposing plants also contained a slightly lower percentage of aromatic carbon than did the starting plant material. The ratio of aromatic to carbohydrate carbon in plant leachates averaged 0.40, within the range found in DOM from reducing pore waters (Orem and Hatcher 1987). Since the leachates were periodically stirred but not continually aerated during the experiment, dissolved oxygen concentrations remained relatively low (<5 ppm, similar to values in Bayou Trepagnier), which may help explain similarities in carbon functionality between leachates and Bayou Trepagnier HMW-DOC.

Aromaticity in plant material appeared to be derived from both tannins and lignin. Peaks were observed at 56 ppm in hackberry and bulrush and at 105 and 130 ppm in hackberry and wax myrtle. These peaks were likely derived from lignin and tannins, respectively (Benner et al. 1990a), although this has not been confirmed, by use of dipolar dephasing, in these samples. Because carbohydrates and aromatic carbon were lost during decomposition, aliphatic and carboxyl carbon accumulated in the dissolved fraction, appearing to resist bacterial decay. Refractory aliphatic carbon in this plant material is likely derived from highly aliphatic biopolymers found in cuticles and cell walls (Tegelaar et al. 1989) and from long-chain lipids or dicarboxylic acids (Hatcher et al. 1985). The short side chains of amino acids in proteins and proteinaceous material were likely less important sources of aliphatic carbon, because there was no positive relationship between the nitrogen content of the samples and spectrum area from 165–220 ppm to indicate a significant contribution from amide groups in proteins (Knicker and Ludemann 1995). Meanwhile, the relative contribution from carboxyl carbon in the leachate was double that of the original plant material, indicating that this material was soluble but not quickly respired. This could indicate formation of new carboxyl groups in the lignin or carbohydrate fractions or formation of amino acids during microbial digestion (Knicker and Ludemann 1995).

The composition of soil organic matter in Bayou Trepagnier reflected its older, more refractory nature. The high aromatic and carboxylic carbon content relative to HMW-DOC reflected the abundance of soil humic substances. Soil organic matter entering the bayou as runoff or groundwater is expected to contribute periodically to the HMW-DOC pool primarily by contributing aliphatic carbon, which was possibly reflected in the slightly higher aliphatic concentrations of the bayou's HMW-DOC when compared with plant leachate

HMW-DOC. The high concentration of aliphatic substances in Bayou Trepagnier HMW-DOC may have also reflected the end products of long-term decomposition of terrestrial material. Terrestrial organic matter in the form of lignin degradation products and carbohydrates has been shown to be mobilized in soils during rainfall (Guggenberger and Zech 1993). Given that the ^{13}C NMR signature of HMW-DOC in Bayou Trepagnier was quite different from soil organic carbon and more similar to plant leachates, allochthonous plant material appeared to be the dominant source of DOC during periods of normal precipitation (Table 3). Soil organic matter adjacent to the bayou appears to contribute a substantial amount of DOC (and thus affect the relative abundance of functional groups) only during episodic flooding events.

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