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Carbon isotopic constraints on the contribution of plant material to the natural precursors of trihalomethanes

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Abstract

The $\delta^{13}C$ values of individual trihalomethanes (THM) formed on reaction of chlorine with dissolved organic carbon (DOC) leached from *maize* (corn; *Zea maize L.*) and *Scirpus acutus* (an aquatic bulrush), and with DOC extracted from agricultural drainage waters were determined using purge and trap introduction into a gas chromatograph-combustion-isotope ratio monitoring mass spectrometer. We observed a 16.8‰ difference between the $\delta^{13}C$ values of THM produced from the *maize* and *Scirpus* leachates, similar to the isotopic difference between the whole plant materials. Both *maize* and *Scirpus* formed THM 12‰ lower in ^{13}C than whole plant material. We suggest that the low value of the THM relative to the whole plant material is evidence of distinct pools of THM-forming DOC, representing different biochemical types or chemical structures, and possessing different environmental reactivity.

Humic extracts of waters draining an agricultural field containing *Scirpus* peat soils and planted with *maize* formed THM with isotopic values intermediate between those of *maize* and *Scirpus* leachates, indicating *maize* may contribute significantly to the THM-forming DOC. The difference between the $\delta^{13}C$ values of the whole isolate and that of the THM it yielded was 3.9‰, however, suggesting diagenesis plays a role in determining the $\delta^{13}C$ value of THM-forming DOC in the drainage waters, and precluding the direct use of isotopic mixing models to quantitatively attribute sources. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Chlorine has been used as a disinfectant in public drinking water supplies for the greater part of this century and more than twenty years ago it was recognized that chlorine (as OCl^-) reacts with the dissolved organic carbon (DOC) present in natural waters to form a variety of halogenated byproducts potentially harmful to human health (Rook, 1976, 1977). The concentration of the most abundant class of byproducts, trihalomethanes (THM; chloroform, bromodichloromethane, dibromochloromethane, and bromoform), is frequently regulated in finished drinking water (US Environmental Protection Agency, 1994). Recent stu-

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dies suggesting that current levels of THM in drinking water supplies may present a chronic health risk have increased interest in reducing the amount of THM in drinking water (Waller et al., 1998). The challenge is to reduce THM levels without significantly increasing the cost of water treatment. Lowering the amount of THM-forming DOC in source waters is thought to be among the most cost-effective means of THM reduction, but little specific information exists about the environmental sources of the specific fraction of DOC that forms THM.

The Sacramento-San Joaquin River Delta supplies drinking water to more than 22 million Californians. Much of the Delta region is a former bulrush (*Scirpus acutus*) marsh that has been drained and intensively farmed because of its rich peat soils and abundant water supply (Atwater, 1980). DOC content in river waters increases as they pass through the Delta before being diverted into the California State Aqueduct for use as drinking water in southern California (California Department of Water Resources, 1994a). The bromide content also increases in the Delta due to tidal mixing of seawater (California Department of Water Resources, 1994a). This simultaneous elevation of bromide and DOC concentrations frequently results in potential THM formation in excess of current regulatory limits when the waters are chlorinated for use as drinking water. Resource managers are currently contemplating environmental changes within the Delta that will affect the extent of THM formation in Delta-derived drinking waters (CALFED, 1998).

The environmental factors that determine the extent to which a particular source water forms THM on chlorination are the concentration of bromide (Br^-), the amount of DOC present in the source waters, and the molar reactivity of the DOC with respect to THM formation (Reckow et al., 1990). The concentration of bromide in solution is related to THM production because it reacts with OCl^- to form OBr^-, a reaction intermediate that forms THM more rapidly from DOC than OCl^- (Reckow et al., 1990). The total amount of THM formed increases with the concentration of DOC, but the relationship is not simple because the molar reactivity of the DOC to form THM varies over a wide range (<5–>15 per 1000 C; California Department of Water Resources, 1994a). Since local geology and proximity to seawater sources largely determine Br^- concentrations, the most effective means of controlling THM may be by changing the quantity and/or quality of DOC released within the watershed. However, effecting such change requires a clear understanding of the sources of the fraction of DOC that forms THM.

Most research indicates THM forms by reaction of OCl^- with the phenolic constituents of humic material dissolved in the source water (Rook, 1977). Rook (1977) suggested that *m*-dihydroxybenzene substituents were most facile in the formation of THM, with the eventual liberation of the C2 carbon to form CX_3. Although the sources of humic material are somewhat obscured by its random polymeric composition (Thurman, 1985), it is generally agreed that the provenance of the phenolic compounds is degraded remains of terrestrial plant materials (Hedges and Mann, 1979). All terrestrial plants contain phenolic constituents, principally within lignin, cutin, and tannin (Sarkanen and Ludwig, 1971). However, the relative abundance of these compounds varies considerably between species (Hedges, 1990b), suggesting that the balance of sources contributing to the bulk DOC may not reflect the balance of sources contributing to the THM-forming fraction of the DOC.

The peat islands in the Delta are largely formed from the remains of *Scirpus acutus* (Atwater, 1980), which uses the Krebs (C3) cycle for photosynthetic fixation of atmospheric CO_2 (Mason, 1957). In contrast, *maize* (corn, *Zea maize L.*), a common agricultural crop within the Delta, uses the Hatch (C4) cycle for the same purpose (Clark and Fritz, 1997). The C3 photosynthetic pathway results in a large fractionation in carbon isotopes between atmospheric and plant tissue carbon (−24–−30‰). The C4 pathway results in a smaller fractionation (−10–−16‰; Clark and Fritz, 1997). This isotopic difference between plant types may prove useful in environmental investigations of the THM-forming fraction of DOC because the isotopic composition of the THM should carry the isotopic signature of its source. However, for the isotopic composition of THM to be an effective tracer of the source of the THM-forming DOC, the isotopic difference among sources must not be obscured by intermolecular isotopic differences within the source materials, nor by fractionation during THM formation or measurement.

This study examines the isotopic compositions of THM formed from leachates of *maize* and *Scirpus*, and from agricultural waters draining a peat island *maize* field. These values are compared to the isotopic composition of bulk plant or isolate material to evaluate the utility of using the $\delta^{13}C$ values of THM as a tracer of environmental sources.

2. Methods

The above-ground portions of live *maize* and *Scirpus* were collected during mid-summer (July) from Twitchell Island (Sacramento-San Joaquin River Delta, California, USA), dried at 75°C in a convection oven, and pulverized in a Tecator mill to pass through a 500 μm screen. Leachates of the plant samples were prepared by adding 0.1265 g of plant material to 200 ml of organic-free water (<0.05 mg/l carbon), and

stirring on a magnetic stirplate at room temperature for 6 h. Resulting solutions were filtered through 0.45 μm pre-combusted (at 450°C) glass fiber filters in a Teflon filtration apparatus. Filtered leachates were freeze-dried to obtain the DOC in solid form. The soil samples are mapped as the Ringe mucky silt loam (Euic, thermic Typic Medisaprists).

DOC was isolated and concentrated from field-collected water samples by sequential extraction on nonionic macroporous resins (XAD-8 and XAD-4: Aiken et al., 1992). Briefly, 15 l of water was acidified to pH 2 with concentrated HCl and loaded onto sequential 295 ml XAD-8 and XAD-4 columns. The fraction adsorbed on each column was then removed separately by elution with 0.1 N NaOH. These eluates were further concentrated and desalted by readsorption on the XAD column, rinsing, and back-elution in base through a cation exchange column. Final eluates were acidified and then freeze-dried. The freeze-dried isolate was reconstituted in 0.001 N $NaHCO_3$ at a concentration of 10 mg/l.

Dissolved organic carbon (DOC) measurements were made with a Shimadzu TOC-5000A total organic carbon analyzer. (The use of trade names in this report is for identification purposes only and does not constitute endorsement by the US Geological Survey.) Each analysis represents the mean of three injections. Reproducibility of replicate analyses was 3% and analysis of organic-free water gave consistent blank values of <0.05 mg/l carbon.

Aliquots of the reconstituted XAD isolate, or plant leachates diluted with organic-free water to a DOC of approximately 4–5 mg/l were reacted with chlorine to form THM using a modified version of EPA Method 510.1. Samples were adjusted to pH 8.3–8.6 with 0.1 N NaOH and then distributed into three 40-ml serum vials. The chlorination solution contained >6000 mg/l free chlorine in an aqueous solution buffered to pH 8.3 with a 1 M H_3BO_3/0.11 M NaOH solution. The samples were dosed with 3–4 mg/l free chlorine per 1 mg/l DOC, following the reactivity-based dosing method (California Department of Water Resources, 1994b; Krasner and Sclimenti, 1993). Chlorinated samples were sealed head-space free in crimp-top vials with teflon-faced septa and incubated for 7 days in the dark at a controlled temperature of 25°C. This method of dosing also permits control of pH and residual chlorine during the reaction. After incubation, pH and free chlorine were measured in one vial per sample. Free chlorine was measured using Hach DPD colorimetric reagents (HACH, 1992). The final pH was 8.3 ± 0.1 and residual free chlorine was 1–6 mg/l. The remaining vials were quenched with 250 μl of 0.16 M Na_2SO_3 immediately prior to loading on the purge and trap unit.

THM concentrations were measured by purge and trap—gas chromatography following a modified version of EPA Method 502.2. All standards and samples were also injected with a surrogate compound, 3 μg of 2-bromo-1-chloropropane dissolved in methanol. Solutions from the trihalomethane formation potential (THMFP) experiments were diluted 5:1 with organic-free water and immediately injected by syringe into the spargers. Solutions were purged for 11 min with ultra-high purity N_2 gas, and the volatile organic compounds trapped on a Tekmar ♯♯3 tenax/silica gel/charcoal trap. Analytes were desorbed for 0.5 min from the trap at 225°C into the N_2 carrier gas and introduced into the 220°C split-splitless injector of the gas chromatograph. Following chromatographic separation, THM were quantified with an electron-capture detector. Full procedural blanks contained <2 μg/l $CHCl_3$ which is insignificant compared to the 30–60 μg/l $CHCl_3$ in the samples. Full procedural replicate analyses of samples yielded $CHCl_3$ concentrations within 3%. Surrogate recovery varied by less than 10% between injections.

Carbon isotopic ratios of THM were measured using compound-specific isotopic analysis (Merritt et al., 1995). THM were introduced into a Hewlett Packard 5890 II Plus gas chromatograph by purge and trap using the Tekmar LSC2000 concentrator. Solutions from the trihalomethane formation potential (THMFP) experiments were loaded undiluted into 5 ml spargers along with 0.6 μg of the internal standard, 2-bromo-1-chloropropane (equivalent to 12 nmole of carbon) dissolved in methanol. Sample solutions were sparged, and analytes desorbed off the trap into the carrier gas flow with ultra-high purity He gas. In order to achieve a completely splitless injection, the transfer line from the purge and trap was directly plumbed into the GC column. Chromatographic separation was achieved using the same column as used for analysis of THM concentrations.

Effluent from the GC was passed through a combustion interface consisting of a quartz tube packed with CuO wire placed in an 850°C furnace. The methanol solvent peak was diverted away from the combustion furnace and into the GC's flame ionization detector (FID) by opening two air-actuated heart split valves controlling ingress of He make-gas for the FID and at the inlet to the furnace. Both valves were closed during passage of the analyte peaks in order to limit dilution by He gas to just the amount required for column flow (~1 ml/min). The analytes were combusted to CO_2 and H_2O, and the H_2O removed by passing the gas stream through a −100°C cold trap.

The purified CO_2 from each analyte was analyzed in a continuous flow of He gas using a Micromass Optima continuous flow stable-isotope mass spectrometer. $^{13}C/^{12}C$ ratios were measured relative to $^{13}C/^{12}C$ ratios in CO_2 gas pulses with known $\delta^{13}C$.

Results are reported as $\delta^{13}C$ values in units of ‰, where

$$\delta^{13}C = \frac{1000 \times [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}]}{(^{13}C/^{12}C)_{standard}}$$

and V-PDB is the standard.

Duplicate analyses of 17 samples yielded a mean deviation between replicates of 0.30‰ (standard deviation = 0.2‰). Full procedural blanks yielded $CHCl_3$ peaks less than 2% the size of $CHCl_3$ in actual samples and with carbon isotopic ratios similar to those in the samples, indicating the insignificant contribution from the blank. All $\delta^{13}C$ values were corrected for the offset between the known and measured $\delta^{13}C$ of the internal standard present in each injection. The average correction was −0.92‰, and the maximum −2.8‰, indicating that isotopic fractionation during analysis was minimal.

$\delta^{13}C$ value of the internal standard was measured using a Carlo–Erba elemental analyzer interfaced with the Micromass Optima continuous flow stable isotope mass spectrometer. 1 μl of neat 2-bromo-1-chloropropane was injected into 3 mg of Chromosorb W (Costech) and rapidly sealed in a small silver cup. This cup was immediately sealed in a second silver cup and the seals were checked by monitoring weight loss from the sample on the balance. If no weight loss occurred, the sample was immediately analyzed (see next paragraph). Six replicate analyses yielded a $\delta^{13}C$ value of −24.07‰ (standard deviation = 0.03‰). Instrument performance was monitored by replicate analyses of an EDTA standard.

Carbon and nitrogen elemental and isotopic compositions of solid materials were also measured using the Carlo-Erba elemental analyzer interfaced with the Optima stable isotope mass spectrometer. Samples consisted of 1.5 mg of plant material, or 1 mg of XAD isolate or freeze-dried leachates, or 1.5–3 mg of soil sealed in tin or silver weigh boats. Carbonate was removed from the soil samples by placing the loaded weigh boats in a desiccator along with a beaker of concentrated HCl. All samples were combusted to CO_2 and N_2 at 1050°C and split with a diluter so that C and N could be measured on the same sample aliquot. The instrument was calibrated against 0.25, 0.5, and 1.0 mg EDTA standards. Calibration standards were analyzed after every 8 samples to correct for instrument drift. Carbon and nitrogen elemental compositions were calculated using data from both the thermal conductivity detector and the peak areas measured by the mass spectrometer. Replicate analyses for soil and isolate samples were within 0.05‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Replicate analyses of plant samples were within 0.04‰ for $\delta^{13}C$, 0.1‰ for $\delta^{15}N$. All sample analyses were within 1% for %C and %N.

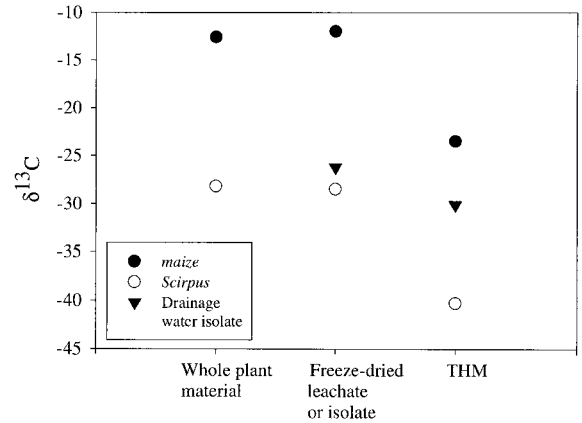

Fig. 1. Comparison of isotopic composition of whole plant material, plant leachates, drainage water isolate, and THM.

The lower precision of $\delta^{15}N$ results is due to sample heterogeneity.

3. Results and discussion

We chose to use *maize* and *Scirpus* as the model THM sources in this study because the agricultural field that is the focus of this study is located in an area of peat-rich soils and has been continuously planted in *maize* for at least the past 6 years. Agricultural practices for *maize* usually result in the return of the majority of biomass to the soil following harvest, and thus residual biomass may contribute substantially to the evolution of DOC into drainage waters. The aquatic bulrush, *Scirpus acutus*, was the dominant species during formation of the peat soils in the Delta, and is the major source of peat carbon (Atwater, 1980).

Lignin, the phenolic polymer common to all higher plants, and putative source of humic-derived THM is more abundant in *maize* than *Scirpus*. *Maize* contains 7.8% by weight lignin (Karunanandaa et al., 1992), while *Scirpus* contains 3.8% (Godshalk and Wetzel, 1978). Lignin in *Scirpus acutus* has been shown to become concentrated during decomposition, resulting in residual mass that is resistant to degradation under the anaerobic conditions prevalent in marshy areas (Godshalk and Wetzel, 1978).

Analysis of the plant samples yielded $\delta^{13}C_{bulk}$ values of −12.6‰ for *maize* and −28.2‰ for *Scirpus*. The $\delta^{13}C_{bulk}$ of *maize* thus is 15.6‰ higher than the *Scirpus*, typical of the isotopic difference between C4 and C3 plants.

We assume that the plant leachates represent the type of organic material potentially liberated, for example, during senescence and diagenesis of plant tis-

Table 1
Measured and calculated parameters for plant leachates and drainage water isolate

Sample	Parameter									
	%OC[a]	%TN[b]	$(OC:TN)_a$[c]	DOC[d]	SUVA-λ_{254}[e]	STHMFP[f]	$\delta^{13}C_{bulk}$[g]	$\delta^{15}N$[h]	$\delta^{13}C_{THM}$[g]	$\Delta\delta^{13}C_{THM}$[i]
Whole *maize*	42.3	4.2	12.6	NA[j]	NA[j]	NA[j]	−12.6	2.3	NA[j]	NA[j]
Maize leachate	29.3	3.0	12.2	59.3	0.013	6.4	−12.0	5.1	−23.5	11.5
Whole *Scirpus*	44.2	1.9	29	NA[j]	NA[j]	NA[j]	−28.2	2.8	NA[j]	NA[j]
Scirpus leachate	22.8	1.0	28.5	30.5	0.010	6.1	−28.5	1.4	−40.3	11.8
Drainage water isolate	57.0	1.5	47.5	NA[j]	0.036	5.8	−26.2	−0.9	−30.1	3.9

[a] %OC—Organic carbon of dried sample as weight percent.
[b] %TN—Total nitrogen content of dried sample weight percent.
[c] $(OC:TN)_a$—Atomic ratio of organic carbon and total nitrogen.
[d] DOC—Dissolved organic carbon (mg/l).
[e] SUVA-λ_{254}—Specific ultraviolet absorbance at a wavelength of 254 nm, normalized to DOC in mg/l.
[f] STHMFP—Specific Trihalomethane Formation Potential; moles of trihalomethane formed in 7 day test potential test × 1000/moles DOC.
[g] $\delta^{13}C = 1000 \times ((^{13}C/^{12}C)_{Sample} - (^{13}C/^{12}C)_{PDB})/(^{13}C/^{12}C)_{PDB}$ of bulk material or THM, as appropriate.
[h] $\delta^{15}N = 1000 \times ((^{15}N/^{14}N)_{Sample} - (^{15}N/^{14}N)_{Atmosphere})/(^{15}N/^{14}N)_{Atmosphere}$.
[i] $\Delta\delta^{13}C_{THM} = \delta^{13}C_{bulk} - \delta^{13}C_{THM}$.
[j] NA—Not applicable.

sues. The leachates do not necessarily reflect the composition of the whole plant material because the leachable materials are the more hydrophilic components of the plant tissues (Benner et al., 1990). Nevertheless, the isotopic compositions of the leachates were similar to those of the whole plant materials (Fig. 1, Table 1), indicating that no significant isotopic discrimination resulted from solubilization. The amount of THM formed upon chlorination of the plant leachates depends on both the quantity and quality of the DOC released (Reckow et al., 1990). The leachate produced from *maize* contained 59 mg/l DOC, twice the mass of DOC (on a weight-normalized basis) as the *Scirpus* leachate (Table 1). *Maize*-derived DOC also formed more THM upon chlorination (6.4 per thousand carbons) as compared to *Scirpus* (6.1 per thousand carbons). Thus, for an equivalent mass of dry plant material, *maize* formed more than twice as much THM as that formed by *Scirpus*.

Table 2
Elemental and isotopic composition of soil core samples. Symbols and abbreviations as in Table 1

Depth (cm)	Parameter				
	%OC	%TN	$(OC:TN)_a$	$\delta^{13}C_{bulk}$	$\delta^{15}N$
0–30	19.6	0.9	25.4	−26.1	−1.0
30–60	18.6	0.9	24.7	−26.3	−0.8
60–90	18.6	0.8	27.4	−26.9	−1.2
90–120	17.8	0.8	26.0	−27.0	−1.4
120–150	31.3	1.3	27.4	−27.2	−1.7
150–180	32.2	1.0	39.5	−27.2	−1.1
180–210	27.9	1.0	31.6	−27.4	−1.3

Quantitative assessment of the contribution of *maize*-derived DOC to drainage waters is problematic because the release of DOC from the *Scirpus* peats is difficult to quantify. In this study, there was little evidence of the presence of *maize*-derived carbon in soil samples taken from the field. The soil samples were very high in organic content (Table 2), varying from >18% OC (weight percent organic carbon) in the surface to >32% OC at depth. The soil carbon isotopic composition also varied with depth, from −26.1‰ in the surface sample, systematically declining to −27.4‰ in the deepest one (195 cm; Table 2). One explanation for the observed change in isotopic composition with depth is the presence of a minor amount of *maize*-derived carbon in the surface samples. If so, the isotopic shift constrains the *maize*-derived contribution to be <10%. Another explanation is that the peat soils are increasingly degraded with depth, which has been shown in other studies to result in isotopic shifts of similar magnitude and direction. Isotopically depleted lignin is concentrated in the residual biomass during peatification (Benner et al., 1987; Spiker and Hatcher, 1988). The contribution of *maize*-derived carbon to DOC in the drainage waters was similarly ambiguous. The $\delta^{13}C_{bulk}$ of the XAD-8 isolate from the drainage waters (−26.2‰; Table 1) was intermediate between the *maize* and *Scirpus* leachate $\delta^{13}C_{bulk}$ values, although the $\delta^{15}N$ (−0.9‰; Table 1) more closely resembled the soil samples (−0.8–−1.7‰; Table 2).

Inferring the source of THM-forming DOC from the isotopic composition of the bulk material, however,

may not be accurate because the isotopic composition of the larger pool obscures that of the smaller (Hedges, 1990b). Preservation of the isotopic difference between *maize* and *Scirpus* in the leachates suggests it may be possible to determine the source of the THM-forming DOC more accurately by examining the $\delta^{13}C$ value of the THM itself. The isotopic composition of the THM may then be used as a tracer to determine the relative contribution of each plant type in environmental studies if it is not obscured by intra- or intermolecular isotopic differences, or fractionated during THM formation reactions, isolation techniques, or measurement procedures.

3.1. Isotopic composition of plant leachates

The $\delta^{13}C$ values of THM that resulted from chlorination of plant leachate reflected the difference in the $\delta^{13}C_{bulk}$ of the two plant materials; the $\delta^{13}C_{THM}$ of *maize* leachates were 16.8‰ higher than *Scirpus* leachates, reflecting the 15.6‰ higher $\delta^{13}C_{bulk}$ value of the *maize* (Fig. 1, Table 1). However, there was a substantial difference between the $\delta^{13}C_{bulk}$ of the leachates and the $\delta^{13}C_{THM}$ of the leachates ($\Delta\delta^{13}C_{THM}$; $\Delta\delta^{13}C_{THM} = \delta^{13}C_{bulk} - \delta^{13}C_{THM}$; see Table 1). The $\Delta\delta^{13}C_{THM}$ of *maize* and *Scirpus* leachates were indistinguishable, with THM averaging 11.7‰ lower than the corresponding leachate (Fig. 1). Since the $\delta^{13}C_{bulk}$ of whole plant material and leachate values were similar in comparison to the $\Delta\delta^{13}C_{THM}$ values (Table 1), we suggest that this difference reflects isotopic differences between whole plant material and the THM precursors themselves.

Low $\Delta\delta^{13}C_{THM}$ values were measured in 60 other isolate samples, with an average $\Delta\delta^{13}C_{THM}$ of 3.5‰ indicating that the high $\Delta\delta^{13}C_{THM}$ observed in the plant leachates are not likely an artifact of the THM formation reaction, nor representative of isotopic inhomogeneities in ^{13}C distribution in a common THM precursor molecule. For example, THM formed from the reconstituted drainage water isolate was only 3.9‰ lower than the $\delta^{13}C_{bulk}$ value (Table 1; Fig. 1; see next section) indicating the large fractionation observed in plant leachates was not characteristic of the reaction or methods of analysis. Lignin, the phenolic polymer found in all higher plants, typically has lower $\delta^{13}C$ values than whole plant material, but the difference is usually <2‰ (Benner et al., 1987). A more likely source of the observed offset is intermolecular differences—different compounds in the DOC that form THM in reaction with chlorine. Because lignin has lower $\delta^{13}C$ values than other compounds in plants, it seems evident that the intramolecular isotopic differences must occur in addition to the intermolecular ones.

3.2. Isotopic composition of agricultural drainage water

Agricultural drainage water was collected from a ditch that accumulates waters draining the experimental plot as well as surrounding fields to investigate the contribution of *maize* to THM-forming DOC in environmental samples. The DOC of the drainage water was high (33 mg/l), and approximately 70% was isolated by serial extraction onto XAD-8 and XAD-4 columns, then reconstituted for subsequent analyses. The isotopic composition of the DOC isolated on the XAD-8 resin ($\delta^{13}C_{bulk} = -26.2$‰, $\delta^{15}N = -0.9$‰; Table 1) similar to that of *Scirpus* (Table 1). The drainage water humic isolate had a much higher specific absorbance at λ_{254} (SUVA; SUVA = absorbance/DOC; Table 1) than the leachates, indicating a higher aromatic content, but formed a similar amount of THM upon chlorination.

The $\delta^{13}C_{THM}$ of the drainage water isolate is -30.1‰, similar to that of whole *Scirpus* or soil organic matter (Tables 1 and 2). The $\delta^{13}C_{THM}$ of the drainage water isolate, intermediate between the leachate $\delta^{13}C_{THM}$ values, constrains the THM-forming DOC to be at least partly *Scirpus* derived, but does not rule out substantial contribution from *maize*. A simple mixing model using the two leachate values indicates that 40% of the THM-forming DOC would be *Scirpus*-derived and the remainder *maize*-derived if the leachates represent the contributing end-members to the drainage water isolate, and if the leachates were chemically representative of the type of DOC found in drainage water isolate.

However, application of this simple mixing model to evaluate contribution of the end-members may not be accurate because the drainage water isolate $\delta^{13}C_{bulk}$ value would have to be near -18‰ to be composed of 60% *maize*-derived DOC. Also, the difference in $\Delta\delta^{13}C_{THM}$ suggests the DOC in the drainage water isolate is chemically different than the DOC in the leachates. The drainage water isolate $\Delta\delta^{13}C_{THM}$ was 3.9‰, much lower than that of either of the leachates. One possible reason for this difference is isotopic fractionation during the process of XAD isolation and concentration. Approximately 70% of the DOC was extracted onto the resin columns. Assuming the fraction of organic carbon extracted onto the XAD columns and that passing the columns forms THM with similar efficiency as the whole water sample, the isotopic value of the 30% of the organic carbon not retained on the columns must possess a $\delta^{13}C$ value of <-65‰ for the isotopic mass balance to result in a $\Delta\delta^{13}C_{THM}$ of near the average value of 11.7 (Table 1)—an unreasonably low value for a fraction of bulk DOC. Direct measurement of the THM formed by organic material not retained by the XAD columns indicates that it had a much lower reactivity (1.2 per 1000

C) than the isolate, so the actual value would need to be much lower still. Also, although there is only one isolate value in Table 1, many other XAD isolate samples from a variety of environments exhibited $\Delta\delta^{13}C_{THM}$ values near 3.5‰ ($\sigma = 1.2$‰), indicating the presence of C4-derived carbon was not the reason for this offset. The lower $\Delta\delta^{13}C_{THM}$ of the isolate compared to the leachates is most probably representative of true chemical differences between the organic material in leachates and that in the drainage water rather than differences in source.

An additional, inescapable inference from the large difference between the $\Delta\delta^{13}C_{THM}$ of the isolate and leachates is that there is a large shift in the isotopic composition of the THM-forming DOC during diagenesis. Therefore, there must be at least two pools of material within plants that form isotopically distinct THM, and these pools degrade at different rates. Isotopically distinct pools of carbon do exist in plants as the result of differing synthetic pathways, but a non-lignin DOC source with a low $\delta^{13}C$ is required as a THM precursor. Thus, a more likely mechanism is that diagenesis concentrates different compositional homologues by, for example, preferentially consuming an isomer that produces low THM isotopic values. Although we are aware of no systematic characterization of isotopic differences in ring carbons in lignin, intramolecular isotopic differences of 3‰ have been reported in the literature for other compounds (O'Malley et al., 1996). Preferential degradation of individual lignin-derived phenols is well established in the literature (Hedges, 1990a; Hedges et al., 1985).

If the degradation of plant material systematically results in a change in the isotopic composition of THM-forming DOC for a variety of source materials, the $\Delta\delta^{13}C_{THM}$—diagnostic of the diagenetic state of DOC—may prove useful in understanding the release of THM-forming DOC from agricultural lands. This information would be valuable in designing best management practices for mitigation of THM-forming DOC in agricultural systems.

4. Conclusions

1. The carbon isotopic composition of individual trihalomethanes (THM) is quantifiable using purge and trap introduction into a gas chromatograph-combustion-isotope ratio monitoring mass spectrometer without apparent fractionation.
2. The natural isotopic difference between the *maize* and *Scirpus* was reflected in the 16.8‰ difference in the $\delta^{13}C$ isotopic composition of THM that results on chlorination of plant leachates. This result indicates carbon isotopes should be a valuable tool for identifying the source of THM precursors in environmental samples.
3. There was a substantial difference ($\Delta\delta^{13}C_{THM}$) between the isotopic composition of fresh plant material and that of THM formed from leachates. Both *maize* and *Scirpus* formed THM with $\delta^{13}C$ values about 12‰ lower than whole plant material. The $\Delta\delta^{13}C_{THM}$ values observed for the plant leachates are interpreted to indicate the presence of distinct pools of THM-forming DOC, representing different biochemical types or chemical structures.
4. The $\Delta\delta^{13}C_{THM}$ of humic extracts of agricultural drainage waters was 3.9‰, much lower than that of plant leachates. This result is evidence that the higher $\Delta\delta^{13}C_{THM}$ observed in plant leachates is not characteristic of the THM formation reaction. Further, when compared to the larger $\Delta\delta^{13}C_{THM}$ of plant leachates, it suggests that chemical alterations during diagenesis of plant material or resin isolation preferentially eliminate isotopically light THM precursors.
5. *Maize* released almost twice as much DOC into solution as the same mass of *Scirpus*. The net result was formation of more than twice the amount of THM from *maize* leachates than *Scirpus* leachates, indicating plant type may be an important variable in managing the amount of THM-forming DOC released into watersheds.

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