

Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela

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Abstract—Stable isotope ratios of carbon and nitrogen in autotrophs and detrital organic matter were investigated during inundation of the Orinoco River floodplain in Venezuela. Sampling emphasized microalgae and fine particulate detritus and included organic matter on submersed surfaces of C₃ and C₄ vascular plants as well as organic matter suspended in the water column and in surficial sediments. Algae and detritus were separated and collected for isotopic analysis by density gradient centrifugation in colloidal silica. Fine particulate organic matter in floodplain waters contains variable proportions of algal material, and the isotopic composition of the algal fraction was often distinct from that of the detrital fraction. Because most floodplain vascular plants assimilate atmospheric CO₂, their δ¹³C values proved to be typical of those reported for terrestrial plants. Algae were quite variable in δ¹³C; phytoplankton were most depleted (δ¹³C, -34.0 to -37.2‰), while epiphytic algae spanned a different and wider range in δ¹³C (-23.4 to -33.0‰). Depletion in ¹³C of organic matter mixtures relative to the range for C₃ vascular-plant sources is caused by the presence of algal carbon, whereas enrichment is caused by algal carbon or by carbon from C₄ vascular plants that grow on the floodplain. Phytoplankton and aquatic macrophytes varied relatively little in δ¹⁵N (1.5 to 4.1‰), but epiphytic algae were more variable (0.1 to 6.5‰). Algal samples in which N-fixing cyanobacteria were abundant were most depleted in ¹⁵N.

INTRODUCTION

THE CYCLING OF ORGANIC matter in floodplains of large rivers involves a combination of aquatic and terrestrial carbon pools and fluxes, the relative importance of which varies over the seasonal cycle of floodplain inundation. Floodplains support high rates of production and consumption of organic matter, and significant quantities of organic matter are transported between the floodplain and the river during inundation. Dissolved oxygen in waters of tropical floodplains, such as those of the Amazon and Orinoco rivers, is characteristically undersaturated with respect to the atmosphere during inundation, indicating that the aquatic portion of the ecosystem is heterotrophic overall (consumption of organic matter exceeds its production: MELACK and FISHER, 1983; RICHEY et al., 1988; HAMILTON and LEWIS, 1990a). However, production of organic matter above the water surface can be high during inundation because most of the floodplain is covered with forest and mats of floating herbaceous plants (JUNK, 1985; PIEDADE et al., 1991). Also, organic matter produced by vascular plants in seasonally dry areas may decompose under water during inundation. The dynamics of organic matter in floodplains are thus complex, and quantitative comparisons are difficult.

An improved comprehension of the organic biogeochemistry of tropical floodplains is needed because (1) most of the biological productivity of rivers appears to be directly dependent on their floodplains (WELCOMME, 1979; BAYLEY, 1989), (2) organic matter transported by rivers to the sea is

modified during its residence within floodplains (RICHEY et al., 1991), and (3) tropical floodplains are an important source of tropospheric methane, a greenhouse gas (ASELMANN and CRUTZEN, 1989). These important biogeochemical functions of tropical floodplains are subject to change as the ecosystems are increasingly modified by hydrologic regulation, deforestation, agricultural conversion, or climate change. Prediction of the direction and magnitude of change requires an understanding of ecosystem biogeochemistry.

Stable isotopes of carbon and nitrogen offer an important tool in the investigation of organic biogeochemistry in floodplains. Two research questions are particularly amenable to the application of stable isotope methodology: (1) What is the relative importance of production of organic matter, compared to allochthonous inputs, in the support of microbial activity and animal food webs in the river and its floodplain, and (2) Which groups of floodplain autotrophs are most important in the supply of labile organic matter to aquatic food webs? The investigation of these questions requires measurements of the isotopic composition of allochthonous organic matter as well as that produced by the various groups of autotrophs on the floodplain.

This study presents measurements of the carbon and nitrogen isotopic composition of organic matter in waters of the Orinoco River floodplain of Venezuela. We emphasize fine particulate organic matter because this fraction is most strongly involved in energy flow through food webs (WOTTON, 1984; HAMILTON et al., 1992) and in advective exchanges with the river (EISMA and CADEÉ, 1991; RICHEY et al., 1991). We collected organic matter from the water column, from submersed plant surfaces, and from sediment surfaces. Most previous measurements of stable isotope ratios in organic matter from various aquatic ecosystems have

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analyzed samples of organic matter that were mixtures of algae and detritus, then attempted to ascertain the dominant component of the samples from other information. In this study, we have developed a method to separate detritus from algae and collect the separated fractions for isotopic analysis, which yields a more accurate measurement of each fraction and is critical in ecosystems such as floodplains where both vascular-plant detritus and algae are abundant. We also present ancillary data on water chemistry and on the taxonomic composition of the algae. The data presented here yield insights into the origin and nature of organic matter in tropical floodplains and provide preliminary information for the design of more specific stable isotope studies in these environments.

THE ORINOCO RIVER AND ITS FLOODPLAIN

The Orinoco River has the world's third largest discharge to the oceans ($36,000 \text{ m}^3 \text{ s}^{-1}$; MEADE et al., 1983). Seasonal variation in discharge results in a regular annual fluctuation in river level of 10–15 m along the main stem. At high water, the river inundates a fringing floodplain of approximately $7,000 \text{ km}^2$ for 4–6 months, with maximum water levels in late August or September (HAMILTON and LEWIS, 1990b). Permanent lakes are abundant on the fringing floodplain and are typically isolated from the river during the season of low water (HAMILTON and LEWIS, 1990a). The other extensive floodplains of the Orinoco, including those of the Delta and the Llanos, are geomorphologically distinct from the fringing floodplain but share some ecological characteristics (HAMILTON and LEWIS, 1990b).

The Orinoco floodplain, like many tropical floodplains, is covered by a mixture of floodplain forest and herbaceous plants that grow primarily in unforested areas (macrophytes). The macrophytes reach their greatest abundance during inundation, when they form extensive floating mats. Potential autotrophic sources of organic matter in floodplain waters thus include trees and shrubs, aquatic macrophytes, suspended algae (phytoplankton), and attached algae that grow on the submersed portions of vascular plants (epiphytic or periphytic algae). Seasonal phases of growth and decomposition for vascular plants and algae coincide with seasonal fluctuations in water level. During inundation, the litter layer and the understory of the forest vegetation are covered with water, and the macrophytes and algae flourish. Some trees and shrubs drop their leaves during flooding, while others retain them. When the water level falls, most of the macrophytes die and decompose rapidly, although both macrophytes and algae may remain abundant in the greatly reduced areas of standing water. The plant ecology of similar floodplains in the Amazon Basin is described by JUNK (1983).

As in most tropical floodplains, the plants that form floating macrophyte mats during inundation in the Orinoco floodplain include both C_4 and C_3 plants (C_4 refers to the dicarboxylic acid pathway of photosynthetic carbon fixation, which is common among tropical grasses, and C_3 refers to the Calvin pathway that is characteristic of most other plants). The C_3 and C_4 plants differ in carbon isotope fractionation during photosynthetic carbon fixation (O'LEARY, 1981; FARQUHAR et al., 1989). The floating mats of C_4 grasses are the only conspicuous C_4 plants in the floodplain.

METHODS

Field Measurements and Sample Collection

We chose to sample during peak inundation and falling water (September–December 1988) because this is the phase of greatest primary and secondary production of the aquatic biota and encompasses the maximum development and subsequent senescence of floating macrophyte mats. The inundation phase is also particularly interesting because at that time the floodplain exchanges water, organic matter, and nutrients with the river, and thus potentially affects the quality and quantity of organic matter transported by the river (HEDGES et al., 1986; LEWIS, 1988).

Samples were collected in the vicinities of the four floodplain lakes

identified in Fig. 1. Sites 2, 3, and 4 form a transect in the downstream direction along a hydrologic flow path across the floodplain, and thus represent a range of hydrologic residence times on the floodplain. Seston (suspended solids) and water for chemical analysis were collected at the center of the open waters (area, $0.1\text{--}2.3 \text{ km}^2$) on the floodplain, and at the point of inflow from the main river channel into the floodplain near Site 2. Most other samples of vascular plants, algae, and detritus were collected from three distinct locations at each site and were then combined to form a composite sample. Samples of filamentous algae, *Utricularia*, and rotten wood are not composites from different locations.

Samples of live vascular plants included tree leaves above the water level and aerial and submersed parts of the floating aquatic plants *Paspalum repens* (a C_4 grass; Gramineae), *Eichhornia azurea*, and *E. crassipes* (C_3 plants; Pontederiaceae). The vascular plant *Utricularia foliosa* (Lentibulariaceae) and large monospecific aggregates of filamentous algae were also collected on occasion from floating mats and from the flooded forest; these were the only completely submersed macroscopic autotrophs. Coarse detritus, composed primarily of recognizable fragments of leaves and wood, was collected from the sediment surface in the flooded forest (grab samples of the litter layer), and from the center of open waters (upper 5 mm of sediment core samples). Samples of vascular plants, filamentous algae, and coarse detritus were dried at 60°C and ground to a fine powder for stable isotope analysis.

Seston was sampled by collection of approximately 100 L of sub-surface water, which was filtered in the field with a $53\text{-}\mu\text{m}$ mesh net to remove zooplankton and larger vascular-plant fragments. At the same time, we also collected water samples for chemical analysis and measured light penetration (Secchi disk) as well as vertical profiles of temperature (thermistor) and dissolved oxygen (polarographic sensor). The water sample containing seston was kept in darkness until the seston was collected at the field laboratory with a continuous-flow centrifuge (simple rotor type: THRONSDEN, 1978). The seston was collected the day of sample collection or, in a few cases, the morning thereafter. Centrifugation retention efficiencies ranged from 25–53% (mean, 41%) for particulate organic carbon and from 67–80% (mean, 73%) for particulate chlorophyll *a* (analytical methods

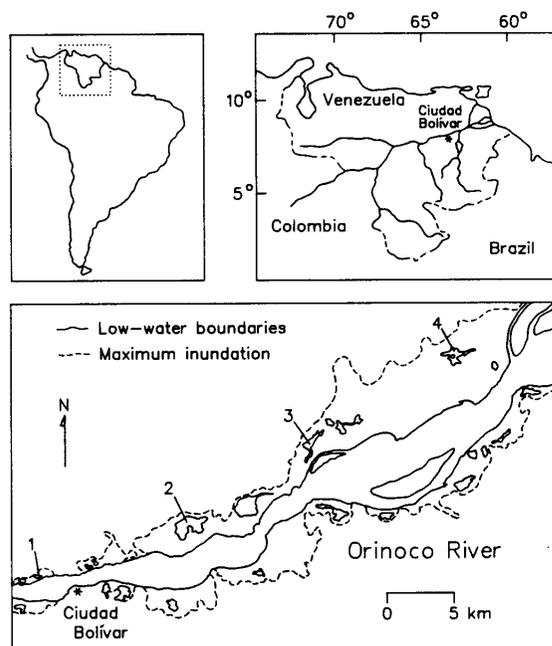


FIG. 1. Location of the study site. Samples were collected from floodplain waters along the north bank of the Orinoco River, in the vicinities of the principal lakes numbered on the map: (1) Orsineria; (2) Tineo; (3) Fundación; and (4) Tasajeras. River water entering the floodplain was sampled at the inflow channel to Lake Tineo.

are given in the following text). The solids (concentrated seston) were diluted with filtered water and refrigerated for 1–3 hours until the separation step, which is described later.

The particulate material adhering to submersed surfaces of vascular plants (epiphyton) was collected from the floating mats and from submersed tree leaves in the flooded forest by gentle agitation and brushing of plant parts in a bucket of water. The resulting slurry of epiphytic material was filtered through sieves of 800- and 275- μ m mesh, which removed larger vascular-plant fragments and macro-invertebrates. Smaller mesh sizes were not used because they retained significant amounts of epiphytic algae. All samples containing algae were kept out of sunlight and were transported to a nearby field laboratory within one hour.

Separation of Algae and Detritus in Organic Matter Samples

Epiphyton slurries washed from surfaces and seston samples concentrated by continuous centrifugation were partitioned into their algal and detrital fractions by centrifugation of the particulate material in pre-filtered colloidal silica, using 50-mL conical bottom tubes. Algae tended to remain in the water just above the silica sol, while other particulates (mostly detrital organic matter and clays) tended to pass through the layer and accumulate in the silica sol at the bottom of the tube. Bacteria and protozoa were present in both fractions, but these would be expected to comprise a negligible proportion of the total carbon in fine detritus from floodplain waters (BOWEN et al., 1984; HEDGES et al., 1986). The optimum density of the silica sol was determined by trial and error with step gradients to provide the best separation of algae from the other material, as judged from microscopic inspection and measurements of chlorophyll *a*. An 80% solution (v/v) of Ludox AM in deionized water usually worked well; the specific gravity of this mixture is about 1.16 (25°C). Observations of particle densities compiled by LAMMERS (1971) indicate that this density would be expected to separate nondiatom algae from most other fine particulates in natural waters, including colloidal organic matter, clays, and mineral precipitates.

Successive centrifugation of 10-mL subsamples of the epiphyton or concentrated seston sample eventually produced algal and detrital fractions of sufficient size for analysis of stable isotopes. Each fraction was removed by pipet, diluted, homogenized in a churn splitter, and collected on pre-combusted glass-fiber filters (Whatman GF/C; effective pore size $\approx 0.7 \mu\text{m}$; SHELDON, 1972), which were then either dried at 60°C (stable isotopes, CHN analysis) or placed in 90% ethanol (chlorophyll *a*). A subsample of the unfractionated seston or epiphyton was also diluted, homogenized, and collected on filters for comparison with the partitioned fractions. Although the colloidal silica passes through the GF/C filters, the silica content of the detrital fraction was reduced by three successive centrifugations through deionized water before the sample was diluted and homogenized. Filters containing the algal or detrital fractions were examined with a dissecting microscope before drying, and any visible invertebrates and vascular-plant fragments were removed with forceps and needles. Such material was not removed from subsamples of the unfractionated particulate material, however. The sample processing described above was performed in low light to avoid loss of chlorophyll, and was completed by the day after sample collection.

The use of colloidal silica in density-gradient centrifugation is described by WOLFF (1975) and PERSSON (1984). Colloidal silica has a negligible osmotic potential and is inorganic, and therefore is unlikely to cause lysis of cells or to contaminate samples of organic matter for isotopic analysis. However, Ludox AM contains a small amount (ca. 25 ppm) of a biocide (PRICE and DOWLING, 1977); isotopic analysis of a sample of oven-dried Ludox AM yielded $\delta^{13}\text{C} = -22.4\text{‰}$ and $\delta^{15}\text{N} = 2.0\text{‰}$. The protocol resulted in dilution of the biocide to insignificant levels before particulates were collected. We also performed the following experiment to ensure that algae did not absorb enough of the biocide to alter their stable isotopic composition. A large sample of filamentous algae (*Mougeotia* sp.: Chlorophyta) was collected, suspended in deionized water, and homogenized in the churn splitter, then divided into nine subsamples of approximately equal size. Triplicate subsamples were soaked in either deionized water (control), 4% Ludox AM (to simulate the medium of the algal fraction), or 80% Ludox AM (to simulate the medium

of the detrital fraction). After 30 minutes, the samples were collected by the centrifugation protocol. The control sample had $\delta^{13}\text{C} = -28.4\text{‰}$ and $\delta^{15}\text{N} = 3.0\text{‰}$. The algae exposed to 4% Ludox AM showed no significant difference in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ relative to the control ($P < 0.05$; Model I ANOVA with planned comparisons), while the algae in 80% Ludox AM showed a significant ($P < 0.05$) but very small enrichment of 0.2‰ in ^{13}C relative to the control (data on $\delta^{15}\text{N}$ are not available for this treatment). These results indicate that contamination of algal samples during exposure to Ludox AM does not present a problem.

Stable Isotope Analyses

Stable isotope analyses were performed in two ways. For approximately half of the samples, pure CO_2 or N_2 gas was obtained by combustion of the organic matter in sealed Vycor tubes at 900°C for 1 h. The gases were collected cryogenically on a manual vacuum line for analysis by isotope ratio mass spectrometry. The remaining samples were analyzed with a newly developed automated analysis system for coupled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements (FRY et al., 1992). Samples were combusted in an elemental analyzer and the gases were cryogenically purified in a custom built stainless steel manifold, which also served as the inlet reservoir for a mass spectrometer. Many samples as well as standards were analyzed by both methods and the results show good agreement. Organic matter samples from the Orinoco floodplain do not contain significant amounts of inorganic carbon and therefore were not acidified before isotopic analysis. The isotope analyses were performed at The Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts.

Stable isotope measurements are expressed as δ values, which are ‰ (parts per thousand) deviations from standard reference materials:

$$\delta X = [(R_{\text{sample}}/R_{\text{std}}) - 1] \times 10^3,$$

where $X = ^{13}\text{C}$ or ^{15}N and $R = ^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. The δ value is higher (more positive) in a sample that is enriched in the heavy isotope. The standards used for mass spectrometry were tank gases: ultra-high-purity N_2 ($\delta^{15}\text{N} = -0.7\text{‰}$) calibrated against atmospheric N_2 and instrument-grade CO_2 ($\delta^{13}\text{C} = -21.9\text{‰}$) calibrated against U.S. National Bureau of Standards reference materials. The data presented here are referenced to the Pee Dee belemnite (PDB) for carbon and atmospheric air for nitrogen. The mean \pm s.d. for the difference between duplicate subsamples of ground material (including samples of animals as well as plants and detritus) was $0.22 \pm 0.21\text{‰}$ for $\delta^{13}\text{C}$ ($N = 42$) and $0.26 \pm 0.23\text{‰}$ for $\delta^{15}\text{N}$ ($N = 39$).

Chemical Analyses of Water Samples

Particulate solids collected on pre-combusted GF/C filters were analyzed for organic C and N with an elemental analyzer. Chlorophyll *a* was determined by a spectrophotometric method with correction for phaeopigments, after extraction in 90% ethanol (NUSCH, 1980). Unfiltered water samples, which were bottled upon collection without gaseous headspace and stored in a dark refrigerator, were analyzed within 24 h for pH (combination electrode) and total alkalinity (incremental titration in an open beaker). Water samples that had been filtered (GF/C) within several hours of collection were analyzed within 24 h for ammonium by the phenolhypochlorite technique. Additional filtered samples were analyzed within three months for Ca, Mg, Na, and K by flame atomic absorption, and for chloride and sulfate by ion chromatography (HAMILTON and LEWIS, 1990a). We calculated the partial pressure and concentration of aqueous carbon dioxide and the calcite saturation index (SI, the \log_{10} of the ratio of the ion activity product to the equilibrium solubility product) from measurements of pH, total alkalinity, ion concentrations, and temperature (GARRELS and CHRIST, 1965; KEMPE, 1982).

Algal Composition and Biomass

Subsamples of the algal fraction of the seston and epiphyton were preserved in Lugol's solution and the algae were identified and counted with an inverted microscope. Morphometric measurements of each species were used in standard geometric formulas to estimate cellular

biovolume. Biovolume was converted to C using the regression equations of STRATHMANN (1967): one equation was used for diatoms and another for all other algae. We also enumerated heterocysts, which are specialized cells of certain filamentous cyanobacteria where nitrogen fixation activity is concentrated, and we determined their biovolumes separately from those of the associated vegetative cells.

RESULTS

Relevant Chemical Characteristics of the Sampling Sites

The physical and chemical characteristics of the sampling sites were typical of the Orinoco floodplain during inundation (HAMILTON and LEWIS, 1990a). Table 1 presents selected variables measured in open-water areas during this study that are relevant to the fractionation of C and N isotopes by aquatic autotrophs. Although thermal stratification was evident, anoxic water was observed only in the hypolimnion of Site 4. Phytoplankton biomass, as indicated by chlorophyll *a*, was higher in floodplain waters than in river water entering the floodplain. The concentration of ammonium was low. Nitrate was not measured immediately but was probably depleted to comparable levels, and was undetectable ($<0.3 \mu\text{M}$) in later analyses by ion chromatography. Concentrations and proportions of major ions indicate that the river was the predominant source of water at all floodplain sites (S. K. Hamilton, unpubl. data).

The pH and total alkalinity varied little during residence of water on the floodplain, as shown by the data for Sites 2–4, which lie along a flow path across the floodplain. The higher

pH and alkalinity of Site 1 may be due to its relatively low flushing rate (TWOMBLY and LEWIS, 1987); it may have contained a significant proportion of water remaining from the previous isolation phase. The partial pressure of aqueous CO_2 (P_{CO_2}) indicates considerable supersaturation at all sites, including the riverine inflow; respiratory CO_2 accumulates largely as $\text{CO}_2(\text{aq})$ in these waters because they are slightly acidic and low in dissolved carbonates (DEVOL et al., 1987). Our unpublished measurements of P_{CO_2} in floodplain waters at other times show that P_{CO_2} is too variable to infer any spatial patterns from the data in Table 1. The saturation index for calcite indicates that carbonates are unlikely to precipitate in these waters. Calcite remains well below saturation in waters of the Orinoco floodplain throughout the year (HAMILTON and LEWIS, 1987).

Evaluation of Algal-Detrital Separations

Measurements of the concentrations of chlorophyll *a* and organic nitrogen in organic matter samples serve as compositional indicators when they are normalized to the concentrations of organic carbon. Table 2 shows these compositional indicators, together with the stable isotope measurements, for the unfractionated (bulk) particulate material and its algal and detrital fractions. Chlorophyll *a* and nitrogen content are expressed in Table 2 in the conventional manner as C:Chl and C:N mass ratios. The C:Chl ratios usually showed a large difference between the algal and detrital fractions, while the C:N ratios showed no consistent difference.

Table 1. Physical and chemical data for the open-water areas of sampling sites identified in Figure 1. D.O. = dissolved oxygen, P_{CO_2} = partial pressure of $\text{CO}_2(\text{aq})$. Calcite SI = calcite saturation index, calculated as the \log_{10} of the ratio of the ion activity product to the solubility product. Precision is given as the standard deviation of a series of replicate analyses.

Variable (Units)	Precision	Site 1	Site 2	Site 3	Site 4	River Inflow
No. sampling dates*		1	3	1	1	1
Surface Temp. ($^{\circ}\text{C}$)	0.6	30.7	29.0	29.8	30.8	--
Bottom Temp. ($^{\circ}\text{C}$)	0.6	30.4	28.3	29.2	29.0	--
Surface D.O. (mg L^{-1})	0.1	5.0	6.8	3.9	3.3	--
Bottom D.O. (mg L^{-1})	0.1	4.6	6.0	3.3	0	--
Total Depth (m)	--	3.0	3.5	2.4	6.1	--
Secchi Depth (m)	0.1	--	0.6	0.5	1.6	--
Total Seston (mg L^{-1})	0.5	10	18	14	4	38
Particulate C (mg L^{-1})	0.2	1.16	1.23	0.91	1.16	--
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	1	16	17	7	7	1
NH_4^+ (μM)	0.2	0.6	0.7	0.5	0.5	1.8
pH	0.1	7.06	6.54	6.55	6.57	6.50
Alkalinity ($\mu\text{eq L}^{-1}$)	1.5	318	182	190	192	195
P_{CO_2} (μatm)	--	2043	3675	3993	3849	4596
$\text{CO}_2(\text{aq})$ (μM)	--	59	121	115	111	133
Calcite SI	--	-2.3	-3.1	-3.1	-3.2	-3.1

* Sampling dates and times: Site 1, 26 Oct 1988 at 1000 hr; Site 2, 21 Sep 1988 at 1100 hr, 10 Nov 1988 at 1000 hr, and 7 Dec 1988 at 1030 hr; Site 3, 9 Oct 1988 at 1640 hr; Site 4, 13 Oct 1988 at 1130 hr; River inflows, 6 Oct 1988 at 1400 hr.

Table 2. Compositional indicators and stable isotope ratios for bulk samples and algal and detrital fractions of seston and epiphyton. C:Chl = mass ratio of organic carbon to chlorophyll *a*; C:N = mass ratio of organic carbon to organic nitrogen. Epi-Pasp. = epiphyton from *Paspalum repens*. Epi-Eich. = epiphyton from *Eichhornia* spp. Epi-forest = epiphyton from submersed tree leaves in the flooded forest.

date	site	sample	code	C:Chl			C:N			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
				algal	bulk	detrital	algal	bulk	detrital	algal	bulk	detrital	algal	bulk	detrital
9 Oct	3	Seston	A	29	183	321	6.0	6.4	7.5	-36.7	-32.0	-30.2	4.1	3.9	3.8
13 Oct	4	Seston	B	57	108	--	7.6	6.8	--	-34.6	-36.1	--	3.4	2.5	--
17 Oct	2	Seston	C	47	86	149	7.4	6.9	8.3	-37.2	-35.6	-34.1	4.0	4.6	4.6
26 Oct	1	Seston	D	--	59	239	7.9	7.4	7.4	-37.2	-35.5	-31.5	3.0	5.6	5.7
10 Nov	2	Seston	E	41	47	384	8.0	6.6	6.9	-34.0	-34.1	-29.7	3.1	5.4	5.8
7 Dec	2	Seston	F	46	--	81	6.4	--	5.6	-34.2	-32.1	-29.0	3.5	4.2	6.2
17 Oct	2	Epi-Pasp.	G	71	99	129	8.9	8.0	8.5	-29.3	-27.9	-27.7	3.6	3.1	3.4
10 Nov	2	Epi-Pasp.	H	57	87	102	7.9	7.2	7.9	-25.2	-23.7	-23.2	1.6	2.1	1.6
7 Dec	2	Epi-Pasp.	I	52	82	138	7.9	7.3	7.8	-23.4	-21.8	-21.7	3.4	3.7	3.5
9 Oct	3	Epi-Pasp.	J	40	161	58	6.7	12.0	8.1	-29.8	--	-26.7	5.7	--	3.8
26 Oct	1	Epi-Pasp.	K	62	107	224	8.2	7.8	8.2	-24.2	--	-23.3	1.9	--	2.6
9 Oct	3	Epi-Eich.	L	41	147	55	7.3	9.1	8.2	-28.8	--	-27.9	4.8	--	5.1
22 Oct	4	Epi-Eich.	M	114	--	--	8.9	--	--	-33.0	--	-33.5	1.9	--	1.8
26 Oct	1	Epi-Eich.	N	29	68	207	5.8	5.9	7.0	-27.0	--	-28.2	0.1	--	1.8
10 Nov	2	Epi-forest	O	54	271	952	8.6	7.7	8.0	-29.8	--	-28.4	5.2	--	3.9
7 Dec	2	Epi-forest	P	52	141	312	6.7	6.9	8.0	-28.3	--	-27.5	6.5	--	4.3

Chlorophyll *a* serves as an indicator of the algal content of the samples because it is rapidly degraded outside of living cells and comprises a negligible fraction of detrital organic carbon, particularly in oxic waters of the euphotic zone (FURLONG and CARPENTER, 1988). For samples of fine particulate organic matter, we adopt an operational definition of detritus as organic matter lacking chlorophyll *a*, although we acknowledge that the distinction between live plant material and detritus is not sharp for material such as senescent algal cells or fresh fragments from vascular plants.

The efficiency of the separations can be compared by plotting theoretical mixing lines using the data in Table 2. If chlorophyll content is expressed as Chl:C rather than as the conventional C:Chl, the mixing line in a plot against $\delta^{13}\text{C}$ is linear. Mixing lines based on Chl:C and $\delta^{13}\text{C}$ are plotted for each separation in Fig. 2. Four of the sixteen separations in Table 2 are not plotted because the necessary data are incomplete. The unfractionated seston does not plot directly along the mixing line between the two endmembers because it contained some invertebrates and larger vascular-plant fragments; these materials were hand-picked from the separated fractions but not from the unfractionated samples. Measurements of unfractionated samples are given in Table 2 but are not plotted in the figure to simplify interpretation of the mixing lines.

Microscopic examination of the detrital fractions revealed that some intact algae were present, and that the amount of algal contamination was not consistent among the samples. The chlorophyll *a* content was used to correct the detrital measurements for the variable degree of algal contamination. Assuming that the Chl:C ratio of detritus is zero, the mixing

lines in Fig. 2 can be extrapolated downward to the abscissa to provide an estimate of the $\delta^{13}\text{C}$ of the detrital endmember of the mixture. If the Chl:C ratio of the algal endmember were known, then we could extrapolate the mixing line upward to estimate the $\delta^{13}\text{C}$ of pure algae. Unfortunately, the Chl:C ratio of algae is quite variable and is subject to short-term changes according to the nutrient and light status of the algae (GEIDER, 1987; RIEMANN et al., 1989). The chlorophyll

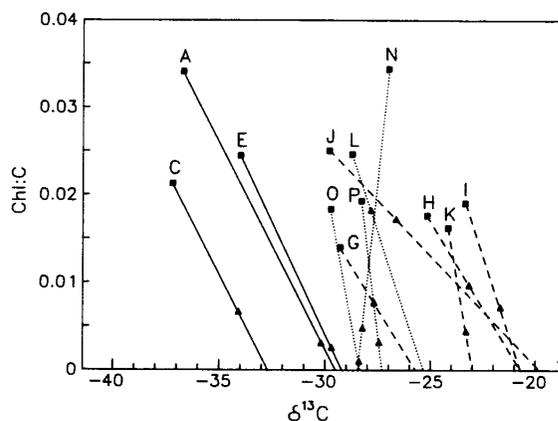


FIG. 2. Carbon mixing lines for the separations of algae and detritus in Table 2, showing extrapolations used to estimate $\delta^{13}\text{C}$ of the pure detritus endmembers. Measurements of the unfractionated samples are not included in the plot. The letters refer to the codes in the table. Solid lines = seston samples; dashed lines = epiphyton from the C_4 grass *Paspalum repens*; dotted lines = epiphyton from C_3 vascular plants (*Eichhornia* spp. and tree leaves).

a contents for the algal fractions in Table 2 are well within the range reported for natural algal assemblages.

Similar mixing lines are plotted based on Chl:N and $\delta^{15}\text{N}$ measurements in Fig. 3. In contrast to $\delta^{13}\text{C}$, there is no consistent difference in $\delta^{15}\text{N}$ between the algal and detrital fractions. For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, corrections based on Figs. 2 and 3 are used to obtain the δ values for the detrital fraction of seston and epiphyton reported below.

Stable Isotope Ratios in Autotrophs

The stable isotopic composition of the major groups of autotrophs in the Orinoco floodplain is shown in Fig. 4. The disparate and narrow ranges of $\delta^{13}\text{C}$ for the two groups of vascular plants are the result of different isotopic fractionations in their photosynthetic pathways (C_3 and C_4); these plants have their leaves above the water level, and thus they utilize atmospheric CO_2 . The $\delta^{13}\text{C}$ values for vascular plants in Fig. 4 are generally consistent with the data of HEDGES et al. (1986) for the Amazon floodplain. Phytoplankton was depleted in ^{13}C relative to vascular plants. The epiphytic algae living on either C_3 or C_4 plant surfaces were more enriched in ^{13}C than the phytoplankton. The $\delta^{13}\text{C}$ values of the epiphytic algae were quite variable, overlapping the $\delta^{13}\text{C}$ values of C_3 vascular plants that utilize atmospheric CO_2 .

With the exception of epiphytic algae and leaves of an unidentified tree from the Leguminosae, the $\delta^{15}\text{N}$ of most of the autotrophs ranged between 1–4‰. The depletion in ^{15}N of the tree leaves is probably due to the presence of fixed nitrogen derived from atmospheric N_2 because nitrogen-fixing bacterial symbionts are commonly associated with trees of the Leguminosae, and microbial nitrogen fixation results in only a small fractionation with respect to atmospheric N_2 (VIRGINIA and DELWICHE, 1982). Possible explanations for the relatively wide variation in $\delta^{15}\text{N}$ of epiphytic algae are considered later.

The aquatic vascular plant *Utricularia foliosa* and large aggregates of filamentous algae do not contribute significant quantities of organic carbon to the floodplain, but were sampled on occasion because they might serve as more easily

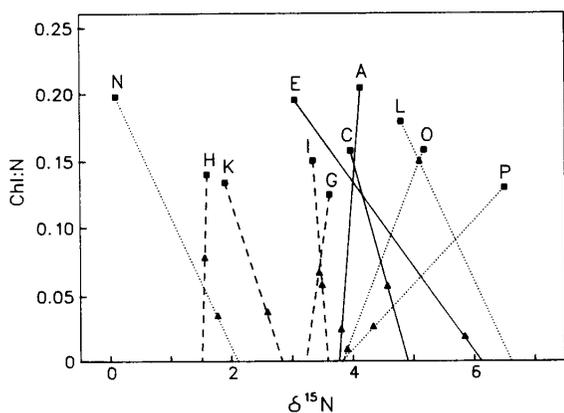


FIG. 3. Nitrogen mixing lines for the separations of algae and detritus in Table 2, showing extrapolations used to estimate $\delta^{15}\text{N}$ of the pure detritus endmembers. The letters refer to the codes in the table. Solid lines = seston samples; dashed lines = epiphyton from the C_4 grass *Paspalum repens*; dotted lines = epiphyton from C_3 vascular plants (*Eichhornia* spp. and tree leaves).

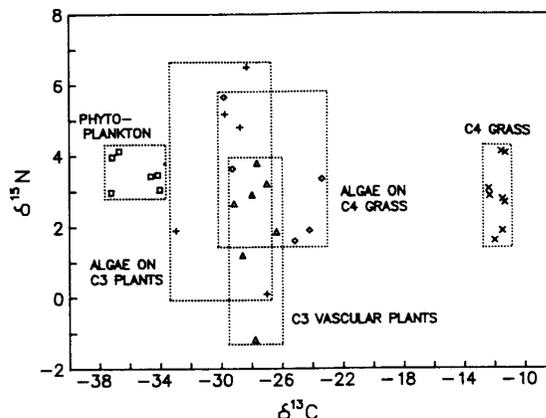


FIG. 4. Stable carbon and nitrogen isotope ratios in the major groups of autotrophs from the Orinoco River floodplain. Each point within a particular group represents a different site or sampling date. C_3 vascular plants include *Eichhornia* spp. and leaves of several tree species. The C_4 grass is *Paspalum repens*. Epiphytic algae were collected from the submersed surfaces of vascular plants. Phytoplankton and epiphytic algae were separated from associated detritus before analysis (see text).

collected isotopic surrogates for the microalgae in their vicinity. However, comparison of the measurements in Table 3 with the isotopic composition of the epiphytic algae from each corresponding site (Table 2) shows no significant correlation for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, indicating that such samples cannot be used as isotopic surrogates for epiphytic algae. In the case of $\delta^{15}\text{N}$, the use of *U. foliosa* as a surrogate for algae is probably not appropriate anyway, because plants of this family (Bladderworts) can supplement their nitrogenous nutrition by trapping and digesting invertebrates in bladders (HUTCHINSON, 1975). The $\delta^{15}\text{N}$ of their tissue may thus be affected by that of their invertebrate prey.

Relation Between Algal Taxonomic Composition and Isotope Ratios

The mean cell size and taxonomic composition of the algae collected from the seston and epiphyton were highly variable (Table 4) but were unrelated to isotopic composition, except for a correspondence between the abundance of nitrogen-fixing cyanobacteria and the more negative $\delta^{15}\text{N}$ values among the epiphytic algal samples (Fig. 5). We use the biomass of heterocysts as an index of nitrogen fixation by the algal assemblage because the nitrogen-fixing capability of these algae is facultative and is directly proportional to the occurrence of heterocystous cells (REYNOLDS, 1984). The data in Fig. 5 are consistent with reports in other studies that cyanobacterial fixation of N_2 can decrease the $\delta^{15}\text{N}$ values of algal assemblages (ESTEP and VIGG, 1985; MACKO et al., 1987).

Stable Isotope Ratios in Detrital Organic Matter

The range in isotopic composition of detrital organic matter (Fig. 6) was narrower than that of the major groups of autotrophs (Fig. 4). Sestonic detritus in floodplain waters was enriched in ^{13}C relative to phytoplankton, but depleted relative to unfractionated particulate organic matter in the riverine inflow (Fig. 6). Epiphytic detritus collected from mats

Table 3. Stable isotope ratios of filamentous algae and of *Utricularia foliosa*, a completely submersed C₃ aquatic vascular plant, collected for comparison with microalgal samples from the same sites.

Date	Site	Sample	Environment	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
9 Oct	3	Filamentous Alga (<i>Spirogyra</i> sp.)	Eichhornia mat	-29.3	6.4
17 Oct	2	Filamentous Alga (<i>Draparnaldiopsis</i> sp.)	Forest	-26.6	7.3
22 Oct	4	Filamentous Alga (<i>Spirogyra</i> sp.)	Forest	-31.9	3.1
13 Oct	4	<i>Utricularia foliosa</i>	Forest	-33.9	4.1
10 Nov	2	<i>U. foliosa</i>	Paspalum mat	-29.0	6.2
7 Dec	2	<i>U. foliosa</i>	Forest	-29.9	7.8

of *P. repens* was enriched in ¹³C compared to epiphytic detritus collected from C₃ plants (Fig. 6).

Coarse detritus from the open-water sediments and from the litter layer of the flooded forest resembled C₃ plants in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 6). Standing dead wood (coded as 8 in Fig. 6) was slightly enriched in ¹³C relative to leaves of C₃ plants; a similar enrichment of wood relative to leaves is found in living trees (LEAVITT and LONG, 1982; HEDGES et al., 1986). Remains of *P. repens* from the previous inundation season (coded as 9 in Fig. 6), which had been trapped on tree branches as the water level fell, were similar in isotopic composition to live tissue. These data agree with the conclusions of numerous studies that the $\delta^{13}\text{C}$ of detrital organic matter tends to resemble that of its autotrophic source (FRY and SHERR, 1984; cf. BENNER et al., 1987).

DISCUSSION

Sources of Organic Matter in Floodplain Waters

The origin and nature of particulate organic matter carried in suspension by the Orinoco River is likely to resemble that carried by the Amazon River, which has been characterized in detail by HEDGES et al. (1986). In the Amazon River, the coarse fraction (>63 μm) is composed of vascular-plant detritus that is mainly derived from tree leaves and is little altered. Much of this coarse fraction probably sediments rapidly from the water column once river water enters the floodplain. The fine fraction (<63 μm) appears to be derived largely from soil humic matter, and is strongly adsorbed to mineral particles. Both fractions have a carbon isotopic composition

Table 4. Algal cell size and taxonomic composition for the phytoplankton and epiphyton samples that were analyzed for stable isotopes. Epi-Pasp. = epiphyton from *Paspalum repens*, Epi-Eich. = epiphyton from *Eichhornia* spp., Epi-forest = epiphyton from submersed tree leaves in the flooded forest.

Date	Site	Sample	Mean Size (pg C ₁ cell ⁻¹)	% of total algal biomass*					
				A	B	C	D	E	F
9 Oct	3	phytopl.	17	0	0	3	5	92	0
13 Oct	4	phytopl.	50	0	0	0	48	11	41
17 Oct	2	phytopl.	10	0	0	0	0	100	0
26 Oct	1	phytopl.	2	0	0	66	1	33	0
10 Nov	2	phytopl.	27	0	0	1	38	61	0
7 Dec	2	phytopl.	--	--	--	--	--	--	--
17 Oct	2	epl-Pasp.	51	1	0	28	51	21	0
10 Nov	2	epl-Pasp.	875	0	0	0	2	98	0
7 Dec	2	epl-Pasp.	62	12	4	5	1	82	0
9 Oct	3	epl-Pasp.	150	39	1	0	0	60	0
26 Oct	1	epl-Pasp.	106	7	2	1	1	91	0
9 Oct	3	epl-Eich.	224	0	0	1	8	91	0
22 Oct	4	epl-Eich.	10	4	1	20	62	14	0
26 Oct	1	epl-Eich.	24	19	18	68	1	11	0
10 Nov	2	epl-forest	2	0	0	100	0	0	0
7 Dec	2	epl-forest	824	0	0	1	0	98	0

* Key to column headings:

- A: N-fixing cyanobacteria (blue-green algae), including all cells
- B: Heterocystous cells of N-fixing cyanobacteria
- C: Non N-fixing cyanobacteria, including all cells
- D: Bacillariophyta (diatoms)
- E: Chlorophyta (green algae)
- F: Euglenophyta

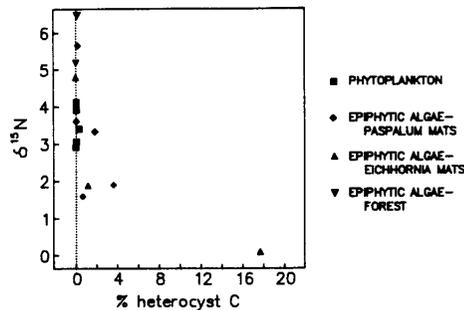


FIG. 5. The relation between $\delta^{15}\text{N}$ and the abundance of heterocysts in algal samples (expressed as the percentage of the total algal biomass). Heterocysts are specialized N-fixing cells of certain cyanobacteria. Algal samples in which heterocysts were abundant were relatively depleted in ^{15}N , presumably reflecting isotopically light nitrogen derived from atmospheric N_2 by biological fixation.

similar to that of terrestrial C_3 plants ($\delta^{13}\text{C}$ -27 – -30% ; HEDGES et al., 1986; CAI et al., 1988). The $\delta^{13}\text{C}$ value of our single sample of suspended organic matter from the Orinoco River was -25% , which is more enriched than samples from the Amazon main stem, but is within the range reported for tributaries of the Amazon River (BIRD et al., 1991).

During residence on the floodplain, the original particulate organic matter contained in the river water is mixed with organic matter from new sources such as algal growth, fresh detritus from vascular plants, and soil humic matter from seasonally inundated areas and the local upland watershed. Detrital organic carbon originating from phytoplanktonic algae is isotopically distinct from terrigenous C_3 carbon, whereas that from epiphytic algae may not be. However, only algal organic carbon can result in substantial depletion of floodplain detritus with respect to the $\delta^{13}\text{C}$ of terrigenous C_3 carbon, and phytoplankton is the most ^{13}C -depleted source of organic matter. Conversely, ^{13}C enrichment of floodplain detritus can result from organic matter produced by epiphytic algae under certain conditions, or from organic matter produced by the C_4 grasses growing on the floodplain.

It is impossible to estimate the proportional contributions of organic carbon sources to detrital material on the basis of stable isotope data alone, unless the detritus has an isotopic composition near one of the extremes of the range of potential sources. Our measurements of detrital $\delta^{13}\text{C}$ fall in the center of the range of autotrophic sources (Fig. 6). Thus, we can only conclude that neither phytoplankton nor C_4 grasses contribute a major proportion of the fine particulate detritus found in the seston, epiphyton, or surficial sediments, although both may be significant components.

Isotopic Fractionation by Algae

The variation in stable isotope ratios of algae in Orinoco floodplain waters is larger than that reported from many other ecosystems (FRY and SHERR, 1984). However, most previous studies analyzed samples of unfractionated seston or epiphyton, which were often assumed to represent the isotopic composition of pure algae, and few studies have sampled such a wide range of autotrophs in a particular ecosystem. Comparison of our isotopic measurements of unfractionated samples with the measurements of separated algal and detrital fractions shows that detrital contamination would tend to

reduce the apparent isotopic variability of algal samples (Table 2). It is therefore possible that the variation that we have documented in algae from the Orinoco floodplain is not unusual.

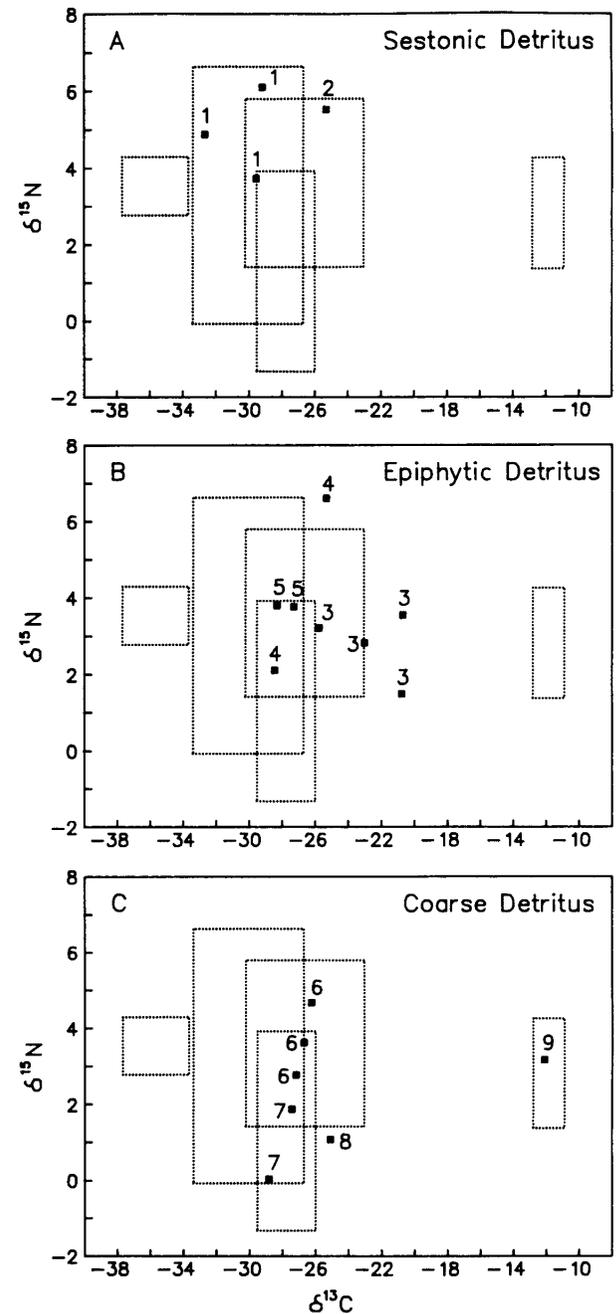


FIG. 6. Stable carbon and nitrogen isotope ratios in detritus from waters of the Orinoco River floodplain. The dotted lines show the ranges for autotrophs from Fig. 4, which were collected concurrently with the detrital samples. Isotope ratios for sestonic and epiphytic detritus have been corrected for algal contamination, as explained in the text. (A) Sestonic detritus: points coded as 1 = seston from floodplain lakes, 2 = seston from river water flowing into the floodplain. (B) Epiphytic detritus: 3 = from the C_4 grass *Paspalum repens*, 4 = from the C_3 plant *Eichhornia* spp., 5 = from submersed C_3 tree leaves. (C) Coarse detritus: 6 = surficial sediments in open-water areas, 7 = leaf litter from flooded forests, 8 = wood from rotten tree trunks, 9 = remnants of *P. repens* stranded in tree branches during the previous inundation season.

Fractionation of carbon isotopes by natural algal assemblages is not well understood, but a variety of factors have been experimentally demonstrated to affect the $\delta^{13}\text{C}$ of aquatic plants (O'LEARY, 1981). The $\delta^{13}\text{C}$ of the dissolved inorganic carbon (DIC) in aquatic environments varies under the influence of aquatic metabolism and atmospheric exchange (DEGENS, 1969; MOOK and TAN, 1991), and such variation would in turn affect algal $\delta^{13}\text{C}$ values. The $\delta^{13}\text{C}$ of DIC in waters of the Orinoco River and its floodplain has not been measured, but the high P_{CO_2} during inundation (Table 1) indicates that respiratory processes contribute large amounts of CO_2 to the DIC pool. Inputs of respiratory CO_2 would result in lighter $\delta^{13}\text{C}$ values of the DIC, but the eventual evasion of a significant proportion of the dissolved CO_2 to the atmosphere would tend to enrich the $\delta^{13}\text{C}$ of the CO_2 remaining in the water. The fractionation of carbon isotopes by algae can also vary with the physiological state of the algae and the extent to which diffusion limits the rate of carboxylation of DIC, as demonstrated by algal culture studies (PARDUE et al., 1976; BEARDALL et al., 1982; KERBY and RAVEN, 1985). Investigation of the causes of variation in algal $\delta^{13}\text{C}$ values in Orinoco floodplain waters would require intensive study of the spatial and temporal variation in the concentrations and $\delta^{13}\text{C}$ values of the DIC, as well as consideration of physiological factors affecting DIC assimilation by the algae.

The $\delta^{15}\text{N}$ values in phytoplankton and floating macrophytes vary over a relatively narrow range (1 to 4‰), while the $\delta^{15}\text{N}$ values in epiphytic algae vary over a wider range (Fig. 4). Autotrophs are known to fractionate nitrogen isotopes under conditions of abundant supply of available nitrogen (MACKO et al., 1987), but when availability is limited, adaptations for active uptake and minimal leakage allow utilization of essentially all of the available nitrogen, and no significant fractionation occurs. CIFUENTES et al. (1989) found that isotopic fractionation of nitrogen by phytoplankton in an estuary did not occur below concentrations of ca. $20\ \mu\text{M}\ \text{NH}_4^+$. Concentrations of dissolved inorganic nitrogen are usually low in floodplain waters during inundation ($<2\ \mu\text{M}$; Table 1 and extensive measurements reported by HAMILTON and LEWIS, 1990a), although river water can contain up to $3\ \mu\text{M}\ \text{NH}_4^+$ and $7\ \mu\text{M}\ \text{NO}_3^-$ before it enters the floodplain during inundation (LEWIS and SAUNDERS, 1989). The $\delta^{15}\text{N}$ values in autotrophs deriving their nitrogen from waters of the Orinoco floodplain should therefore resemble those of the available nitrogen pool (principally ammonium and nitrate). The more variable $\delta^{15}\text{N}$ values in epiphytic algae are partly explained by the occurrence of N-fixing cyanobacteria, which contribute N that is depleted in ^{15}N (see Results section). Four samples of epiphytic algae were particularly enriched in ^{15}N (Fig. 4), suggesting that there may be significant spatial variability in the isotopic composition of available nitrogen.

Implications for Stable Isotope Studies in Floodplain Environments

This study has documented the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that occurs in autotrophs and detrital organic matter of the Orinoco River floodplain during inundation. Algal growth produces organic matter that is particularly variable in $\delta^{13}\text{C}$. The isotopic composition of fine particulate organic

matter in the seston or epiphyton cannot be considered to represent phytoplankton or epiphytic algae because of the presence of significant amounts of detritus from other sources. The data reported here will be useful in the design of future stable isotope studies of the biogeochemistry of organic matter in floodplain environments. Our method for separation and harvest of algae and detritus from mixtures of fine particulates provides improved measurements of stable isotope ratios of these fractions and could be adapted and refined for use in other aquatic ecosystems in addition to floodplains.

Our study was restricted to the inundation phase, which is the most important period from the standpoint of overall production by the aquatic biota and of understanding fluxes of organic matter between the river and the floodplain. However, seasonal variability in the isotopic composition of algae and other aquatic autotrophs is likely to be large in floodplain waters. HAMILTON and LEWIS (1987 and 1990a) showed that during isolation from the river, many Orinoco floodplain lakes diverge considerably from their inundation-phase chemistry. Inflows of nutrients from the river cease during isolation, and nutrient regeneration becomes more important in sustaining the growth of aquatic autotrophs. N-fixing cyanobacteria dominate the phytoplankton during isolation, and high algal productivity can result in elevated pH and undersaturation of CO_2 with respect to the atmosphere in lake waters. Rainfall and groundwater become more important to the water budgets of lakes during isolation. Major differences in isotope geochemistry would thus be expected in these waters during the isolation phase, and these differences might increase over the course of isolation from the river.

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