TRANSFORMATION OF ALLOCHTHONOUS DISSOLVED ORGANIC CARBON IN A TROPICAL BLACKWATER RIVER AS MEASURED BY FLUORESCENCE ANALYSIS: APPLICATION TO FOODWEB ECOLOGY

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INTRODUCTION

The Cinaruco River is a blackwater ecosystem in the Venezuelan llanos (savana) with low pH, nutrient concentrations, and apparent autochthonous primary production. The river has strong seasonal hydrology and supports large populations of ecologically diverse fishes (Winemiller and Jepsen, in press). Undetermined are the relative contributions of autochthonous (aquatic) and allochthonous (terrestrial) production sources supporting high stocks of secondary consumers.

Excitation-emission fluorescence spectroscopy of colored dissolved organic matter has been used successfully for the characterization of water types (Coble, 1996) and for the tracking of river plumes (Del Castillo et al., 2000). Total dissolved organic matter is strongly correlated with fluorescence intensity. Therefore, fluorescence can be used as a proxy for dissolved organic matter concentration. The transfer of carbon along trophic levels is one of the most important mechanisms driving the carbon cycle. In forested areas, carbon fixed by vegetation accumulates in litter. Some is returned to the atmosphere through biodegradation. Some leaves fall directly into the rivers, and some material is leached by rainwater and transported to rivers by runoff. Here, we evaluate the use of excitation emission fluorescence spectroscopy to trace the transport of organic carbon from forest leaves to rivers in a tropical forest. The goal is to determine the relative contribution of allochthonous carbon to the river environment.

METHODS

We collected fresh leaves from branches of the different species of trees growing along Laguna Larga, a lagoon emptying into the Cinaruco River. In addition, we collected fallen leaves from the exposed forest floor, and submerged leaves from the bottom of the lagoon. These three pools of leaves were dried to a constant weight at 70° C and partitioned into triplicate subsamples. Each subsample was leached into 1 liter of ultra pure (18 mega ohm) de-ionized water for a period of 48 hours and then filtered

(Whatman GF/F) to remove any large particles (>0.7 μm). We also collected water samples from Laguna Larga and from the Cinaruco River, at a location upstream from the lagoon. All water samples were filtered (GF/F), stored in opaque vials, and frozen until analysis.

Fluorescence spectroscopy was performed using a SPEX Fluorolog II fluorescence spectrophotometer running in ratio mode with a bandpass of 5 nm. Three-dimensional excitation-emission matrices (EEMs) were created by measuring the emission spectra from 270 to 710 nm at forty separate excitation wavelengths ranging between 260 and 455 nm. The emission scans were concatenated to form the EEMs. Data processing and corrections for optical biases of the instrument were performed according to Coble *et al.* (1993). The fluorescence intensities at each emission maximum were transformed to equivalents of quinine sulfate dihydrate and expressed in parts per billion (ppb QSE). Absorption spectroscopy was done using a Hitachi U-3300 dual beam spectrophotometer equipped with 1 cm quartz cells. Milli-Q water was used as a reference. Values are reported as absorbance (A). Samples that reported high absorption values were diluted with Milli-Q water before making fluorescence measurements to avoid self-shading. Dilution factors were used to calculate final fluorescence intensities.

RESULTS

Here we only present results from duplicate samples collected in this study (Table 1). These are described in terms of their excitation and emission maxima (EX-EM) and the fluorescence intensity at each maximum.

Table 1. Excitation, emission wavelength, and fluorescence intensity for the main fluorophores found in this study. We arbitrarily named the fluorophores 1 to 4 to facilitate the discussion.

Sample ID	Excitation (nm)	Emission (nm)	Fluorescence @ EM max (QSE)	Peak Region
Attached Leaves (a)	275	344	16562	1
	360	440	2468	2
	300	439	9127	3
	250	436	10379	4
Attached Leaves (b)	275	*315**	*	1
	360	439	1881	2
	300	439	6669	3
	250	438	7449	4
Fallen Leaves (a)	275	*315**	*	1
	360	440	2605	2
	305	441	10574	3
	255	441	11399	4
Fallen Leaves (b)	275	*313**	*	1
	**	**	**	2
	310	433	3000	3
	240	430	922	4
Submerged Leaves (a)	**	**	**	1
	**	**	**	2
	320	455	165	3
	255	436	152	4
Submerged Leaves (b)	**	**	**	1
	**	**	**	2
	325	443	207	3
	255	437	185	4
Laguna Larga	**	**	**	1
	**	**	**	2
	305	435	40	3
	255	444	54	4
Ciranuco River	**	**	**	1
	**	**	**	2
	310	432	10	3
	255	448	14	4

*** In doubt due to overlapping with Water Raman**
**** Peak not present**

The peaks were arbitrarily named 1 to 4 to facilitate the discussion, and to avoid confusion with other naming methods used in the literature (see Coble, 1996). The designations of the peaks do not refer to an exact EX-EM, but to a narrow spectral region where several fluorophores with similar EX-EM were found.

The fluorescence contour plots and the absorption spectra for samples from attached leaves showed four strong fluorophores (Table 1). Absorption spectra showed one absorption peak at ~285 and a shoulder at ~330 nm (Fig 1).

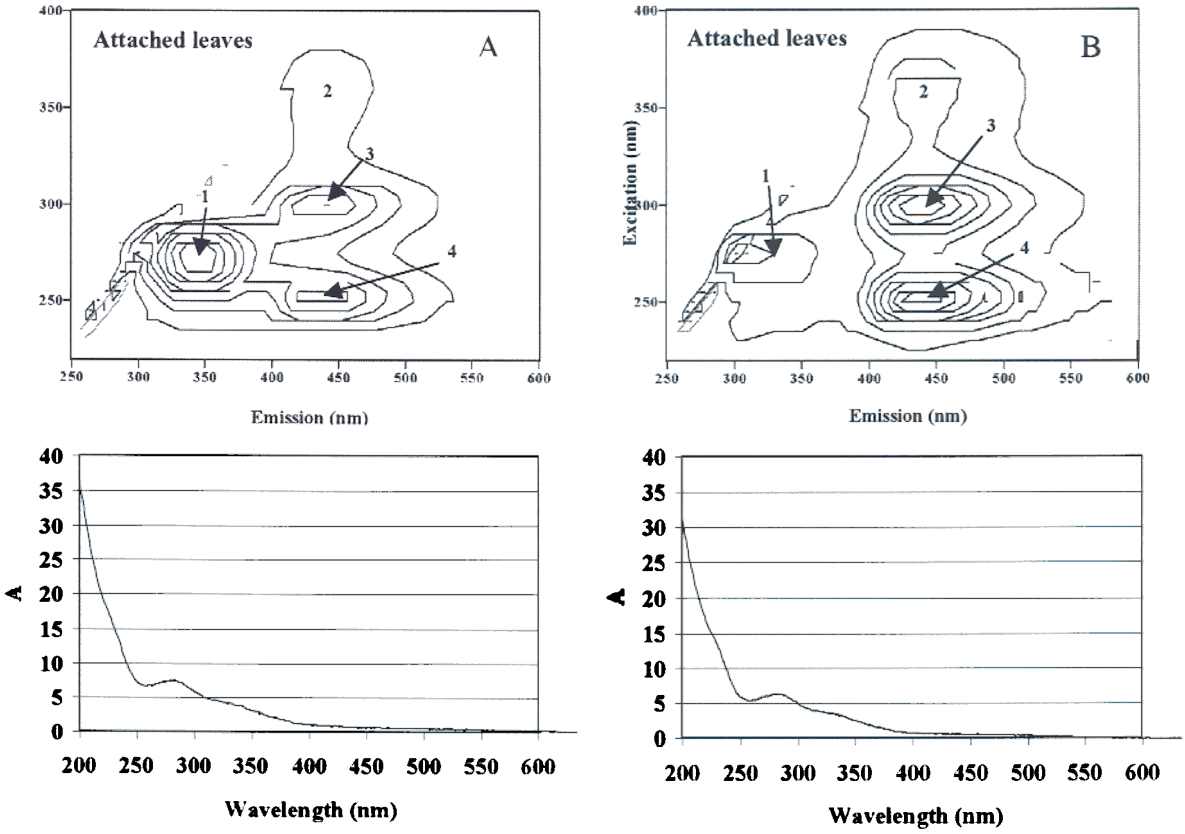

Figure 1. Fluorescence and absorption spectra for leachates from attached leaves. Note that the shape and position of the fluorescence peaks shown are primarily by the display parameters selected in the contouring software. Therefore, refer to Table 1 for the exact emission and excitation wavelengths, and fluorescence intensities.

Leachates from fallen leaves showed a similar degree of complexity and fluorescence intensity (Fig 2). Sample *b*, however, does not contain fluorophore 2 and had lower fluorescence intensity. Absorption spectra for both samples showed a single peak at ~275 nm.

Peak 1 was preponderant in leachates from attached leaves. However, the location of this peak at excitation ~275 nm is problematic because it overlaps part of the Water Raman emission. As a result, the shape of the peak can be distorted by imperfect water-blank corrections so its fluorescence cannot be quantified with certainty. Nevertheless, the intensity of the fluorescence in this region, relative to the Water Raman, indicated a reduction in peak 1 fluorescence in the detached leaves. Although this is not a rigorous quantitative analysis, it suggested that peak 1 corresponds to the most labile fluorophore.

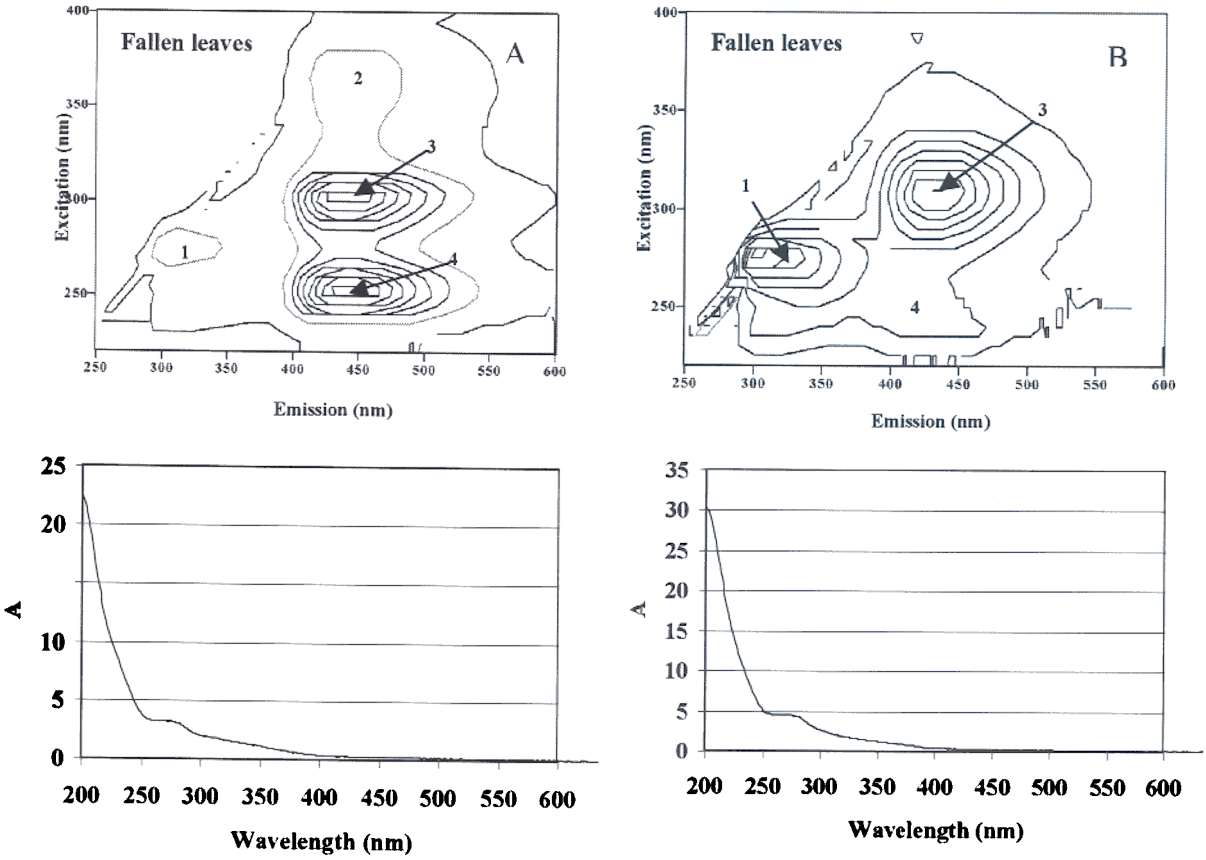

Figure 2. Fluorescence and absorption spectra for leachates from detached leaves. Note the absence of peak 2 in sample b and the loss of the absorption shoulder at ~330 nm.

The fluorescence and absorption spectra of leachates from submerged leaves are very similar to those from water samples collected in Laguna Larga and Cinaruco River (Fig. 3). The fluorescence spectra show only peaks 3 and 4. Samples from the lagoon and the river are blue shifted with respect to the leachates from submerged leaves suggesting fluorophores with lower molecular complexity. It is not possible to compare the fluorescence intensities between water bodies and the leachates. However, we can compare the fluorescence intensity between Laguna Larga and the Cinaruco River. Fluorescence intensities were higher in the lagoon, indicating longer water residence time or higher input of leaves. Absorption spectra for the leachates and water samples show the same absorption peak at ~ 275 nm. Duplicates (a,b) from the leachates are nearly identical in absorption intensity and spectral shape. Water samples from the lagoon showed stronger absorption than the river water, consistent with the fluorescence data.

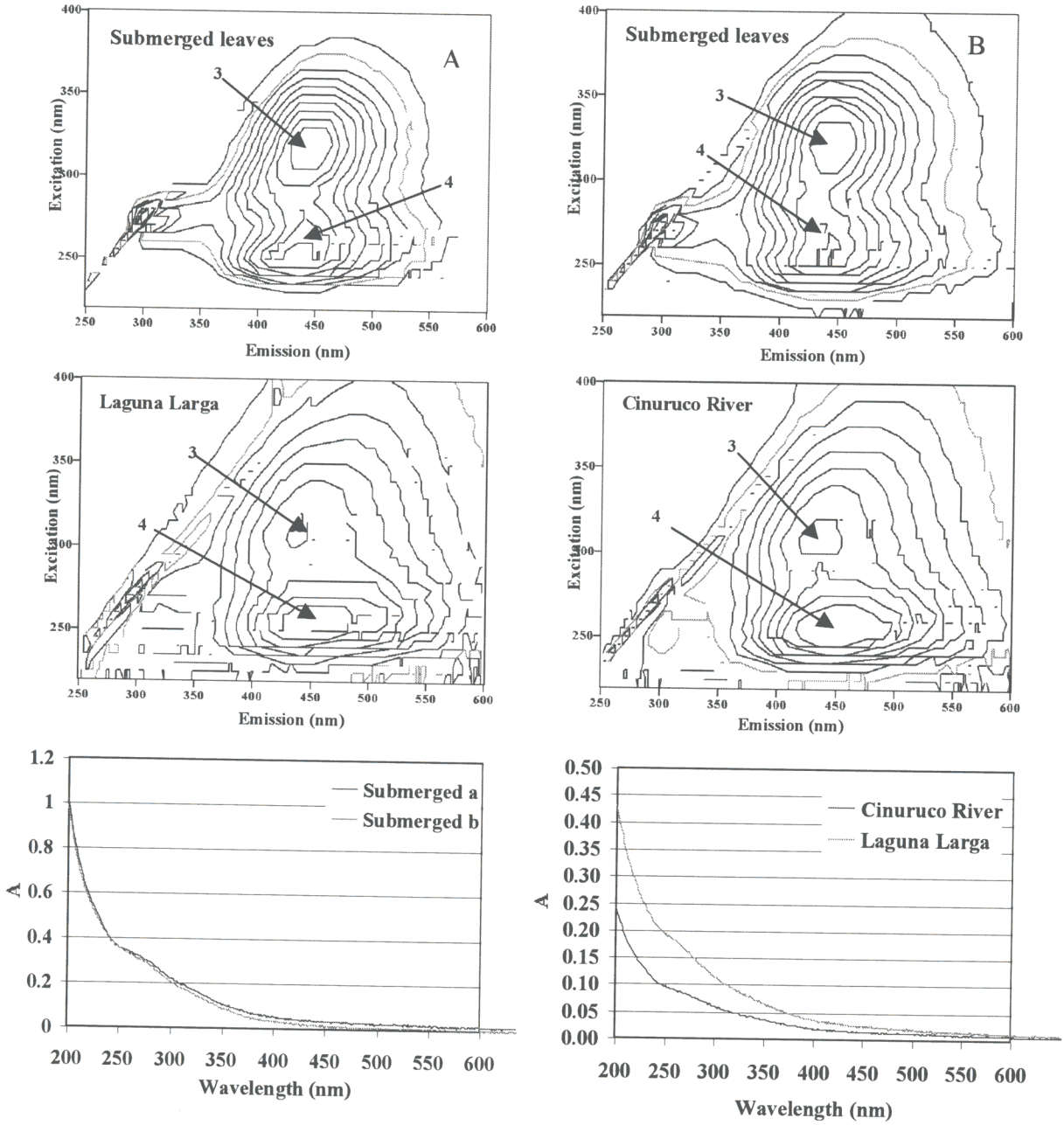

Figure 3. EEMs and absorption spectra for leachates from submerged leave, and from waters collected in Laguna Larga and the Cinaruco River.

DISCUSSION

Fluorescence and absorption spectra from attached and fallen leaves showed higher complexity and signal intensity than those from submerged leaves. The reduction in complexity and signal intensity in submerged leaves suggested that attached and fallen leaves contained organic material that is degraded quickly in the river water. Note that there was a 40-fold difference in fluorescence intensity (peak 3) between the leachates from fallen and submerged leaves. Moreover, peaks 1 and 2 were not present in the submerged leaves. This indicated that a large amount of organic matter is degraded quickly in river waters.

The similarities between the leachates from submerged leaves and those from Laguna Larga and the Cinaruco River suggested that most of the fluorescent organic matter in the river originated from the decomposition of fallen leaves.

CONCLUSIONS

Excitation-emission fluorescence spectroscopy can be used to trace the transport of organic carbon from leaves into rivers. Our results suggest that a large fraction of fluorescent organic matter contained in leaves is degraded quickly in river water. This represents a strong allochthonous contribution of carbon into the system. The spectral similarities between leachates from submerged leaves and river and lagoon water suggests that most of the fluorescent organic matter originates from the fallen leaves. It may also suggest that this organic matter is resistant to degradation. Future experiments should include collection of runoff water and sub-sampling during leaching experiments over longer periods. Sub-sampling for total organic carbon concentrations will provide a way for quantifying the input of carbon from leaves into the river waters.

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