J.E. Birdwell and A.S. Engel – Variability in terrestrial and microbial contributions to dissolved organic matter fluorescence in the Edwards Aquifer, Central Texas. *Journal of Cave and Karst Studies,* v. 71, no. 2, p. 144–156.

VARIABILITY IN TERRESTRIAL AND MICROBIAL CONTRIBUTIONS TO DISSOLVED ORGANIC MATTER FLUORESCENCE IN THE EDWARDS AQUIFER, CENTRAL TEXAS

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Abstract: Most cave and karst ecosystems are believed to be dependent on an influx of allochthonous organic carbon. Although microbes are largely responsible for the fate of dissolved organic matter (DOM) in karst, the role of microbes in chemosynthetic (autochthonous) production and processing of DOM has received limited attention. Chromophoric dissolved organic matter (CDOM) is the fraction of DOM that absorbs ultraviolet and visible light, and differences in the fluorescence spectral characteristics of humic-like (terrigenous) and protein-like (microbially-derived) CDOM allow for tracing the relative contributions of allochthonous or autochthonous carbon sources, respectively, in water. We investigated CDOM in karst-aquifer well and spring waters along the fresh- to saline-water transition zone of the Edwards Aquifer, Central Texas, over a four year period. The groundwater fluorescence spectral characteristics were distinct from those generally observed in surface waters and soil porewaters. The dominant source of organic carbon in the aquifer waters may be a product of chemolithoautotrophic primary production occurring in situ. It is possible that the absence of a strong terrestrial CDOM signature may be due to filtering effects in the epikarst or rapid utilization by heterotrophs in the aquifer. Our results indicate that intense recharge following periods of drought may influence the intensity of microbial activity, either due to an influx of DOM or nutrients from the surface that was not quantified by our analyses or because of increased in situ autotrophic activity, or both. The variable contributions of allochthonous and autochthonous DOM during and after recharge events call into question whether karst aquifer ecosystems are necessarily dependent on allochthonous organic matter.

INTRODUCTION

For most karst aquifers, meteoric water enters the subsurface from the surface (e.g., at sinkholes, fractures), moves for some distance underground in conduit- or in diffuse-flow systems, and often resurges at the surface as diffuse or spring discharge (White, 1988). The surface-derived water can carry with it a signature of its origin, including particulate and dissolved organic matter (DOM) from plant and soil material, but also anthropogenic contaminants. As a direct result of this hydrological connectivity between the surface and subsurface, karst aquifers are one of the most susceptible habitat types to disturbance due to their responsiveness to changes in the surface-water balance and possible contaminant release (e.g., Chen et al., 2001; Butscher and Huggenberger, 2009). In recent years, climatic conditions and widespread urbanization have led to increased stress on karst aquifer systems, which can be important drinking water sources due to their potential for significant permeability and porosity.

One way to monitor water quality and ecosystem integrity for management and conservation purposes is by evaluating the nature and behavior of DOM (Spizzico et al., 2005; Green et al., 2006). DOM is important in the global carbon cycle and ecosystem nutrient balance, as well as in the transport of trace metals and organic contaminants in natural and engineered systems (e.g., McKnight et al., 1992; Johnson and Amy, 1995). DOM is found throughout the biosphere as a complex mixture of freely dissolved and aggregated organic molecules at various stages of chemical, physical, or biological compositional change from parent compounds; the sum of these processes is known as diagenesis (e.g., Peuravuori and Pihlaja, 2004). Unfortunately, there have been limited investigations on the nature of DOM in karst (e.g., Baker and Genty, 1999; Farnleitner et al., 2005; Green et al., 2006; Einsiedl et al., 2007).

Chromophoric (also known as colored) dissolved organic matter (CDOM) is the fraction of DOM that

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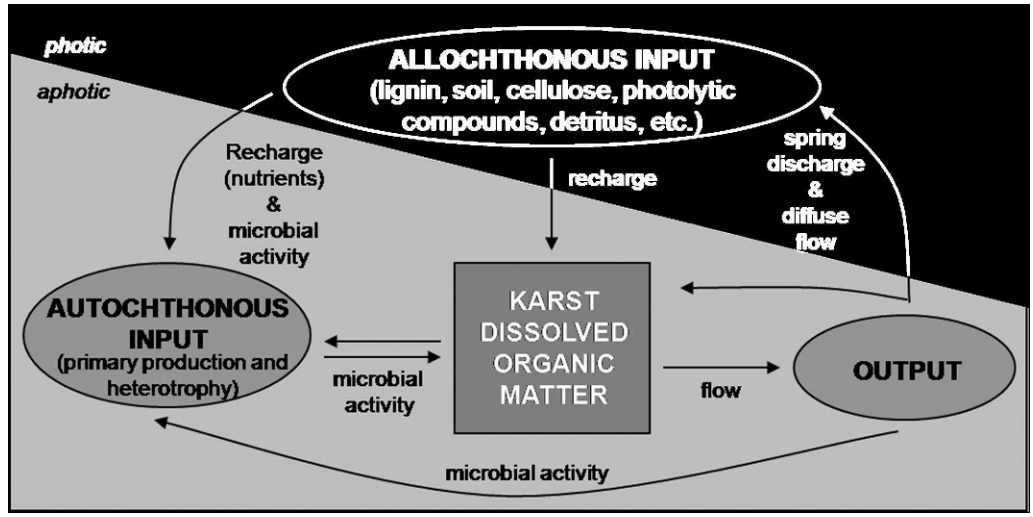

Figure 1. Schematic representation of dissolved organic matter (DOM) in karst aquifers based on the surface and subsurface being hydrologically connected. For this study, the square box represents the karst aquifer, which is sampled via wells, and the composition and nature of DOM at a specific point in time within the karst aquifer. Organic matter in the aquifer can be sourced from allochthonous and/or autochthonous inputs. Allochthonous input can enter the aquifer directly; this material is often processed by microbes and may take on a signature of autochthonous input. Some DOM may remain in the aquifer system because of in situ physical, chemical, or microbiological breakdown (i.e., diagenesis). Freshwater springs are one type of output from the aquifer system, whereby the DOM from the aquifer will mix with, and potentially become part of, an allochthonous input source at another location.

absorbs ultraviolet (UV) and visible light and represents a wide range of structures within the compositional range of DOM. Essentially, CDOM is what causes the pale yellow to brown color of filtered natural water (i.e., water not containing suspended particles like clays). The absorbance and fluorescence properties of CDOM, measured at natural abundance concentrations via spectroscopic methods, can provide information on the nature of the DOM present in a water sample, as well as DOM parent materials. By measuring the fluorescent properties of CDOM, it is possible to distinguish between DOM derived from terrestrial sources, which are dominated by the degradation products of photosynthetic primary production, and other microbial sources, which are characterized by fluorescent amino acids (McKnight et al., 2001). Various modes of degradation can affect CDOM characteristics, including photodegradation due to exposure to UV radiation from the sun and biodegradation through microbial heterotrophic metabolism. Fluorescence can also be used to identify the presence of various types of contaminants, such as polycyclic aromatic hydrocarbons (combustion byproducts) and fluorescent brightening agents (often present in wastewater and sewage).

In uncontaminated natural waters, two types of CDOM fluorophores are typically observed and are manifested as humic-like and protein-like peaks or features in collected fluorescence spectra (Baker and Lamont-Black, 2003; Chen et al., 2003; Hudson et al., 2007). Humic substances, primarily fulvic acids, are the dominant component of DOM in most surface waters. These materials represent a large fraction of the total dissolved organic carbon (DOC) because their refractory nature and high solubility allow them to accumulate in solution (Frimmel, 1998). In general, humic-like fluorescence is a term used to describe spectral features that resemble those of isolated humic and fulvic acids, while the term protein-like fluorescence applies to spectral peaks attributed to the fluorescent amino acids tryptophan and tyrosine. Although both fluorophore types absorb and are excited by UV light (240–360 nm), humics emit primarily in the violet to near-green wavelengths (400–500 nm), which is attributed to complex photophysical interactions between quinone-like structures from degraded terrestrial material, such as lignin (Ariese et al., 2004; Del Vecchio and Blough, 2004; Cory and McKnight, 2005; Boyle et al., 2009; Cook et al., 2009), In contrast, microbially-derived CDOM emits in the UVB to UVA wavelength range (300–380 nm). In karst, CDOM spectral features are expected to be derived from several sources, including material brought into the system by surface recharge, wind, speleothem drip-waters, or as guano, produced from microbial processing of allochthonous material within the aquifer, and in situ microbial activity (e.g., Griebler and Lueders, 2008) (Fig. 1).

Healthy aquatic environments contain high concentrations of microbial biomass (e.g., Whitman et al., 1998, Griebler and Lueders, 2008). In the absence of allochthonous input, autochthonous CDOM produced by microbial activity is the dominant form of DOM in groundwater,

which is supported from examinations of CDOM that possess a relatively greater portion of biologically labile materials relative to surface waters, including polysaccharides, alkyl alcohols, aldehydes, ketones, and amides that are produced in situ (Leenheer, 1981). However, except for some studies of CDOM in groundwater (Baker and Lamont-Black, 2003), cave drip waters (e.g., Baker and Genty, 1999), and the open ocean (Murphy et al., 2008) that identified significant protein-like fluorescence associated with microbial activity, there have been few investigations of systems where terrigenous DOM sources or photosynthetically-driven microbial activity are lacking (e.g., McKnight et al., 2001). It is clear that fluorescence signatures indicative of microbial activity are often not observed in water samples because biomolecules are more susceptible to enzymatic attack relative to more refractory fulvic acids (e.g., Moran and Hodson, 1990; Claus et al., 1999; Ogawa et al., 2001; Hertkorn et al., 2002; Yamashita and Tanoue, 2003; Anesio et al., 2004; Judd et al., 2006).

Therefore, our current understanding of the nature and behavior of CDOM primarily derived from microbes and microbial activity is limited. These issues make investigating the relative contribution of different sources of organic matter in caves and karst aquifers unique. In cave and karst settings, it is clear that water from the surface contributes to the majority of recharge. Organic matter from terrestrial sources is expected to enter into these waters via leaching from vadose soils and epikarst. In addition, subsurface environments are devoid of sunlight, and therefore two important processes in the terrestrial carbon cycle (photosynthesis and photodegration) do not directly affect DOM in these systems. Because of the combination of distinct CDOM signature anticipated for surface-derived recharge and the lack of photophysical processes in caves and karst, any CDOM produced in situ, for example by chemolithoautotrophic microbial activity, should possess fluorescent characteristics that are readily distinguishable.

In this study, our goal was to characterize CDOM from fresh and saline water sampled from the karstic Edwards Aquifer in Central Texas, one of the most spatially extensive, permeable, and productive aquifers in the United States (e.g., Hovorka et al., 1995; Sharp and Banner, 1997). Based on what is known of aquifer geochemistry, hydrology, and microbiology, we hypothesized that allochthonous CDOM input would be distinguishable from an autochthonous CDOM generated by in situ microbial activity, and that microbial signatures would be more prevalent than terrestrial signatures in the deep, more saline portions of the aquifer. Additionally, the samples were available over a four-year window that included periods of severe drought and intense recharge in the Edwards Aquifer region. This allowed for an assessment of the potential use of fluorescence to examine changes in CDOM sources during and following climatic events that might perturb the subsurface hydrology and ecosystem.

MATERIALS AND METHODS

THE EDWARDS AQUIFER

The Edwards Aquifer is located in south-central Texas and extends across a narrow, 8- to 60-km-wide tract approximately parallel to the Balcones Fault Zone that arcs from north of the city of Austin to south of the city of Brackettville. The aquifer is within extensively karstified Cretaceous carbonate rock units, with a predominant groundwater flow path from southwest to northeast (e.g., Hovorka et al., 1995; Groscehen and Buszka, 1997; Kuniansky et al., 2001; Lindgren et al., 2004). The aquifer is subdivided into different segments based on discharge sites and regional structural controls, as well as by water type. In general, recharge to the aquifer takes place where limestone outcrops in the recharge zone along the Balcones Fault Zone and on the Edwards Plateau in the contributing zone (Fig. 2) (Lindgren et al, 2004). Edwards Plateau soils are mollisols that support grasses, savanna vegetation, and some trees; these terrigenous sources would be expected to contribute soil humics and photosynthetically-derived organic material, like lignin and cellulose, to the aquifer. In comparison, regional soils are generally described as thin and stony and have been characterized as dominantly calcareous, clayey, and loamy (e.g., Musgrove and Banner, 2004).

Downdip of the recharge and contributing zones, the aquifer is artesian and is predominately freshwater, with total dissolved solids (TDS) of 1000 mg L^{-1} or less (Groscehen and Buszka, 1997). Permeability within the freshwater zone is multimodal and varies over eight orders of magnitude, with a significant percentage of the porosity represented by a complex network of fractures (fracture flow), macroscopic caves, and underground solution-enlarged features (conduit flow) (Hovorka et al., 1995). Natural freshwater discharges from the artesian zone along faults, including at San Pedro Springs, Comal Springs, and San Marcos Springs, from southwest to northeast, respectively (Fig. 2).

TDS values in excess of 1000 mg L^{-1} constitute saline water, and the transition between the freshwater and saline-water zone, locally known as the bad-water line (Schultz, 1993), trends parallel to the Balcones Fault Zone, and is encountered at depth and downdip of the freshwater (Fig. 2). The saline-water zone has high concentrations of hydrogen sulfide (H_2S) as a result of leakage of brine and H_2S gas from oil-fields along the eastern edge of the aquifer (e.g., Sharp and Banner, 1997). Although there is still much to be learned about the saline-water zone, flow is hypothesized to be in tight fractures or through intercrystalline or intergranular porosity (matrix flow) (e.g., Hovorka et al., 1995). The freshwater–saline-water interface is generally stable through time, although historical data from times of regional drought indicate that saline water may have discharged from freshwater springs and

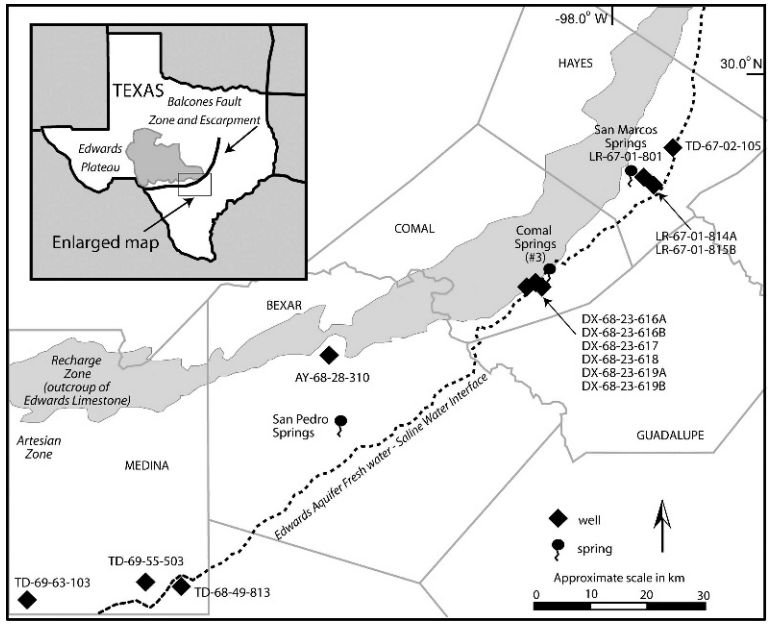

Figure 2. Detailed Edwards Aquifer sampling locations in Central Texas. The freshwater and saline water transition zone line is based on Groscehen and Buszka (1997) and recent estimates from Edwards Aquifer Authority online reports (http://www.edwardsaquifer.org/).

that there is possible updip movement of the transition zone (e.g., Harden, 1968; Perez, 1986).

The classic model for karst development in carbonates involves carbonic acid dissolution, where carbon dioxide (CO_2) is sourced from infiltrating meteoric water and dissolution is focused at a local to regional base level. This would imply that most karst development takes place at or above the water table. However, for the Edwards Aquifer, karstification due to carbonic acid is juxtaposed with the possibility of dissolution within the saline-water zone that some have suggested is due to abiotic or microbial sulfide oxidation to sulfuric acid (e.g., Grubbs, 1991; Schindel et al., 2000; Randall, 2006; Engel and Randall, 2008). It is unclear how much of Edwards Aquifer development may be a result of this type of process or to what extent microbes influence the nature of porosity and permeability between the fresh and saline-water zones.

Sample Acquisition and Geochemical Characterization

We focused our investigation during a time period extending from May 2005 to April 2009, and only in Hayes, Comal, Bexar, and Medina counties. We collected water from wells drilled into the Edwards Aquifer carbonates within the saline-water zone and at freshwater springs (Table 1; Fig. 2). When collected from wells, water was purged a minimum of one well volume prior to sample acquisition (purge volumes depended on well volumes). Grab samples were collected at springs. Physiochemical properties were measured immediately in the field using standard electrode methods (e.g., APHA, 1998), including temperature and pH on an Accumet AP62 meter with a double junction electrode (Accumet, Fisher Scientific, USA), and TDS and temperature on a YSI-85 meter (YSI Inc., Yellow Springs, OH, USA). Dissolved hydrogen sulfide was measured in the field using the methylene blue colorimetric method on a portable V-2000 multi-analyte photometer (CHEMetrics, Inc., Calverton, VA, USA) (APHA, 1998). Alkalinity, as total titratable bases, here dominated by bicarbonate, although acetate can also be high in some saline waters (Groscehen and Buszka, 1997), was determined in the field from a filtered sample by titration to pH 4.3 (APHA, 1998). Each water sample was filtered simultaneously into HDPE bottles through a 0.45-μm Whatman glass fiber filter (GF/F, precombusted at 500 °C) and a 0.2-μm polyvinylidene-fluoride membrane (PVDF) filter (Millipore, Bedford, Mass.). Filtered water was stored on ice for transport and maintained at 4 °C until analysis, at which point samples were brought to room temperature (~22 °C).

Fluorescence Spectroscopy

Fluorescence measurements on samples from May 2005, January 2007, and June 2007 were made using a SPEX Fluorolog-3 spectrofluorometer (Jobin Yvon, Edison, NJ, USA). Spectra for samples from March 2008 and April 2009 were made using a SPEX Fluoromax-4 spectrofluorometer. Identical settings were used with both instruments. Slits for both excitation and emission monochromators were set to 5 nm, and a 0.1 second integration time was used. Analyses were done in a 1-cm quartz cuvette at room temperature (22 °C). Instrument stability was

Table 1. General descriptions of Edwards Aquifer water samples, including well depth, either as the depth interval of the open-hole (^{O}) or the screened interval depths (^{S}), and total dissolved solids (TDS), dissolved sulfide concentrations, and total alkalinity.

Sampling Site Name	Well Number	Collection Period	Well Depth (m)	Temp (°C)	pH	TDS (mg L^{-1})	Sulfide (mg L^{-1})	Alkalinity (mg L^{-1})
Paradise Alley well	DX-68-23-616A	May 2005	209–224^{S}	23.6	7.20	1140	3.5	233
Paradise Alley well	DX-68-23-616A	Jan. 2007		23.0	7.80	1559	0	199
Paradise Alley well	DX-68-23-616A	June 2007		25.0	7.23	1149	3.3	253
Paradise Alley well	DX-68-23-616A	March 2008		25.4	7.25	1159	4.6	266
Paradise Alley well	DX-68-23-616A	April 2009		24.3	7.25	1443	4.2	327
Comal Spring #3		Jan. 2007		23.3	7.18	372	0	250
Comal Spring #3		March 2008		23.1	7.07	302	0	248
LCRA field deep well	DX-68-23-617	June 2007	214–226^{S}	25.0	7.31	369	0	271
LCRA field deep well	DX-68-23-617	March 2008		25.2	7.03	373	0	262
LCRA field shallow well	DX-68-23-618	June 2007	166–180^{S}	24.5	7.46	438	0.3	239
LCRA field shallow well	DX-68-23-618	March 2008		23.9	7.50	459	0.3	235
Girl Scout shallow well	DX-68-23-619A	June 2007	185–198^{S}	24.9	7.32	375	trace	289
Girl Scout deep well	DX-68-23-619B	June 2007	228–240^{S}	24.4	7.42	353	trace	229
Sonterra well #8	AY-68-28-310	May 2005	103–373^{O}	26.0	7.40	990	trace	219
Aquarena Golf Course well	LR-67-01-814A	Jan. 2007	154–169^{S}	22.0	6.87	12,440	38.5	290
Aquarena Hotel spring	LR-67-01-801	June 2007		21.6	7.12	401	0	331
South Medina well	TD-69-63-103	June 2007	801–1038^{O}	38.9	7.31	366	trace	223
Farm well	TD-69-55-503	June 2007	137–610^{O}	27.7	7.26	332	0	240
SE Medina well	TD-68-49-813	June 2007	783–973^{O}	41.4	7.16	817	5.9	346

Table 2. Fluorescence characteristics of Edwards Aquifer water samples, including maximum emission intensity (I_{max}), maximum emission wavelength ($\lambda_{Em,\ max}$), maximum excitation wavelength ($\lambda_{Ex,\ max}$), FI, and HIX (letters in parenthesis by Sampling Site Names correspond to spectra labels in Fig. 4).

Sampling Site Name	I_{max} (RU)	$\lambda_{em,\ max}$ (nm)	$\lambda_{ex,\ max}$ (nm)	FI	HIX
Paradise Alley well (a)	3.4	395	240	2.16	4.30
Paradise Alley well (c)	3.3	395	240	2.14	3.48
Paradise Alley well (e)	37.0	340	270	1.93	0.39
Paradise Alley well (i)	2.0	382.5	240	2.01	5.00
Paradise Alley well (l)	1.7	385	240	1.90	2.98
Comal Spring #3	1.0	412.5	240	1.79	4.33
Comal Spring #3 (j)	0.8	415	240	1.74	2.93
LCRA field deep well	12.5	305	240	1.58	1.15
LCRA field deep well (l)	1.6	435	240	1.51	9.03
LCRA field shallow well (g)	16.0	342.5	240	1.84	0.62
LCRA field shallow well (k)	1.6	375	240	1.75	3.40
Girl Scout shallow well	10.5	307.5	240	1.54	1.12
Girl Scout deep well (f)	3.7	340	275	1.90	0.55
Sonterra well #8 (b)	2.2	395	250	2.65	2.52
Aquarena Golf Course well (d)	2.7	397.5	240	2.38	2.72
Aquarena Hotel spring (h)	10.5	342.5	240	1.90	0.61
South Medina well	16.5	337.5	275	1.72	0.17
Farm well	3.9	310	240	1.92	0.24
SE Medina well	4.6	305	240	2.41	0.51

determined using the Raman peak of deionized water excited at 348 nm with emission monitored at 395 nm (position of Raman peak indicated by the white circle in Fig. 3, panel a). Raman intensities were consistent during each session, with values varying less than 2% between runs on both instruments. Samples were analyzed in signal/reference mode with the fluorescence emission intensity normalized to the intensity of the xenon lamp at the particular excitation wavelength applied. Absorbance spectra for May 2005, January 2007, and June 2007 samples were collected using a double-beam UV-3101PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan), while spectra for those samples from March 2008 and April 2009 were collected using a double-beam Lambda-850 spectrophotometer (Perkin Elmer, Waltham, Mass.). For both instruments, absorbance spectra were obtained using a 1-cm quartz cuvette over the range of 200–700 nm with deionized water as the reference.

Excitation-emission matrix (EEM) fluorescence spectra were obtained by collecting a series of forty-three emission scans (λ_{Em} = 250–550 nm, 2.5-nm steps) at 5-nm excitation wavelength (λ_{Ex}) intervals between 240 and 450 nm. An EEM spectrum provides a fluorescence spectral signature of a sample containing CDOM within the UV and visible range of the electromagnetic spectrum. Examples of EEM spectra are shown in Figure 3 (panels a, b, and e–h) and Figure 4. Spectral corrections for primary and secondary inner filter effects were made using absorbance spectra (Lakowicz, 1999). Raman scattering was mitigated by subtracting a blank spectrum collected on pyrogen-free deionized water from each corrected EEM. Rayleigh scattering effects were edited from each spectrum following correction and blank subtraction. Differences between uncorrected and corrected EEMs can be seen by comparing panels a and b of Figure 3 and the effects on individual emission scans are illustrated in panels c and d. EEM contour plots were assembled by combining the individual emission spectra using SigmaPlot 10 (Systat Software, Inc., San Jose, CA, USA). Each contour line represents between 5% and 10% of the maximum emission intensity, depending on the number of contour lines shown.

CDOM spectra are often described in terms of characteristic peaks that have been identified in studies of surface waters from a wide range of environments. The system described by Coble (1996) is one of the most commonly used for discussing CDOM fluorescent features. The peaks include UVC-excited humics (peak A, $\lambda_{Ex} \leq$ 260 nm. λ_{Em} = 400–460 nm), UVA-excited humics (peak C, λ_{Ex} =320–360 nm, λ_{Em} = 420–460 nm), marine humics (peak M, λ_{Ex} = 290–310 nm, λ_{Em} = 370–410 nm), tyrosine-like (peak B, λ_{Ex} = 275 nm, λ_{Em} = 305 nm) and tryptophan-like (peak T, λ_{Ex} = 275 nm, λ_{Em} = 340 nm). The locations of these peaks are illustrated in Figure 3, panel b.

Two diagnostic metrics based on specific emission scans were used to assess ecologically relevant properties of the CDOM in the aquifer samples. The Zsolnay Humification Index (HIX) (Zsolnay et al., 1999) is used to estimate the degree of CDOM humification, which can be considered an indicator of DOM bioavailability within a natural system because highly humified organic substances are expected to

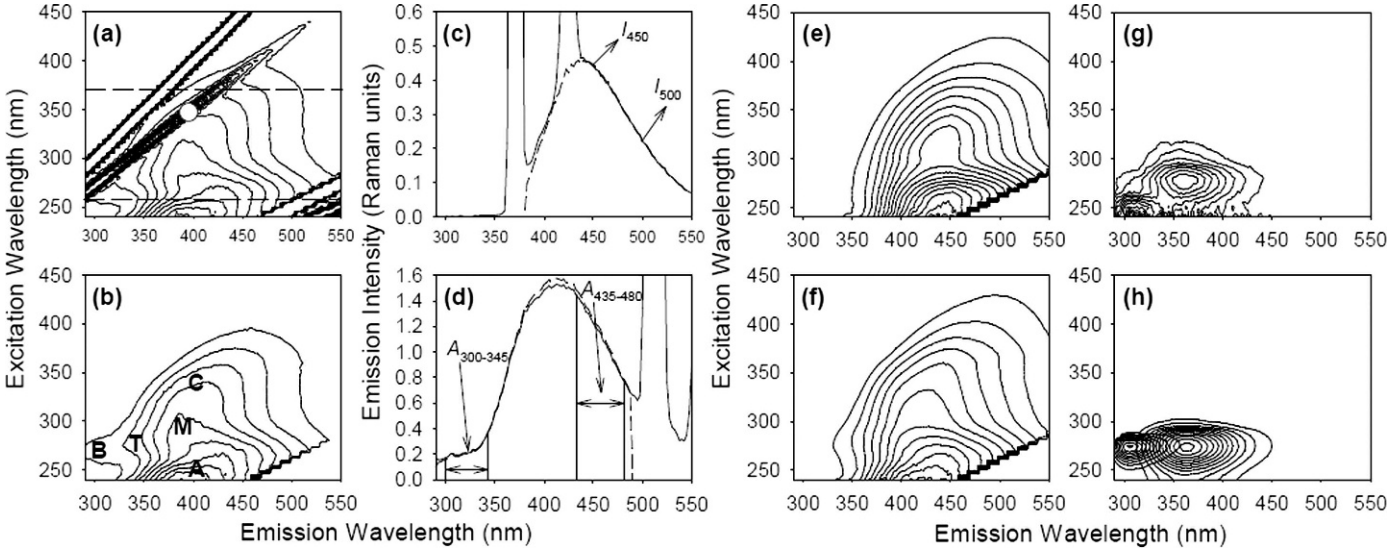

Figure 3. Overview of fluorescence excitation-emission matrices (EEMs) and scans along with corrected spectra of surface water fulvic acids and biological fluorophore standards. (a) Uncorrected EEM spectrum for Paradise Alley well water (March 2008) showing scattering bands relating to (from upper left to lower right) 1st order Rayleigh, 1st order Raman, 2nd order Rayleigh and 2nd order Raman; dashed lines indicate positions of emission scans for the Humification Index (HIX) (bottom) and Fluorescence Index (FI) (top); white circle indicates position of the reference Raman peak ($\lambda_{Ex} = 348$ nm, $\lambda_{Em} = 395$ nm). (b) Corrected EEM spectrum for Paradise Alley well water (March 2008) following inner filter effect corrections, removal of Raman scattering by DI water blank subtraction and editing to remove Rayleigh scattering bands. Positions of Coble (1996) peak designations indicated by letters A, C, M, T and B (see Results section for details). (c) Uncorrected (solid line) and corrected (dashed line) emission scan for FI calculation (intensities at 450 and 500 nm indicated by arrows). (d) Uncorrected (solid line) and corrected (dashed line) emission scan for HIX calculation (integration areas indicated). (e) Suwannee River fulvic acid EEM spectrum (terrestrial humic-like standard). (f) Pony Lake fulvic acid EEM spectrum (microbial humic-like standard). (g) EEM spectrum of tryptone (protein-like standard). (h) EEM spectra of the fluorescent amino acids tyrosine (left peak) and tryptophan (right peak).

be less labile, and therefore, persist in the environment longer than substances with a low degree of humification (Zsolnay et al., 1999; Ohno, 2002). Soil and terrestrially-derived CDOM is expected to have higher HIX values than CDOM from microbial sources. The HIX was calculated by dividing the sum of the emission intensities between 435 and 480 nm by the sum of the intensities between 300 and 345 nm with excitation at 254 nm. The McKnight Fluorescence Index (FI) (McKnight et al., 2001) was used to assess the relative contributions of allochthonous (terrestrially-derived material) and autochthonous (microbial material produced in situ) CDOM. FI values are determined from an emission scan with excitation at 370 nm by calculating the ratio of the emission intensity at 450 nm to that at 500 nm (McKnight et al., 2001). In a study of fulvic acids derived from terrestrial and microbial sources, McKnight et al. (2001) determined that FI values of 1.4 or less indicated CDOM of terrestrial origin, while values of 1.9 or higher corresponded to microbially-derived CDOM. Moreover, when applied to filtered whole water samples, similar diagnostic values resulted (McKnight et al., 2001). Examples of the emission scans used to calculate these indices are shown in panels c (FI) and d (HIX) of Figure 3.

STANDARDS AND MODEL COMPOUNDS FOR FLUORESCENCE SPECTROSCOPY

A set of reference fluorescence spectra was obtained to represent CDOM from different environments and compounds whose fluorescence characteristics are similar to CDOM spectral features observed in other studies. Samples from the International Humic Substances Society (IHSS) served as proxies for dissolved humic substances derived from terrestrial plants (Suwannee River fulvic acid, Georgia, USA, IHSS catalog #2S101F) and photosynthetic microorganisms (Pony Lake fulvic acid, Antarctica, IHSS catalog # 1R109F). Other standards included L(-) tryptophan (Acros Organics, Thermo Fisher Scientific, Inc., NJ, USA) and L(-) tyrosine (Acros Organics). Tryptone (Fisher Bioreagents, Fisher Scientific, Inc., NJ, USA), a casein digest commonly used as an additive in microbial culture media, was included to obtain a protein-like signature. All comparison samples were prepared to concentrations of ~10 mg carbon L^{-1} using pyrogen-free deionized water. Corrected EEM spectra for these materials are shown in Figure 3 (panels e–h) and illustrate the different regions of the excitation-emission fluorescence landscape occupied by humic-like

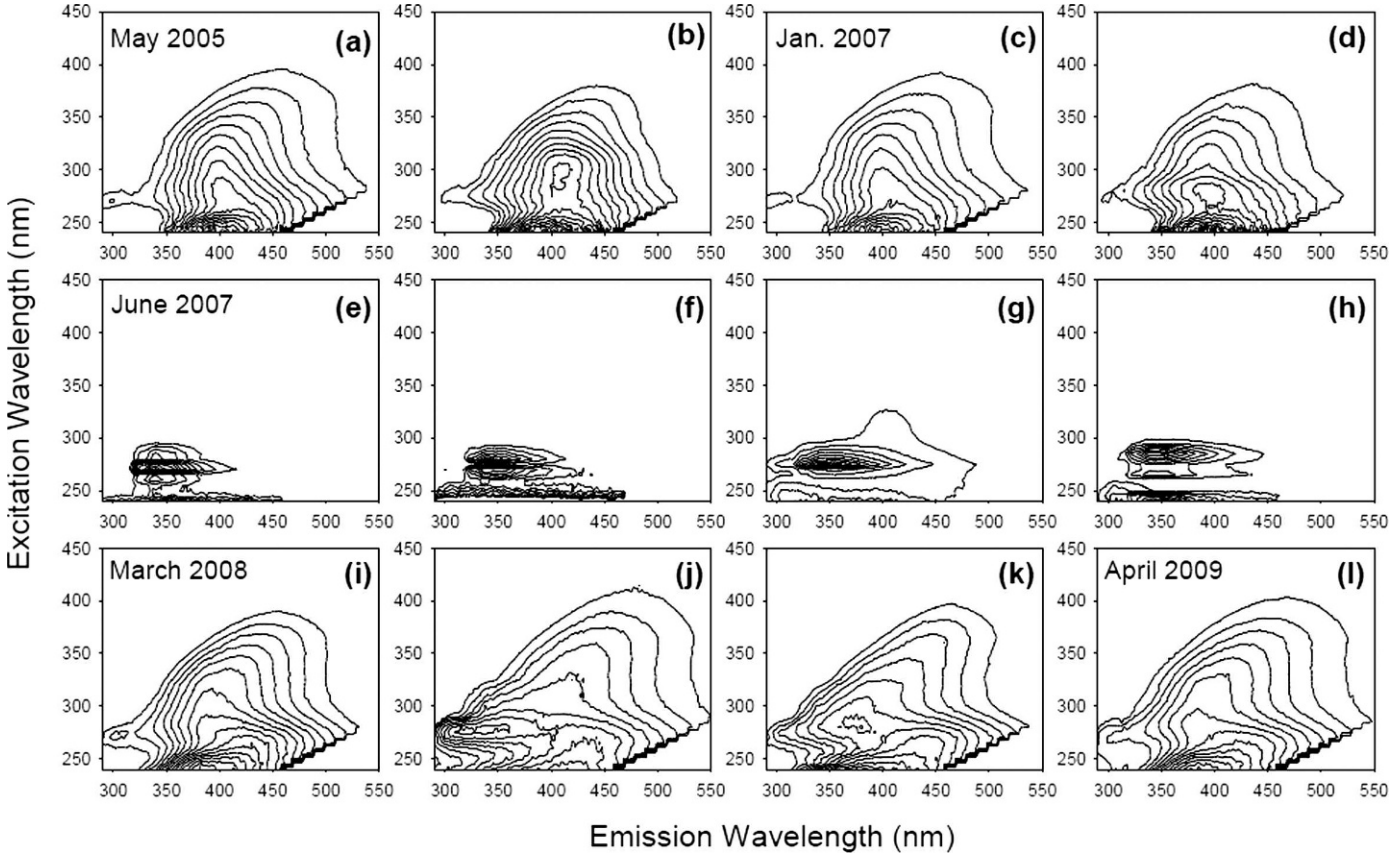

Figure 4. EEM spectra of various waters from sampling sites in the Edwards Aquifer, Central Texas; see Table 2 for sample information and correspondence to panel letter.

(Fig. 3, panels e and f) and protein-like (Fig. 3, panels g and h) fluorophores.

Hydrological and Drought Data for the Edwards Aquifer

Climatic and hydrologic extremes of Central Texas lead to significant fluctuation in annual recharge to the aquifer, primarily in response to variation in regional precipitation. To compare the fluorescence characteristics of aquifer-water samples to hydrological conditions relevant to recharge, data were obtained from online databases maintained by local and federal agencies charged with monitoring important aspects of the hydrology of Edwards Aquifer. We used the Palmer Drought Severity Index (PDSI) (Palmer, 1965) for Texas Divisions 6 and 7 to assess the relative availability of recharge water for the Edwards Aquifer system. The National Oceanic and Atmospheric Administration, through the Climate Prediction Center, publishes PDSI data for specific regions throughout the United States[1]. Division 6 encompasses the Edwards Plateau (i.e., recharge and contributing zones) and Division 7 contains the locations of the wells and discharge springs. The PDSI is based on a water balance approach, taking into account precipitation and soil moisture supply. PDSI values below zero correspond to drier than normal conditions and values below −4 indicate extreme drought.

For a direct indicator of water storage in the aquifer, we used information on well levels and spring flow rates, as well as the estimated annual net extraction or influx of aquifer water, during the study period. Water levels for three regional wells and the flow rates for two springs were acquired from Edwards Aquifer Authority (EAA) reports and online data[2] (Schindel, 2008). We focused on well-level data from the Hondo Index Well, Medina County, and flow-rate data for Comal Springs, Comal County, which were representative of the others. Annual total recharge to, and discharge from, the Edwards Aquifer for most of the study period was found in a recent report from the EAA (Schindel, 2008). Net annual water influx or extraction from the aquifer was calculated for 2005, 2006, and 2007 using these values.

Statistical Analyses

Student's t-test (2-tail, type 2) analyses were performed to assess whether FI, HIX, and emission maximum wavelength ($\lambda_{Em,\ Max}$) values determined for samples

[1] http://www.cpc.noaa.gov/products/monitoring; ftp://ftp.ncdc.noaa.gov/pub/data/cirs/

[2] http://www.edwardsaquifer.org/pages/waterlevels_sa.asp

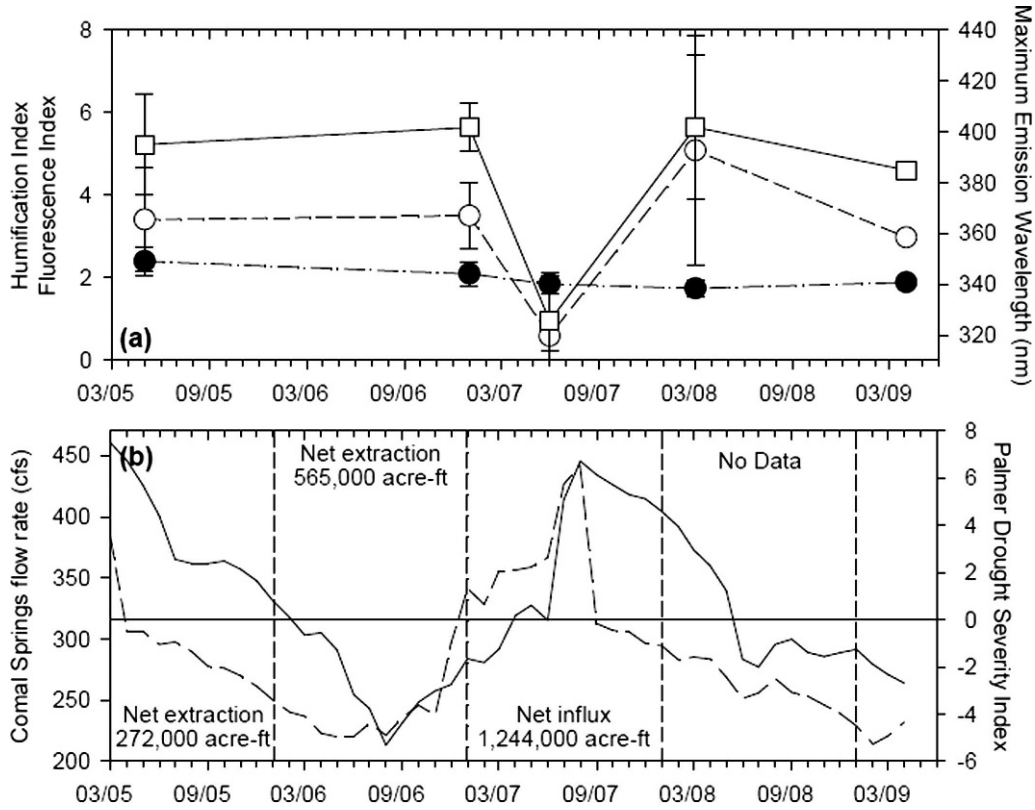

Figure 5. (a) Average values of Fluorescence Index (●, dash-dot line), Humification Index (○, dashed line) and Emission Maximum wavelength (□, solid line) values for each sampling period (error bars represent standard deviation). (b) Discharge flow rate (cubic feet per second, cfs) for Comal Springs, Comal County, Texas (solid line), and Palmer Drought Severity Index (PDSI) for Division 7 (as defined by the National Climate Data Center), Texas (dashed line), from 2005 through April 2009.

collected during the four sampling events were significantly correlated. Calculations were made using Microsoft Excel (Redmond, WA). *P*-values below 0.05 were considered indicative of significant differences between the measured and calculated fluorescence parameters determined during each sampling period. Statistical comparison was not possible for the April 2009 sampling event, as water could only be obtained from a single well.

RESULTS

CHARACTERIZATION OF CHROMOPHORIC DISSOLVED ORGANIC MATTER

Maximum fluorescence emission intensities (I_{Max}) of CDOM in Edwards Aquifer samples were between 1 and 40 Raman Units (RU) (Table 2). The EEM spectra contained many of the characteristic peaks observed in other studies of marine and terrestrial CDOM (Fig. 3, panel b). The dominant features in the CDOM spectra for water samples collected during May 2005, January 2007, March 2008, and April 2009 were peaks A and M, with some samples containing contributions from peaks C, T, and B. The June 2007 samples were dominated by peak T, which had fluorescent intensities that were an order of magnitude higher than any other feature present. It should be noted that the fluorescence response of CDOM is sensitive to pH and temperature; however, this was not an issue for the aquifer waters, which all had circumneutral pH and moderate temperatures that were not significantly different among the sampling times (Table 1).

FLUORESCENCE INDICES

Average values and standard deviations for HIX, FI, and $\lambda_{Em,\ Max}$ determined for each sampling event are shown in Figure 5a. The two fluorescence indices, HIX and FI, provide a clear contrast among the reference materials and the well and spring waters (Table 2). The IHSS reference fulvic acids had HIX values greater than 25. The HIX value for tryptone was 0.05. All but one of the Edwards Aquifer water samples from May 2005, January 2007, March 2008, and April 2009 had HIX values of 5 or lower and none of the June 2007 samples had values greater than 1.2. FI values are rarely lower than ~1.0, and the highest reported values are less than ~3.0 (McKnight et al., 2001). As an example of typical surface water humic substances, Suwannee River fulvic acid exhibited a FI value of 1.25, while for tryptone, the value was 2.45. Pony Lake fulvic acid, representing the most humified portion of

the DOM from a lake dominated by photosynthetic microbial activity, had a FI of 1.52. FI values for samples collected in May 2005 and January 2007 were the highest observed and were generally greater than 2, while those from June 2007, March 2008, and April 2009 were between 1.5 and 2. *P*-values determined for comparisons of HIX and $\lambda_{Em,\ Max}$ values for the May 2005, January 2007, and March 2008 sampling events were all $\geq$0.37, while comparisons of those samples to the HIX and $\lambda_{Em,\ Max}$ from the June 2007 samples yielded *P*-values $<$0.001. These results indicate that the fluorescent properties of the June 2007 samples were significantly different than the other sampling periods. For the FI data, the *P*-values were 0.37 between May 2005 and January 2007 and 0.50 between June 2007 and March 2008; all other comparisons had *P*-values between 0.01 and 0.12. These results indicate that there were not significant differences in FI among the different sampling times. While statistical testing was not possible for the April 2009 sample from the Paradise Alley well, it was very similar to the sample collected from that site in March 2008, but with a 40% lower HIX value.

Drought and Recharge Data

The PDSI values for Division 7 from May 2005 through April 2009 indicate that the region experienced variable drought conditions until August 2006 (Fig. 5b). Similar conditions also existed for Division 6 (data not shown). Lower discharge rates at Comal Springs and lower well levels at the Hondo Creek Index well (data not shown), corresponded with PDSI values that were indicative of drought conditions. From August 2006 until September 2007 the region experienced wetter conditions, leading to greater spring discharge and higher (positive) PDSI values. The summer of 2007 had the most intense wetting in both Divisions 6 and 7, and many places in the two regions experienced major flood events. Since September 2007, the Divisions have had lower PDSI values, indicating worsening drought conditions (Fig. 5b).

The net volume of water entering or being extracted from the Edwards Aquifer system was calculated from recharge and discharge estimates, as a way to assess whether net removal of water from the aquifer could exacerbate drought effects on the subsurface ecosystem. During 2005 and 2006, estimated net volumes of 33,551 and 69,692 hectare meter (272,000 and 565,000 acre-feet) of water were removed from the aquifer, respectively. In 2007, the aquifer system had a net estimated recharge of 153,446 hectare meter (1,244,000 acre-feet) of water. These estimates are consistent with trends in the PDSI, well level, and spring discharge data for the same periods.

Discussion

In this study, we set out to characterize the nature of CDOM in freshwater and saline water from the karstic Edwards Aquifer in Central Texas, both from the standpoint of understanding the relative contributions of terrigenous and autochthonous (e.g., microbial) CDOM to the aquifer, but also from the viewpoint of determining if there would be different CDOM sources because of variability in surface hydrology that could contribute to, or perturb, the aquifer ecosystem. We used fluorescence spectral characteristics of CDOM to characterize the relative contributions of organic matter into the Edwards Aquifer from May 2005 through April 2009. Regardless of being freshwater or saline water, the FI and HIX values determined for the water samples indicate that the CDOM present in the aquifer waters has a significant microbial component and does not possess a signature indicative of DOM dominated by soil or other surface sources. The high FI values indicate that most of the CDOM is likely of microbial origin, though some sites had values that suggest a mixture of terrestrial and microbial sources. The low HIX values from the water samples are consistent with fluorescent, water soluble, extracellular substances excreted by microorganisms or organic matter extracted from plant biomass and animal manure (Zsolnay et al., 1999; Ohno and Bro, 2006; Hunt and Ohno, 2007; Ohno et al., 2007). The lack of humification implies that much of the CDOM is labile and likely to be an active constituent in the local carbon cycle. This supports the suggestion by Simon et al. (2007) that there must be significant DOM processing in subsurface environments, as well as the results from Einsiedl et al. (2007) indicating that DOM transformation in karst is rapid and occurs on the order of decades, unlike many soil organic matter pools that persist for centuries (e.g., Trumbore, 1997).

Collectively, the results are not surprising, as CDOM in cave drip waters (e.g., Baker and Genty, 1999) has been linked to microbial activity and Goldscheider et al. (2006) recently concluded that DOM in karst lacked a significant plant- or soil-derived humic signature. However, in addition to the general indices, the overall patterns of CDOM fluorescence spectral features from our work suggest that recharge into different portions of the aquifer may have had a significant impact on the subsurface ecosystem. These results are unique. Samples from May 2005, January 2007, March 2008, and April 2009 were all from times of low recharge and regional drought (Fig. 5a–b) and shared similar humic-like spectral characteristics that had shorter maximum emission wavelengths, compared to spectra for reference humic substances like Suwannee River and Pony Lake fulvic acids (Fig. 3e–f). Hence, although the fulvic acid data do not necessarily differentiate between allochthonous or autochthonous DOM, we attribute the fulvic acid-like fluorescence to a humic substance that is primarily derived from heterotrophic processing of other types of DOM. The shorter wavelengths could also be due in part to the lack of exposure to solar radiation.

In contrast, nearly all of the June 2007 fresh and salinewater samples, which corresponded to a period of

high precipitation and recharge to the aquifer (Fig. 5b), had intense tryptophan peaks and fluorescence intensities in the other peak areas that were comparable to those observed from the other sampling times. The signature indicates that there was an increase in microbial activity because of autochthonous DOM produced by chemolithoautotrophs, along with in situ heterotrophy, or because of a combination of in situ processes and surface infiltration. Based on the fluorescence spectra, any possible contribution of surface DOM appears to be small relative to the in situ production, as the average FI values from the June 2007 data set indicate persistent microbial CDOM influences (Fig. 5a). It is possible that an influx of water from the surface could have stimulated microbial activity because of nutrient input, leading to an increase in the observed intensity of a microbial CDOM signature. But, it seems unlikely that surface recharge would transport DOM with other dissolved nutrients to the sampled aquifer depths, so another possible explanation for the variability is that terrestrial organic carbon did infiltrate into the subsurface but that most of the DOM was sequestered in the epikarst and shallower portions of the aquifer. The results are not conclusive enough to support either explanation for the June 2007 data set, as it is possible that a combination of processes occurred concomitantly. The samples collected in March 2008 and April 2009 contained prominent tryptophan-like peaks with similar intensities to those from May 2005 and January 2007, as well as more pronounced features in the peak C area. The intensity of the biological peak could represent baseline microbial activity, although at a much lower level than observed in the June 2007 samples. The presence of the more pronounced peak C feature suggests that some terrestrial CDOM did enter the aquifer waters during the period of greater recharge (December 2006 through September 2007), which is consistent with the slightly lower FI values observed in the June 2007 and March 2008 datasets.

The average FI values were nearly constant throughout the entire sampling period because the relative distribution of allochthonous and autochthonous CDOM in the wells does not vary significantly. The implication is that, even when there is an influx of surface water, terrestrial CDOM is not likely to become the dominant DOM fraction in the aquifer waters (as sampled from the wells). The differences in spectral features and indices between the low and high recharge periods are directly related to the appearance of an intense tryptophan peak in most of the samples from June 2007. This spectral change could not be the result of wastewater contamination or some other surface source of amino acid fluorescence. We propose that the shift in CDOM fluorescence properties is due to changes in climatic conditions and related changes in the hydrological properties and ecological conditions in the aquifer at the wells and springs, which may lead to an increase in microbial activity in the aquifer. If this conjecture is correct, then these results support that cave and karst ecosystems, even at significant depth in karst aquifers, are influenced by disturbances such as recharge events.

CONCLUSIONS

Karst aquifers are an exceptional type of groundwater system where the subsurface can be in direct hydrological communication with the surface. However, based on this and other recent studies, it is becoming increasingly apparent that the link between the surface and subsurface may also be blurred in karst. The most significant finding from our study is that CDOM in the Edwards Aquifer groundwater carries a signature suggestive of being sourced primarily from microbial activity. We understand that aquifer recharge rarely occurs over the entire spatial scale of the recharge area, and therefore epikarstic flow in one area may rapidly flush through the system, whereas another part of the aquifer may receive less or no infiltration. This makes it difficult to estimate recharge, and so it is possible that we did not sample locations that might be influenced more directly by terrigenous organic matter contributions. There are also many questions related to how much material, including organic carbon, infiltrates into the Edwards Aquifer through the epikarst, or how organic matter is temporarily stored, and even retarded, in the unsaturated zone. The physical nature of the epikarst should cause highly variable flow path interconnectiveness, as well as geochemically diverse aqueous- and gas-phase input (Musgrove and Banner, 2004; Taucer et al., 2005). Consequently, ecosystems that develop in karst aquifers must sustain themselves through times of limited epikarstic input (i.e., recharge), as well as survive times of intense and extreme input.

Another important result from our study is that the persistent signatures of microbial CDOM in the aquifer call into question the dependence of karst aquifer ecosystems on terrigenous carbon because there may be processes interfering with communication with the surface, even following extreme recharge events. In the absence of significant contributions of allochthonous DOM and because photosynthesis is not possible in the aquifer, the source of CDOM that we identified is associated with microbial processing of (chemolitho)autotrophically-produced organic matter (Randall, 2006). Cave and karst waters can have high microbial cell abundances, and there has been significant effort to understand the types of microbial processes, including autotrophy, in cave and karst systems (e.g., Sarbu et al., 1996; Sarbu et al., 2000; Simon et al., 2003; Farnleitner et al., 2005; Goldscheider et al., 2006; Opsahl and Chanton, 2006; Simon et al., 2007; Porter et al., 2009), although not in the Edwards Aquifer (Engel and Randall, 2008). The issue of microbial contributions to the ecosystem carbon cycle may indicate a significant departure from our current understanding of karst aquifer ecosystem dynamics (e.g., Pronk et al., 2006),

whereby differences in the nature and composition of DOM between surface and karst groundwater have been attributed to retention (selective or not) of terrigenous DOM by soil as water percolates into the subsurface, biotic molecular transformation of terrestrial inputs, or in situ microbial production (e.g., Einsiedl et al., 2007). In conclusion, scientists who manage karst aquifers for water quality and resource allocation should consider future work developing methods to discriminate between differences in DOM due to epikarstic filtering and in situ primary productivity linked to microbial diversity.

Acknowledgements

We thank G. Schindel and other scientists at the Edwards Aquifer Authority for access to wells and field assistance and J. Waugh and D. Mahula from the San Antonio Water System, who also assisted in field work. The authors thank R. Cook, M. Lowry, I. Warner, and E. D'Sa for equipment access. C. Schulz and S.A. Engel assisted in field data collection and analyses, and K. Brannen, B. Donnelley, and S. White helped with laboratory analyses. The manuscript benefited from insightful comments provided by K. Lavoie, C. Wicks, and two anonymous reviewers. The research was funded by Louisiana Board of Regents Support Fund (contracts LEQSF (2006-09)-RD-A-03).

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