

ABSTRACT

BARNETT, EMILY LYN. The Influence of Regional Groundwater on the Dissolved Organic Matter in Costa Rican Streams. (Under the direction of Dr. Christopher Osburn).

To determine the influence of different groundwater sources on dissolved organic matter (DOM) cycling we used a paired watershed approach and focused on two small streams in Costa Rica, the Arboleda and the Taconazo. Streams play an important, but not well-understood role in the global carbon (C) cycle. The hydrology of a stream, including the groundwater chemistry, can have a huge impact on the DOM in a stream. These two streams are located in La Selva Biological Research Station and have adjacent watersheds; therefore the streams have very similar characteristics, except for one key difference of groundwater contribution. Local groundwater is the only groundwater input that influences the Taconazo, but the Arboleda has the additional input of regional groundwater. The regional groundwater discharging into the Arboleda has very different characteristics than the local groundwater. In this study, questions of how quality and quantity of the DOM differed between and within the Arboleda and the Taconazo at baseflow and stormflow are addressed. DOM quality was investigated using colored dissolved organic matter (CDOM) absorbance and fluorescence. CDOM was quantified using the absorption coefficient at 254 nm (a_{254}), its molecular weight estimated using the slope ratio (S_R), and its aromaticity estimated using the specific UV absorbance at 254 nm ($SUVA_{254}$). Additionally we used parallel factor analysis (PARAFAC) to elucidate source of FDOM and DOC concentrations were measured to determine concentration. We found that the Arboleda and the Taconazo had more similar DOM, in terms of a_{254} , DOC concentration, S_R , and maximum fluorescence for each of the modeled PARAFAC components, at stormflow as compared to baseflow, due to the decreased influence of the regional groundwater in the Arboleda and the increased influence of storm

throughflow. The quality of the DOM differed between the Arboleda and the Taconazo, the DOM in the Taconazo was 29% more aromatic than the DOM in the Arboleda. Quantity of DOM was also different between the two streams. Average baseflow a_{254} in the Taconazo was 61% higher, at 6.72, than the average baseflow a_{254} in the Arboleda, at 2.62. The average DOC concentration in the Taconazo was 1.84 mg L^{-1} , 32% higher than the 1.26 mg L^{-1} average measured in the Arboleda. Despite the Arboleda having far less DOC and DOM at baseflow than the Taconazo did due to its dilution with heavily degraded, low-DOC regional groundwater. However, despite these distinctions at baseflow, we calculated a DOC export of $23.96 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Arboleda and $3.06 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Taconazo. The carbon export in the Taconazo was within a range of calculated carbon exports from other studies, both in the tropics and in temperate regions. However, the Arboleda carbon export far exceeded the other systems compared in this study. The results of this study showed that stream systems heavily influenced by groundwater-surface water interactions can play a significant role in carbon export from watersheds that often may be unrealized, despite having comparatively low DOM concentrations. This study shows that groundwater inputs should be taken into account in studies of stream carbon exports.

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The Influence of Regional Groundwater on the Dissolved Organic Matter in Costa Rican
Streams

by
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DEDICATION

To my family for everything, especially your constant support. Without you none of this would have been possible.

BIOGRAPHY

I grew up in Henrico, Virginia and received my Bachelor's of Science degree from the College of William and Mary in geology with a minor in environmental science and policy in 2014. I completed an undergraduate thesis where I studied the quality of drinking water in the Jamestown aquifer. This research included measuring the dissolved organic carbon in the groundwater and I became interested in studying organic matter in other environments, which led me to pursuing a Master's at NCSU.

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INTRODUCTION

Dissolved organic matter (DOM) is operationally defined as any organic material that passes through an average filter with a pore size of less than 0.7 μm (Aitkenhead-Peterson et al., 2003). DOM is comprised of a very diverse mixture of soluble and colloidal organic compounds composed mainly of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) (Aitkenhead-Peterson et al., 2003). DOM is a major component of the organic matter transported to the coastal zone by rivers (Meybeck, 1982; Ludwig et al., 1996). It plays a major role in aquatic food webs, controls the availability of dissolved nutrients and metals, affects the optical properties of aquatic systems (Findlay and Sinsabaugh, 2003), and constitutes an important pathway for carbon (C), nitrogen (N), and phosphorus (P) transport from land to the sea. Rivers play a large, but understudied, role in the global carbon cycle (Cole et al., 2007). Additionally, an understanding of how small streams influence the carbon cycle is needed.

An important component of DOM is the dissolved organic carbon (DOC). Rivers and streams transport or store about 2 Pg of terrestrial organic carbon annually (Cole et al., 2007) and at least half of the C inputs to fluvial systems are outgassed to the atmosphere before reaching the oceans (Cole et al., 2007; Aufdenkampe et al., 2011). About 50% of the total organic carbon exported from rivers is in the form of DOC (Raymond and Bauer, 2001). DOC concentrations in freshwater range from about 1 to 60 mg C L^{-1} . However, high values in this range are unusual; in many systems the range is 1 to 5 mg C L^{-1} (McDonald et al., 2004). Riverine DOM not only varies in quantity but also in quality or lability, which refers to how readily microorganisms can use this carbon pool. A small amount of riverine DOM is biodegradable and quickly used by microorganisms but most of it is partially oxidized

because it is more difficult for microorganisms to use (McDonald et al., 2004). DOM has a humic fraction and a non-humic fraction. The non-humic fraction includes biopolymers such as lipids, carbohydrates, polysaccharides, amino acids, proteins, and resins. Humics constitute a large portion of the DOM pool and are generally considered more refractory or less biodegradable than the non-humic fraction of the DOM.

The four main sources of DOC to fluvial systems are soil organic matter, in-situ primary production, atmospheric precipitation, and groundwater (Lloret et al., 2011). Although precipitation is generally a minor contributor (Lloret et al., 2011), precipitation events are likely to change the relative percent contribution the different sources have on stream DOC, and play an important role in DOC transport in streams. For example, the total concentration of any OC entering the streams from soils would likely increase during a rain event. In a study of 30 forested watersheds, Raymond and Saiers (2010) determined that precipitation events led the system to a greater DOC flux than baseflow conditions. Additionally, during stormflow events, rivers are mainly sourced from storm runoff, as opposed to being sourced from groundwater during baseflow (Lloret et al., 2011). DOC concentrations increase with discharge (Raymond and Saiers, 2010). There is a pattern of DOC concentrations increasing on the rising limb of a hydrograph and decreasing on the falling limb; this likely represents that water-soluble soil organic matter is depleted after a certain amount of flushing from rain via hydrologic throughflow (Hornberger et al., 1994). Previous studies have also found a link between the hydrology and organic matter export of streams (Mulholland, 1997, Schiff et al., 1998).

There are also qualitative changes that occur in the dissolved organic matter (DOM) pool in a stream during a rain event. These events tend to flush more terrestrial DOM from

the land surface into the stream and the resulting exported DOM is of a more terrestrial origin than is observed during baseflow (Hood et al., 2006; Lloret et al., 2011). A number of studies have shown that during flushing events more labile DOC may be transported from terrestrial sources into waters (McClain et al., 2003, Buffam et al., 2001). Vidon et al. (2008) found that in agricultural watersheds DOC exported during storm events tended to be of higher aromaticity. Isotopic composition of the DOC can also change during storm events. Lloret et al. (2011) found $\delta^{13}\text{C}$ -DOC, or a measure of the ratio of stable carbon isotopes (^{13}C to ^{12}C), to be lower during baseflow than during stormflow. A previous study in the French West Indies found that ignoring the input from extreme storm events (for example, flash floods), which account for over 60% of the annual flux in some streams, can lead to a large underestimation in the total net DOM flux of a stream (Lloret et al., 2011). Even this number may be conservative, as some streams have seen storm DOM account for 86% of the annual flux (Raymond and Saiers, 2010).

It is of particular interest to study DOM in tropical systems, such as are present across La Selva Biological Research Station, Sarapiquí, Heredia Province, Costa Rica. The role of tropical ecosystems in the inland carbon cycle is understudied. Closing this knowledge gap is an essential task as hydrology, vegetation, and biological activity are expected to change with the increasing climate variability, particularly in the tropics. The aim of this project was to examine stream DOM dynamics within tropical rainforest watersheds at La Selva Biological Station in Costa Rica (Fig. 1). A particular emphasis was placed on the role of regional groundwater transport of DOM into headwater streams. We focused on DOM transport between groundwater and surface water by comparing and contrasting sources of DOM in two watersheds, the Arboleda and the Taconazo. Both watersheds contain similar soils,

vegetation, and geography but the Arboleda receives a significant input of regional groundwater, while the Taconazo does not (Genereux et al., 2009).

DOC in runoff from forested areas comes predominantly from soil organic matter (Battin et al., 2008). These differences in soil organic matter properties could impact DOC quantity and quality (Strohmeier et al, 2013). Therefore it is relevant in any study in which watersheds are compared to attempt to take these variables into consideration. For this reason, the Arboleda and Taconazo are an ideal place to conduct a paired-watershed comparison study such as the one done here because they share similar soils and vegetation. However, there is one key difference in the groundwater that discharges into the Arboleda, as compared to the Taconazo. Local groundwater discharges into the Taconazo, whereas both local groundwater and regional groundwater discharges into the Arboleda. Regional groundwater flow does not conform to surface water drainage divides; it is often much older than shallow, local groundwater and is very important because it can influence the chemistry of the surface water as this groundwater likely has different characteristics than the local groundwater (Genereux et al., 2002, Genereux and Jordan, 2006).

The purpose of this study was to explore three main questions:

- 1) Are there differences in DOM quantity and quality between the Arboleda and the Taconazo?
- 2) What is the difference in DOM quantity and quality within each stream at baseflow and stormflow? Is the DOM in the Arboleda and the Taconazo more similar at stormflow as opposed to baseflow?
- 3) What do these potential differences tell us about the carbon cycle within this ecosystem and how can this be applied to tropical ecosystems in general?

The main hypothesis of this thesis is that differences in DOM quality and quantity between the Arboleda and the Taconazo are pronounced at baseflow, but that these differences would be lessened at stormflow. To evaluate DOM quality and quantity, samples were collected weekly over a 2-year period and during several rain events. DOC concentration, PARAFAC fluorescence components, CDOM absorption, and $\delta^{13}\text{C}$ -DOC values were paired with stream discharge to determine the aromaticity, molecular weight, and carbon flux of these systems at baseflow and stormflow. Results were placed in context of stream DOM quality and export among temperate and tropical ecosystems.

STUDY SITE

The work done in this study was conducted at La Selva Biological Station (LSBS), a field research station managed by the Organization for Tropical Studies (OTS). LSBS is a 1536-hectare research preserve in the Heredia province of Costa Rica. LSBS is near Braulio Carrillo National Park and Barva Volcano. The geology of the site is mainly volcanic rocks of Quaternary age and intermediate composition (McDade et al., 1993). The two streams studied at LSBS were the Arboleda and the Taconazo (Fig. 1). These two streams were chosen for a paired watershed study because they have many similar characteristics, including the vegetation, relief, soil type, rainfall, temperature, and evapotranspiration (Genereux et al., 2005). The streams are slightly different in size; the watershed of the Arboleda is 47.14 ha while the watershed of the Taconazo is 27.94 ha (Genereux et al., 2005). The main difference between these two streams is the water chemistry. The Arboleda has much higher inorganic solute concentrations than the Taconazo, a product of a high influx of interbasin groundwater flow which contributes older, regional groundwater to the Arboleda and none to the Taconazo (Genereux et al., 2002).

Interbasin groundwater flow is often much older than any local groundwater that is input to streams. Additionally, this water is often different chemically from local groundwater due to traveling further and deeper into the ground and coming into contact with different rocks or minerals than local groundwater. High solute concentrations (due to magmatic outgassing or weathering of volcanic rock) in the Arboleda shows the input of regional groundwater; additionally, the discharge of water from the Arboleda is larger than the discharge due solely to precipitation, further suggesting the input of regional groundwater (Genereux et al., 2009; Genereux and Jordan, 2006). A small regional groundwater

contribution to the Taconazo was suggested by previously calculated watershed water budgets, but this was not reliably distinguishable from zero, and the chemical data (solute concentration) suggest no input to the Taconazo (Genereux et al., 2005; Genereux et al., 2002).

MATERIALS AND METHODS

Field Methods:

Water samples for the analysis of DOC concentration and isotopic composition as well as optical properties of the DOM were collected approximately weekly from the Arboleda and Taconazo streams from January 2012 through March 2014 (Table 1). Samples were also taken at Guacimo Spring, a spring south of La Selva with water representative of IGF. Additionally, we sampled storm events at high frequency in the Arboleda and the Taconazo in June 2013. For the weekly sampling stream water samples were collected in 1-L polycarbonate bottles. The sample bottles were cleaned in detergent (Sparkclean 1) and rinsed with ultrapure water before use. Before sample collection, the sample bottle was rinsed three times with the stream water and the rinses discarded downstream of the sample collection site. Bottles were transported immediately to the laboratory where they were treated as described below. Three storm events were sampled using Teledyne ISCO 3700 samplers near the weirs in both the Arboleda and the Taconazo (Fig. 1). These samplers collected a 300 mL stream water sample every hour for twelve hours. ISCO bottles were transported to the laboratory at the end of the 12-hour collection and treated as described below.

Discharge data from V-notch weirs were taken every 15 minutes in both the Arboleda and the Taconazo (Genereux et al., 2013). Some dates do not have recorded data due to instrument malfunction; in this case TOPMODEL (Beven et al., 1995) was used to estimate the discharge based on the regional precipitation measured at a rain gauge that is within 1.5 km of both weirs] (Zanon et al. 2014). For data analysis purposes, we divided the samples into categories of baseflow and stormflow. We did this with a visual examination of the

plotted discharge data and the precipitation data. We observed that all discharges below $9 \text{ m}^3 \text{ min}^{-1}$ in the Arboleda were baseflow samples and all discharges above $11 \text{ m}^3 \text{ min}^{-1}$ were stormflow samples. All samples taken at discharges in between these numbers we classified as stormflow if there had been measured precipitation a maximum of five hours before the sample was collected. The same procedure was followed for the Taconazo, with all discharges below $0.5 \text{ m}^3 \text{ min}^{-1}$ taken as baseflow samples and all discharges above $1.5 \text{ m}^3 \text{ min}^{-1}$ taken as stormflow samples.

Laboratory Methods:

In the laboratory facilities at La Selva, weekly samples were transferred to 125-mL polycarbonate bottles and then frozen. Samples were transported frozen from Costa Rica to the United States and stored in a freezer upon arrival at North Carolina State University (NCSU). We thawed samples and allowed them to come to room temperature before we used a Barnant Company vacuum pump to filter the samples using a 500-mL side arm flask through a pre-combusted $0.7\text{-}\mu\text{m}$ glass fiber filter within 24 hours of thawing. The volume filtered was recorded. Absorbance and fluorescence on filtered thawed samples was measured within one week of thawing and filtering (see details below). The remaining filtrate was transferred to a DOC vial and acidified with 85% phosphoric acid to a pH of 2 for further DOC concentration and isotopic composition analysis (see details below). High-frequency samples collected during storm events were filtered at La Selva laboratory facilities as described above and transported to NCSU for analysis. Optics measurements (absorbance and fluorescence) were taken on these samples. Additionally, we analyzed these samples for DOC and $\delta^{13}\text{C}$ -DOC. From these measurements, SUVA_{254} (the specific UV

absorption at 254 nm) and S_R (the slope ratio) were also calculated. Precipitation and discharge were also measured and plotted for both the Arboleda and the Taconazo (Figs. 2 and 3).

Optical Data:

Colored dissolved organic matter (CDOM) refers to DOM that also absorbs blue light. CDOM has been shown to be a useful carbon tracer and in identifying mixing between bodies of water (Chen et al., 2010). CDOM Absorbance spectra of the samples were measured from 200 to 800 nm on a Varian Cary 300UV spectrophotometer in quartz cells that were 10-cm in length. These samples were corrected by applying an ultrapure lab water blank. The blank-corrected values were converted to Napierian absorption coefficients (m^{-1}) (Osburn et al., 2012). Several methods of using CDOM absorbance exist for studying the quality of DOM. Absorbance at 254 nm (a_{254}) of a water sample has been used as a proxy for its DOC concentration (Coble, 2007) and the ratio of a_{254} to DOC concentration, termed the *specific UV absorbance*, ($SUVA_{254}$) can be used to estimate the relative amount of the aromatic groups in its DOM (Weishaar et al. 2003). The specific UV absorbance at 254 nm, $SUVA_{254}$, of a sample was computed as its decadic absorption coefficient at 254 nm divided by its DOC concentration.

Absorbance measurements were also used to calculate the slope ratio (S_R), the ratio of the slope of absorbance and wavelength from 275 – 295 nm and from 350- 400 nm when plotted on a Cartesian coordinate system (Helms et al., 2008). High values of S_R have been interpreted as low molecular weight and less aromatic CDOM, while the inverse is true for

smaller values of S_R (Helms et al., 2008, Spencer et al., 2009). During precipitation events, organic matter in streams tends to have a higher aromaticity (Vidon et al., 2008).

Fluorescence excitation-emission spectra were measured on a Varian Eclipse spectrofluorometer, sampled from 240 to 450 nm in the excitation mode (Ex) and 300 to 600 nm in the emission mode (Em). These samples were corrected by subtracting an ultrapure lab water blank. Samples were also corrected for the inner filtering effect and were corrected to the spectrofluorometer's Raman signal and converted to quinine sulfate units (QSU). Corrections of absorbance and fluorescence spectra were done with in-house processing scripts using MATLAB 2014a (MathWorks). Fluorescence spectroscopy is an advantageous way to examine the quality of the DOM because it is inexpensive and non-destructive (Murphy et al, 2014). The intensity and shape of the excitation-emission matrix (EEM) can help quantify and characterize DOM (Stedmon and Bro, 2008). Fluorescence spectra for a sample were processed into excitation-emission matrix (EEM), a data form compatible with the DOMFluor toolbox (Murphy et al., 2013) in MATLAB, which allowed a parallel factor analysis (PARAFAC) model to be fit to the data.

PARAFAC is a tool to analyze EEMs; it is a statistical technique that allows for decomposition of a series of EEMs into mathematical components. Previous studies have found PARAFAC to be a useful tool in differentiating between sources of CDOM (Walker et al., 2009). The PARAFAC modeling approach involves a series of steps: (1) create the dataset, (2) preprocess the results, (3) develop preliminary models, (4) validate the final model, and (5) interpret the results. In starting to develop this model, first outliers were removed and the results were examined to ensure that appropriate number of components had been modeled. When too few components are included in a given model, chemically distinct

components are often combined. On the other hand, if too many components are fit to the data, over-fitting of the data may result, usually because two or more components represent one signal and that signal is combined with instrument noise (Murphy et al, 2013).

Examining and minimizing the residuals ensured that the appropriate number of components had been fitted. The residuals resemble randomly distributed scattering; however, a pattern of a peak next to a trough in the residuals of many samples is an indication that an incorrect number of components were fit to the data (Stedmon and Bro, 2008). To ensure the robustness of the model, a split-half analysis was conducted on the data. In this analysis, data is split into halves and modeled. If the same model was fit to each part of the data then the model was robust. These steps led to the final selection of a validated model. PARAFAC components can be interpreted as derived from a specific source (for example, terrestrial humic material, protein-like amino acid fluorescence, and others; Murphy et al, 2013).

Next, the output was compared to the OpenFluor database, at a 95% similarity. OpenFluor is an online database where researchers can upload published, peer-reviewed PARAFAC models. OpenFluor allows users to search and compare results of other PARAFAC models and examine matched components in the context of other environments for which PARAFAC models have been created (Murphy, 2014). It was also useful to compare components of a new model because they may match previously identified peaks. OpenFluor has the additional benefit of offering a quantitative way to compare components across systems, as opposed to the method of visual comparison that has previously been used to characterize FDOM components (Murphy, 2014). It was also be useful to examine the F_{\max} values of the PARAFAC components (essentially the concentration of each component standardized to quinine sulfate equivalents, QSE). Plotting F_{\max} values allowed for a

visualization of how the amount of fluorescence in these samples changed or how different the values were between the Arboleda and the Taconazo.

Dissolved Organic Carbon Measurements

DOC concentration was measured using an OI Analytical Aurora Model 1030 Total Organic Carbon Analyzer while carbon isotope data was obtained with a Delta V Isotope Ratio Mass Spectrometer. The method used was originally created by St-Jean (2003) and has been modified by Osburn and St-Jean (2007). To remove dissolved inorganic carbon (DIC) from the sample before measuring DOC concentration, samples were sparged with argon for thirty minutes (low DIC samples) to three hours (high DIC samples). Reference carbon dioxide pulses were sent through the system before analysis until an acceptable standard deviation (<0.05) was observed. Before samples were analyzed, 12 blanks (MilliQ water) were analyzed followed by a calibration curve of an appropriate range. A standard curve consisting of six caffeine standards, which ranged from 0.25 mg L^{-1} to 25 mg L^{-1} DOC, and two sucrose standards, which ranged from 0.5 mg L^{-1} to 5 mg L^{-1} from the International Atomic Energy Agency (IAEA) were analyzed. The caffeine standards had isotope values of $\delta^{13}\text{C}_{\text{VPDB}} = -27.77 \pm 0.04 \text{ ‰}$ and sucrose standards had isotope values of $\delta^{13}\text{C}_{\text{VPDB}} = -10.45 \pm 0.03 \text{ ‰}$, based on the Vienna Pee Dee Belemnite (VPDB). Sample analysis followed the blank and standard runs. After every five samples an instrument “cleanup” was completed to ensure the best function of the system. Samples and standards were blank-corrected and a linear regression of the area of the curve of the caffeine standards was created to relate area under the curve to DOC concentration.

Blank-corrected $\delta^{13}\text{C}$ -DOC values were normalized to known $\delta^{13}\text{C}$ -DOC values of caffeine and sucrose standards. The isotopic composition of the DOC can be used to help identify sources of DOM. Stream stable carbon isotopes ($\delta^{13}\text{C}$) of DOC are sensitive to organic matter sources in the watershed and modulated by primary production within the stream. Typical ranges of $\delta^{13}\text{C}$ -DOC are -33 to -23‰ in many freshwaters (Raymond and Bauer, 2001). These values are typically more depleted (i.e., lower or more negative) during baseflow (Lloret et al., 2011).

RESULTS

Hydrology in the streams

The aim of this study was to determine how hydrology (groundwater, stormflow) influenced the dissolved organic matter composition and export in two watersheds that differ in terms of source of base flow. Therefore, hydrologic results for the streams are briefly presented (Genereux and Jordan, 2006). Precipitation (mm) was measured at the OTS Meteorological Station at La Selva with a model TB4-L tipping bucket every 30 minutes (Zannon, 2011). Precipitation, measured in m^3 per 30 min, was plotted with time. Visually, we observed that precipitation and discharge spikes occur at similar times within each individual watershed (Figs. 2 and 3). The rainfall would increase the discharge of local groundwater; because the Arboleda has a mixture of both regional and local groundwater, after rainfall we would expect the ratio of local to regional groundwater in the Arboleda to increase. Increasing this ratio would cause the stream water in the Arboleda during stormflow to be more similar to the stream water in the Taconazo. Figures 2 and 3 also show that baseflow appeared relatively consistent over time with some cycling that likely corresponded to seasonal variability in the water table (Genereux et al., 2002). We observed minimum baseflow values of $8.7 \text{ m}^3 \text{ min}^{-1}$ and maximum baseflow values of $10.6 \text{ m}^3 \text{ min}^{-1}$ in the Arboleda. In the Taconazo we observed minimum baseflow values of $0.05 \text{ m}^3 \text{ min}^{-1}$ and maximum baseflow values of $1.3 \text{ m}^3 \text{ min}^{-1}$. These baseflow values can be compared to a maximum observed stormflow of $93.7 \text{ m}^3 \text{ min}^{-1}$ in the Arboleda and $35.4 \text{ m}^3 \text{ min}^{-1}$ in the Taconazo.

DOM concentrations between the two watersheds

Differences in the amounts of DOM between the Arboleda and Taconazo were visualized in a boxplot of the a_{254} value and DOC concentration of the three sampling sites, with comparison to the DOM measured in the Guacimo Spring, (Figs. 4 and 5). These values are summarized in Table 2. An ANOVA with Rank's (Dunn's Test) was completed on the metrics used to measure quantity and quality of the DOM in these streams to determine significance (Table 3). There was a statistically significant difference between the baseflow a_{254} and DOC in the Taconazo and Arboleda, but not in the stormflow samples in the Arboleda compared to the Taconazo. We also compared boxplots of the a_{254} values and DOC concentrations measured in the Arboleda, Taconazo, and the Guacimo Spring. Overall, Taconazo had the highest values and the Guacimo Spring the lowest values. Both stormflow and baseflow samples were included, but often stormflow samples were outliers.

Values that were greater than 2.7σ of the mean were defined as outliers as per Williamson et al. (1989) and interpreted as more likely to be stormflow samples in the Arboleda. Of the Arboleda a_{254} values, 17 points were outliers; of these 16 were stormflow samples (there were 31 stormflow samples that were not outliers) (Fig. 4). For DOC measurements taken from the Arboleda 11 of the 14 values that were greater than 2.7σ were stormflow samples (there were 36 stormflow samples that were not greater 2.7σ) (Fig. 5). There were 20 values that were outliers of the Taconazo a_{254} values; of these 19 were stormflow samples (there were 43 stormflow samples that were not outliers) (Fig. 4). DOC values in the Taconazo had 8 values that were greater than 2.7σ of which only 3 were stormflow samples (there were 58 stormflow samples that were not greater than 2.7σ) (Fig. 5). Guacimo Spring samples had a much smaller degree of variation and far fewer values that

were outliers. Guacimo Spring a_{254} values had 3 points that were outliers and DOC values in Guacimo Spring had only 1 value that was an outlier (Figs. 4 and 5). The relatively small variation in the Guacimo Spring samples and the small number samples outside the box and whiskers in the boxplot showed that this spring as a very stable DOM concentration.

Guacimo Spring represented the regional groundwater that flows into the Arboleda, and therefore we infer that this IGF was a stable contribution to the Arboleda without much variation. In both streams, stormflow has different DOM than baseflow, and because there are more baseflow samples, stormflow samples tend to be outside the whiskers of the boxplot. However, there are some stormflow samples that fell within the whiskers of these plots and they were more common in the Taconazo than in the Arboleda.

There were differences between the amounts of CDOM and DOC in the Arboleda and the Taconazo (Fig. 4). We next investigated the differences in CDOM absorption properties using the qualitative measurements S_R and $SUVA_{254}$ (Table 4). S_R was generally higher in the Arboleda than the Taconazo. In the Arboleda, baseflow S_R values (mean 1.07 ± 0.14) tended to be higher than stormflow (mean 0.97 ± 0.15); we saw the reverse trend in the Taconazo where the mean value at baseflow was 0.80 ± 0.18 and the mean value at stormflow was 0.84 ± 0.38 (Table 4). However, the difference between stormflow and baseflow S_R values was less pronounced in the Taconazo than it was in the Arboleda. $SUVA_{254}$ values were higher in the Taconazo than in the Arboleda and values in both streams were higher at stormflow than at baseflow (Table 4).

EEM fluorescence was used to describe the quality of DOM in the streams and spring water (Fig. 6). EEM spectra generally resemble natural organic matter from many freshwater aquatic ecosystems with most intense fluorescence peaking at $E_x=240 \text{ nm}/E_m = 440 \text{ nm}$ and

tapering off to least fluorescence >350 nm excitation. Emission spectra were generally broad and Gaussian in shape, which is common for DOM derived from humic substances (Fellman et al. 2010). Despite the Arboleda and Taconazo at baseflow and stormflow exhibiting similar fluorescent patterns (Fig. 6A-D), there were many key differences between the Arboleda, Taconazo, and Guacimo Spring. Comparing the Arboleda at baseflow (Fig. 6A) and the Taconazo at baseflow (Fig. 6B) we found that the fluorescent peaks were similar. However, there was a difference in intensity between the two streams. The fluorescence intensity of the Arboleda was only 4 QSE, but the fluorescence intensity of the Taconazo was 8 QSE. Moreover, we noted a secondary peak at $Ex = 310$ nm and $Em = 440$ nm in the Taconazo that was nearly absent from the Arboleda.

As compared to the baseflow samples (Fig. 6A, 6B), the stormflow samples (Fig. 6C, 6D) had greater fluorescence intensity, and the peak shapes were generally similar to each other. However, it was clear that the Arboleda at stormflow (Fig. 6C) and the Taconazo at stormflow (Fig. 6D) were identical in intensity range and nearly double the intensity of the Taconazo at baseflow and five times the intensity of the Arboleda at baseflow. This supported the hypothesis that the DOM in the Arboleda and the Taconazo was more similar at stormflow as opposed to baseflow. Additionally, a sample EEM of Guacimo Spring (Fig. 6E) showed that there was very little fluorescent DOM in the Guacimo Spring (<1 QSE across the entire EEM), and because Guacimo Spring is representative of the regional groundwater discharging into the Arboleda these EEMs support the assertion that the FDOM in the Arboleda is being diluted by input from regional groundwater. We determined this when examining the lower fluorescence intensity in the Arboleda (Fig. 6A) as compared to the Taconazo (Fig. 6B). It bears mentioning too that the Arboleda appeared to develop the

secondary peak at $Ex=310/Em=440$ that was observed in the Taconazo at baseflow, albeit at lower intensity. This suggested that DOM in the streams during storm events was nearly identical.

To further explore the characteristics of the DOM in these streams, a PARAFAC model was fit to the stormflow and baseflow DOM fluorescence measurements from the Arboleda and the Taconazo (Figs. 7 and 8). Guacimo Spring samples lacked fluorescence, despite having some absorption (not all CDOM fluoresces, Stedmon and Murphy 2014) and were not included in the model. A six-component model was validated using split-half analysis, where the set of EEMs was split in half and each half was modeled separately to ensure that a robust model was being fit to the dataset (Murphy et al. 2013).

Each component exhibited key fluorescence properties that partition DOM into seemingly allochthonous (external to stream) and autochthonous (internal to stream) sources (Fig. 7, Table 5). Component 1 was noticeably blue-shifted to shorter wavelengths in both the Ex and Em modes. This signal had a well-defined secondary peak at $Ex = 320$ nm centered on $Em = 440$ nm. Component 2 (C2) was centered on $Ex=400$ and also had a secondary peak at $Ex=300$. C2 was indicative of reprocessed material (Osburn et al., 2016). Component 3 (C3) had the broadest fluorescence extending well into the longer wavelengths for excitation (>350 nm) and centered on 500 nm emission. Component 4 (C4) seemed to encompass the A and C peak and had a primary and secondary peak (Coble, 2007). This fluorescence is typical of highly conjugated molecules that would originate from soil organic matter (Del Vecchio and Blough 2004; Fellman et al. 2010). Components 5 and 6 exhibited fluorescence that often is attributed to protein-like substances but also shares characteristics with phenolic material from lignin (Hernes et al., 2009; Wünsch et al 2015).

Next the excitation and emission values from the PARAFAC loadings were compared to the OpenFluor database (<http://www.openfluor.org>). As of May 2016, 63 models were available on OpenFluor. Table 5 summarizes the results of comparing our six PARAFAC components to the OpenFluor database. Component 1 (C1) was identified as a terrestrial, humic-like component and matched models including North American estuaries and the South Atlantic Bight. Component 2 (C2) was identified as reprocessed terrestrial, humic-like, and this component matched signals seen in North American Estuaries, Coastal and Shelf Seas, and the Zambezi River Basin, among others. Component 3 (C3) was identified as terrestrial, soil fulvic-like and matched models such as Coastal and Shelf Seas, Tropical Rivers in Venezuela, and the Florida Keys. The last two components, C5 and C6, were identified as amino acid or protein-like fluorescence, consistent with tryptophan and tyrosine. Component 5 was seen in two Florida Keys models and C6 was seen in the South Atlantic Bight model (Table 5).

Comparisons of DOM Properties and Relationship to Groundwater and Discharge

The differences in DOM properties showed that the groundwater heavily influences OM, as evidenced by the differences between the Arboleda and Taconazo, and that an input of precipitation led to more similar OM between the Arboleda and Taconazo. As shown in Figures 4 and 5, there were differences in the a_{254} and DOC values within the Arboleda and Taconazo streams, and the Guacimo Spring. We next determined if discharge was responsible for these differences in a_{254} values and DOC concentrations of the Arboleda and the Taconazo. Both the Arboleda and Taconazo showed that DOC and a_{254} tended to increase

with increasing discharge (Figs. 9A, 9B, 10A and 10B) which was made clear in the stormflow samplings with multiple data points collected over a many hour period (Fig 11).

The spikes of a_{254} and DOC were more pronounced in the Arboleda than in the Taconazo (Fig. 11). The spike of DOC with stream flow was likely due to the influence of the relatively optically clear and fairly DOC free Guacimo Spring (mean DOC $0.5 \pm 0.16 \text{ mg L}^{-1}$) discharging into the Arboleda stream (mean DOC at baseflow, $1.26 \pm 1.51 \text{ mg L}^{-1}$), in effect diluting its CDOM and DOC concentrations at baseflow and making the increase at stormflow appear more pronounced. The DOC concentrations of the Taconazo were more variable during baseflow and stormflow compared to the Arboleda without as large of a change in from baseflow to stormflow as was observed in the Arboleda (Fig. 11).

We used a mixing model to determine if IGF is the only source of dilution between the Arboleda and the Taconazo. For this mixing model, we assume that there are only two sources of water, IGF and the local groundwater. IGF is represented by the Guacimo Spring samples and local groundwater is represented by the Taconazo baseflow samples. The average Guacimo Spring chloride concentration and a_{254} was used as one end member and the average Taconazo chloride concentration and a_{254} was used as the other end member. If the Arboleda represents a mix of local groundwater and IGF, the Arboleda samples would fall along a mixing line between the Taconazo and the Guacimo Spring. We used the rationale that if the Arboleda would be identical to the Taconazo, unless it was being diluted by IGF in which case the Arboleda samples must fall along the line draw between the points.

Due to the variability in the a_{254} and DOC values we created a mixing triangle. If the Arboleda samples fall within this triangle then we assumed that the Arboleda is a mix of only IGF and local groundwater. Combining the techniques used in prior mixing model analyses

with baseflow and stormflow observations in this study elucidated clear trends that showed the connection between groundwater and surface in the La Selva streams (Nagy, 2012). We used the chloride concentrations (mM) of the samples as a conservative tracer to distinguish between high-solute IGF and low-solute local groundwater. Previous studies found that Guacimo Spring, our representative IGF source, has high chloride concentrations and the Taconazo, our representative local groundwater, has low (not distinguishable from zero) chloride (Genereux et al., 2002 and Nagy, 2012). We used chloride concentrations and the a_{254} values of each sample to determine if the Arboleda represented a mix between Guacimo Spring (regional groundwater) and the Taconazo (taken as the local groundwater) (Fig. 12). The model supported the claim that Arboleda represented a mix between the Taconazo (representative of local groundwater) and Guacimo Spring (representative of IGF) (Fig. 12).

The mixing model showed that the Arboleda samples fall within the mixing lines between the Taconazo and Guacimo Spring, meaning that the Arboleda does represent a mix of local groundwater and IGF (Fig. 12). We also found a separation between baseflow and stormflow in the Arboleda. In the Arboleda the baseflow samples tended to have a lower a_{254} than the stormflow samples. These results show that the low CDOM water in Guacimo Spring was diluting the CDOM in the Arboleda. These results were very similar previous results from this system (Nagy, 2012). A second mixing model was also created using the Cl concentration (mM) and DOC concentration (Fig 13). The mixing model with DOC also showed that the stormflow samples in the Arboleda tended to fall more towards the Taconazo than the Guacimo Spring.

It was also useful to examine the F_{\max} values of the PARAFAC components (essentially the concentration of each component standardized to quinine sulfate equivalents,

QSE) that were modeled for each stream (Figs. 14 and 15). F_{\max} values for the Arboleda (Fig. 14) and the Taconazo (Fig. 15) were plotted along with discharge of each stream. These values are used to show how much of each component occurs in the Arboleda and the Taconazo and to compare trends between stormflow and baseflow. The F_{\max} values follow generally similar patterns in both the Arboleda (Fig. 14) and the Taconazo (Fig. 15). The components in the Arboleda increased with stormflow and decreased back to baseflow (Fig. 14). However, in the Taconazo there was a trend of the components increasing during a drier period (Fig. 15). We attributed this to the local groundwater having a greater concentration of DOC in the drier period. In both the Arboleda (Fig. 14) and Taconazo (Fig. 15) the F_{\max} values for C6 showed the least amount of variation. During the storm sample collection of June 2013 we observed a pattern of F_{\max} increase during the rising limb of the discharge and decrease during the falling limb was seen (Figs. 14 and 15). It was also reasonable to conclude that the F_{\max} values in the Taconazo are generally higher than in the Arboleda due to the dilution of the Arboleda by low CDOM and low DOC groundwater measured for the Guacimo Spring, which best represents the groundwater discharging into the Arboleda.

$\delta^{13}\text{C}$ -DOC values can be useful in determining source of OM at the bulk level beyond the use of DOM optical properties, which measure only a portion of the total DOM. If isotope values were different between baseflow and stormflow they could be used as a way to identify source. In the Arboleda and the Taconazo there was not a significant trend of isotope value change with discharge (Fig. 9C and Fig. 10C). This lack of trend was due to the wide range of isotope values seen in the data, with no clear separation between baseflow and stormflow (Fig. 16). $\delta^{13}\text{C}$ -DOC values have been used to identify sources of DOC (C3 or C4 plants) and autochthonous versus allochthonous inputs. C3 plants have more depleted isotope

values ($\delta^{13}\text{C}$ -26 to -35‰) compared to C4 plants ($\delta^{13}\text{C}$ -8 to -18‰) and soils and forest litter have isotope values of $\delta^{13}\text{C}$ -23 to -27‰ (Bianchi and Canuel, 2011). The more depleted $\delta^{13}\text{C}$ -DOC values (<-30‰) tend to suggest primary production by freshwater phytoplankton and periphyton. It appeared little variation existed in the results that could be attributed to differences between stormflow and baseflow.

SUVA₂₅₄ and S_R are both proxies for aromaticity (Weishaar et al., 2003, Spencer et al., 2009) and are plotted along with discharge in Figure 17. In general, SUVA₂₅₄ did increase with discharge in both the Arboleda (Fig. 17A) and the Taconazo (Fig. 17B). However, none of SUVA₂₅₄ values also appear to increase during 2013 which also suggests a localized climatic signal. However, neither of these trends were significantly different from zero (Figs. 9E and 10E).

The average S_R values for both stormflow and baseflow were higher in the Arboleda than they were in the Taconazo (Table 4). There was a significant difference between S_R in the Arboleda at baseflow and the Taconazo at baseflow, as well as a significant difference between the Arboleda at stormflow and the Taconazo at stormflow (Table 3). At baseflow S_R in the Arboleda was 1.07 ± 0.14 and in the Taconazo was 0.80 ± 0.18 . At stormflow S_R in the Arboleda was also higher, at 0.97 ± 0.15 and in the Taconazo was 0.84 ± 0.38 (Table 4). However, the values at stormflow were more similar to each other than the values at baseflow. It is important to note that both the linear regression of the S_R values in the Arboleda and the Taconazo with discharge were significant (Figs. 9D and 9E). If we examined the absorbance spectra of representative samples we also observed the stormflow sample in the Arboleda increased in CDOM and followed a similar pattern to the stormflow

sample in the Taconazo (Fig. 18). Figure 18 also showed the very low CDOM Guacimo Spring, demonstrating how IGF dilutes the Arboleda.

There was a higher mean S_R at baseflow than at stormflow in the Arboleda, but the reverse was true in the Taconazo (Table 4). In general, low S_R is associated with higher molecular weight DOM, which is likely allochthonous (Spencer et al., 2009, Helms et al., 2008). However, in both the Taconazo and the Arboleda the mean values at stormflow and baseflow were similar; therefore, it was difficult to determine if there was a large difference between allochthonous and autochthonous DOM between baseflow and stormflow. It is important to note that in Figure 17B three samples were excluded from this figure because they were very different values than the rest of the samples, and excluding them allowed for a better pattern of S_R variation with discharge to be distinguished. Any values below 0.7 or above 1.05 were excluded from the plot, so that any trends with discharge could be observed. However, these data points were still included in all analyses of S_R . There was a range of high and low S_R values at both stormflow and baseflow in the Arboleda and Taconazo (Fig. 17).

Principle components analysis of DOM in the Arboleda and Taconazo streams

A Principal Component Analysis (PCA) was conducted to determine if there was any separation between the Arboleda and Taconazo with respect to DOM properties. (Fig. 19). PCA can explain the variation in a dataset by decomposing the multivariate data into principal components (Bro and Smilde, 2014). Principal Component 1 (PC1) was able to explain 57.47% of the variation in the dataset and PC2 was able to explain 13.95% of the variation in the dataset, thus PCA captured >70% of the variability. PC1 explained the largest

amount of variation in the dataset and PC2 explained the second largest. We included in the PCA Q (discharge), S_R , a_{254} , DOC, $\delta^{13}\text{C}$ -DOC values, and the F_{max} values for all fluorescent components.

PCA showed a separation between the Arboleda and the Taconazo based on DOM quantity and discharge and loadings of the components showed what each principal component represents (Fig. 19). The component loadings are useful because they are comparable to correlation coefficients. The square of the loadings add up to the amount of correlation that is explained by that particular principal component. This means that the loadings also show how much of the given principal component is explained by each of the metrics included in the PCA (Bro and Smilde, 2014). The results of our PCA was consistent with other results from this study, because PC2 showed positive loadings with discharge, and scores for the Arboleda, which has consistently higher discharge than the Taconazo at both baseflow and stormflow, plot closer to loading for discharge than scores for the Taconazo (Fig. 19). DOC concentration and a_{254} were higher at stormflow than at baseflow in both the Arboleda and the Taconazo (Tables 2 and 3). The stormflow samples were more likely to be positive values on the PC1 axis, also showing that DOC concentration, a_{254} , and F_{max} of all six components also tended to be higher at stormflow than at baseflow. Additionally, S_R in the Taconazo was higher at baseflow than at stormflow (Table 4). It is also notable that the loading for $\delta^{13}\text{C}$ -DOC plots closer to the origin than any of the other loadings. Loadings closer to the origin influence the dataset less than points further away, and this is consistent with other study results, demonstrating an insignificant difference in $\delta^{13}\text{C}$ -DOC values between the two streams and at baseflow and stormflow (Fig. 19).

Dissolved Organic Carbon Fluxes:

DOC fluxes were computed to compare the Arboleda and the Taconazo to each other and to other systems. LOADEST, or Load Estimator, (Runkel et al., 2004) was used to calculate fluxes in the Arboleda and in the Taconazo. LOADEST is a FORTRAN program to calculate exports and fluxes in a body of water, given discharge and concentration of the wanted variable (Runkel et al., 2004). LOADEST uses three statistical methods to estimate loads, the Adjusted Maximum Likelihood Estimation (AMLE), the Maximum Likelihood Estimation (MLE), and the Least Absolute Deviation (LAD). These three models are appropriate for different datasets. The first two are best suited for datasets where the residuals of the model are normally distributed. LAD is useful when residuals are not normally distributed. The program uses ordinary least squares regression to estimate loads. In addition, steps are taken to correct for retransformation bias by applying bias correction factors (Runkel et al., 2004). For the Arboleda DOC an estimated annual flux of 8.09×10^6 g C yr⁻¹ was calculated. The carbon export (flux per year divided by watershed area) was 17.52 g C m⁻² yr⁻¹. The estimated annual flux of DOC in the Taconazo was 7.78×10^5 g C yr⁻¹ or an export of 2.79 g C m⁻² yr⁻¹ (Table 6). Additionally, method M5 (Equation 1) from Birgand et al. was used to compute fluxes for the Arboleda and Taconazo as a comparison to LOADEST (2010). This method is thought to be the best annual flux estimate (Birgand et al., 2010). In Equation 1, V is the annual cumulative flow rate (in m³ yr⁻¹), C_i is the concentration measured at the i th day and time (m³ min⁻¹), Q_i is the discharge measured at the i th day and time, and K is a conversion factor to correct for different units.

$$Flux = KV \frac{\sum_{i=1}^n C_i Q_i}{\sum_{i=1}^n Q_i} \quad (\text{Eq. 1})$$

This calculation yielded similar results to calculations made with LOADEST (Table 6). Using Equation 1 we computed a carbon flux in the Taconazo of 9.23×10^5 g C yr⁻¹ and 8.65×10^5 g C yr⁻¹ in 2012 and 2013, respectively. In the Arboleda for 2012 and 2013 we calculated a carbon flux of 1.05×10^7 g C yr⁻¹ and 1.45×10^7 g C yr⁻¹, respectively. Fluxes were higher in the Arboleda due to the larger watershed and but primarily due to the larger discharge, even despite smaller DOC concentrations in this stream. Export was also higher in the Arboleda, which we attributed to the much larger discharge of the Arboleda compared to the Taconazo.

It was important to compute exports because this allowed us to constrain the carbon cycle in these systems to better understand the carbon cycle as a whole. Comparing these results at baseflow and at stormflow allowed us to determine whether storms were as important for DOC export in these systems as they were in others (Lloret et al., 2011, Yoon and Raymond, 2012). It is important to know whether storms are responsible for the largest export of DOC from a system because that can lead to a better understanding of the carbon cycle as a whole and allow for more accurate data in any carbon cycle model.

The DOC export results computed in the present study for the Taconazo were similar to DOC exports from other systems. As a comparison, the Taconazo carbon export values fell within a range seen in other systems, from 0.74 g C m⁻² yr⁻¹ to 6.59 g C m⁻² yr⁻¹, both tropical and temperate. Additionally the Taconazo exported similar amounts of carbon as calculated in a 2006 study of this area. The 2006 Taconazo carbon export was 4.0 g C m⁻² yr⁻¹ (Table 7) (Genereux et al., 2013).

DOC export for the Arboleda was greater than many other studies, including the Q. Sonadora in Puerto Rico, which exported 6.59 g C m⁻² yr⁻¹ (Table 7). However, the carbon

export of the Arboleda was similar to Guadeloupe (French West Indies), particularly during the flood level exports. In Guadeloupe the low water level exports ranged from 0.4 to 1.7 g C m⁻² yr⁻¹ and the flood level exports ranged from 11.3 to 42.2 g C m⁻² yr⁻¹ (Lloret et al., 2011).

DISCUSSION

Groundwater dilution of surface water DOM in the Arboleda and the Taconazo

Differences in the groundwater source to the Arboleda and the Taconazo influenced the surface water DOM. In the Arboleda regional groundwater diluted the DOM in the surface water. As shown in Figures 4 and 5, the Taconazo had higher a_{254} values and DOC concentrations than the Arboleda or Guacimo Spring. The small variation in value of the Guacimo Spring data suggests IGF is stable and provides similar inputs to the Arboleda during all seasons. Additionally, much of the CDOM and DOC largely entered streams during storm events, resulting in CDOM and DOC concentrations in the streams that were much higher than the background levels in the stream during baseflow. Guacimo Spring a_{254} and DOC values were far more similar to the Arboleda a_{254} and DOC than the Taconazo (Figs. 4 and 5). These results support the idea of this regional groundwater (represented by Guacimo Spring) discharging into the Arboleda and being diluted by precipitation and local groundwater (Genereux et al., 2002).

The evidence for mixing of bedrock groundwater and local groundwater was further seen by noting that the Arboleda a_{254} and DOC values fall in between the Taconazo and Guacimo Spring values (Figs. 4 and 5). Creation of mixing models showed that the a_{254} values and DOC concentrations in the Arboleda fell within the triangular mixing space created between the local groundwater (represented by the average chloride concentration and a_{254} or DOC of all Taconazo samples) and the IGF (represented by the average chloride concentration and a_{254} or DOC of all Guacimo Spring samples); therefore, these samples were viewed as a mixture between the local groundwater (Taconazo) and the IGF (Guacimo Spring) (Fig. 12 and 13). These results were consistent with work done in the past; previous

results demonstrated that the DOM in the Arboleda supported the assertion that the Arboleda is a mix between the local groundwater seen in the Taconazo and the regional groundwater seen in the Guacimo Spring (Genereux et al., 2005, Nagy, 2012).

The regional groundwater discharging into the Arboleda contained less DOM than the local groundwater discharging into the Taconazo (Genereux et al., 2009). The DOM concentrations in the Arboleda were also found to be more dilute than the Taconazo in this study. Mean DOC concentration in the Arboleda at baseflow was 68% of the mean DOC concentration in the Taconazo at baseflow; mean a_{254} in the Arboleda at baseflow was 39% of the mean a_{254} value in the Taconazo at baseflow. However, at stormflow the mean DOC in the Arboleda was 131% that of the mean DOC concentration in the Arboleda and the mean a_{254} value in the Arboleda at stormflow was 91% of the mean a_{254} value in the Taconazo at stormflow (Table 2). Thus, the DOM in these streams is clearly being influenced by storm inputs.

Any runoff or precipitation entering the Arboleda or Taconazo would be similar because the characteristics (vegetation, soil type) of the area surrounding the streams were similar. Therefore, we expected the Arboleda and the Taconazo to be more similar in terms of DOM quality at stormflow than at baseflow. However, despite the similarities of the Arboleda and the Taconazo at stormflow we expected that the stormflow samples were more likely to be outliers in the Arboleda than in the Taconazo due to the baseflow concentrations of a_{254} and DOC for the Taconazo being higher than they were for the Arboleda (i.e. due to the dilution of the regional groundwater in the Arboleda). Therefore, any increased stream discharge due to storm inputs with CDOM and DOC would result in a larger change to the Arboleda than to the Taconazo and storms would have a greater impact on the DOM in the

Arboleda than on DOM in the Taconazo. The effect of precipitation was seen when comparing the a_{254} values and DOC concentrations with discharge (Fig. 11). For the Taconazo DOC concentrations, more outliers occurred during baseflow than during stormflow. The Taconazo had a more extreme cyclical pattern of discharge which could also explain why there were more outliers at baseflow than at stormflow (Figs. 4 and 5).

Five components of the PARAFAC model fit to all Arboleda and Taconazo samples (including stormflow and baseflow samples) showed sources that are expected in this system (Fig. 7). We modeled a component (C1) ubiquitous to freshwater streams (Osburn et al., 2016, Kowalczyk et al., 2009). C2 was indicative of reprocessed terrestrial material and has been seen in other tropical systems, such as the Zambezi River Basin (Lambert et al., 2016). It is likely that soil is being leached of organic matter, particularly during storms, and this would contribute to the fluorescence of these water samples as a whole. C3 was identified as a terrestrial, humic-like or soil component (Murphy et al., 2014, Yamashita et al., 2010). The fifth and sixth components were also seen in previous studies and were associated with protein-like, amino acid fluorescence (Kowalczyk et al., 2009, Yamashita et al., 2010) (Table 5). However, it is noted that this protein-like fluorescence, particularly C5, also can overlap with hydroxylated aromatic compounds such as tannins, which might indicate recent plant material rather than autochthonous production (Maie et al. 2007; Hernes et al. 2009).

Typically the protein-like or amino acid-like fluorescence seen in C5 is associated with primary production, but there currently exists ambiguity in the literature concerning the chemical identity of this component. Previous work has found that protein-like fluorescence decreases during stormflow in upland streams and that during storms upland streams receive a large input of humic-like material; however in wetlands protein-like fluorescence increases

during storm events (Fellman et al., 2009). Past studies suggested that protein-like fluorescence can represent a variety of sources but in terrestrial-dominated systems the protein-like fluorescence could derive from tannin. However, because we found an increase during stormflow (Figs. 14, 15, and 20) and there is a concentration of protein-like fluorescence in the leaf drip and bromeliad EEM (Fig. 21), it is likely that the protein-like fluorescence represented fresh land plant material. The leaf drip EEM (Fig. 21A) more closely resembled the Taconazo baseflow and the Taconazo and Arboleda stormflow EEMs in intensity (Fig. 6B-D). The bromeliad sample was visibly colored like brewed tea, the latter of which is dominated by tannins.

The so-called protein-like fluorescence is indicated in Figure 21 with red boxes and while a less noticeable feature than the humic fluorescence in the leaf drip (Fig. 21A) or bromeliad (Fig. 21B) DOM, its magnitude still was far greater than the magnitude of fluorescence in either stream's baseflow EEM. Therefore, we believe C5 represents a fresh tannin-like signature that is far more consistent with our hydrologic results. This result is consistent with land plant primary production, even if there is ambiguity between its chemical origin (i.e. protein or tannin). We hypothesized that this increase in C5 was specifically due to an input of throughfall. Throughfall is rain that falls through the canopy and can be different from rainfall because it could wash away DOM from leaves. We expected that an increase in C5 during stormflow would be caused by increased input from throughfall and we observed an increase in C5 during stormflow in Figures 14, 15, and 20), along with an increase in the other components as well. We also observed higher F_{max} values of all components with higher discharges, although the relationship was not linear

(Figs. 22 and 23). This increase makes sense during storm events because the first three components were identified as terrestrial humic, with the third one including a soil signal.

We also observed an increase from baseflow to stormflow in the absorption spectra of the data (Fig. 18). Guacimo Spring has the lowest absorption and the Arboleda at baseflow is the most similar to the absorption values of Guacimo Spring. Additionally the Arboleda and the Taconazo both had increased CDOM at stormflow as opposed to baseflow (Fig. 18). This is similar to the results seen in fluorescent DOM (Fig. 6) where the intensity of the fluorescence was greater in the Taconazo at baseflow than in the Arboleda at baseflow, while both streams increased in fluorescence intensity at stormflow. Thus we had good agreement between CDOM absorption and fluorescence indicating the qualitative similarity of the stream DOM during stormflow and again supporting our assertion that throughfall is a key input of DOM to these streams during storm events. Another input of DOM during storms could be stemflow, which is precipitation that was traveled down the stem of a plant and has the potential to remove different DOM from the plant than throughfall alone.

Comparisons with Discharge

We found that the Arboleda and the Taconazo were more similar at stormflow than at baseflow. There did not seem to be a clear separation of DOC concentration between baseflow and stormflow in the Taconazo, especially as compared to the Arboleda (Fig. 11). Also, there was not a clear distinction between baseflow and stormflow DOC concentration in the Taconazo as there was in the Arboleda, which led to the conclusion that the local groundwater DOC was likely higher and more variable in the Taconazo than in the Arboleda.

Figure 11 supported these results, as we observed a much more variable background DOC concentration in the Taconazo than in the Arboleda.

An ANOVA with Ranks (Dunn's Test) was also completed on many of the metrics we used in this study to determine statistical differences between the streams and to incorporate stormflow versus baseflow (Table 3. In this case, a_{254} values and DOC values were compared (respectively) between the Arboleda at baseflow, the Arboleda at stormflow, the Taconazo at baseflow, and the Taconazo at stormflow. A $P < 0.05$ was considered a statistically significant difference. Results showed a statistically significant difference of DOC concentration and a_{254} values between baseflow and stormflow in the Taconazo, baseflow and stormflow in the Arboleda, and the Arboleda and Taconazo at baseflow.

Due to the input of throughfall and runoff into both the Arboleda and the Taconazo during stormflow, we expected that the differences in DOM concentration between the groundwaters discharging into the Arboleda and Taconazo would be reduced, in effect making these streams more similar in terms of DOM. As expected, there was not a significant difference between the Taconazo at stormflow and the Arboleda at stormflow. These results led to the conclusion that again, the Arboleda and Taconazo are far more similar when the influence of regional groundwater is minimized.

The similarities in DOM between the streams occurred when both the Arboleda and Taconazo were at stormflow. Additionally, the Arboleda at stormflow is similar to the Taconazo at baseflow, suggesting that inputs to the Taconazo due to local groundwater were potentially similar to any runoff, precipitation, or throughfall that enters the streams during stormflow. Similarities between the Taconazo at baseflow and stormflow are expected

because the local groundwater was fed by the same precipitation and throughfall that the streams are fed by during storm events.

Figure 16 lends support to the idea of throughfall heavily influencing these streams during storm events because F_{\max} of C5 did increase during storm events, a time in which little primary production in the stream would be occurring. It was interesting that there was an increase in baseflow F_{\max} values of these components during a drier period (Fig. 15). This increasing of baseflow DOM in the Taconazo during a dry period may have been due to increasing DOM concentrations in groundwater during drier periods. As this pattern was not seen in the Arboleda, it suggested that the Arboleda was better able to buffer against local changes due to the very consistent input of regional groundwater that is absent in the Taconazo. The F_{\max} values of all six components increase during stormflow due to more FDOM in the streams during storms (Fig. 20).

An ANOVA Test with Ranks (Dunn's Test) shows that there was a statistically significant difference ($P < 0.05$) in F_{\max} C1, C2, C3, C4, and C5 between the Arboleda at stormflow and the Arboleda at baseflow, between the Taconazo at baseflow and the Taconazo at stormflow, and at the Taconazo at baseflow and the Arboleda at baseflow. However, there was not a significant difference between the Taconazo at stormflow and the Arboleda at stormflow (Table 6). These results showed that the Arboleda and the Taconazo were more similar to each other with regard at stormflow than at baseflow. These results supported the idea of precipitation and throughfall being a large influence on C5, because for a variety of DOM qualitative parameters, no significant differences were found between the Arboleda and the Taconazo at stormflow. Therefore, C5 has the potential to be used as a throughfall tracer in this environment.

A slightly different result was found doing an ANOVA on C6 F_{max} values in these instances. There was not a significant difference between the Taconazo at stormflow and the Arboleda at stormflow or between the Taconazo at stormflow and the Taconazo at baseflow. Our ANOVA results also strongly support the conclusion that the Taconazo and Arboleda are very similar to each other at stormflow. Overall this suggests a very different quality of organic matter that dominates these systems during stormflow as opposed to baseflow.

We expected that isotope values would be enriched with increasing discharge based on the general isotope value of soils because these isotope values are typically enriched relative to C3 plant material isotope values (Bianchi and Canuel, 2011). It is logical to expect that during precipitation events, plant material was more likely to contribute to the DOC due to throughfall and stemflow contributions to overland flow and surface runoff into streams. A large throughfall component was expected in time periods of high discharge. However, there appeared to be a great deal of scatter in this relationship and so it was difficult to distinguish any trends. Previous research into the $\delta^{13}\text{C}$ values of *Peperomia*, a common plant species in La Selva, found $\delta^{13}\text{C}$ -DOC values of -22.3 to -33.8‰ (Ting et al., 1985). The variation in $\delta^{13}\text{C}$ -DOC within the same species could be due to some natural variation between plants of the same species or these values could change seasonally (Tieszen, 1991). Another study, which encompassed a wider variety of plant species, was conducted in Guyana, a rainforest country similar to the environment at La Selva (Hammond et al., 2005). That study found $\delta^{13}\text{C}$ -DOC values of -25.9 to -30.0‰ for fresh plant material. Therefore, the heterogeneity of plant types that are contributing material to the streams appears to explain the underlying variability in our $\delta^{13}\text{C}$ -DOC values. While the heterogeneity was surprising, it indicates a very strong linkage between terrestrial primary production (plant tissues) and the fluvial

carbon cycle in tropical watersheds. Further work is needed to constrain the isotopic variability of stemflow and throughfall as well as resulting effects on stream DOM values.

There did not seem to be as clear of a separation between the isotope values between baseflow and stormflow, or between the Arboleda and the Taconazo as was observed for CDOM absorbance and fluorescence. In fact, an ANOVA on Ranks (Dunn's Test) determined that there was no statistically significant difference between the $\delta^{13}\text{C}$ -DOC values. Further, no relationship existed between $\delta^{13}\text{C}$ -DOC values and discharge. This result could be due to the aforementioned control on $\delta^{13}\text{C}$ -DOC values by tissue type and plant physiology, especially if throughfall and stemflow are important controls on DOM in these streams rather than soil organic matter. PC2 showed positive loadings of $\delta^{13}\text{C}$ -DOC with discharge, which would indicate that enriched isotope values occur during high stream flow (Fig 14).

Moreover, the lack of a relationship of $\delta^{13}\text{C}$ -DOC values with discharge or differences between baseflow and stormflow could simply mean that the entire system is dominated by relative amounts of the same source material. For example, our $\delta^{13}\text{C}$ -DOC values cover the range of values expected for fresh land plant material. This is a wide range from -24 to -34‰. Fresh plant litter likely is quickly metabolized in humid tropical environments hence isotopic signals may shift much more quickly than do CDOM signals. Isotope values only inform on the source of material rather than its concentration. Hence, if the source of the material was relatively constant, then no clear trends between the streams would have been determined. Moreover, the general lack of DOM in Guacimo Spring meant establishing its $\delta^{13}\text{C}$ -DOC value was very difficult. Thus, presence of just one source signal (tropical rainforest vegetation) explains the lack of a pattern with our isotope results. The

input of throughfall and stemflow into these streams would be similar between streams and the similar isotope values among tropical rainforest vegetation make it likely that we would be unable to differentiate a source. Given that these systems likely have a large throughfall component, it makes sense that the isotope values would be similar and that the DOC in the stream is representative of fresh throughfall and stemflow material.

Even though a statistical difference in source could not be elucidated using the isotope values, differences in aromaticity could still be used to inform ideas about the nature of the OM found in the streams, likely based on the nature of the tropical plant litter reaching the streams. The average values for S_R of the Taconazo at stormflow were 0.84 ± 0.38 and at baseflow 0.80 ± 0.18 . For the Arboleda at stormflow the average S_R was 0.97 ± 0.15 and at baseflow 1.07 ± 0.14 (Table 4). These values fell within the range of those seen in other systems. Helms et al. (2008) found a range of 0.5 to 2.5 in systems ranging from the Great Dismal Swamp, Suwannee River, and the Atlantic Ocean. Lower S_R values were found in the Great Dismal Swamp and higher values were found in the rivers (Helms et al., 2008). Flushing events in Alaska resulted in a lower S_R , values of 0.79-0.86 (Spencer et al., 2009). Larger S_R values tended to indicate low molecular weight CDOM and smaller values indicated high molecular weight CDOM, which was likely allochthonous (Helms et al., 2008). Our results suggested that the Arboleda DOM had a higher molecular weight than the DOM in the Taconazo.

In addition to the Arboleda having higher S_R values during baseflow and stormflow (Fig. 17), the values also varied more than in the Taconazo. The larger variability of S_R in the Arboleda has been suggested to represent the mixing of regional groundwater and local groundwater in the Arboleda, but this mixing of two groundwaters does not occur in the

Taconazo; therefore, the range and variation of values is less (Genereux et al., 2013). These results were consistent with their assessment of mixing in the Arboleda.

The $SUVA_{254}$ results were consistent with the results from S_R values. The Arboleda had lower $SUVA_{254}$ values than the Taconazo, meaning that the Taconazo samples were more aromatic. For the Arboleda in stormflow, the average $SUVA_{254}$ was $2.00 \pm 1.00 \text{ L mg}^{-1} \text{ C m}^{-1}$ and at baseflow it was $1.52 \pm 0.84 \text{ L mg}^{-1} \text{ C m}^{-1}$. For the Taconazo at stormflow the average was $2.42 \pm 2.42 \text{ L mg}^{-1} \text{ C m}^{-1}$ and at baseflow it was $2.14 \pm 0.95 \text{ L mg}^{-1} \text{ C m}^{-1}$ (Table 4). Both streams increased in aromatic C during stormflow, suggesting that much of the aromatic C entered the streams during storm events. Additionally, this result supported the hypothesis that the Arboleda and the Taconazo are more similar to each other during stormflow than they are during baseflow. Further, the $SUVA_{254}$ and S_R values support the idea that more distinct difference in the optical properties of DOM were found as compared to the stable C isotope values of DOM.

An ANOVA also was performed on the $SUVA_{254}$ and S_R results (Table 3). For S_R , it was determined that there was a statistically significant difference between Arboleda baseflow and Taconazo baseflow, and Arboleda stormflow and Taconazo stormflow. There was not a significant difference between the Arboleda baseflow and Arboleda stormflow or the Taconazo at stormflow and Taconazo at baseflow. It is of interest to note that there was a significant difference between the Arboleda and the Taconazo at stormflow. The S_R results were in contrast to results using $SUVA_{254}$, despite both S_R and $SUVA_{254}$ being proxies for aromaticity.

The ANOVA results for $SUVA_{254}$ values showed that there was a significant difference between the Arboleda at baseflow and the Taconazo at baseflow (Table 3).

However, there was not a statistically significant difference between the Taconazo at stormflow and the Arboleda at stormflow or between the Arboleda at stormflow and the Arboleda at baseflow. $SUVA_{254}$ results thus indicated that the aromaticity of the DOM at stormflow in the streams is very similar. These results supported the assertion that more DOM is likely mobilized and entered the streams due to storm events and this DOM is likely to be very similar, or the same, in both streams.

In other streams, $SUVA_{254}$ values have been shown to increase with stormflow by 9-36% (Hood et al., 2006). In the Arboleda and the Taconazo $SUVA_{254}$ increased by about 20%, within the range shown by (Hood et al., 2006). The $SUVA_{254}$ values in the Arboleda and the Taconazo also fell within a range of values seen in other environments, $1.09 \text{ L mg}^{-1} \text{ C m}^{-1}$ in the Midwestern United States (Vidon et al., 2008) to $3.44 \text{ L mg}^{-1} \text{ C m}^{-1}$ in the Arctic (Mann et al., 2015). Vidon et al. (2008) also showed an increase of $SUVA_{254}$, and therefore DOM aromaticity, during storms, which is what was observed in the Arboleda and the Taconazo.

PCA was able to separate the Arboleda and the Taconazo streams based on DOM quality, quantity, and discharge (Fig. 19). Along PC1 loadings for F_{\max} , DOC, and a_{254} were positive and the storm samples trended closer to these loadings as well, indicating the mobilization of organic matter into the streams during storms. Along PC2, positive loadings for discharge corresponded to positive loadings for S_R (Fig. 19). The Arboleda and Taconazo samples were separated based on discharge. Additionally, the Arboleda Baseflow samples appeared to contain be the most autochthonous DOM, indicated by these values trending closest to the S_R loading. This supports the idea that terrestrial material is exported to the streams during stormflow. It is interesting to note that the Taconazo samples do not separate

as much along stormflow and baseflow as the Arboleda samples do. This is likely due to the regional groundwater in the Arboleda heavily influencing the baseflow DOM quality of this stream (Fig. 19).

Carbon Fluxes

Finally, we compared carbon export of these streams relative to each other and to other systems. Fluxes and export of DOC were found to be higher in the Arboleda than in the Taconazo (Table 6). We attributed this result of higher C flux to the larger watershed and larger discharge of the Arboleda, and the larger export to the larger volume of water in the Arboleda.

To assess the influence of storms on DOC flux we sampled three storms. These storm samples were collected every hour for 12 hours on three days in June 13, 2013, June 14, 2013, and June 25, 2013 (Table 8). The M5 method was used to compute storm fluxes (Equation 1). However, in this instance K represented the volume of water discharged per storm. In the Arboleda the estimated fluxes for these dates were 2.37×10^4 g C storm⁻¹, 7.91×10^4 g C storm⁻¹, and 5.83×10^4 g C storm⁻¹. All three storms are have a higher estimated flux (in only 12 hours) than the average daily flux of 2.22×10^4 g C d⁻¹. In the Taconazo these fluxes were 2.38×10^3 g C storm⁻¹, 1.98×10^4 g C storm⁻¹, and 5.66×10^3 g C storm⁻¹, respectively. One of these values was lower than the average daily flux of 2.13×10^3 g C d⁻¹; however, the storm samples were collected over a period of 12 hours, suggesting that precipitation and runoff inputs did increase carbon fluxes for the Taconazo and the Arboleda beyond what would be expected in a single day.

The paradox of DOC flux results was also shown if we visually examine the DOC concentration and discharge (Fig. 11B). The baseflow DOC is higher in the Taconazo than in the Arboleda and the stormflow DOC does not spike in the Taconazo as it does in the Arboleda (Fig. 11). Figure 11 suggested that in the Taconazo the local groundwater had much more of an impact on DOC concentration than precipitation and runoff inputs do, however, in the Arboleda storm flow inputs increase the DOC concentrations much more than the groundwater does. However, there is a positive correlation of discharge and DOC concentration in the Arboleda and the Taconazo (Fig. 9B and 10B). Additionally, an increase in POC during stormflow was seen in previous work done at this site (Rojas-Jimenez, 2014). Previous work done in this system calculated a POC export of $9.82 \times 10^{-3} \text{ kg C m}^{-2} \text{ yr}^{-1}$ in the Arboleda and $1.43 \times 10^{-3} \text{ kg C m}^{-2} \text{ yr}^{-1}$ in the Taconazo (Rojas Jimenez, 2014).

CONCLUSIONS

The major conclusions of this study are:

- 1) The main differences in OM between the tropical streams in this study at baseflow were that a_{254} values in the Taconazo were 61% higher than in the Arboleda and the DOC concentrations in the Taconazo were 32% higher than in the Arboleda, both statistically significant differences. Moreover, the Taconazo also had 29% more aromatic DOM than did the Arboleda. However, there was no statistically significant difference between the DOM concentrations in the Arboleda and the Taconazo at stormflow.
- 2) With respect to DOM, the Arboleda and the Taconazo were more similar to each other at stormflow than at baseflow. However, there was still a significant difference found in S_R of the two streams at stormflow. Stormflow exerted clear changes on the quality of DOM in streams by mobilizing more aromatic DOM and increasing the concentration of DOM. The mean Arboleda a_{254} values increased from 2.62 m^{-1} at baseflow to 10.22 m^{-1} at stormflow and the Taconazo increased from 6.72 m^{-1} at baseflow to 11.22 m^{-1} at stormflow. The source of the DOM in the Arboleda and Taconazo at stormflow and baseflow was very similar, as evidenced by $\delta^{13}\text{C}$ -DOC values showing no statistically significant difference at flow regime or between streams. We believe this is evidence of stronger linkages of plant tissue, as opposed to soil organic matter, to DOC and therefore that the DOC in the stream is comprised primarily of fresh throughfall and stemflow.
- 3) Differences between the Arboleda and the Taconazo showed that regional groundwater was very influential in the amount of carbon exported in surface water

stream systems ($23.96 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Arboleda as compared to $3.06 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Taconazo); streams with lower DOC concentrations can have a comparatively large flux of carbon and export more C than streams with a higher DOC concentration or larger streams.

There were differences at baseflow in the quality of the OM in the Arboleda compared to the Taconazo. The OM in the Taconazo was 29% more aromatic than the OM in the Arboleda. DOC export out of the Arboleda was 8x greater than the DOC export out of the Taconazo, attributed to the larger discharge of the water in the former stream compared to the latter stream and resulting from IGF.

DOM in Arboleda and the Taconazo does appear to become more similar during stormflow than during baseflow. Among the metrics used to measure DOM quantity and quality of the Arboleda and Taconazo such as: a_{254} , DOC, and F_{max} of C1, C2, C3, C4, C5, C6, and SUVA_{254} , none were significantly different. However, at baseflow there was a statistically significant difference between these metrics and between the S_R values in the Arboleda and the Taconazo. These results were due to the influence of low-DOC, degraded, and thus optically-clear regional groundwater in the Arboleda that dilutes the largely terrestrial signals in that stream. By taking the paired watershed approach as was done in this study, these differences could be elucidated. The impact of this low-DOM groundwater was greater at baseflow than it was at stormflow when other inputs (e.g., throughfall and/or runoff) were also contributing to the discharge of the stream.

Clearly, streams can be influenced by the underlying groundwater, especially in terms of the impact on the amount of carbon exported from their watersheds. Future studies should

consider groundwater-surface water interactions when determining carbon export from watersheds. It has been shown in this study that stream DOM quality as well as quantity can be greatly affected by a groundwater-surface water connection. Our values for DOC export match previous work in this study site: $4.0 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Taconazo and $14.1 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the Arboleda (Genereux et al., 2013). This can be compared to exports of 3.06 and $23.96 \text{ g m}^{-2} \text{ d}^{-1}$ in the Taconazo and Arboleda calculated in this study. Despite the Arboleda and Taconazo being relatively small watersheds, the carbon export of these streams exceeds the carbon export of some of the larger mountainous streams in New Zealand, with carbon exports of 1.1 to $5.2 \text{ g C m}^{-2} \text{ d}^{-1}$ (Carey et al., 2005). These samples represent a temperate environment, with the lowest carbon export indicative of almost no indigenous vegetation loss and the highest carbon export indicative of nearly 100% indigenous vegetation loss and replaced by agriculture or other vegetation (Carey et al., 2005). The Arboleda carbon export also exceeded that of other tropical and temperate streams, beyond just those in New Zealand (Table 7).

In contrast to groundwater, there was evidence that throughfall could contribute a large amount of DOM to a stream system. In stream ecosystems with sufficient canopy in their watersheds, the connection of carbon biogeochemistry to forest dynamics may be stronger than previously realized by considering the effect of DOM inputs from throughfall in addition to regional groundwater. Both clearly need consideration.

Future Directions

The role of throughfall on DOM biogeochemistry and cycling in these and other forested stream ecosystems should be examined further. This study showed that the difference in regional groundwater between the Arboleda and the Taconazo played a huge

role in carbon fluxes; therefore, future considerations of stream DOM should include an investigation of the groundwater. There was some evidence that throughfall has a large contribution to DOM in the stream. High-resolution data, such as Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), a technique that allows for the determination of compounds in DOM (Mopper et al., 2007), should be used as a technique to further study these systems because it can be used to further determine similarities and differences between the DOM in these three sample sites.

A comparison of DOM in precipitation, throughfall, and stem flow could further elucidate OM sources to the Arboleda and Taconazo. Experiments were conducted toward the end of the work for the present study to capture both throughfall and stemflow. Future work at La Selva and elsewhere should quantify the carbon input to streams due to these processes. It is highly anticipated that the quality of DOM contributed both in throughfall and stemflow are highly bioavailable and contribute to the carbon demand in stream ecosystems as well as to the CO₂ fluxes from them. The DOM in throughfall and stemflow should be studied; depending on the characteristics of this DOM compared to the stream DOM we could determine whether or not it is likely that throughfall and stemflow DOM make it into the streams. The Arboleda and Taconazo streams are uniquely appropriate settings to investigate this new frontier of stream carbon biogeochemistry.

Soil-leaching experiments could be done to study soil OM inputs to streams in these systems, where storms are so frequent. One of the key uncertainties in this study was how soil organic horizons in contact with local groundwater were influencing the DOM quantity and quality in these streams. It was beyond the scope of this study to investigate soil DOM properties, but clearly a focus on soil DOM biogeochemistry will clarify perhaps the

variability observed in DOM properties both as stormflow and at baseflow as evidenced by the scatter in results presented in the mixing model analysis.

Finally, this study has shown the importance of high resolution monitoring of surface water properties of CDOM absorption and fluorescence as proxies for DOM biogeochemistry because we can see the change in DOM between stormflow and baseflow. Future work in this and other regions could integrate a variety of geospatial analyses of hydrology with CDOM properties, incorporating remotely sensed information with carbon biogeochemistry information. For example, geographical information systems (GIS) could possibly be used to assess the canopy flux of DOM via throughfall and stemflow based on canopy cover and tree community composition and soil properties. In stream measurements of CDOM optical properties are now being used to quantify DOM biogeochemistry (Etheridge et al. 2014). Together these observational platforms promise important advances in understanding stream biogeochemistry within the context of regional to global carbon cycling.

TABLES

Table 1: Summary of samples collected and used in this study at the Arboleda and Taconazo watersheds at LSBS, Costa Rica.

	Frequency	Start date	End date
Automated stream discharge	15 minutes	1 Jan 2012	14 Mar 2014
Absorbance	Weekly	30 Jan 2012	14 Mar 2014
Fluorescence	Weekly	30 Jan 2012	14 Mar 2014
Dissolved organic carbon (DOC)	Weekly	30 Jan 2012	14 Mar 2014
Stable isotope data ($\delta^{13}\text{C}$)	Weekly	30 Jan 2012	14 Mar 2014
Storm sampling (Absorbance, fluorescence, DOC, isotopes)			
Storm 1	Every hour for 12 hours	13 Jun 2013	14 Jun 2013
Storm 2		14 Jun 2013	15 Jun 2013
Storm 3		25 Jun 2013	25 Jun 2013

Table 2: Mean, standard deviation, median, minimum, and maximum of a₂₅₄ and DOC for the following four categories: Arboleda at baseflow, Arboleda at stormflow, Taconazo at baseflow, and Taconazo at stormflow.

		Arboleda Baseflow	Arboleda Stormflow	Taconazo Baseflow	Taconazo Stormflow
a₂₅₄	Mean	2.62	10.22	6.72	11.22
	Standard Deviation	1.23	7.20	1.76	5.44
	Median	2.23	9.39	6.25	9.71
	Minimum	1.65	1.86	4.17	2.27
	Maximum	9.93	25.95	14.25	23.06
DOC	Mean	1.26	3.08	1.84	2.36
	Standard Deviation	1.51	2.80	1.51	1.16
	Median	0.83	2.04	1.26	2.25
	Minimum	0.22	0.45	0.52	0.65
	Maximum	9.25	11.52	8.25	5.81

Table 3: Summary of ANOVA (Dunn’s tests) conducted and whether or not there was a significant difference between the two sets compared. A blank cell is indicative of no significant difference

	Arboleda Baseflow vs. Arboleda Stormflow	Taconazo Baseflow vs. Taconazo Stormflow	Arboleda Baseflow vs. Taconazo Baseflow	Arboleda Stormflow vs. Taconazo Stormflow
a ₂₅₄	Significant	Significant	Significant	
DOC	Significant	Significant	Significant	
F _{max} C1	Significant	Significant	Significant	
F _{max} C2	Significant	Significant	Significant	
F _{max} C3	Significant	Significant	Significant	
F _{max} C4	Significant	Significant	Significant	
F _{max} C5	Significant	Significant	Significant	
F _{max} C6	Significant		Significant	
SUVA ₂₅₄			Significant	
S _R			Significant	Significant

Table 4: Mean, standard deviation, median, minimum, and maximum of SUVA₂₅₄, SR, and $\delta^{13}\text{C}$ -DOC for the following four categories: Arboleda at baseflow, Arboleda at stormflow, Taconazo at baseflow, and Taconazo at stormflow.

		Arboleda Baseflow	Arboleda Stormflow	Taconazo Baseflow	Taconazo Stormflow
SUVA₂₅₄	Mean	1.52	2.00	2.14	2.42
	Standard Deviation	0.84	1.00	0.95	1.07
	Median	1.36	2.03	2.12	2.45
	Minimum	0.46	0.47	0.44	0.31
	Maximum	3.56	4.04	4.74	5.36
SR	Mean	1.07	0.97	0.80	0.84
	Standard Deviation	0.14	0.15	0.18	0.38
	Median	1.05	0.93	0.77	0.78
	Minimum	0.85	0.81	0.73	0.73
	Maximum	1.56	1.49	2.08	3.92
$\delta^{13}\text{C}$-DOC	Mean	-27.83	-26.78	-27.69	-27.07
	Standard Deviation	4.03	2.82	3.42	2.53
	Median	-27.58	-26.69	-27.72	-27.15
	Minimum	-36.98	-32.81	-34.51	-34.39
	Maximum	-21.04	-21.78	-21.65	-21.20

Table 5: PARAFAC Component, maximum excitation and emission, number of models matched on OpenFluor, source of fluorescence, and other environments where the component has been found.

	Ex/Em Maxima	Matches	Probable Source	Matched Models
C1	240/442	10	Terrestrial, humic-like	North American Estuaries ¹ , South Atlantic Bight ²
C2	240/400	13	Reprocessed, terrestrial, humic-like	North American Estuaries ¹ , Coastal and Shelf Seas ³ , Zambezi River Basin ⁴
C3	245/504	17	Terrestrial, soil fulvic-like	Costal and Shelf Seas ³ , Tropical Rivers in Venezuela ⁵ , Florida Keys ⁶
C4	240/438			
C5	270/302	14	Protein-like, amino-acid	Florida Keys ^{6,7}
C6	240/350	2	Protein-like	South Atlantic Bight ²

¹ Osburn, C.L., Boyd, T. J., Montgomery, M. T., Bianchi, T. S., Coffin, R. B, and Paerl, H. W.(2016) Optical Proxies for Terrestrial Dissolved Organic Matter in Estuaries and Coastal Waters. *Front. Mar. Sci.* 2:127. doi: 10.3389/fmars.2015.00127

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³ Murphy, K.R., Bro, R., Stedmon, C.A., 2014, Chemometric analysis of organic matter fluorescence. in: Coble, P., Baker, A., Lead, J., Reynolds, D., Spencer, R. (Eds.). *Aquatic organic matter fluorescence*. Cambridge University Press, New York.

⁴ Lambert T., Teodoru C. R., Nyoni F., Bouillon S., Darchambeau F., Massicotte, P., and Borges A.V., 2016, Along-stream transport and transformation of dissolved organic matter in a large tropical river, *Biogeosciences*, 13, 2727-2741.

⁵ Yamashita, Y., Maie, N., Briceno, H., Jaffe, R. 2010. Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. *J Geophys Res* 115, G00F10.

⁶ Yamashita, Y., Scinto, L.J., Maie, N., Jaffe, R., 2010. Dissolved Organic Matter Characteristics Across a Subtropical Wetland's Landscape: Application of Optical Properties in the Assessment of Environmental Dynamics. *Ecosystems* 13, 1006-1019.

⁷ Yamashita, Y., Boyer, J.N., Jaffe, R. 2013. Evaluating the distribution of terrestrial dissolved organic matter in a complex coastal ecosystem using fluorescence spectroscopy. *Continental Shelf Research* 66, 136-144

Table 6: Carbon export for the Arboleda and Taconazo computed using LOADEST and the M5 method for 2012 and 2013

	LOADEST Export	2012 M5 Export	2013 M5 Export	Average Export
Arboleda	17.53	22.85	31.51	23.96
Taconazo	2.79	3.3	3.1	3.06

Table 7: Table of Carbon export comparisons from this study, a previous study in these watersheds, and other locations (tropical and temperate).

Site	Export gC m ⁻² yr ⁻¹
Arboleda (this study)	23.96
Taconazo (this study)	3.06
Arboleda (2006 study)	14.10
Taconazo (2006 study)	4.00
Deshaies (French West Indies)	1.60
Q. Sonadora (Puerto Rico)	6.59
Juruena headwaters (Brazil)	3.15
Big Hollow Creek (Ohio)	4.40
Satellite Branch (Charlotte, NC)	0.74

Table 8: Summary of estimated carbon flux from each storm sampled

	Arboleda Flux (gC storm ⁻¹)	Taconazo Flux (gC storm ⁻¹)
Storm 1	2.37×10^4	2.38×10^3
Storm 2	7.91×10^4	1.98×10^4
Storm 3	5.83×10^4	5.66×10^3

FIGURES

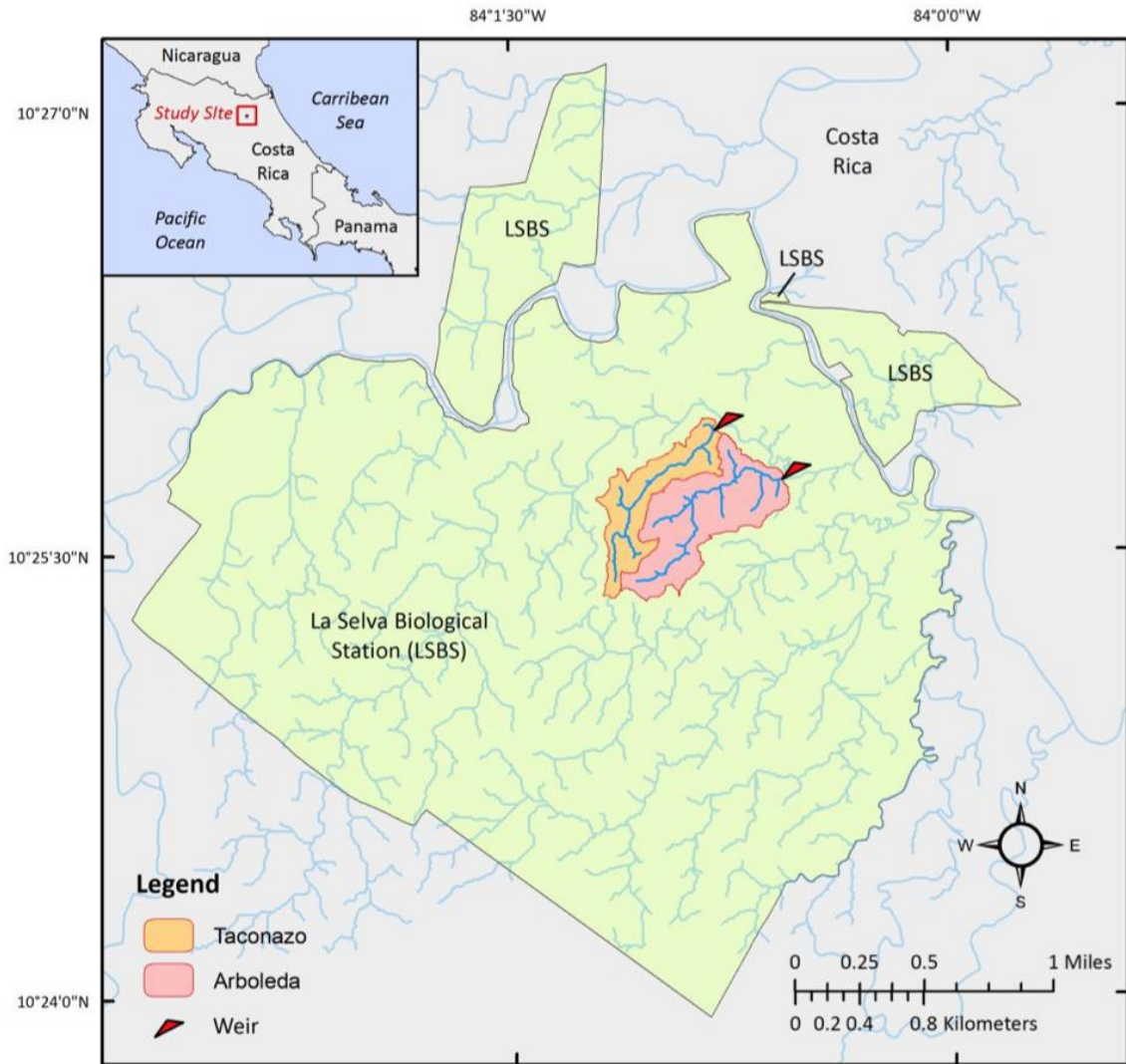


Figure 1: Map of study site at La Selva Biological Station showing location of the Taconazo and the Arboleda watersheds (from Nagy, 2012). Guacimo Spring is located south of La Selva Biological Station.

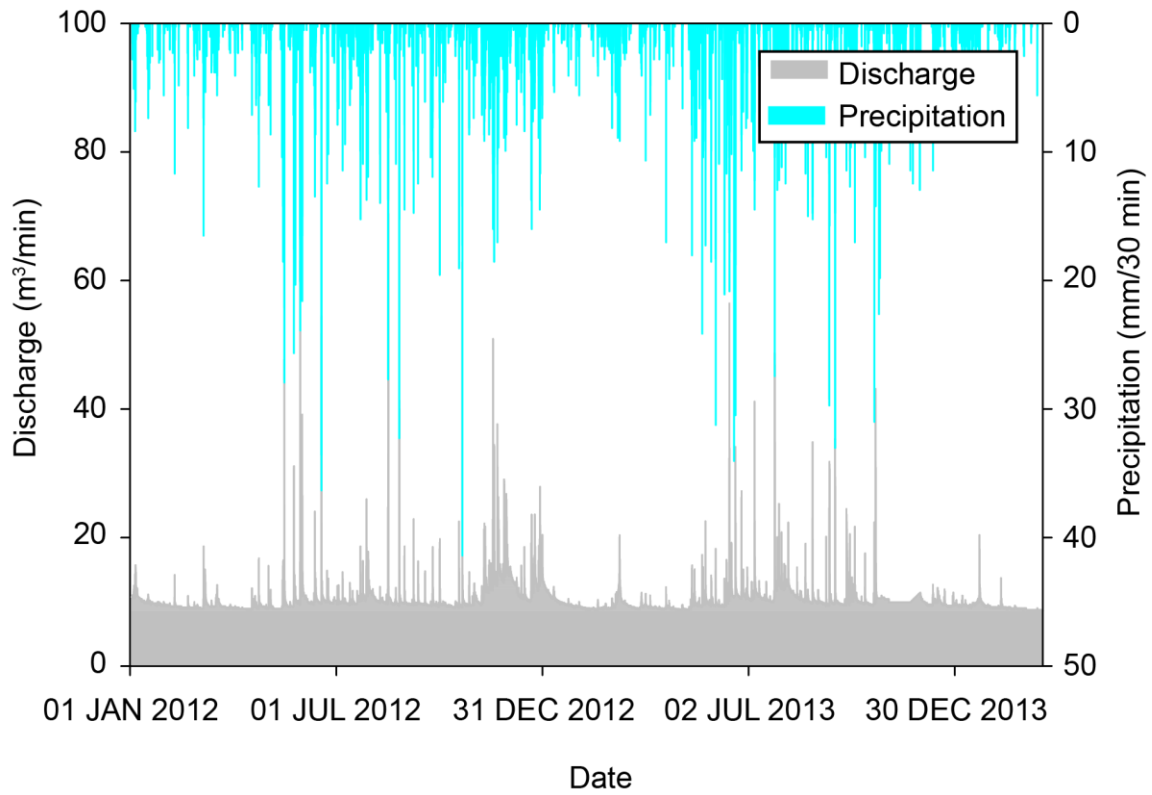


Figure 2: Discharge in $\text{m}^3 \text{min}^{-1}$ for the Arboleda stream (data from D.P. Genereux) and precipitation in mm per 30 min (data from La Selva Biological Station, <http://www.ots.ac.cr/meteoro/default.php?pestacion=2>).

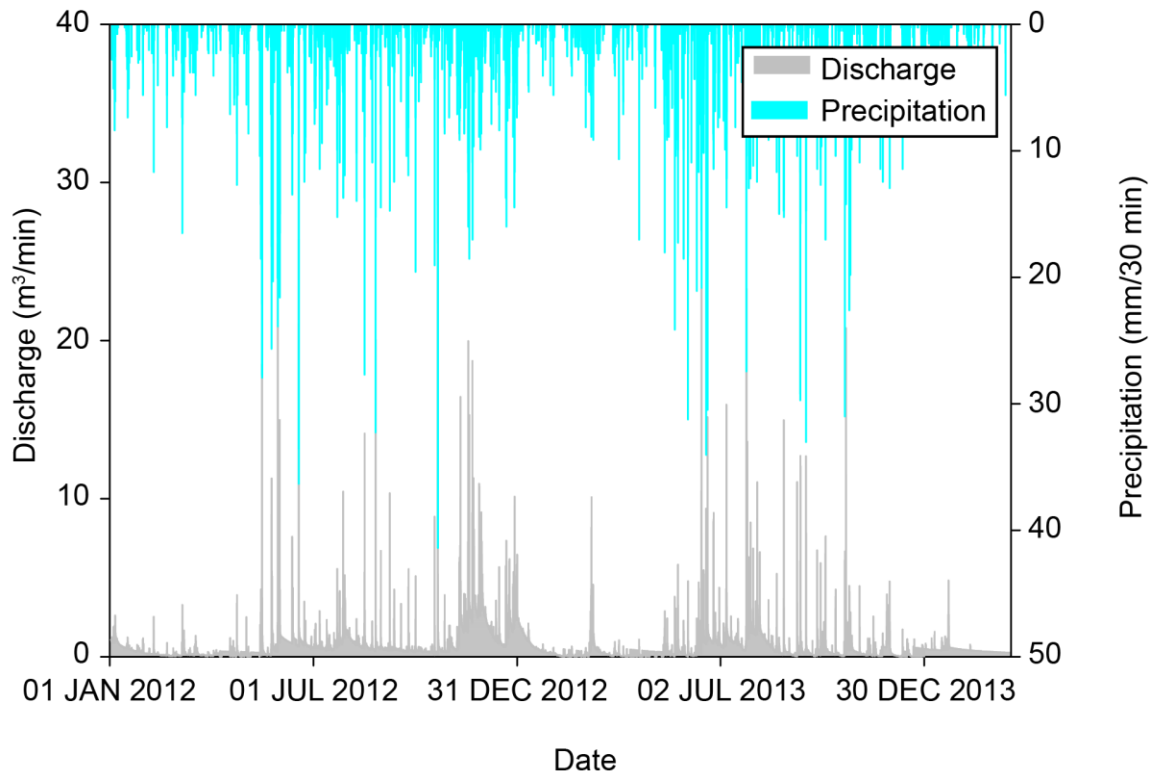


Figure 3: Discharge in $\text{m}^3 \text{min}^{-1}$ for the Taconazo stream (data from D.P. Genereux) and precipitation in mm per 30 min (data from La Selva Biological Station, <http://www.ots.ac.cr/meteoro/default.php?pestacion=2>).

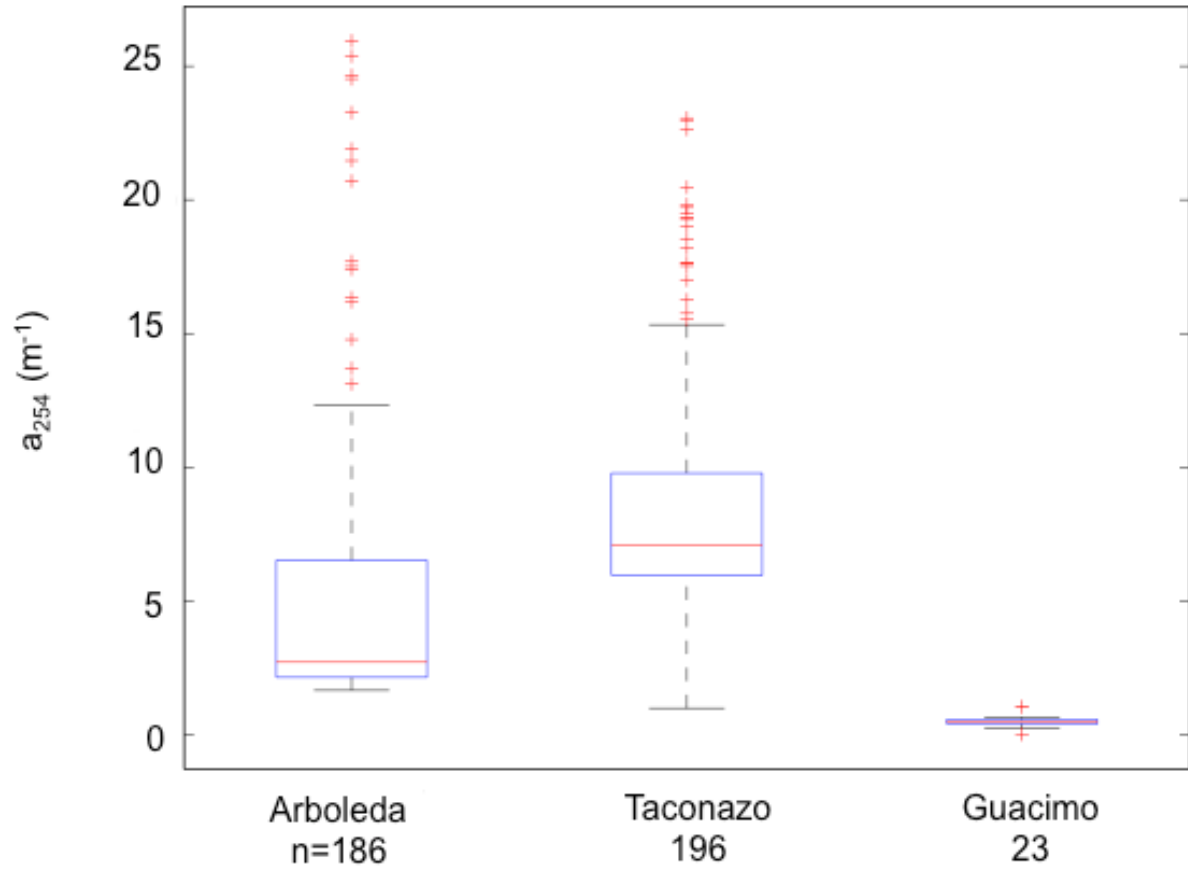


Figure 4: Boxplot of a_{254} (m^{-1}) for the Arboleda, Taconazo, and Guacimo Spring. The median value is a red line, the 25th and 75th quartiles are the box edges and red crosses are outliers. The whiskers extend to the furthest data point that was not an outlier. The number of samples in each plot is indicated below the label.

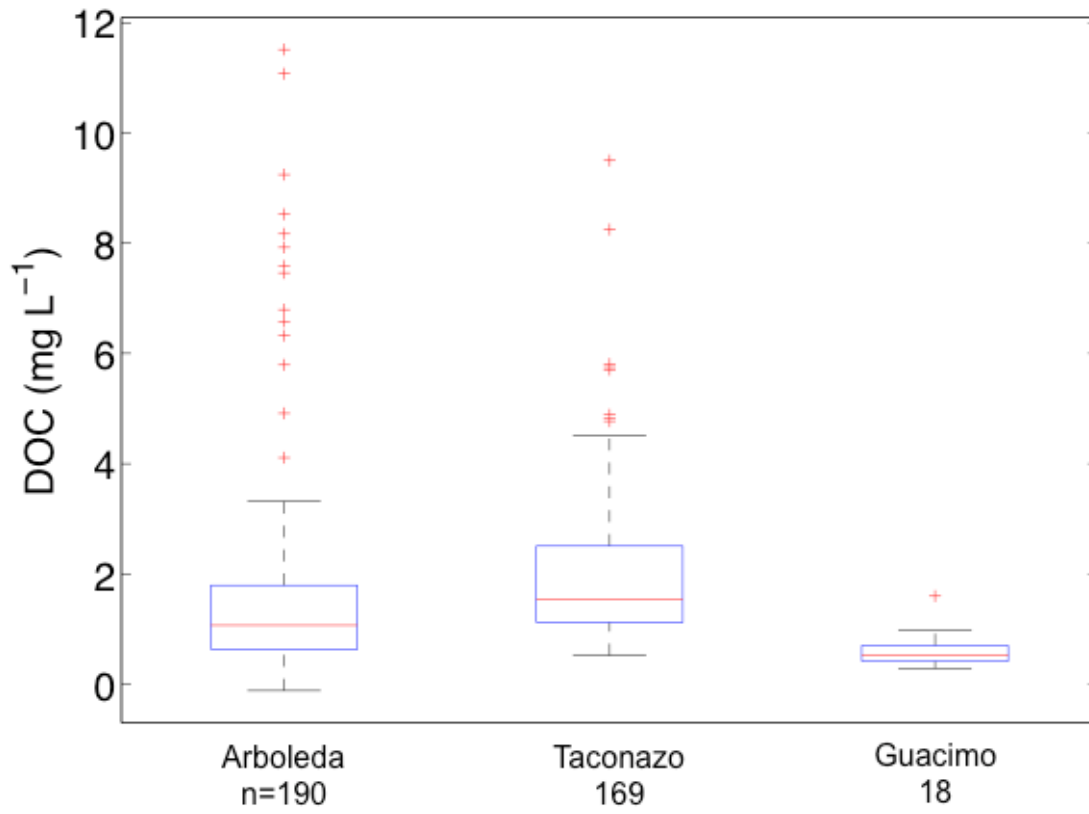


Figure 5: Boxplot of DOC concentrations (mg/L) for the Arboleda, Taconazo, and Guacimo Spring. The median value is a red line, the 25th and 75th quartiles are the box edges and red crosses are outliers. The whiskers extend to the furthest data point that was not an outlier. The number of samples in each plot is indicated below the label.

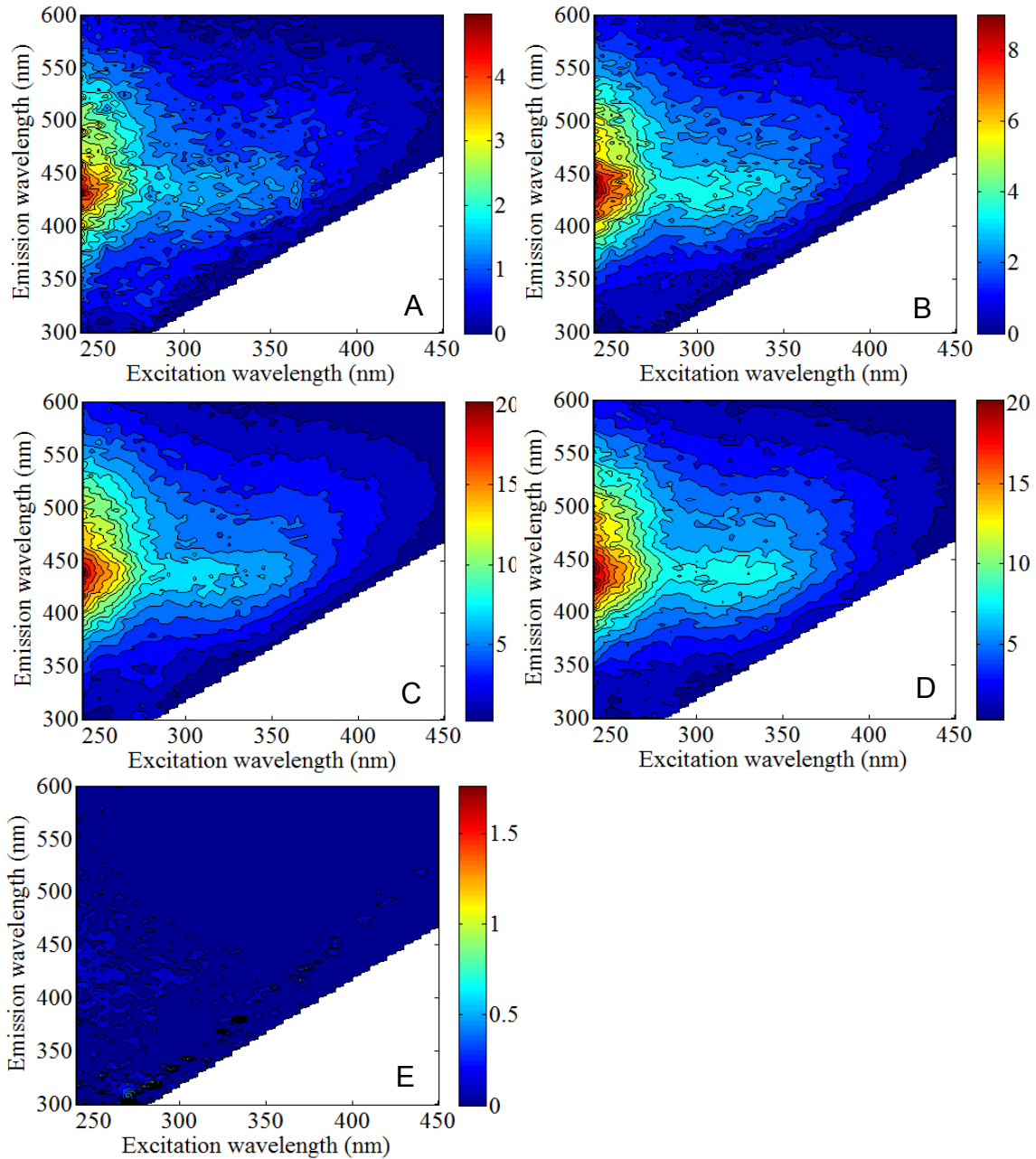


Figure 6: Representative EEMs of this study: A) Arboleda at baseflow; B) Taconazo at baseflow; C) Arboleda at stormflow; D) Taconazo at stormflow; E) Guacimo Spring Note the intensities are different.

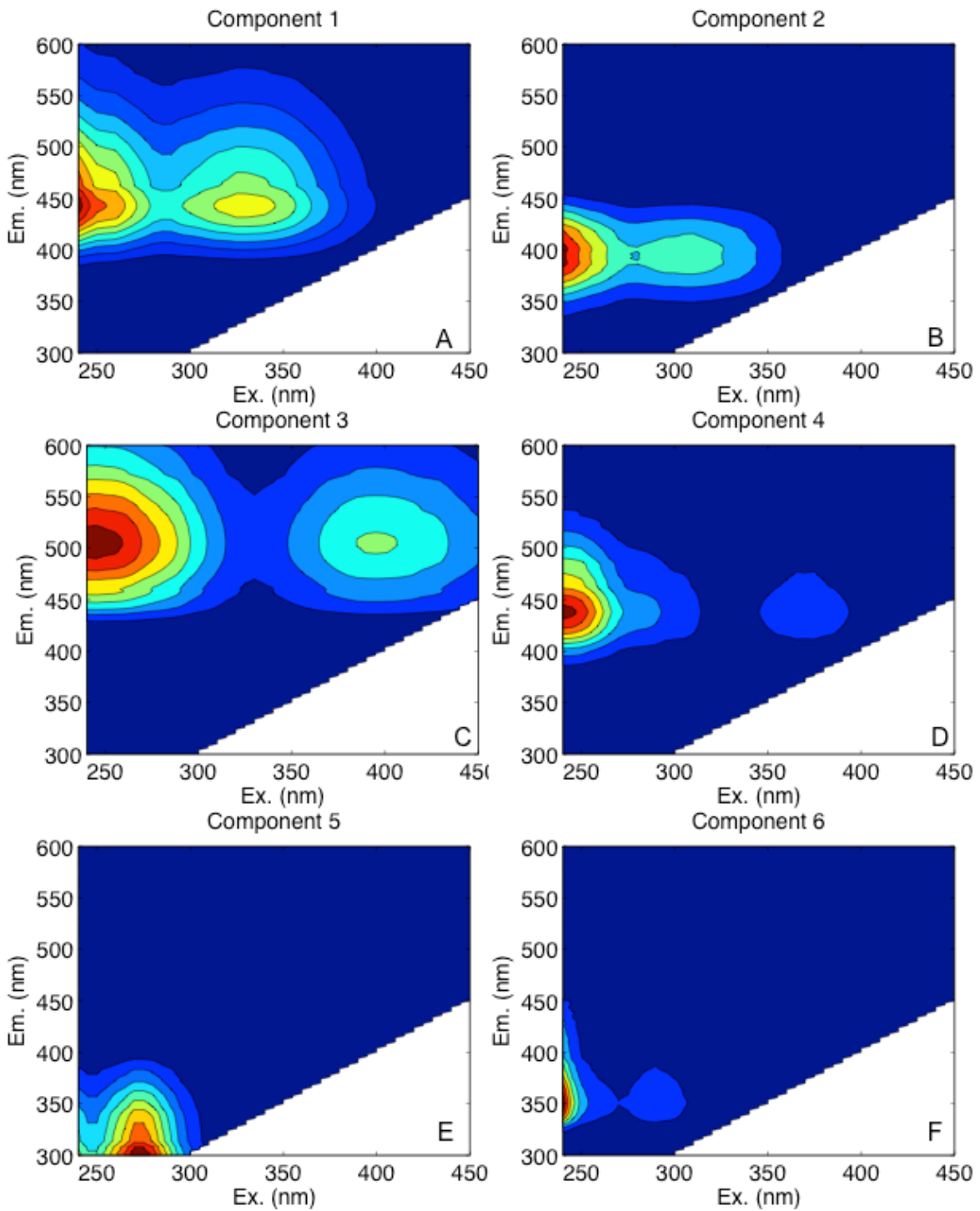


Figure 7: Fluorescent components of the Arboleda and Taconazo modeled using PARAFAC .

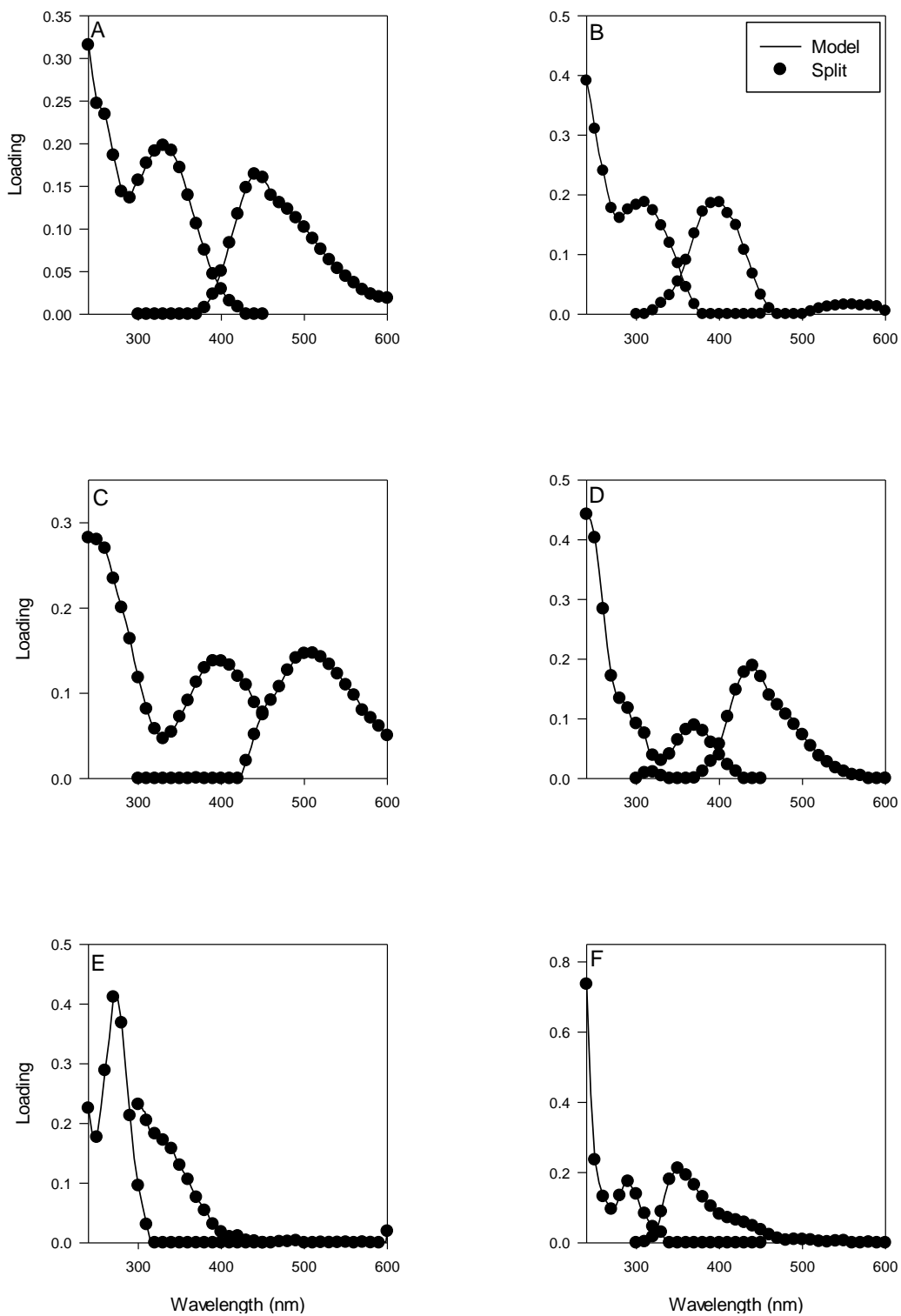


Figure 8: Graphs of the model and split of each PARAFAC component (labels correspond to Fig. 7 above) with excitation and emission wavelength. These graphs demonstrate how well the model fits the split of the data.

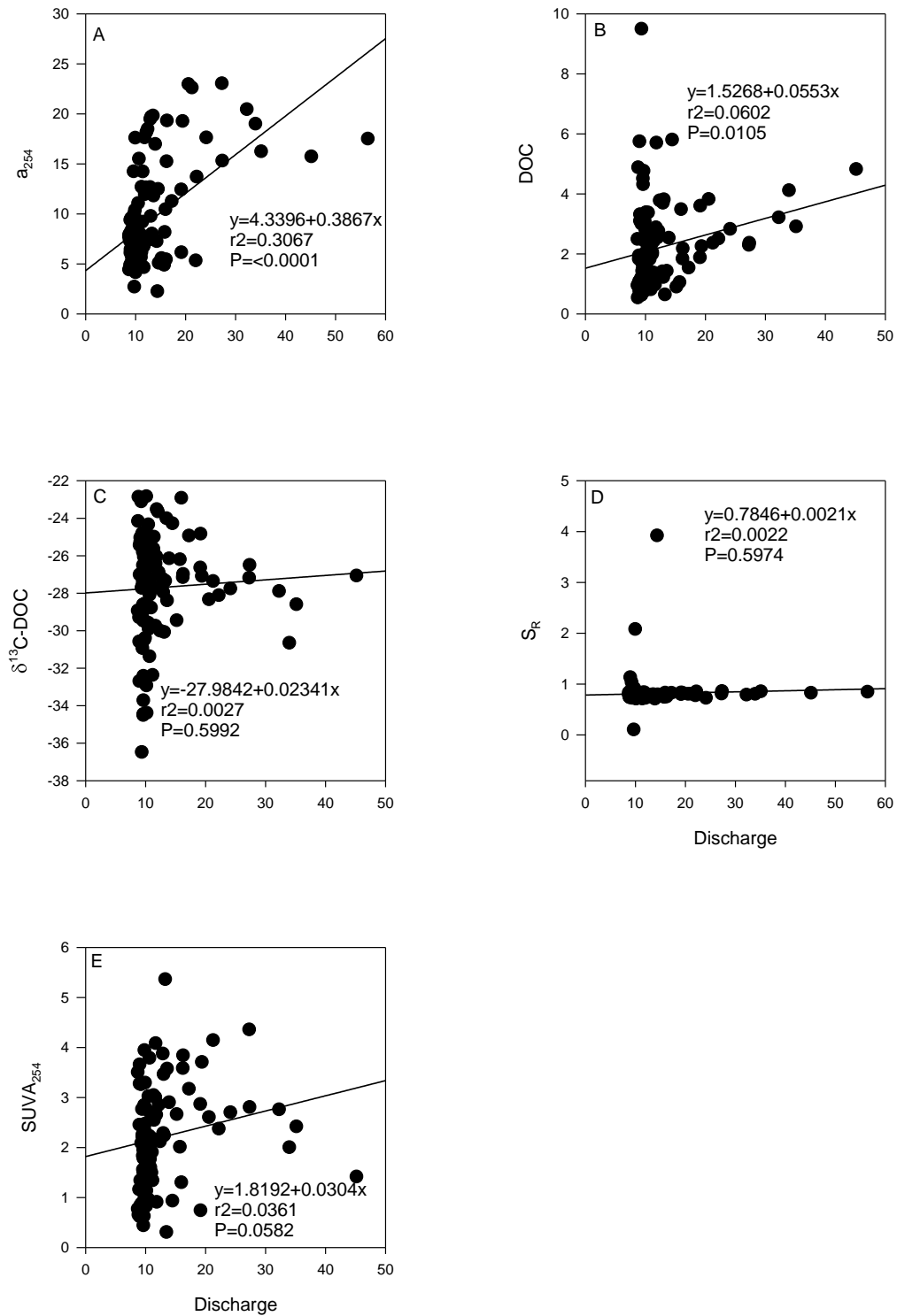


Figure 9: Linear regressions showing the relationship between discharge and measured metrics including: A) a_{254} ; B) DOC, C) $\delta^{13}\text{C-DOC}$, D) S_R , and E) SUVA_{254} measured in the Arboleda.

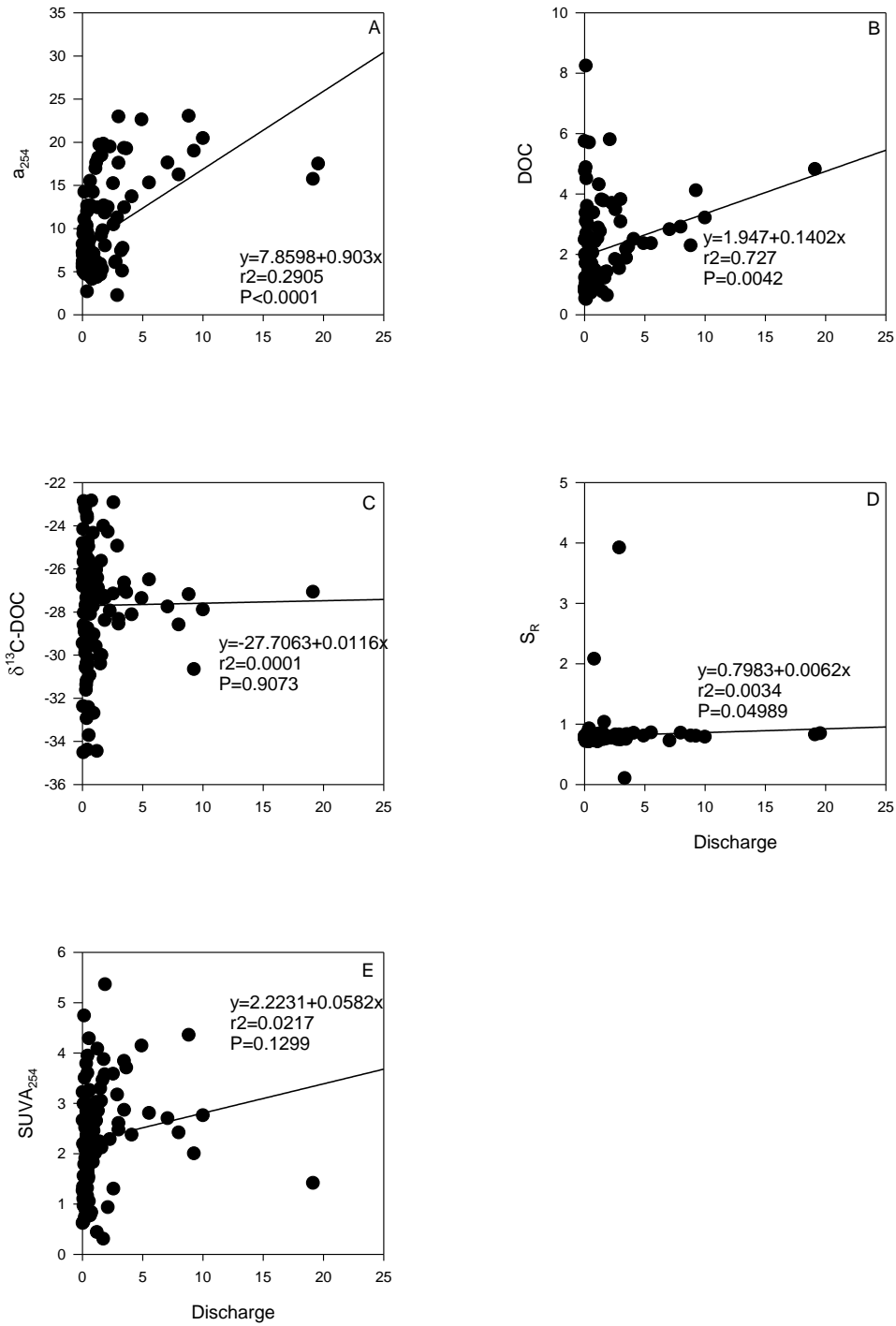


Figure 10: Linear regressions showing the relationship between discharge and measured metrics including: A) a_{254} ; B) DOC, C) $\delta^{13}\text{C-DOC}$, D) S_R , and E) SUVA_{254} measured in the Taconazo.

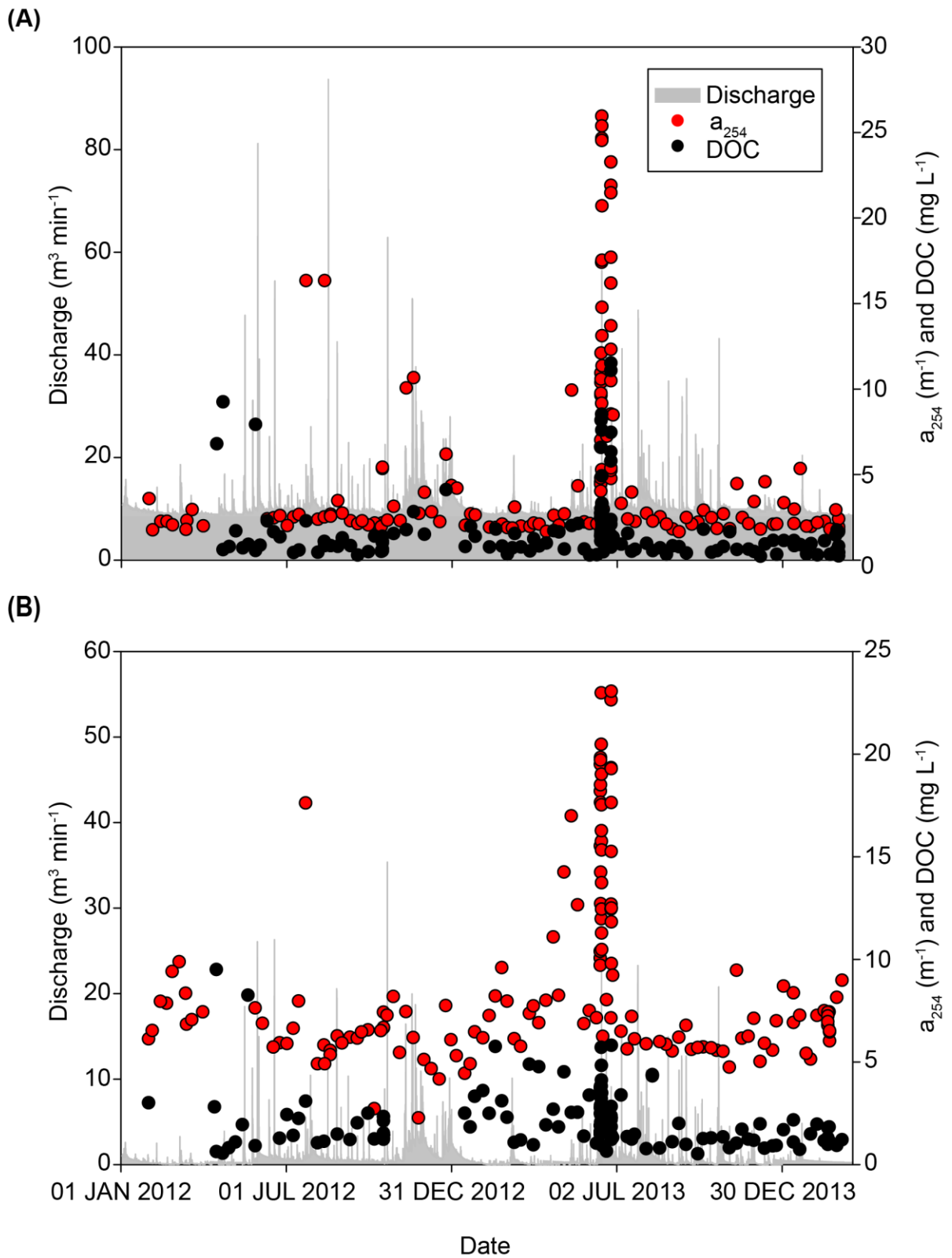


Figure 11: Absorbance at 254 nm and DOC concentration plotted with discharge in the background for the Arboleda (A) and Taconazo (B)

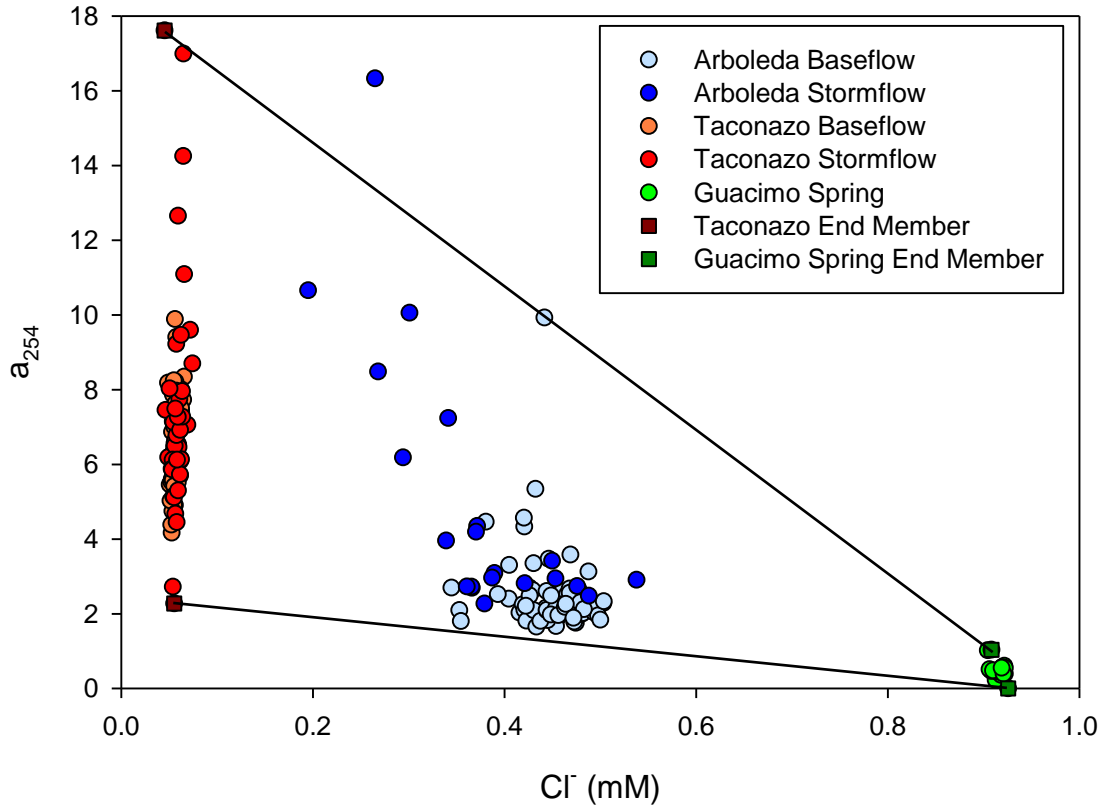


Figure 12: A mixing model using chloride (mM) as a conservative tracer and a_{254} for the Arboleda and Taconazo at baseflow and stormflow to demonstrate that the Arboleda is a mix between the end members of local groundwater (represented by the Taconazo average values) and regional groundwater (represented by the average Guacimo Spring values).

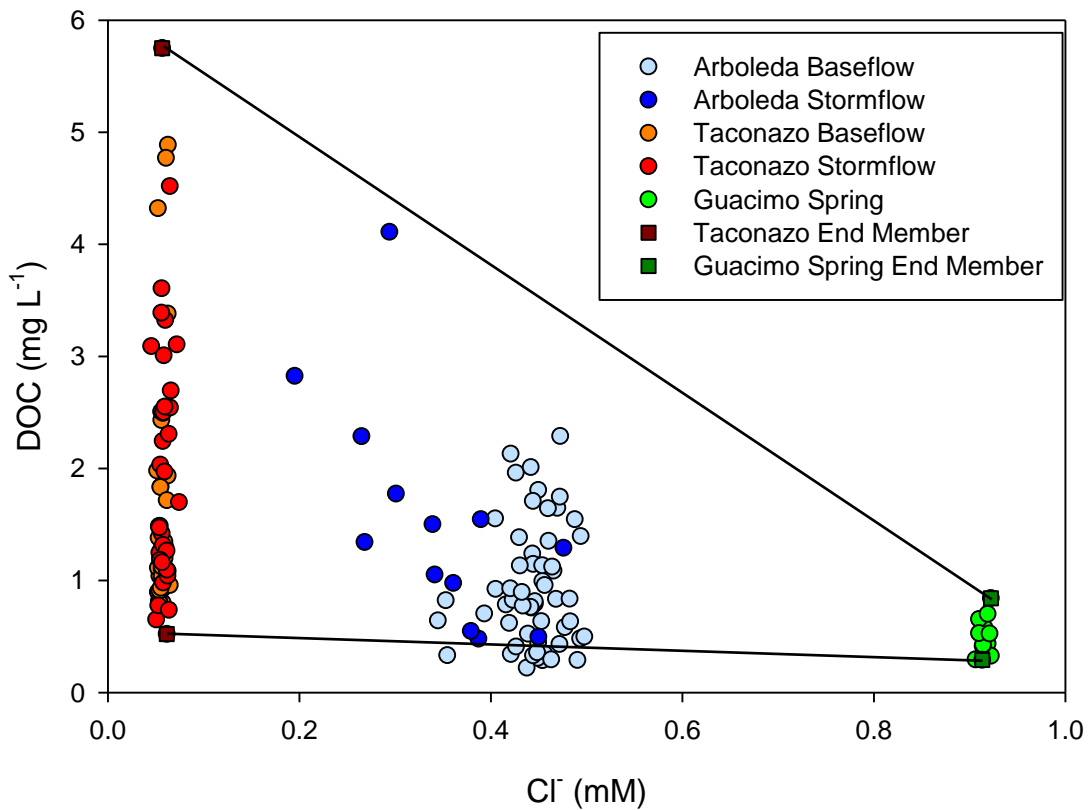


Figure 13: A mixing model using chloride (mM) as a conservative tracer and DOC concentration for the Arboleda and Taconazo at baseflow and stormflow to demonstrate that the Arboleda is a mix between the end members of local groundwater (represented by the Taconazo average values) and regional groundwater (represented by the average Guacimo Spring values).

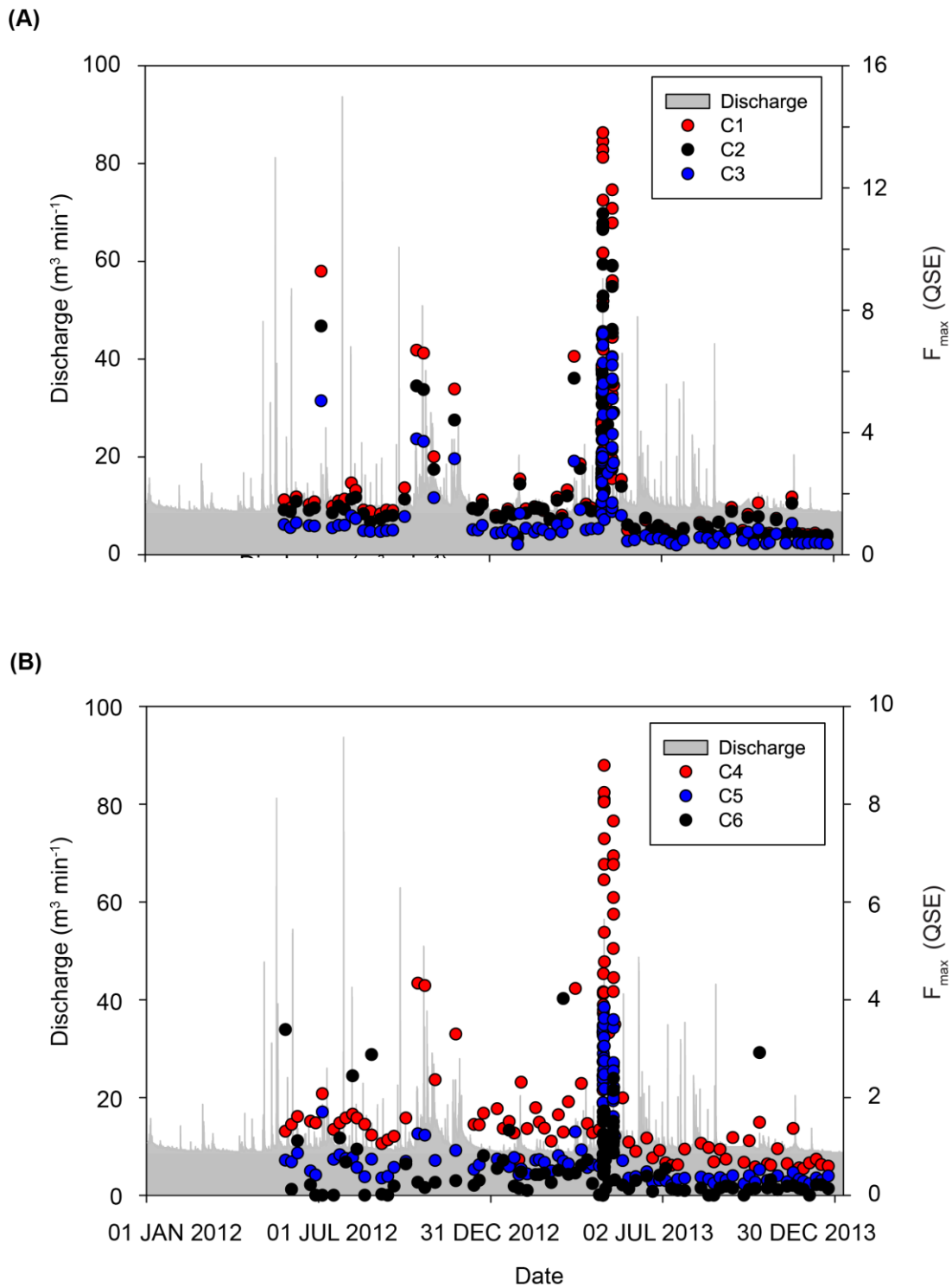


Figure 14: F_{\max} values of the PARAFAC components for the Arboleda plotted with discharge in the background: A) C1-C3; B) C4-C6 .

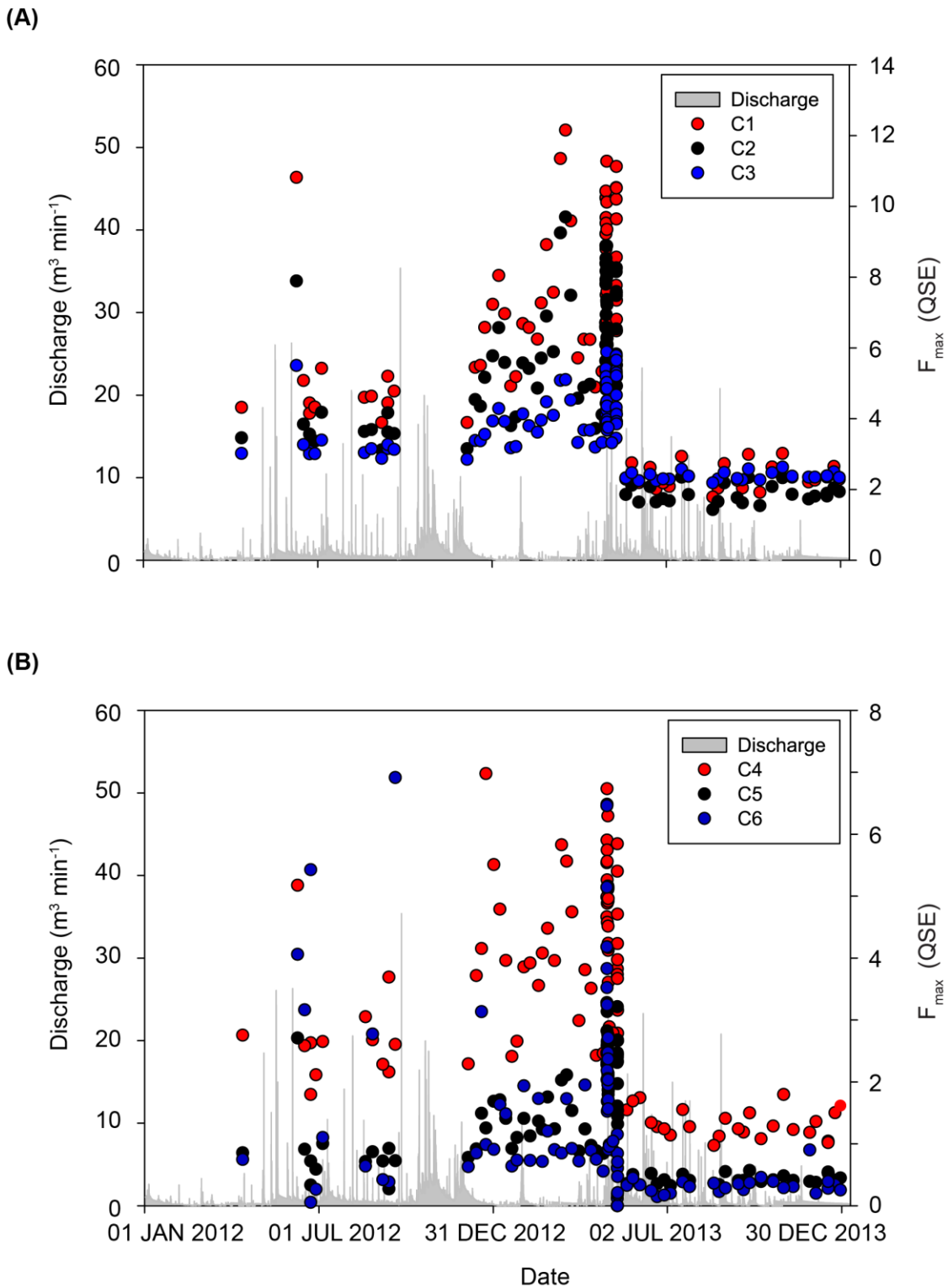
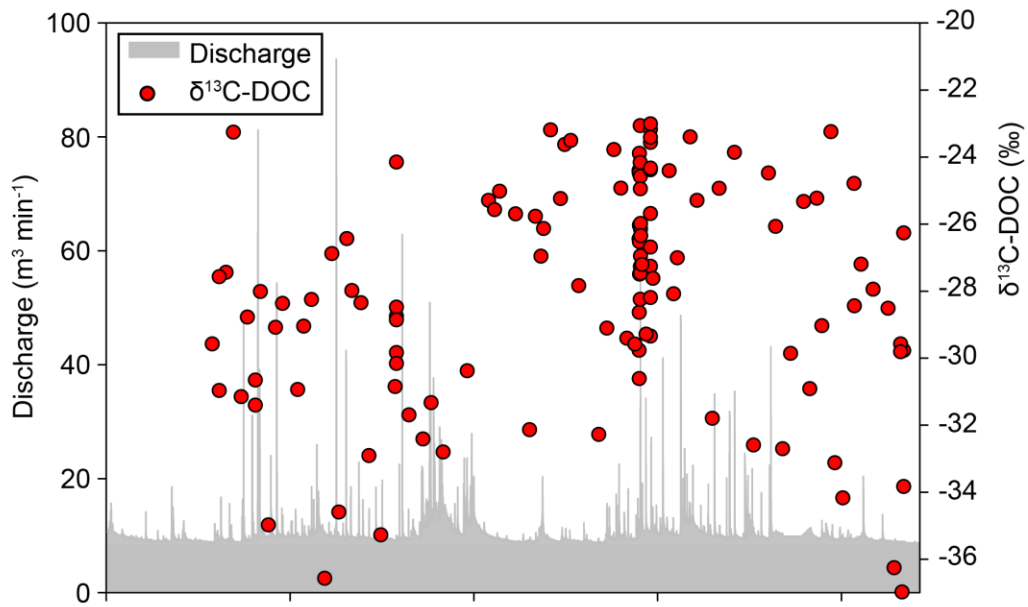


Figure 15: F_{\max} values of the PARAFAC components for the Taconazo plotted with discharge in the background: A) C1-C3 and B) C4-C6 .

(A)



(B)

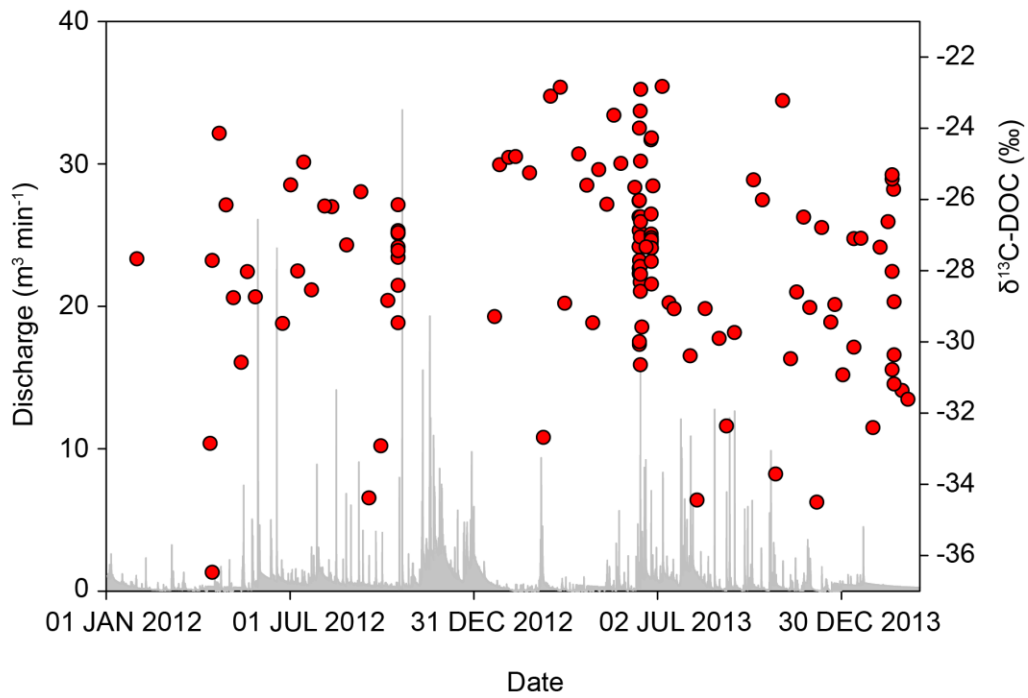


Figure 16: Stable carbon isotopes ($\delta^{13}\text{C}$) plotted with discharge in the background for the: A) Arboleda and B) Taconazo

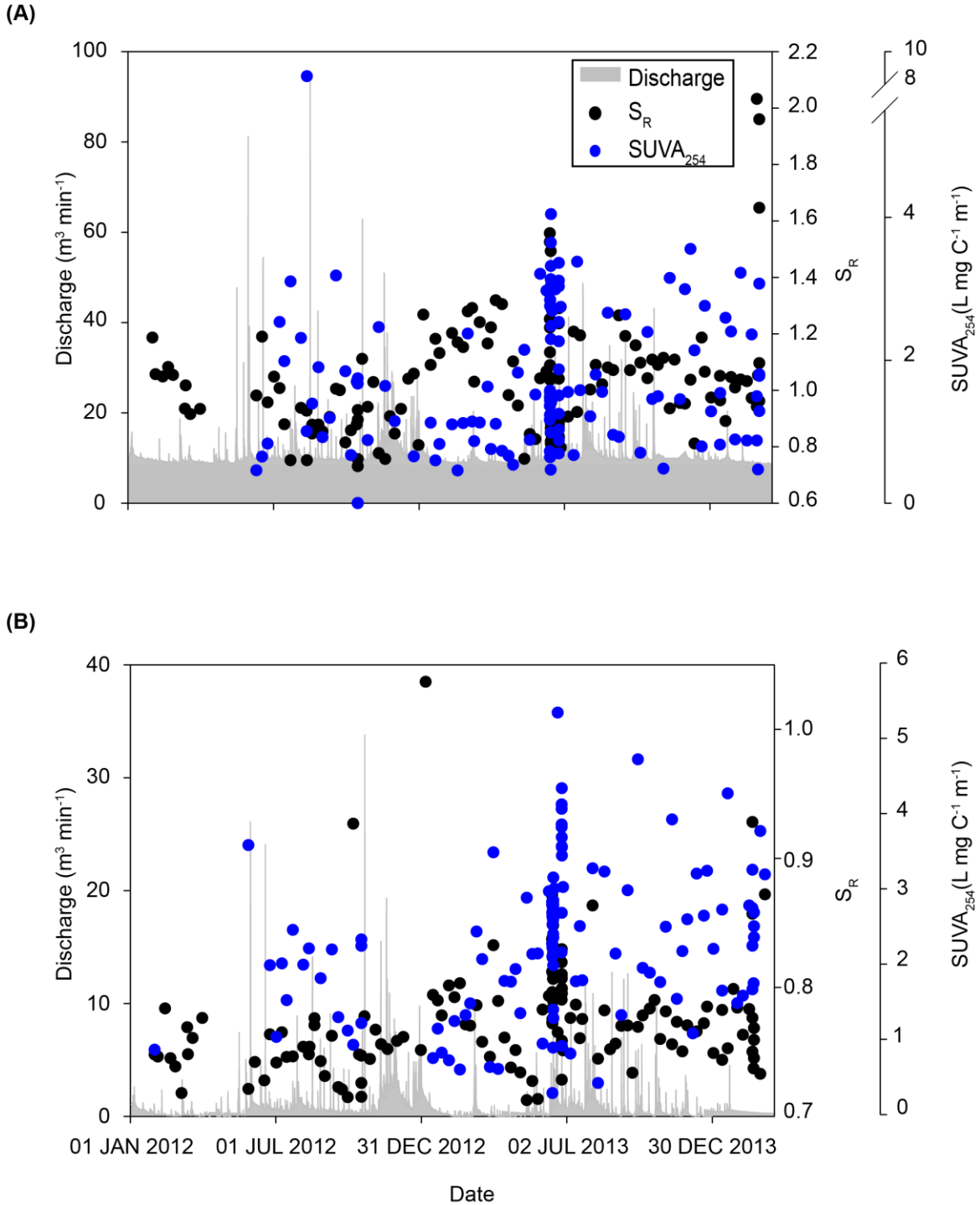


Figure 17: Slope ratio and $SUVA_{254}$ plotted with discharge in the background for the Arboleda (A) and Taconazo (B). In 9B, any values below 0.7 or above 1.05 were excluded. Due to this exclusion three data points are not seen in Figure 8B, two samples at stormflow and one at baseflow, with S_R values of 0.11, 3.92, and 2.08, respectively.

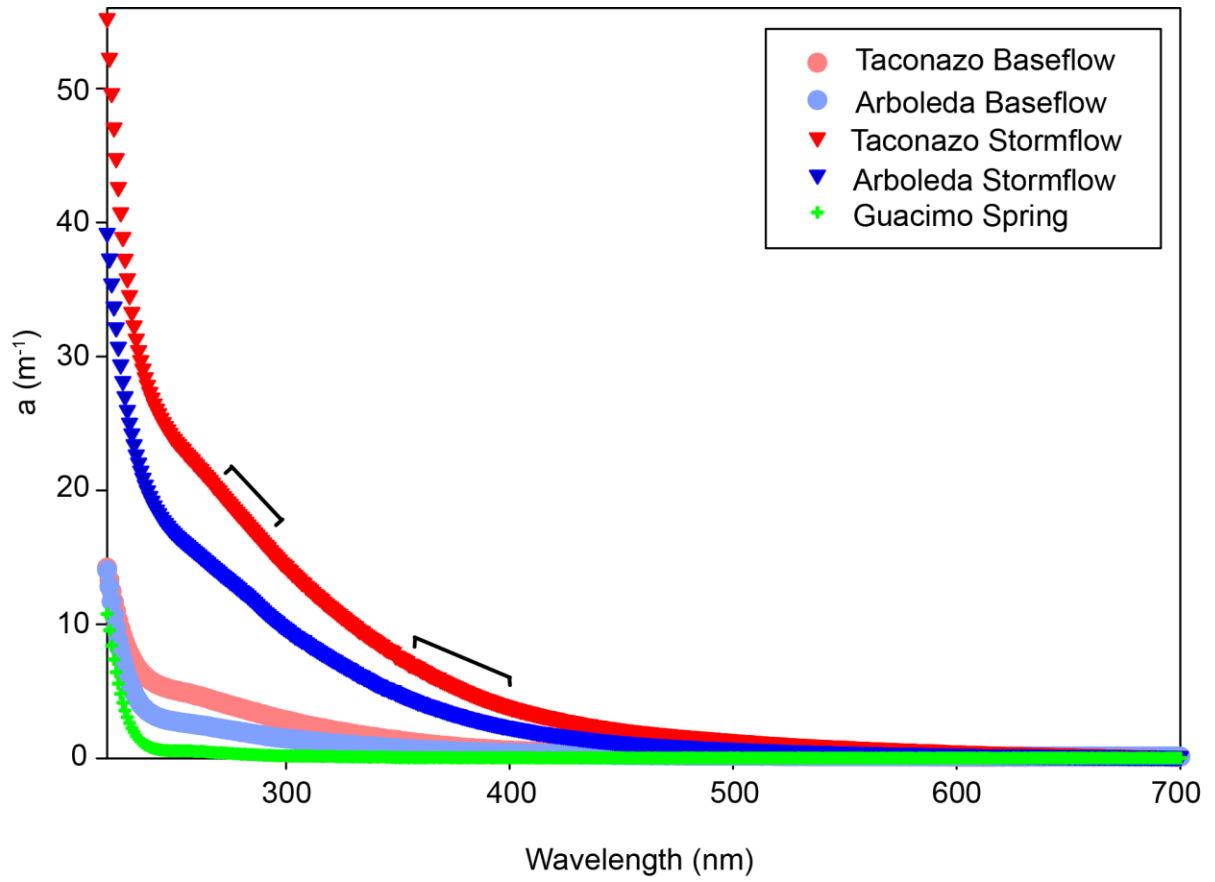


Figure 18: Representative absorbance spectra for the Arboleda and Taconazo at baseflow and stormflow, and Guacimo Spring. Brackets indicate the ratios over which SR was computed.

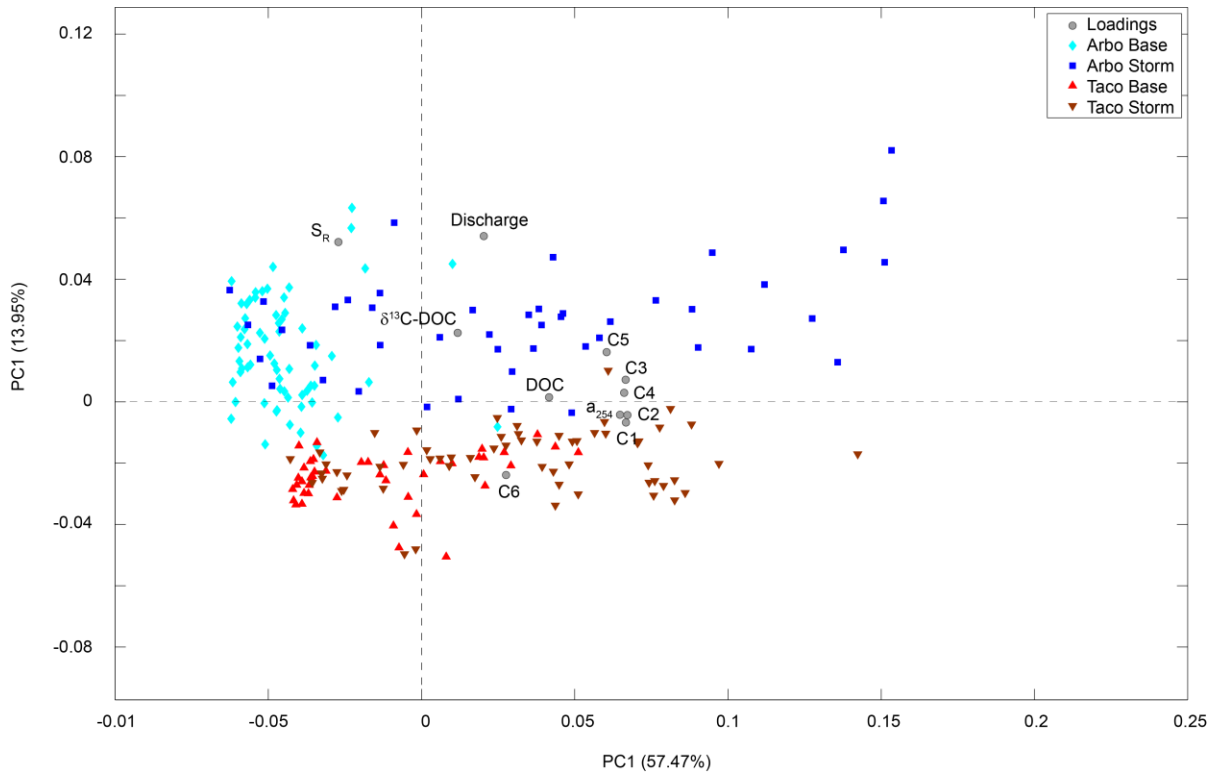


Figure 19: PCA of samples. PC1 and PC2 show some separation along PC2 for the Arboleda (blue) and Taconazo (red). Darker blue or red symbols indicate that the sample was collected at stormflow. Loadings are plotted in gray.

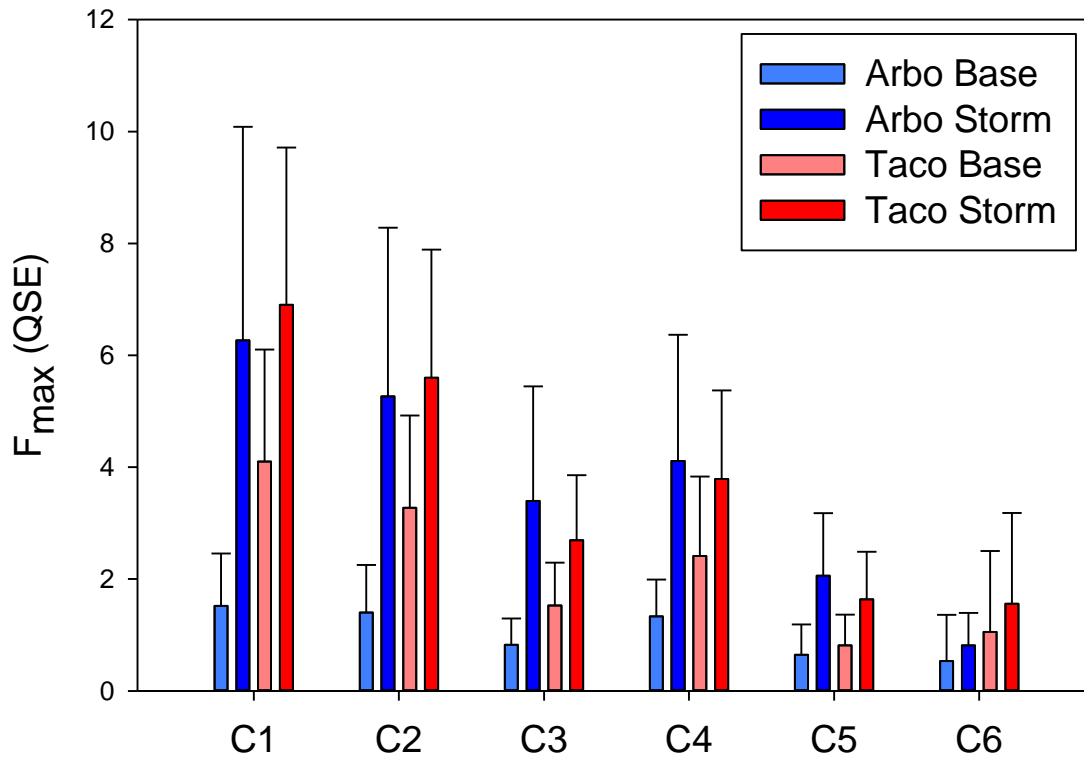


Figure 20: Average Fmax Values (QSE) for the six components identified in the PARAFAC model in the four sample sets: Arboleda at baseflow, Arboleda at stormflow, Taconazo at baseflow, and Taconazo at stormflow.

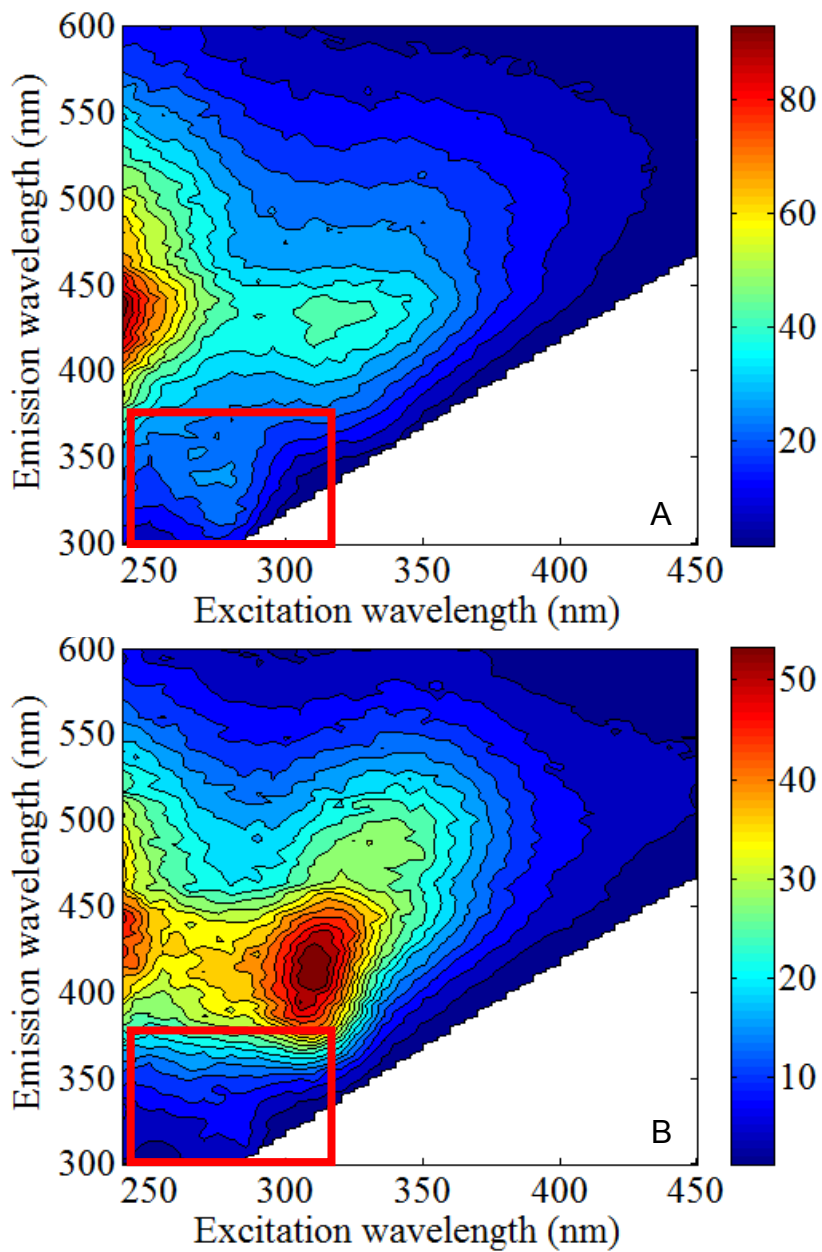


Figure 21: Sample EEM of leaf drip (A) and bromeliad water (B), the red box shows the enriched protein-like fluorescence (referred to as component 3 (C3) in the PARAFAC model) of these samples

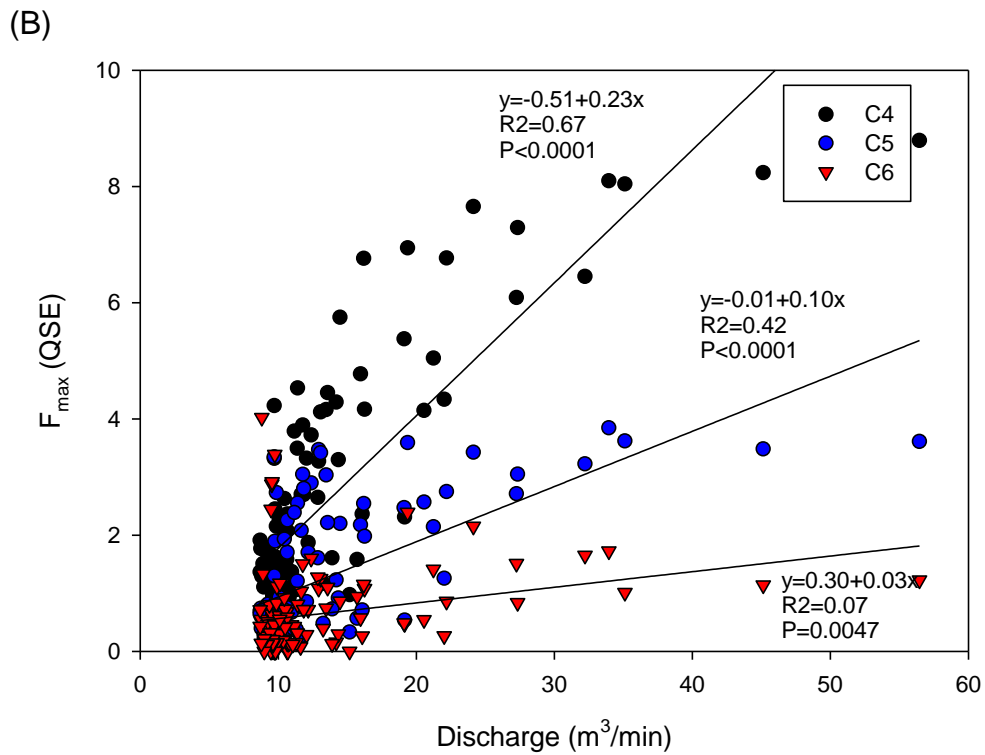
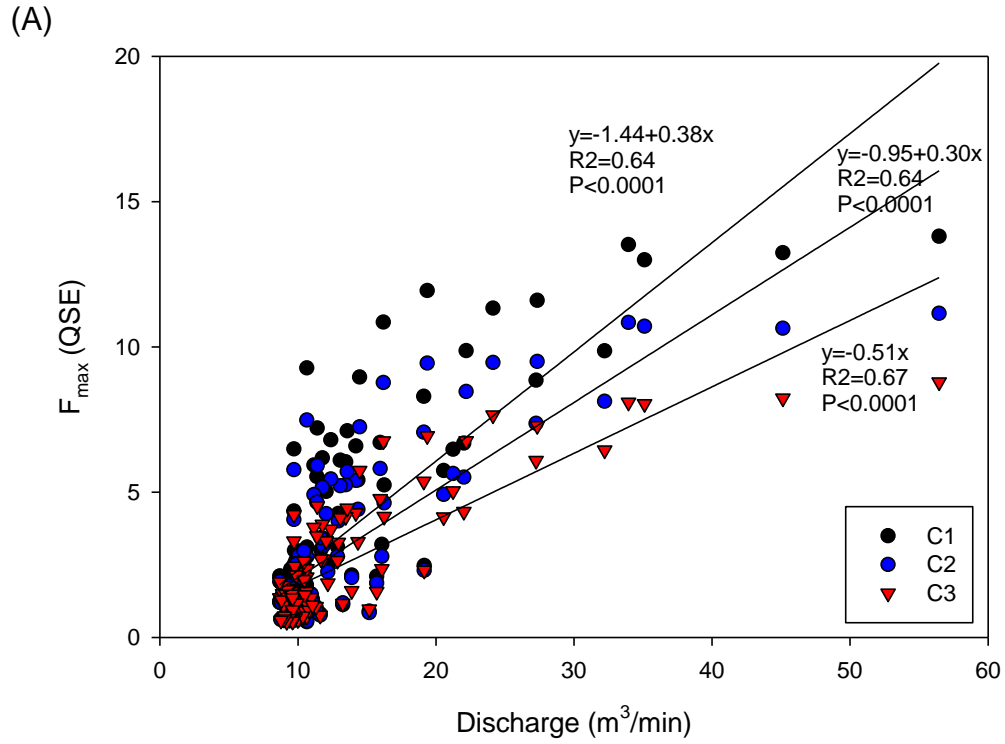


Figure 22: Linear regressions of discharge with the first three PARAFAC component F_{\max} values (A) and the last three PARAFAC component F_{\max} values (B) measured in the Arboleda

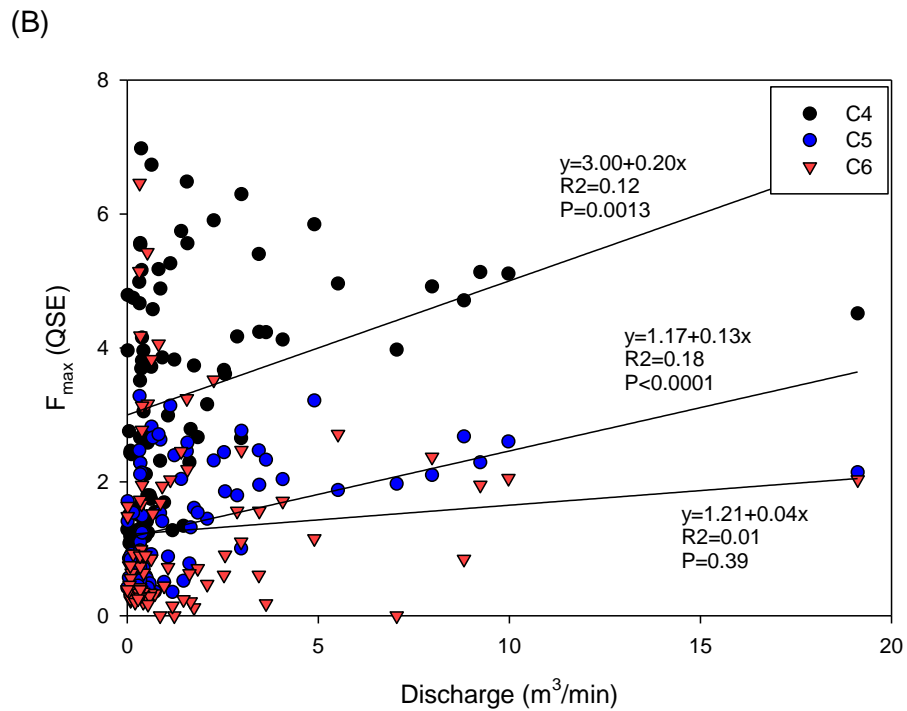
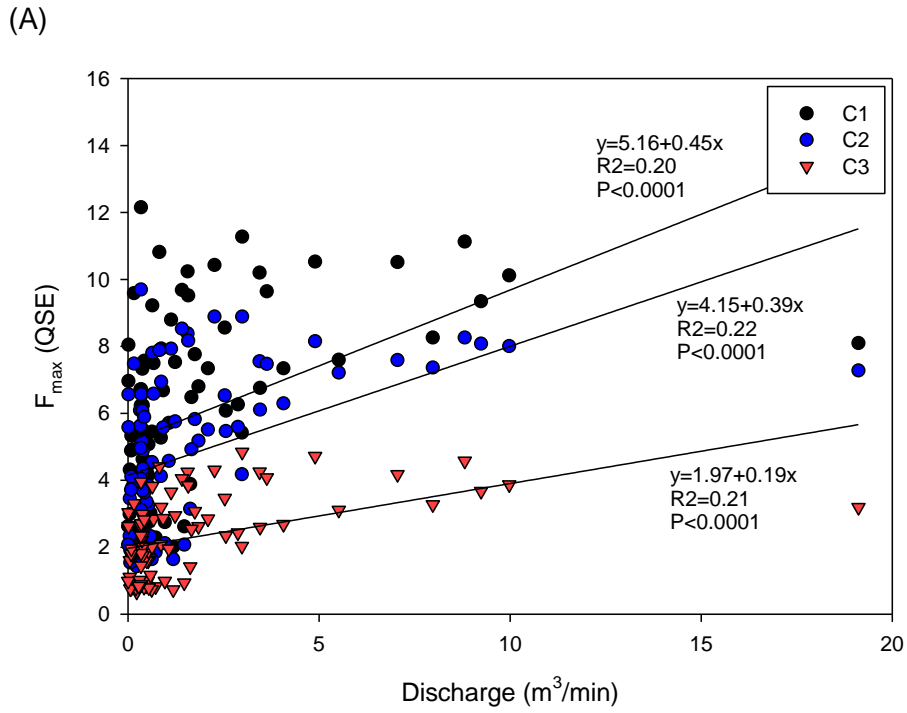


Figure 23: Linear regressions of discharge with the first three PARAFAC component F_{\max} values (A) and the last three PARAFAC component F_{\max} values (B) measured in the Taconazo

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