Flow, nutrients, and light availability influence Neotropical epilithon biomass and stoichiometry

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Abstract. Light, nutrient availability, and flow are strong factors controlling the elemental composition and biomass of epilithon in temperate stream ecosystems. However, comparatively little is known about these relationships in tropical streams. We investigated how gradients of light and nutrient availability, seasonality, and habitat influenced epilithon biomass, chlorophyll *a*, and nutrient ratios in montane streams of Trinidad, West Indies. We sampled 4 focal tributaries of a single river, 2 of which had canopies experimentally thinned, every other month over a 2-y period to observe temporal dynamics and light effects on epilithon. We also sampled 18 sites across Trinidad's Northern Range Mountains once each in a wet and dry season to examine the effects of naturally occurring differences in light and dissolved nutrient availability on epilithic characteristics. We found greater chlorophyll *a* concentrations in habitats with greater light availability, but the effect of light on epilithon stoichiometry differed between the site-survey and focal-tributary data. In general, epilithic C:nutrient ratios decreased with increasing dissolved nutrient concentrations, but relationships between nutrient availability and biomass probably were obscured by naturally high dissolved N and P concentrations in many of the streams. Season and habitat type had profound effects on epilithon variables. Biomass and % C generally decreased in riffles and under wet-season conditions. These results suggest multiple controls for the quantity and quality of stream epilithon and have important implications for in-stream consumers.

Key words: streams, light:nutrient hypothesis, periphyton, algae, disturbance, Trinidad.

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Ecological stoichiometry is the study of the mass balance of essential elements as they cycle through the biosphere (Sterner and Elser 2002). Investigators have applied a stoichiometric framework to gain insight into evolution (Jeyasingh and Weider 2007), population dynamics (Nakazawa 2011), trophic interactions (Dickman et al. 2008), and ecosystem function (Sterner and Elser 2002, Schade et al. 2005). Epilithon is a rock-bound biofilm comprising algae, fungi, bacteria, extracellular exudates, and detritus. It is an important basal resource in streams, and its elemental composition is plastic (Sterner and Elser 2002, reviewed by Cross et al. 2005). Algae constitute only a portion of biofilm assemblages, but shifts in algal nutrient quality affect epilithon stoichiometry as a whole, even when algae are at low densities (Frost et al. 2005). Nutrient imbalances often arise between grazing

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macroinvertebrates and their food resources because of algal stoichiometric plasticity. These imbalances have consequences for foodweb structure and nutrient cycling (Dickman et al. 2008, Small and Pringle 2010).

Light availability controls epilithon community composition, quantity, and quality. Increasing light availability often leads to increased algal biomass (e.g., Sanches et al. 2011) and higher C:nutrient ratios because of increased C fixation relative to nutrient acquisition (Sterner et al. 1997, Sterner and Elser 2002, Dickman et al. 2006). Phytoplankton adapted to low-light environments have higher chloroplast densities and elevated chlorophyll concentrations relative to those adapted to high-light environments (Geider et al. 1998, reviewed by Arrigo 2005). Chlorophyll has relatively high N and low P content, so greater intracellular chlorophyll concentrations lead to higher N:P ratios in low-light-adapted cells relative to those growing under high-light conditions (Geider et al. 1998, reviewed by Arrigo 2005). P content of algae also may increase at low light levels because of increased phospholipid production resulting from photoacclimation (Dickman et al. 2006).

Nutrient availability strongly affects epilithon stoichiometry (Sterner and Elser 2002) and biomass (Kohler et al. 2011), but above certain concentrations, nutrients may no longer stimulate primary production in streams (Newbold 1992). Algal cells assimilate N and P in excess of their metabolic requirements when nutrient concentrations are high, presumably as a means of coping with variable resource availability (Sterner and Elser 2002, Dodds and Whiles 2010). As a result, epilithon nutrient content can increase in response to increasing nutrient concentrations, thereby causing C:nutrient ratios to decline (Fanta et al. 2010, Small and Pringle 2010, Kohler et al. 2011). Stream nutrient concentrations vary with local and regional vegetation patterns, geologic conditions, and a suite of human activities in watersheds (Carpenter et al. 1998, Kemp and Dodds 2001, Neill et al. 2001, Wetzel 2001). Combined, these factors create considerable heterogeneity in nutrient concentrations across streams in relatively close proximity to one another.

Spatial and temporal heterogeneity of light and nutrients create a diverse resource template across which nutrient limitation and algal stoichiometry can vary (Hill et al. 2011). The Light:Nutrient Hypothesis (LNH; Sterner et al. 1997) predicts that epilithon nutrient content will increase with increasing nutrient availability and decrease with increasing light availability as a result of the mechanisms outlined above. Most tests of the LNH have been restricted to lentic (Hillebrand et al. 2004, Dickman et al. 2006, Sanches et al. 2011) or artificial habitats/substrata (Fanta et al.

2010, Guariento et al. 2011, Hill et al. 2011) and have provided mixed support for LNH predictions (Hill and Fanta 2008, Hill et al. 2009, Liess et al. 2009). Thus, considerable uncertainty exists regarding how light and nutrients interact to shape epilithon properties.

In lotic ecosystems, high and low flows have the potential to alter ecosystem structure and function profoundly through scour and accrual of epilithon (Biggs and Thomsen 1995, Dodds et al. 1996, Francoeur and Biggs 2006). Variation in discharge and flow velocity influence the flux of nutrients to epilithic communities (Dodds and Biggs 2002) and shape algal community structure (Biggs et al. 1998, Francoeur and Biggs 2006). By extension, it is reasonable to predict that flow also affects epilithon stoichiometry, and if so, both spatial and temporal variation in flow should be taken into consideration when assessing epilithon communities in natural streams. Spatial variation can be manifested in differences between pools and riffles, which have very different benthic flow characteristics. In tropical streams, temporal variation may result from distinct wet–dry seasons (Wantzen et al. 2006), providing a unique opportunity to investigate how habitat and seasonality combine to influence epilithon biomass and stoichiometry.

We examined how epilithon is influenced by light, nutrients, and flow in Neotropical streams. Specifically, we: 1) conducted a 2-y experiment in 4 focal streams to assess the effects of flow variability and manipulated canopy cover on epilithon biomass, chlorophyll *a*, and stoichiometry and 2) sampled 18 sites in Trinidad's Northern Mountain Range to examine the effects of natural gradients of light availability and dissolved nutrient concentrations on epilithon characteristics. Based on the LNH, we predicted that increased light availability would result in epilithon with greater C:nutrient ratios and lower N:P because of increased photosynthetic rates and lower intracellular chlorophyll *a* concentrations (Sterner et al. 1997). We also expected C:nutrient ratios to decrease with greater nutrient concentrations in the water column because of alleviation of limitation (Hillebrand and Kahlert 2001). Last, we predicted that epilithon biomass and C:nutrient ratios should decrease in the wet season because of more frequent scouring, which would decrease the relative amount of detritus associated with epilithon.

Methods

Study sites

We worked in the Northern Range of Trinidad, West Indies, and used 2 field approaches. First, we quantified temporal trends in epilithon stoichiometry
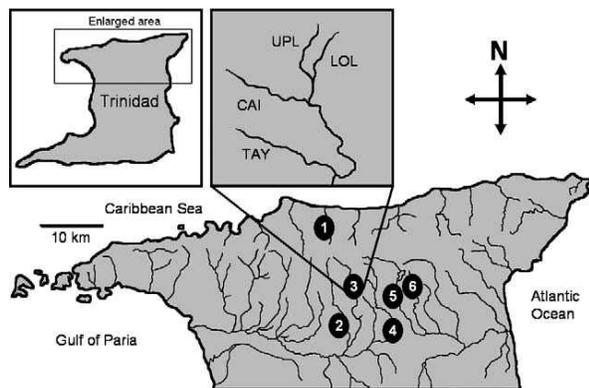
FIG. 1. Map of drainages sampled in the Northern Range of Trinidad (1 = Marianne, 2 = Arima, 3 = Guanapo, 4 = Aripo, 5 = Quare, 6 = Turure). Top center is detail of Heights of Guanapo from the Guanapo drainage, which contains the 4 focal sites (Lower La Laja = LOL, Upper La Laja = UPL, Taylor = TAY, and Caigual = CAI).

and biomass in 4 first-order streams in the Guanapo drainage: Lower La Laja (LOL), Upper La Laja (UPL), Taylor (TAY), and Caigual (CAI) (Fig. 1). These 4 headwater streams, collectively termed *focal streams*, are relatively undisturbed, although they drain small areas of active and inactive citrus, coffee, and cocoa plantations (Helson et al. 2006) and are similar in geology and floral communities. We sampled streams every other month from September 2007 to September 2009. In July 2007 and July 2008, we experimentally thinned riparian canopies at UPL (4% canopy reduction) and TAY (28% canopy reduction) to create elevated light conditions. We maintained canopy treatments throughout the sampling period.

Second, we surveyed 18 stream sites from 6 river basins in Trinidad's Northern Range (Fig. 1). These streams represented a broad range of natural canopy cover and nutrient concentrations. Thus, we were able to examine the effects of light and nutrients on epilithon biomass and stoichiometry across larger gradients than existed in focal streams. Three of these drainages, the Turure, Quare, and Aripo, have prominent $CaCO_3$ formations (*tufa*; see Day and Chenoweth 2004), and consequently have low natural concentrations of soluble reactive P (SRP; <10 µg/L) caused by Ca–P precipitation, but varying levels of dissolved inorganic N (DIN). The Arima and Guanapo Rivers have relatively high DIN and SRP concentrations from natural processes (Guanapo) and agricultural activity (Arima). In contrast, the Marianne River system is relatively pristine (Helson et al. 2006) with low levels of DIN and SRP. In each of the 6 basins, we sampled epilithon from headwaters (UP), midreaches (MD), and downstream (DN) reaches to

examine natural canopy effects. We sampled once in the wet season (June–December) and once in the dry season (January–May) to evaluate seasonal differences. The Turure, Arima, and Quare rivers were sampled in July 2008 (wet season) and March 2009 (dry season), whereas the Aripo, Marianne, and Guanapo were sampled in July 2007 and March 2009.

Sampling procedure

Focal streams.—In each focal stream, we established 3 transects 30 to 50 m apart, and sampled epilithon from pools and riffles separately along each transect on each date. We took ≥5 samples at randomly chosen locations in each habitat with a modified Loeb sampler (Loeb 1981), which is a cylinder (5.07 cm^2) with a brush-fitted plunger, to remove epilithon from substrata. We combined all samples from a single habitat type into a single slurry and kept slurries in a cooler until they could be processed that evening. At each transect, we measured canopy cover periodically with a hemispherical densiometer (method of Lemmon 1956), and we measured light continuously with Hobo® light loggers (Onset Computer Corp., Bourne, Massachusetts). We used conversions provided by Thimijan and Heins (1983) to convert lux to moles quanta m^{-2} d^{-1} and averaged daily totals for each stream for each month. In August 2008, a major flood carried away many of our light loggers, some of which were not recovered. For UPL and LOL, we averaged the available values from 2008 to substitute for missing data (May–August 2008). For CAI, we averaged later values because no previous data were available. We are confident that values were comparable based on densiometer estimates and historical trends. For TAY, we lost all prethinning light values, so we were unable to use previous or subsequent data. However, we placed loggers in the unthinned reach immediately below the experimental site in April 2009 and used these data to estimate light conditions before the canopy was reduced. We based flow measurements on stage, which was measured at the bottom of the LOL reach with a Solinst pressure transducer (Solinst Canada, Georgetown, Ontario) every 30 min.

Site survey.—We established 10 transects 10 to 20 m apart at each survey site. We alternately sampled pool and riffle habitats, resulting in 5 pool and 5 riffle samples per site per season. In 2008 and 2009, we sampled epilithon at each transect by scrubbing the tops of 3 rocks with a toothbrush to yield a single aggregated slurry for each transect. We traced the outline of each rock onto waterproof paper and calculated rock surface area in the laboratory. At

streams sampled in the wet season of 2007 and at the MD Turure site where loose rocks were not present because of carbonate precipitation, we collected pool and riffle samples with a Loeb sampler as explained above. Loeb samplers yielded higher estimates of ash-free dry mass (AFDM) and chlorophyll *a* than rock scrapings (TJK, unpublished data), so we used only stoichiometric data from Loeb samples. Last, we used a densiometer to estimate % canopy cover at each sampling site, and we used the method of Gore (2006) to estimate stream discharge.

Epilithon processing

We took quantitative subsamples from each epilithon slurry to estimate chlorophyll *a*, AFDM, and C:N:P stoichiometry. We filtered chlorophyll *a* subsamples through precombusted 25-mm GF/F filters and froze the filters until analysis. Within 30 d of sampling, we extracted samples in 90% reagent-grade ethanol for 24 h in the dark. We used an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, California) to estimate active chlorophyll *a* and corrected for phaeophytin (Parsons et al. 1984). We collected a 2nd subsample for estimation of AFDM on a preweighed, precombusted 47-mm GF/F filter. We combusted the subsample in a muffle furnace at 500°C and reweighed to calculate AFDM/m^2. We divided chlorophyll *a* values by corresponding AFDM values to create an alternative formulation of the autotrophic index (AI), which is an indicator of trophic state (Flotemersch et al. 2006).

We dried subsamples for C:N:P analysis in an oven at 50–55°C and ground them to a fine powder. We measured C and N content with a COSTECH Analytical ECS 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, California) and corrected for inorganic C with a fumigation method similar to that used by Hedges and Stern (1984). We combusted the P subsample in a muffle furnace at 500°C for 1 h, digested it with 1 N HCl, and analyzed it as for SRP (Murphy and Riley 1962) with a Cary 100 UV–visible spectrophotometer (Agilent Technologies, Palo Alto, California). The resulting values were then converted to molar C:N, C:P, and N:P ratios.

Nutrients

We collected stream water for water chemistry analyses on each sampling date at focal and survey streams. We used an Aquafluor handheld fluorometer to measure NH_4^+ immediately by the method of Holmes et al. (1999) as modified by Taylor et al. (2007). We quantified NO_3^- and NO_2^- with a Dionex ICS-90 Ion Chromatography System equipped with a

Dionex Automated Sampler and Chromeleon software (Dionex Corporation, Sunnyvale, California). NO_2^- was never detectable in samples (<5 µg/L). Therefore, we calculated DIN as the sum of NH_4^+ and NO_3^-. We measured SRP with the method developed by Murphy and Riley (1962) on a Pharmacia LKB Ultraspec III spectrophotometer (model: 80-2097-62; Pharmacia Biotech, Uppsala, Sweden) with a detection limit of 1.5 µg/L.

Statistical analysis

Prior to analyses, we conducted an exploratory data analysis (Zuur et al. 2010) to identify potential outliers, lack of variance homogeneity, and temporal independence. We $\log_{10}(x)$-transformed all variables that were not normally distributed to satisfy the assumption of normality. We used generalized least squares (GLS) regression models to analyze epilithon chlorophyll *a*, AFDM, AI, and molar nutrient ratios because this method allowed us to account for heterogeneity of variances among levels and for temporal autocorrelation in the focal-stream data (Pinheiro and Bates 2001).

The global model (i.e., models with all explanatory variables) for both the focal and survey streams had fixed effects that included stream identity, habitat (pool or riffle), season (wet or dry), and dissolved nutrients (NH_4^+, NO_3^-, and SRP). We included light in the model as photosynthetically active radiation (PAR) for focal streams and as % open canopy for survey streams. In our analysis of the focal stream reaches, we also included the number of days since stage increases (flow events) of different sizes to examine the effect of flow and associated scour on epilithon characteristics. In cases where multiple flow magnitudes were significant, the final model included the flow condition that produced the lowest Akaike Information Criterion (AIC) value. Occasionally, variation differed between streams or habitats. Therefore, we used the *varIdent* variance structure in our GLS regressions to allow levels to vary independently within categorical variables, thereby avoiding violation of assumptions of homogeneity of variance (Zuur et al. 2009). Last, we used an autoregressive moving average (ARMA) serial correlation structure for our focal site data to remove temporal nonindependence by modeling each date as a function of previous dates (Zuur et al. 2009).

We investigated optimal model structure for each variable by using restricted maximum likelihood (REML) on global models to test for the best combination of random effects (i.e., variance and correlation structures), followed by maximum likeli-

TABLE 1. Minimum adequate generalized least squares models for epilithon variables in focal streams (see Fig. 1 for stream names). df = degrees of freedom, L-ratio = log likelihood ratio, vf = variance function, AFDM = ash-free dry mass, AI = autotrophic index, SRP = soluble reactive P, since.8 = days since an 8-cm increase in stream stage, since.16 = days since a 16-cm increase in stream stage.

Epilithon variable	df	L-ratio	vf	Coefficients	Value	SE	t-value	p-value
Chlorophyll a	10	−259.85	~1: stream × month	Intercept	2.573	0.777	3.312	0.001
				Stream (LOL)	0.790	0.151	5.222	0.000
				Stream (TAY)	0.470	0.140	3.345	0.001
				Stream (UPL)	0.185	0.139	1.328	0.186
				Season (wet)	−1.588	0.157	−10.099	0.000
				Habitat (riffle)	−0.435	0.094	−4.645	0.000
				Since.8	−0.387	0.077	−5.050	0.000
				Light	0.442	0.093	4.764	0.000
				SRP	−0.624	0.161	−3.869	0.000
AFDM	12	−199.0	~1: month × habitat	(Intercept)	4.164	0.640	6.505	0.000
				Habitat (riffle)	−1.289	0.103	−12.486	0.000
				Since.16	0.174	0.043	4.006	0.000
				NO_3^-	−0.741	0.124	−5.986	0.000
				SRP	0.600	0.126	4.767	0.000
AI	17	−127.14	~1: stream × month	Intercept	2.424	0.381	6.369	0.000
				Stream (LOL)	0.506	0.117	4.312	0.000
				Stream (TAY)	0.083	0.105	0.787	0.432
				Stream (UPL)	0.291	0.117	2.483	0.014
				Habitat (riffle)	0.595	0.049	12.065	0.000
				Season (wet)	−0.368	0.105	−3.499	0.001
				SRP	−0.568	0.114	−4.989	0.000
C:N	21	146.49	none	Intercept	2.966	0.092	32.360	0.000
				Habitat (riffle)	−0.180	0.015	−12.283	0.000
				Light	−0.032	0.010	−3.116	0.002
				NH_4^+	−0.044	0.018	−2.418	0.017
C:P	14	−81.12	~1: month × habitat	Intercept	6.305	0.197	31.990	0.000
				Stream (LOL)	0.396	0.070	5.632	0.000
				Stream (TAY)	0.083	0.072	1.156	0.249
				Stream (UPL)	0.262	0.062	4.201	0.000
				Season (wet)	−0.180	0.046	−3.873	0.000
				SRP	−0.302	0.060	−5.028	0.000
N:P	16	−38.09	~1: month × habitat	Intercept	3.951	0.201	19.696	0.000
				Stream (LOL)	0.370	0.057	6.541	0.000
				Stream (TAY)	0.195	0.054	3.615	0.000
				Stream (UPL)	0.192	0.050	3.814	0.000
				Habitat (riffle)	0.103	0.045	2.319	0.021
				Season (wet)	−0.259	0.050	−5.136	0.000
				Since.8	−0.054	0.022	−2.403	0.017
				SRP	−0.299	0.051	−5.877	0.000

hood (ML) for fixed effects (Diggle et al. 2002). Model improvement was assessed based on a reduction in the AIC value for a given model and likelihood ratio (L-ratio) tests (Quinn and Keough 2002). Once we had created GLS models and assessed structure, we refined models by eliminating nonsignificant terms (i.e., backward selection) using ML to retrieve the minimum adequate model and used REML to report final models (West et al. 2006). We conducted an L-ratio test with ML to identify significance of individual variables. This test compared the minimum adequate model with the same model without the given variable. We set significance at α = 0.05. We ran all statistical analyses with the *nlme* package (Pinheiro et al. 2006) in R (version 2.9.2; R Development Core Team, Vienna, Austria). The complete output for the full models is presented in Table 1 for the focal streams and Table 2 for the survey streams.

Results

Focal streams

Light, stage, and dissolved nutrients.—Scouring flow events were more frequent during the wet season, with fewer days between events raising stream stage ≥8 cm (t-test, $t = 16.81$, $p < 0.001$) or 16 cm (t-test, $t = $

TABLE 2. Minimum adequate generalized least squares models for epilithon variables in survey streams (see Fig. 1 for drainages). Chlorophyll *a*, ash-free dry mass (AFDM), and autotrophic index (AI) values from samples collected with Loeb samplers were excluded (see text for explanation). df = degrees of freedom, *L*-ratio = log likelihood ratio, vf = variance function, SRP = soluble reactive P.

Epilithon variable	df	*L*-ratio	vf	Coefficients	Value	SE	*t*-value	*p*-value
Chlorophyll *a*	10	204.11	~1: site × season × habitat	Intercept	2.578	0.198	13.033	0.000
				Stream (Aripo)	−0.138	0.136	−1.009	0.314
				Stream (Guanapo)	0.093	0.137	0.681	0.497
				Stream (Marianne)	−0.522	0.146	−3.569	0.000
				Stream (Quare)	−0.215	0.118	−1.825	0.069
				Stream (Turure)	−0.544	0.122	−4.469	0.000
				Season (wet)	−1.588	0.102	−15.549	0.000
				Open canopy	0.469	0.059	7.933	0.000
				SRP	−0.406	0.061	−6.682	0.000
AFDM	13	−122.59	~1: site × season	Intercept	2.173	0.111	19.523	0.000
				Stream (Aripo)	0.284	0.098	2.899	0.004
				Stream (Guanapo)	0.103	0.098	1.051	0.295
				Stream (Marianne)	−0.226	0.098	−2.302	0.022
				Stream (Quare)	0.264	0.066	3.980	0.000
				Stream (Turure)	−0.008	0.073	−0.112	0.911
				Season (wet)	−0.190	0.060	−3.141	0.002
				Habitat (riffle)	−0.702	0.054	−13.089	0.000
				Open canopy	−0.153	0.035	−4.409	0.000
AI	22	−130.81	~1: stream × habitat	Intercept	1.013	0.118	8.573	0.000
				Stream (Aripo)	−0.073	0.087	−0.839	0.402
				Stream (Guanapo)	0.102	0.089	1.140	0.255
				Stream (Marianne)	0.102	0.088	1.151	0.251
				Stream (Quare)	−0.283	0.063	−4.514	0.000
				Stream (Turure)	−0.245	0.059	−4.156	0.000
				Season (wet)	−0.801	0.058	−13.716	0.000
				Habitat (riffle)	0.522	0.048	10.805	0.000
				Open canopy	0.367	0.036	10.222	0.000
				SRP	−0.231	0.033	−7.057	0.000
C:N	45	−664.89	~1: stream × habitat	Intercept	13.682	0.312	43.787	0.000
				Stream (Aripo)	0.372	0.388	0.958	0.339
				Stream (Guanapo)	−0.977	0.314	−3.114	0.002
				Stream (Marianne)	−1.622	0.318	−5.106	0.000
				Stream (Quare)	1.491	0.328	4.551	0.000
				Stream (Turure)	2.881	0.290	9.919	0.000
				Season (wet)	−1.483	0.193	−7.678	0.000
				Habitat (riffle)	−2.164	0.218	−9.929	0.000
				NH_4^+	−2.011	0.229	−8.798	0.000
C:P	35	−112.09	~1: stream × habitat	Intercept	7.392	0.227	32.542	0.000
				Stream (Aripo)	0.184	0.076	2.434	0.016
				Stream (Guanapo)	−0.030	0.067	−0.441	0.660
				Stream (Marianne)	0.299	0.114	2.635	0.009
				Stream (Quare)	−0.033	0.077	−0.433	0.665
				Stream (Turure)	0.022	0.073	0.297	0.767
				Season (wet)	−0.187	0.056	−3.344	0.001
				Habitat (riffle)	−0.363	0.039	−9.368	0.000
				Open canopy	0.179	0.037	4.888	0.000
				SRP	−0.251	0.033	−7.542	0.000
				NH_4^+	−0.502	0.065	−7.661	0.000
				NO_3^-	−0.180	0.034	−5.241	0.000
N:P	23	−83.61	~1: stream × season	Intercept	5.030	0.205	24.569	0.000
				Stream (Aripo)	0.001	0.069	0.016	0.987
				Stream (Guanapo)	−0.099	0.058	−1.702	0.090
				Stream (Marianne)	0.387	0.104	3.727	0.000
				Stream (Quare)	−0.207	0.070	−2.951	0.003
				Stream (Turure)	−0.147	0.062	−2.361	0.019

TABLE 2. Continued.

Epilithon variable	df	L-ratio	vf	Coefficients	Value	SE	t–value	p–value
				Season (wet)	−0.108	0.050	−2.147	0.033
				Open canopy	0.127	0.035	3.661	0.000
				SRP	−0.172	0.028	−6.193	0.000
				NH_4^+	−0.452	0.061	−7.427	0.000
				NO_3^-	−0.217	0.033	−6.662	0.000

16.22, $p < 0.001$) than during the dry season (Fig. 2). DIN and SRP concentrations ranged from 76 to 382 and from 10 to 68 µg/L, respectively (Fig. 3A, B). NH_4^+ (t-test, $t = 3.99$, $p < 0.001$) and SRP (t-test, $t = 6.78$, $p < 0.001$) concentrations were greatest in the dry season in all focal streams, but NO_3^- concentrations did not differ between seasons. As a result, DIN:SRP ratios were generally greater in the wet than in the dry season, but this relationship was not statistically significant. Fluxes of PAR were greater at streams with experimentally thinned canopies than at control streams. Thinning increased fluxes in UPL by 30% and TAY by 800% on average (Fig. 3C).

Epilithon biomass and stoichiometry.—Chlorophyll *a* was positively related to PAR (L-ratio = 22.49, df = 9, $p < 0.001$), negatively related to SRP concentrations (L-ratio = 15.07, df = 9, $p < 0.001$) and days since a ≥8-cm increase in stage (L-ratio = 25.14, df = 9, $p < 0.001$), and differed among streams (L-ratio = 28.08, df = 7, $p < 0.001$). Chlorophyll *a* was lower in riffles than in pools (L-ratio = 21.43, df = 9, $p < 0.001$) and in the wet than in the dry season (L-ratio = 87.81, df = 9, $p < 0.001$) (Fig. 4A). AFDM increased with SRP concentrations (L-ratio = 19.99, df = 11, $p < 0.001$) and with days since a 16-cm increase in stage (L-ratio

FIG. 2. Stage measured at the bottom of Lower La Laja (a focal site) throughout the study period. Grey bars indicate dry-season months.

= 13.81, df = 11, $p < 0.001$) and decreased with increasing NO_3^- concentration (L-ratio = 25.86, df = 11, $p < 0.001$). AFDM was lower in riffles than in pools (L-ratio = 84.24, df = 11, $p < 0.001$; Fig. 4B). AI differed among streams (L-ratio = 23.15, df = 14, $p < 0.001$) and was negatively related to SRP concentration (L-ratio = 17.42, df = 16, $p < 0.001$). AI was greater in riffles than in pools (L-ratio = 89.81, df = 16, $p < 0.001$) and was lower in the wet than in the dry season (L-ratio = 11.24, df = 16, $p < 0.001$) (Fig. 4C).

C:N of epilithon was lower in riffles than in pools (L-ratio = 67.82, df = 20, $p < 0.001$; Fig. 4D) and was negatively related to PAR (L-ratio = 7.34, df = 20, $p = 0.007$) and NH_4^+ concentration (L-ratio = 4.77, df = 20, $p = 0.029$; Fig. 5A). C:P (L-ratio = 39.51, df = 11, $p < 0.001$) and N:P (L-ratio = 37.77, df = 13, $p < 0.001$) differed among streams. C:P was lower in the wet than in the dry season (L-ratio = 12.80, df = 13, $p < 0.001$; Fig. 4E) and was negatively related to SRP concentration (L-ratio = 22.08, df = 13, $p < 0.001$; Fig. 5B). N:P was higher in riffles than in pools (L-ratio = 4.42, df = 15, $p = 0.036$) and lower in the wet than in the dry season (L-ratio = 21.98, df = 15, $p < 0.001$) (Fig. 4F), and negatively related to SRP concentration (L-ratio = 27.30, df = 15, $p < 0.001$; Fig. 5C) and days since an 8-cm increase in stage (L-ratio = 0.024, df = 15, $p = 0.024$).

Site survey

Dissolved nutrients and canopy cover.—Water chemistry was strongly influenced by basin and stream position (i.e., UP, MD, DN) (Fig. 6A, B). Nutrient concentrations varied seasonally. SRP concentrations were significantly greater in the dry than in the wet season (t-test, $t = 4.86$, $p < 0.001$), and NH_4^+ concentrations were greater in the wet than in the dry season (t-test, $t = -4.8853$, $p < 0.001$). However, NO_3^- concentrations did not differ between seasons. Consequently, molar DIN:SRP was greater in the wet than in the dry season. DN sites had the most open canopies (Fig. 6C) and greatest discharges, averaging 25% open canopy and 326 L/s, respectively. MD and UP sites were similar, with mean % open canopy of
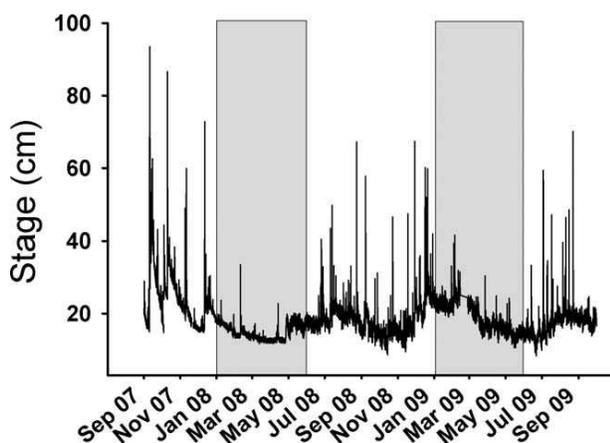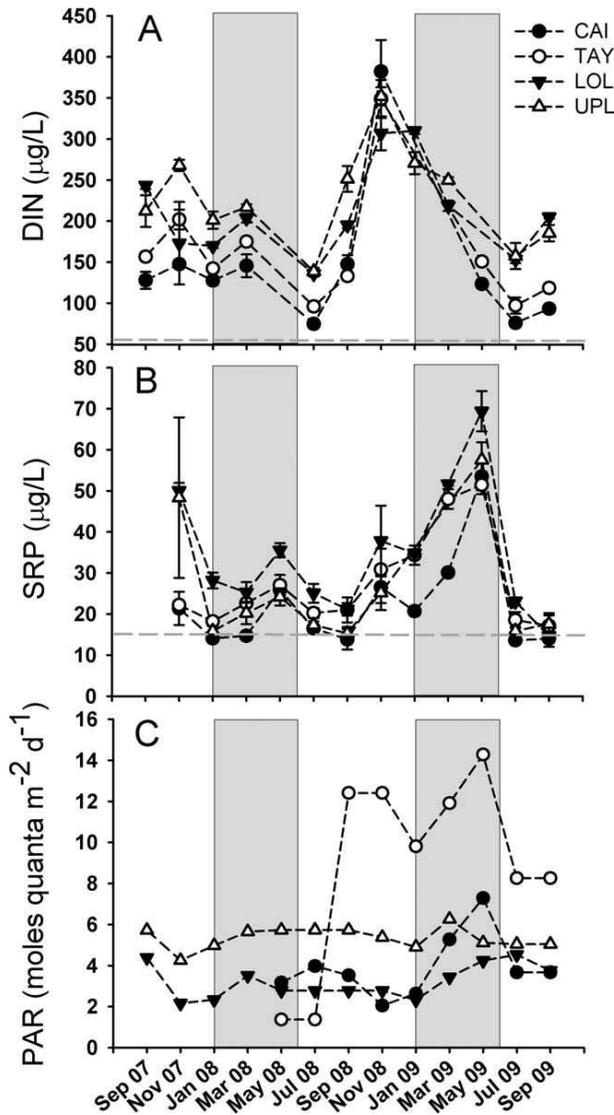
FIG. 3. Mean (±1 SE) dissolved inorganic N (DIN) (A), soluble reactive P (SRP) (B), and the average daily total photosynthetically active radiation (PAR) (averaged over each sampled month) (C) for the 4 focal streams. Canopy was thinned at UPL in July 2007 and at TAY in July 2008. Dotted lines indicate predicted concentrations of epilithon saturation for DIN and SRP (Newbold 1992). Grey bars indicate dry-season months. See Fig. 1 for site codes.

8.4 and 7.0% and mean discharge of 43.4 and 35.7 L/s, respectively.

Epilithon biomass and stoichiometry.—Chlorophyll *a* differed among streams (*L*-ratio = 30.77, df = 5, *p* < 0.001) and was negatively related to SRP concentration (*L*-ratio = 42.46, df = 9, *p* < 0.001), positively related to % open canopy (*L*-ratio = 57.93, df = 9, *p* < 0.001), and was lower in the wet than in the dry

season (*L*-ratio = 172.98, df = 9, *p* < 0.001; Fig. 7A, B). AFDM differed among streams (*L*-ratio = 40.69, df = 8, *p* < 0.001), was lower in the wet than in the dry season (*L*-ratio = 9.223, df = 12, *p* = 0.002) and in riffles than in pools (*L*-ratio = 117.69, df = 12, *p* < 0.001) (Fig. 7C, D), and decreased with % open canopy (*L*-ratio = 15.95, df = 12, *p* < 0.001). AI differed among streams (*L*-ratio = 34.16, df = 17, *p* < 0.001) and was lower in the wet than in the dry season (*L*-ratio = 90.58, df = 21, *p* < 0.001) and higher in riffles than in pools (*L*-ratio = 76.41, df = 21, *p* < 0.001) (Fig. 7E, F). AI was negatively related to SRP concentration (*L*-ratio = 37.11, df = 21, *p* < 0.001) and increased with % open canopy (*L*-ratio = 65.10, df = 21, *p* < 0.001).

C:N was lower in riffles than in pools (*L*-ratio = 46.22, df = 44, *p* < 0.001) and lower in the wet than in the dry season (*L*-ratio = 35.54, df = 44, *p* < 0.001) (Fig. 7G, H). C:N differed among streams (*L*-ratio = 90.67, df = 40, *p* < 0.001) and was negatively related to NH_4^+ concentrations (*L*-ratio = 29.21, df = 44, *p* < 0.001; Fig. 5D). C:P was lower in riffles than in pools (*L*-ratio = 50.87, df = 34, *p* < 0.001) and in the wet than in the dry season (*L*-ratio = 8.10, df = 34, *p* = 0.004; Fig. 7I, J). C:P differed among streams (*L*-ratio = 16.89, df = 30, *p* = 0.005), increased with % open canopy (*L*-ratio = 10.98, df = 34, *p* < 0.001), and decreased with NH_4^+ (*L*-ratio = 27.00, df = 34, *p* < 0.001), NO_3^- (*L*-ratio = 18.87, df = 34, *p* < 0.001), and SRP (*L*-ratio = 36.27, df = 34, *p* < 0.001; Fig. 5E) concentrations. In pools, N:P was higher in the wet than in the dry season (Fig. 7K, L). However, the wet season had a negative effect on modeled epilithon N:P (*L*-ratio = 4.13, df = 22, *p* = 0.042). N:P differed among streams (*L*-ratio = 39.01, df = 18, *p* < 0.001), was positively related to % open canopy (*L*-ratio = 8.12, df = 22, *p* = 0.004), and was negatively related to NH_4^+ (*L*-ratio = 29.22, df = 22, *p* < 0.001), NO_3^- (*L*-ratio = 31.08, df = 22, *p* < 0.001), and SRP (*L*-ratio = 24.55, df = 22, *p* < 0.001; Fig. 5F) concentrations.

Discussion

Light, nutrients, and flow significantly affected epilithon biomass and stoichiometry in Trinidadian streams. Greater chlorophyll *a* concentrations were associated with higher light levels as predicted, but the relationship between light and epilithon stoichiometry was not consistent between focal and survey streams. Nutrient content of epilithon was significantly greater in streams with greater dissolved nutrients. However, we did not detect strong relationships between nutrients and epilithon biomass, possibly because of light limitation, varying
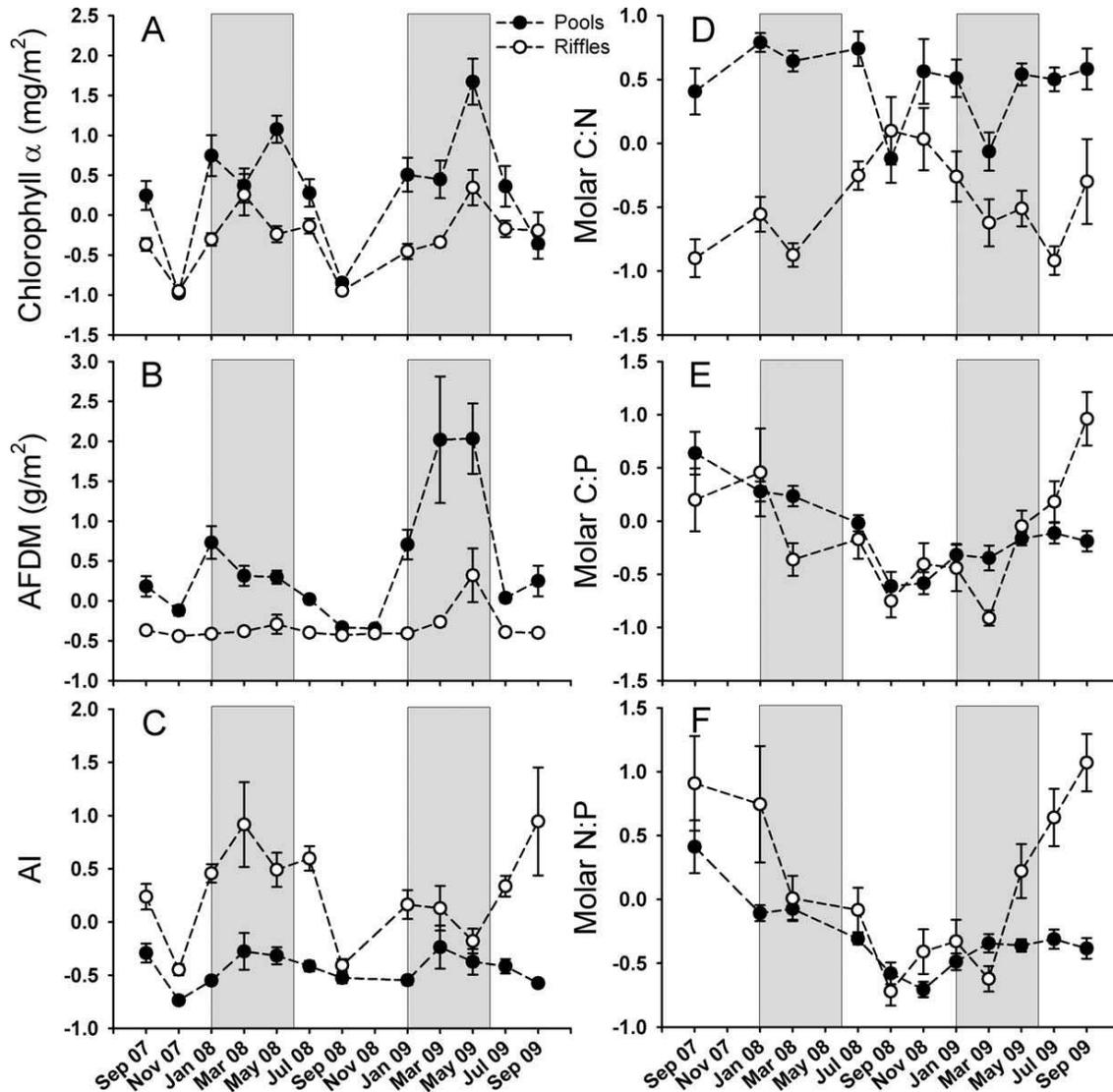
FIG. 4.   Mean (±1 SE) *z*-transformed focal-stream epilithon chlorophyll *a* (A), ash-free dry mass (AFDM) (B), autotrophic index (AI) (C), molar C:N (D), C:P (E), and N:P (F) averaged by pool and riffle habitats. Grey bars indicate dry-season months. *z*-transformations defined by $z = (X - \overline{X})/SD$.

periods between sampling times and scouring flows, or high nutrient availability at some locations. Our data suggest that epilithon communities in these Neotropical streams are influenced by numerous factors that structure epilithon biomass and affect their stoichiometry differentially through space and time.

Flow regime, habitat, and epilithon variability

Tropical streams are characterized by relatively consistent irradiance and temperature, which potentially enable epilithic growth throughout the year.

However, seasonal changes in flow could strongly affect the benthic ecology of these systems. Flow can be an important factor controlling algal biomass in temperate streams (Biggs and Close 1989, Dodds et al. 1996, Francoeur et al. 1998), and flow variables (wet/dry seasons, days since flow events) were strong predictors of epilithon dynamics in our study. Congruent with our predictions, epilithon AFDM was almost always greater when sampled following a period of relatively stable flow conditions or in habitats with reduced flow velocity (dry seasons and pool habitats, respectively) than during or soon after periods of high flow or in riffles. Wet-season flow
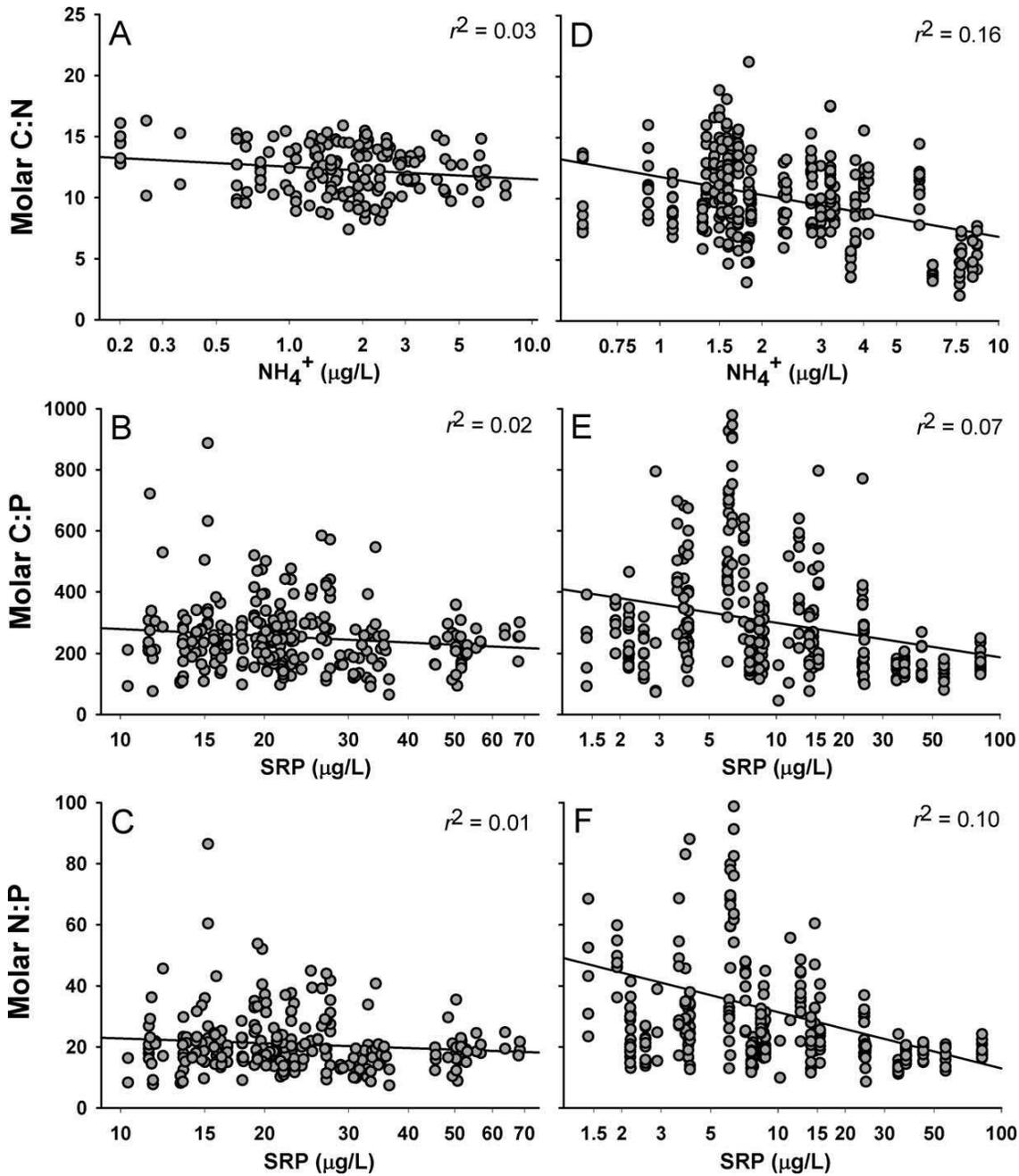
FIG. 5.   Simple linear regressions for molar C:N vs log-transformed NH_4^+ concentration (A, D), molar C:P vs log-transformed soluble reactive P (SRP) (B, E), and molar N:P vs log-transformed SRP (C, F) at focal (A, B, C) and survey (D, E, F) streams.

conditions reduced AFDM in pool habitats to levels observed year-round in riffle habitats. In contrast, chlorophyll *a* decreased with increasing days since an 8-cm increase in stage. This result might have been caused by accumulation of fine detritus and associated shading of epilithic algae during low-flow periods or by increased foraging by invertebrate grazers and

fish, but our data do not permit us to discriminate between these potential mechanisms.

Season and habitat type affected epilithon nutrient ratios in focal and survey streams. All epilithon nutrient ratios except focal-stream C:N were lower in the wet than in the dry season, and focal- and survey-stream C:N and survey-stream C:P were lower
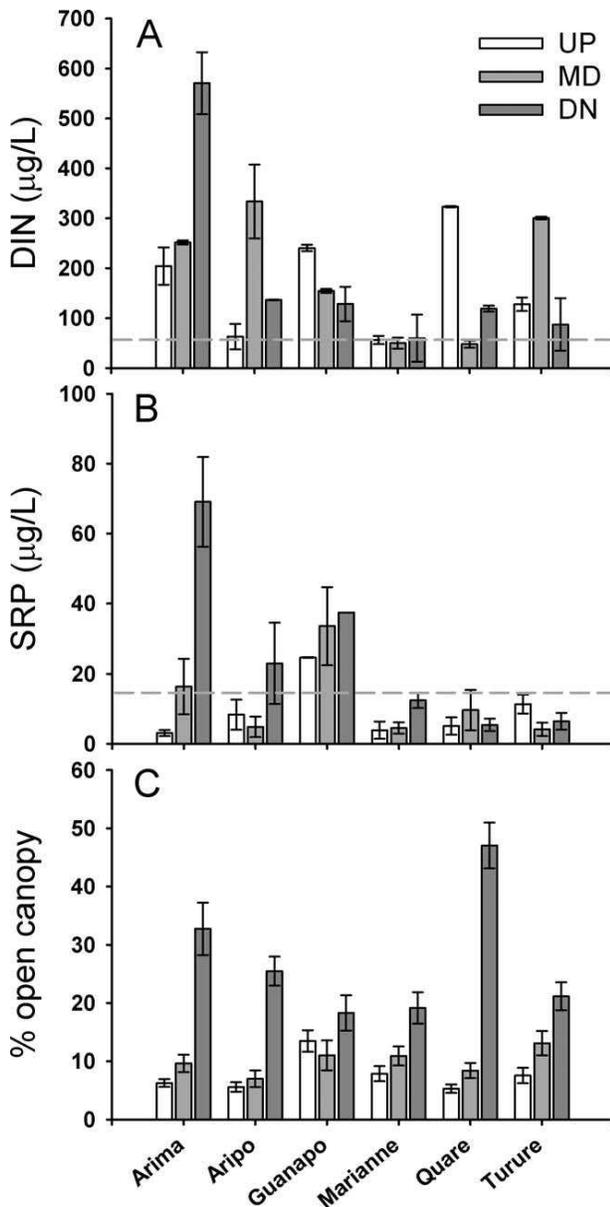
FIG. 6. Mean (±1 SE) dissolved inorganic N (DIN) (A), soluble reactive P (SRP) (B), and % open canopy (C) in headwater (UP), midstream (MD), and downstream (DN) reaches of survey streams in 6 drainages in the Northern Range of Trinidad. Dotted lines indicate predicted limits of saturation (Newbold 1992).

in riffle than in pool habitats. However, our prediction that epilithic nutrient ratios would decrease as the proportion of active algae increased was not supported. AI (the ratio of chlorophyll *a* to AFDM) was greater in the dry than in the wet season, but we should have observed lower C:nutrient ratios in the dry season if nutrient ratios reflected increased active

algae relative to AFDM. Instead, the higher dry-season C:nutrient ratios that we observed probably reflected increased C fixation by algae rather than a proportional increase in nutrient-poor senescent algae and detritus.

Nutrient availability

Dissolved nutrient concentrations affected epilithon biomass and stoichiometry in focal and survey streams, but relationships between nutrients and epilithon variables did not always agree with our predictions. For example, focal-stream AFDM decreased with greater NO_3^-, whereas chlorophyll *a* content decreased with greater SRP concentrations in both data sets. Newbold (1992) suggested that N may limit productivity in streams at concentrations ≤ 50 to 60 µg N/L, whereas P limitation might be alleviated at concentrations >15 µg P/L. SRP in the 4 focal streams averaged ~26 µg/L over the course of the study. This range is similar to the range of benthic saturation thresholds reported in previous studies (reviewed by Hill et al. 2009). DIN averaged ~191 µg/L, a concentration well above previously suggested thresholds. Thus, nutrient concentrations were relatively high in these streams. Some of the unexpected relationships between nutrients and epilithon biomass observed in our study might be indicative of light limitation or might be linked to seasonal changes in hydrology rather than to temporal patterns in water-column nutrients. SRP concentrations at focal and survey sites were greatest in the dry season, whereas NO_3^- concentrations were either stable across seasons or greater in the wet than in the dry season (e.g., focal sites in 2008). Our study was not designed to identify the catchment-scale mechanisms behind seasonality in nutrient concentrations, but our data are consistent with dilution of SRP in high-flow conditions (e.g., Triska et al. 2006) and leaching of soil N under wet conditions (Goller et al. 2006, Singh and Kashyap 2007). Links between hydrology and nutrient concentrations may explain why AFDM was negatively associated with NO_3^- and positively correlated with SRP in our study. Significantly lower chlorophyll *a* in both the focal and survey sites with increasing SRP might be a result of factors associated with stable hydrology rather than a direct effect of P availability on epilithon abundance.

In line with our hypotheses, the P content of epilithon increased with increasing SRP concentrations, and C:N ratios decreased with increasing concentrations of NH_4^+. However, other results were not consistent with stoichiometric theory. For example, NO_3^- and NH_4^+ were positively correlated with
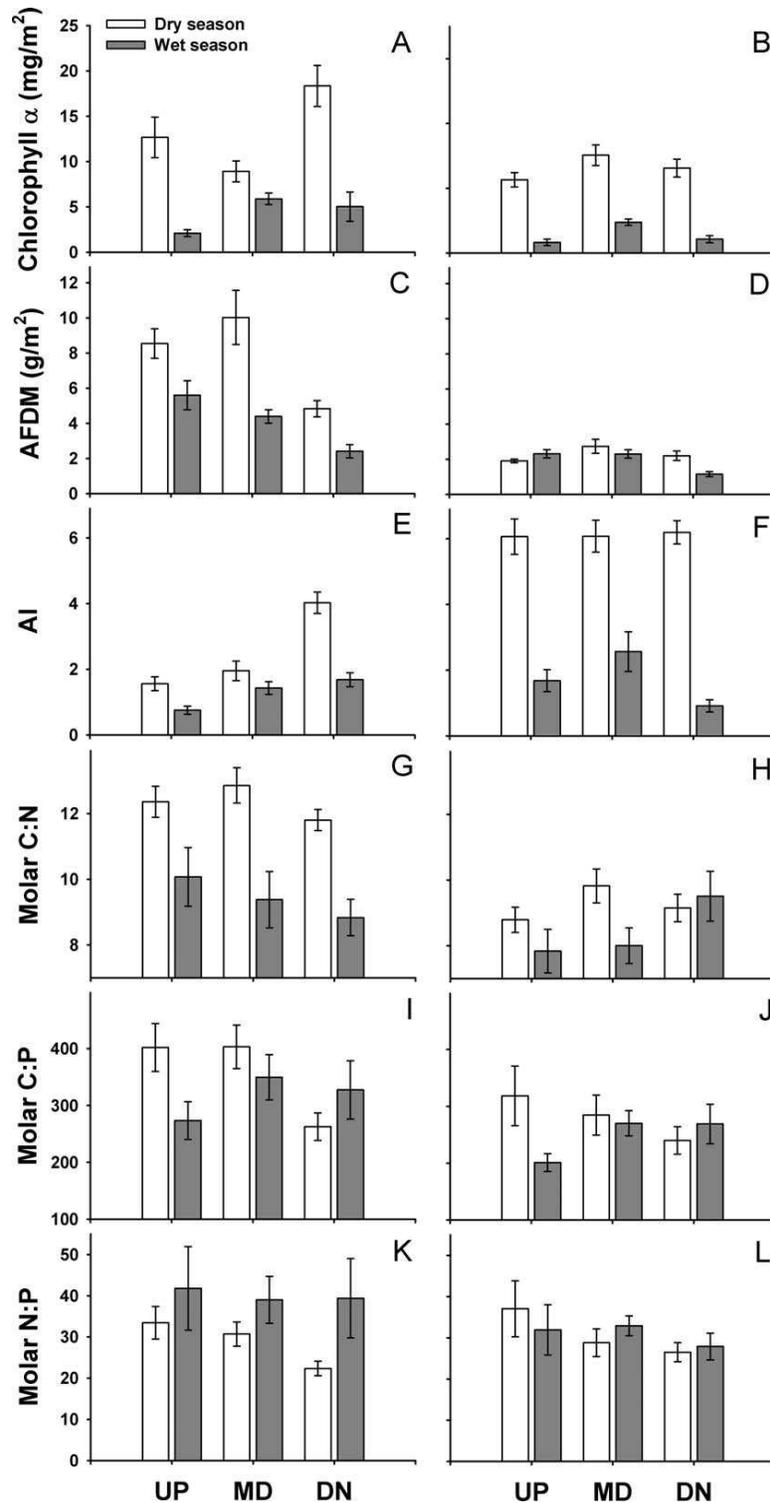
FIG. 7. Mean (±1 SE) epilithon chlorophyll *a* (A, B), ash-free dry mass (AFDM) (C, D), autotrophic index (AI) (E, F), molar C:N (G, H), C:P (I, J), and N:P (K, L) in pool (A, C, E, G, I, K) and riffle (B, D, F, H, J, L) habitats at survey streams in wet and dry seasons. 2007 wet-season biomass data (chlorophyll *a*, AFDM, AI) were removed (see text for explanation).

epilithon P content in the survey streams (decreased N:P and C:P ratios) but not in focal streams. The survey streams had a larger gradient of P availability than did focal streams (where SRP was often <15 μg P/L), so available NO_3^- and NH_4^+ in the water column may have increased P assimilation via production of enzymes required for P acquisition (Arrigo 2005). However, this hypothesis will require further investigation.

Light availability

Primary production in Trinidadian streams (Grether et al. 2001, TNH, unpublished data) and in other small forested tropical streams is often light limited (Davies et al. 2008). Variation in light among focal streams was quite large, especially when sites were compared with the experimentally thinned reach at TAY, which was 2 to 5× more open than the other focal streams. For comparison, Hill et al. (1995) found that a temperate Tennessee stream was light-limited until ~7 moles quanta m^{-2} d^{-1}, and this value was exceeded among focal streams only at the cleared reach of TAY. These results are consistent with our observations that chlorophyll a increased in focal and survey streams with light availability and did not appear to be directly linked to nutrient availability.

Available light was positively related to epilithon C:P only in survey streams, where the range of P concentrations was greater than in the focal streams. However, available light was negatively related to epilithon C:N in the focal streams, and epilithon N:P increased with % open canopy in survey streams. These results are contrary to the predictions of the LNH that C:N should increase with light, C-fixation rates, and reduced cellular chlorophyll a concentrations (Sterner et al. 1997, Sterner and Elser 2002). Ours is not the first study to produce results that conflict with LNH predictions. For example, Liess et al. (2009) found that increased light was associated with decreased C:N, and Hill and Fanta (2008) found no relationship between light and P content. The stoichiometric changes we observed may be the result of changes in epilithon community composition rather than changes in cellular physiology.

Synthesis and implications

Primary production is an important energy source in temperate and tropical streams (Minshall 1978, Brito et al. 2006, Lau et al. 2009), but more research is required to make meaningful comparisons across broad latitudinal ranges (Wantzen et al. 2006, Boyero et al. 2009). The seasonal and habitat-specific shifts in C:N:P ratios we observed suggest that flow dictates

epilithon abundance and nutrient quality, presumably by removing detrital C or by decreasing C fixation. One implication of our results is that organisms that cannot or do not feed selectively on epilithon may be susceptible to seasonal variation in food quality and may shift from energy to nutrient limitation during periods of stable flow. For organisms capable of selective feeding, habitat choice may mediate seasonal shifts in quality because epilithon had consistently higher chlorophyll a and nutrient content in riffles than in pools, thereby providing habitat-dependent variation in food quality.

Variability in epilithon composition also may affect the elemental composition of consumers. Vertebrate consumers are generally considered to be homeostatic, but Small and Pringle (2010) showed that increased resource quality elevated the nutrient content of secondary producers in Costa Rican streams. They suggested that this shift might have important implications for organisms at higher trophic levels. In support of this hypothesis, Dickman et al. (2008) demonstrated that C:P ratios of carnivorous fish were significantly related to the nutrient quality of phytoplankton, which in turn was mediated by light and nutrients. In Trinidadian streams, local site conditions explain stoichiometric variation in Trinidadian guppies (El-Sabaawi et al. 2012), which use epilithon as a food source (Zandonà et al. 2011), and influence N recycling (Palkovacs et al. 2009, Bassar et al. 2010). The effect of food quality on higher trophic levels in these systems is poorly understood, but our data indicate that spatial and temporal variation in epilithon quality responds to physical and chemical drivers and provide an initial step toward understanding how bottom-up factors influence food quality in tropical streams.

Conclusions

Our results suggest that explicit and integrated consideration of flow, nutrient, and light variables is necessary to provide a predictive framework for understanding epilithon biomass and nutrient fluxes between inorganic nutrient pools, primary producers, and higher trophic levels. Flow and habitat variables were useful for modeling epilithon biomass and stoichiometry, but our predictions based on the LNH were only partially supported. Nutrient availability consistently increased the nutrient content of epilithon as predicted, but the effects of light were often insignificant or contrary to our hypotheses. This result suggests that the effects of light on epilithon stoichiometry in field conditions are complex and may differ through time and space by interacting with

other unmeasured variables. More effort is needed to elucidate the effects of these interacting controls on epilithon, especially in natural settings, to refine predictions and improve existing stoichiometric frameworks such as the LNH.

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