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DO NUTRIENTS LIMIT ALGAL PERIPHYTON IN SMALL BLACKWATER COASTAL PLAIN STREAMS?[1]

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ABSTRACT: We examine the potential for nutrient limitation of algal periphyton biomass in blackwater streams draining the Georgia coastal plain. Previous studies have investigated nutrient limitation of planktonic algae in large blackwater rivers, but virtually no scientific information exists regarding how algal periphyton respond to nutrients under different light conditions in smaller, low-flow streams. We used a modification of the Matlock periphytometer (nutrient-diffusing substrata) to determine if algal growth was nutrient limited and/or light limited at nine sites spanning a range of human impacts from relatively undisturbed forested basins to highly disturbed agricultural sites. We employed four treatments in both shaded and sunny conditions at each site: (1) control, (2) N (NO_3-N), (3) P (PO_4-P), and (4) N + P (NO_3-N + PO_4-P). Chlorophyll *a* response was measured on 10 replicate substrates per treatment, after 15 days of *in situ* exposure. Chlorophyll *a* values did not approach what have been defined as *nuisance* levels (i.e., 100-200 mg/m^2), even in response to nutrient enrichment in sunny conditions. For Georgia coastal plain streams, algal periphyton growth appears to be primarily light limited and can be secondarily nutrient limited (most commonly by P or N + P combined) in light gaps and/or open areas receiving sunlight.

(KEY TERMS: algae; periphyton; periphytometer; nutrient limitation; light limitation; water quality.)

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INTRODUCTION

Dark-colored, low-gradient blackwater river systems in the southeastern United States (U.S.) originate in swamps, bogs, and marshes and often contain instream swamps (Smock and Gilinsky, 1992). These rivers are common throughout the lower Atlantic Coastal Plain where water chemistry is heavily influenced by the flat topography, typically sandy soils and elevated levels of dissolved organic carbon (DOC) that causes water to appear black (Wharton, 1978). Dissolved organic acids originate from swamp soils or decomposing litterfall from heavily vegetated floodplains and riparian zones (Beck *et al.*, 1974; Meyer, 1990).

Pristine blackwater streams have low nutrient levels because of soil type and nutrient retention in floodplains (Meyer, 1992; Smock and Gilinsky, 1992), but increased anthropogenic nutrient inputs from

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intensive agricultural operations can alter stream dynamics. Nutrient enrichment studies in large coastal plain blackwater rivers (e.g., Phlips *et al.*, 2000; Mallin *et al.*, 2001) show that dense phytoplankton blooms can adversely affect benthic organisms in these systems. However, no nutrient bioassay studies have examined algal periphyton response to nutrients in smaller blackwater tributaries.

In this study, we used passive nutrient-diffusion periphytometers modified from the design developed by Matlock *et al.* (1998) to investigate algal periphyton response to nutrient enrichment in Georgia's coastal plain streams. We modified the periphytometers to operate in shallow, low-flow conditions, which are typical of many blackwater streams during the summer. We chose this specific technique over other alternatives (e.g., clay pots, tiles, and mesh in combination with nutrient-enriched agar solutions) (Pringle and Bowers, 1984; Fairchild *et al.*, 1985; Corkum, 1996; Mosisch *et al.*, 2001; Tank and Dodds, 2003), because it allows complete recovery of algal assemblages attached to nutrient-diffusing filters, reducing treatment variability and increasing the likelihood of detecting significant differences (Morin and Cattaneo, 1992). Our objective was to determine if nutrients and/or light are factors limiting algal standing crop in streams draining a range of different land use types, from relatively undisturbed forest/wetland basins to highly disturbed agricultural sites. We hypothesized that nutrient concentrations and light levels in sometimes heavily shaded coastal plain streams would not support nuisance algal biomass (chlorophyll *a* values between 100 and 200 mg/m^2) (Horner *et al.*, 1983; Welch *et al.*, 1988) even across different land uses.

METHODS

Study Sites

We selected nine blackwater stream sites located within the Ocmulgee, Suwannee, and Satilla river basins (Figure 1). Human impacts within study stream basins (Table 1) ranged from relatively undisturbed (73-95% forest/wetland and 0.24-5% agriculture; $n = 4$), to moderately disturbed (51-67% forest/wetland; 17-29% agriculture; $n = 3$) to highly

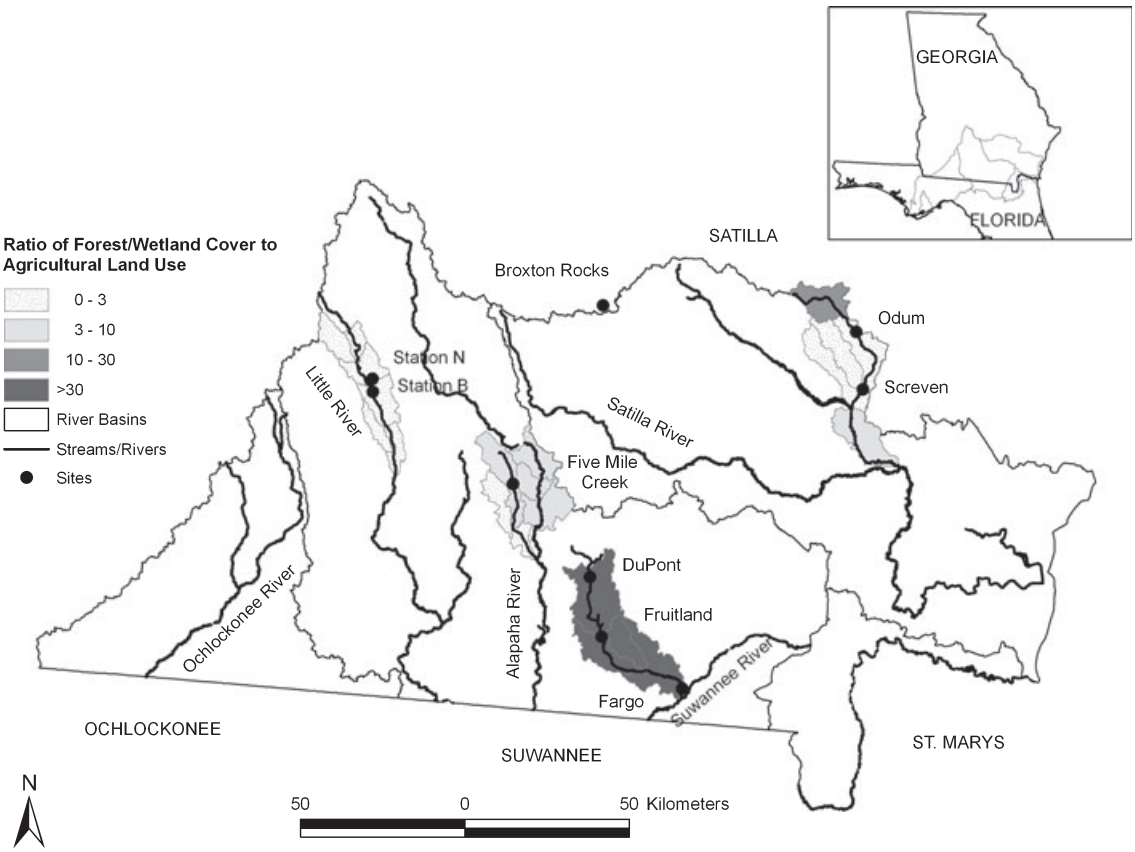

FIGURE 1. Map of the Southern Coastal Plain of Georgia Showing Study Watersheds and Nine Blackwater Stream Sites.

TABLE 1. Watershed Area and Percent Land Use (forest/wetland and agriculture).

Site	Watershed			Stream Order	NO_3-N * (μg/l)	NH_4-N (μg/l)	SRP (μg/l)	DIN:SRP Ratio		Limiting Nutrients	
	Size (ha)	For/Wet %	Ag %					Day 0	Day 15	Expected	Observed
1	910	95.00	5.00	2	17	45-89	4-21	13	124	P	P, N + P
2	79,933	82.00	0.16	5-6	26-28	368-409	19-33	104	67	P	None
3	17,279	80.84	0.81	5-6	21-93	176-403	13-16	60	175	P	None
4	40,690	73.07	0.24	5-6	30-30	67-1530	7-37	55	219	P	P
5	5,197	66.95	17.24	2-3	19-31	80-308	9-21	52	79	P	N + P
6	15,986	56.23	24.87	2-3	17-33	121-201	29-39	28	24	N	N, N + P
7	29,045	50.59	28.63	2-3	21-36	414-738	25-82	89	48	P	None
8	33,430	34.33	41.07	5-6	20-33	56-240	0-17	428	51	P	N + P
9	1,570	34.28	54.14	5-6	28-306	101-641	7-47	131	37	P	P

*Stream samples were collected on periphytometer deployment and retrieval dates at Sites 1-7 (n = 2) and throughout the experiment at Sites 8 and 9 (n = 9).

disturbed (34% forest/wetland; 41-54% agriculture; n = 2).

Relatively undisturbed (Site 1: Broxton Rocks – Broxton; Sites 2-4: Suwannoochee Creek – Fargo, DuPont and Fruitland) and moderately disturbed (Site 5: Five Mile Creek – Weber; Sites 6 and 7: Little Satilla Creek – Odum and Screven) forested basins consisted primarily of pine plantations managed for pulp and timber production in the uplands and hardwood forests in the floodplains (Figure 1). The two highly disturbed stream sites (Sites 8 and 9: U.S. Department of Agriculture-Agricultural Research Service gauging Stations N and B, Little River, Tifton, Georgia) were in a basin dominated by a cotton-peanut crop rotation, but generally contained mature hardwood riparian forest buffers. With the exception of the Broxton Rocks stream site, located within the Broxton Rocks Preserve, all stream study sites were on the public right-of-way and accessible at road crossings.

Periphytometers

Two periphytometers were deployed at each of the nine study sites between April and June 2004: one under tree canopy cover in the shade (typical conditions of each study stream) and the other in full sunlight (i.e., within a light gap of the riparian canopy). Each periphytometer consisted of a primary and secondary frame (Figure 2). Primary frames were constructed with lawn fence wire (7.6 cm by 5 cm grid) attached by plastic ties to 5 cm diameter schedule 40 PVC pipes. Forty 20 ml scintillation vials were attached to the wire grid in a completely randomized design because preliminary experiments revealed treatment proximity did not affect chlorophyll a accrual (Carey, 2005). There were 10 replicates within each of the following treatments: (1) control (C) – deionized water, (2) nitrate (N) – 87.5 mg/l NO_3-N (using 632 mg/l KNO_3), (3) phosphate (P) – 12 mg/l PO_4-P (using 103.8 mg/l $Na_2HPO_4 \cdot 7H_2O$), and (4) nitrate + phosphate (N + P).

Vial caps were drilled to produce 1.6 cm holes and both a membrane filter (25 mm diameter, 0.45 μm pore size, Millipore® Catalog No. HVLP02500, Billerica, Massachusetts) and a glass fiber filter (1.5 μm pore size, Whatman® 934-AH Catalog No. 1827–105, Brentford, Middlesex, United Kingdom) were installed across the top of each vial. Glass fiber filters functioned as artificial growth substrates for periphyton, while membrane filters regulated diffusion of nutrient solutions.

Secondary frames (3.8 cm diameter schedule 40 PVC pipes) had the same dimensions as primary frames (Figure 2). A fiberglass insect screen, taped to the bottom of each secondary frame, protected glass fiber filters from invertebrate grazers and prevented floating debris from settling on top of the nutrient-diffusing vials, which could potentially result in shading of filters. The fiberglass screen on each secondary frame was approximately 2.5 cm below vials on the primary frame. High-density foam weatherstrip (MD Building Products®, 1.3 cm × 1.9 cm, Oklahoma City, Oklahoma) closed the gap between primary and secondary frames (Figure 2) and several holes were drilled into each secondary frame to ensure that 20 ml vials were immediately beneath the water surface (Figure 2).

Periphytometers were prepared in a laboratory and transported to sites in a custom-built cooler. Each periphytometer was carefully placed in streams with glass fiber filters parallel to streamflow. The periphytometer frames were anchored with rope, attached to metal rods, and allowed to move with the current and adjust to fluctuating water levels

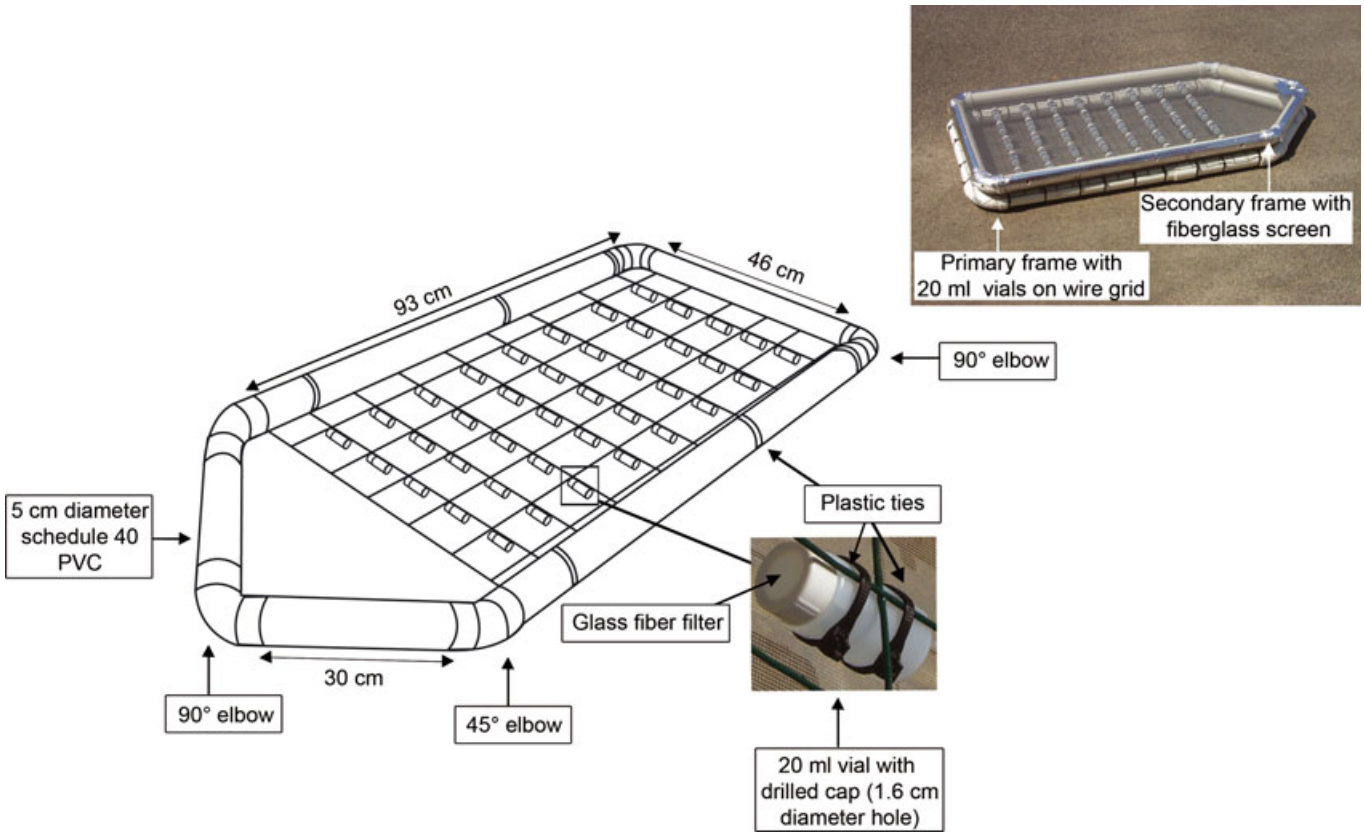

FIGURE 2. Schematic Diagram of Periphytometer Modified From Matlock *et al.* (1998) Showing Primary Frame With Forty 20 ml Scintillation Vials and Inset Showing an Inverted Picture of Primary and Secondary Frames Together; When Deployed, the Secondary Frame Lies Beneath the Primary Frame.

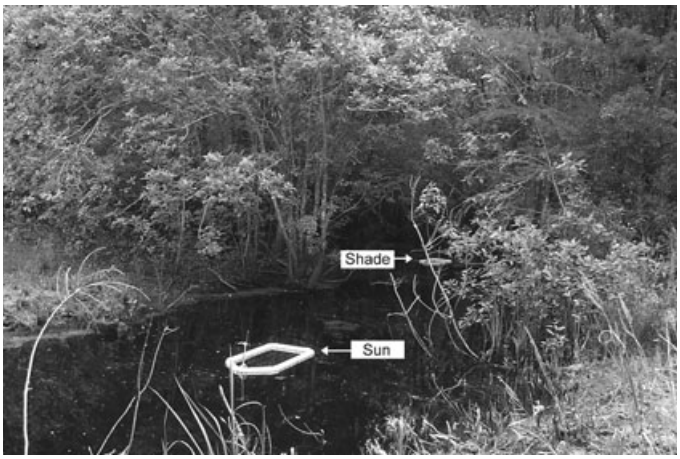

FIGURE 3. Modified Periphytometers Deployed in Shaded and Sunny Conditions at Site 6 (Little Satilla Creek – Odum) in the Southern Coastal Plain of Georgia. Secondary frames are not visible.

(Figure 3). Periphytometers were retrieved after 15 days, placed in the cooler and transported to the laboratory. Glass fiber filters from each vial were then carefully removed, sorted by treatment groups, transferred to Petri dishes, covered in foil and frozen for at least 24 hours. USEPA Standard Method 10200H.3 was used to extract chlorophyll *a* from filters (APHA, 1998) and chlorophyll *a* content (mg) was determined by analysis on a Turner Designs® TD 700 laboratory fluorometer (Sunnyvale, California). Chlorophyll *a* values were expressed as mg/m^2 by relating mass to the exposed surface area of glass fiber filters (1.7 cm^2).

Physicochemical and Statistical Analyses

Water samples were collected on periphytometer deployment and retrieval dates at each site. Dissolved oxygen (DO) and temperature were measured with a YSI® 550 DO meter (Yellow Springs, Ohio) and a LI-COR® quantum sensor (LI-190SA, Lincoln, Nebraska) measured photosynthetically active radiation (PAR). We did not measure PAR values for Sites 2 and 6 because of overcast conditions. Streamflow rates were well below the threshold (0.02 m/s) of our current velocity measurement instruments during the experiments as our study streams typically

TABLE 2. Dissolved Oxygen, Temperature, pH, Dissolved Organic Carbon, Total Suspended Solids, Solar Radiation, and Flow During Periphytometer Experiments at Nine Blackwater Stream Sites in the Southern Coastal Plain of Georgia Between April and June 2004.

						Solar Radiation (μmol/s/m^2)		Flow (m^3/s)	
Site	DO (mg/l)*	Temp (°C)	pH	DOC (mg/l)	TSS (mg/l)	Shade	Sun	Day 0	Day 15
1	4.95-9.08	15.80-17.35	5.49-6.38	16.60-20.55	8.80	85.30	1,038.10	Low flow	Low flow
2	2.97-6.70	16.80-20.20	4.05-4.09	52.09-52.36	1.29-8.41	–	–	Low flow	Low flow
3	3.41-5.68	18.00-19.60	4.26-4.77	40.80-46.11	4.86-10.62	55.62	1,662.10	Low flow	No visible flow
4	2.68-7.91	20.00-20.40	4.04-4.16	48.19-62.72	3.63-15.33	46.40	974.00	Low flow	Low flow
5	1.70-2.50	17.40-19.00	4.22-4.24	31.47-35.01	4.86-6.29	87.50	1,162.01	Low flow	No visible flow
6	0.30-0.87	21.00-22.00	4.76-5.07	32.35-36.72	9.00-33.64	400.00	1,950.00	No visible flow	No visible flow
7	0.42-0.54	19.90-20.80	5.10-5.66	31.74-32.73	6.00-8.00	–	–	No visible flow	No visible flow
8	0.35-0.89	22.60-23.80	6.69-7.24	11.71-18.45	7.37-8.12	20.70	1,350.00	0.00	0.00
9	1.23-2.19	21.80-22.90	6.64-6.97	12.02-16.86	2.64-2.99	60.50	1,354.10	0.03	0.02

Notes: pH and DOC were measured on periphytometer deployment and retrieval dates at Sites 1-7 (n = 2) and throughout the experiments at Sites 8 and 9 (n = 9).

*DO, temperature and TSS were measured on periphytometer deployment and retrieval dates at all sites (n = 2).

had extremely low flows or no visible flow (Table 2). At Sites 8 and 9, daily flow measurements were available and water samples were collected daily throughout periphytometer deployment. Water samples from all stream sites were analyzed for suspended solids, dissolved inorganic nitrogen (DIN: nitrate-N + ammonium-N), orthophosphate (soluble reactive phosphorus, SRP), chloride, potassium and DOC using standard analytical techniques (APHA, 1998). A pH meter (Orion® Model SA720, Allometrics Inc., Seabrook, Texas) measured water sample pH in the lab. Residual solutions in 20 ml vials were also analyzed for nitrate and phosphate concentrations using standard colorimetric techniques (APHA, 1998).

Treatments were compared using either an analysis of variance (ANOVA) or Kruskal-Wallis procedures in Statistical Analysis Systems (SAS Institute, Cary, North Carolina). The ANOVA procedure was used when assumptions of normality and homoscedasticity could be met (raw data or log transformations). Chlorophyll *a* treatment means, for individual periphytometers, were compared using an ANOVA (where necessary, values were log-transformed). *T*-tests were also used to compare treatment means in the shade at each site to corresponding treatments in the sun. Tukey's multiple comparison procedure was used to determine which means were significantly different at $\alpha = 0.05$. Linear regressions were used to determine whether residual treatment concentrations in 20 ml vials were significantly related to chlorophyll *a* values. For each treatment group (N, P, and N + P), mean chlorophyll *a* values from each site in the shade and sun were compared to corresponding mean residual nutrient concentrations.

RESULTS

Site Variables

Stream temperatures ranged between 15 and 24°C and except for Site 6, where trees were cut during the experiment, PAR values in the shade were below 100 μmol/s/m^2 but generally above 1,000 μmol/s/m^2 in the sun (Table 2). Mean stream pH values for Sites 9 (6.80) and 8 (6.95) were significantly greater than all other sites ($p < 0.01$), while Sites 4 (4.10) and 2 (4.07) had significantly lower pH values ($p < 0.01$) than every site except Site 5 (4.23; Table 2). DO concentrations were well below regulatory standards (4 mg/l) at several sites (Table 2). Sites 6-8, for example, all had DO concentrations below 1.00 mg/l on both deployment and retrieval dates.

Except for Sites 3 and 9, mean NO_3-N concentrations on deployment and retrieval dates at each site were below 45 μg/l (Table 1). Site 9 (112 μg/l) had significantly higher NO_3-N concentrations ($p < 0.05$) than Site 1 (17 μg/l), while Site 8 (7 μg/l) had significantly lower mean SRP concentrations ($p < 0.01$) than both Site 6 (35 μg/l) and Site 7 (53 μg/l; Table 1). NH_4-N concentrations for Site 3 (289 μg/l), Site 2 (389 μg/l), and Site 7 (576 μg/l) were significantly greater ($p < 0.05$) than Site 1 (67 μg/l; Table 1). DOC levels for Site 4 (55.46 mg/l) and Site 2 (52.22 mg/l) were significantly greater than all other study sites except Site 3 ($p < 0.01$; Table 2). Site 8 (14.93 mg/l), Site 1 (18.57 mg/l) and Site 9 (13.76 mg/l) had significantly lower DOC concentrations than all other sites ($p < 0.01$).

Chlorophyll a *Analyses*

Chlorophyll *a* values among treatments in the shade were significantly different at only Sites 1, 6, and 8 (Table 3). Except for N treatments at Site 1, mean chlorophyll *a* values for treatments in the sun at all sites were significantly greater than corresponding treatments in the shade ($p < 0.05$). For both periphytometers at Site 1, N + P treatments produced significantly higher chlorophyll *a* values than all other treatments ($p < 0.01$), but P treatments were significantly higher than both N treatments and controls ($p < 0.01$). There were no significant differences between controls and treatments at Sites 2, 3, or 7 ($p > 0.05$), but in the sun at Site 4, chlorophyll *a* values for P and N + P treatments were significantly greater than the control ($p < 0.01$). Chlorophyll *a* values for the control, N, and P treatments in the sun at Site 5 were not significantly different ($p > 0.05$), but these treatments were lower than the N + P treatment ($p < 0.01$). In the shade at Site 6, the only significant difference between the treatments was the higher chlorophyll *a* values for the N + P treatment ($p < 0.01$). However, in the sun at Site 6, the N treatment was significantly greater than both the P treatment and the control ($p < 0.01$), while the N + P treatment produced significantly greater chlorophyll *a* values than all other treatments ($p < 0.01$). The P treatment in the shade at Site 8 was significantly greater than both the control and N treatment ($p < 0.05$) but chlorophyll *a* values in the shade at Sites 8 and 9 were the lowest in the study (Table 3). Similar to Site 5, chlorophyll *a* values for the N + P treatment in the sun at Site 8 were significantly greater than remaining treatments ($p < 0.01$). Chlorophyll *a* values for P and N + P treatments in the sun at Site 9 were not significantly different ($p > 0.05$), but both produced significantly higher values than the N treatment and control ($p < 0.01$).

Residual Concentrations

Average residual nitrate concentrations in N and N + P treatments were between 0.09 and 11.17 mg NO_3-N/l (Table 4). Residual PO_4-P concentrations in P and N + P treatments ranged from 0.23 to 3.72 mg PO_4-P/l. Regression analyses of N, P, and N + P

TABLE 4. Average Residual Nutrient Concentrations (i.e., NO_3-N and PO_4-P) for N, P, and N + P Treatment Vials From Retrieved Periphytometers Deployed for 15 Days at Nine Blackwater Stream Sites in the Southern Coastal Plain of Georgia Between April and June 2004.

		Shade (mg/l)			Sun (mg/l)		
Site	**Nutrient**	**N**	**P**	**N + P**	**N**	**P**	**N + P**
1	NO_3-N	4.24	–	5.24	3.64	–	6.42
	PO_4-P	–	3.29	2.70	–	3.70	3.14
2	NO_3-N	0.72	–	0.76	0.68	–	0.69
	PO_4-P	–	1.17	0.96	–	1.24	0.89
3	NO_3-N	1.18	–	1.13	1.30	–	1.34
	PO_4-P	–	1.07	1.18	–	1.44	1.21
4	NO_3-N	2.98	–	2.00	6.42	–	11.17
	PO_4-P	–	2.53	1.57	–	3.30	3.72
5	NO_3-N	1.15	–	1.34	1.39	–	4.00
	PO_4-P	–	2.18	1.19	–	1.93	2.19
6	NO_3-N	0.96	–	0.62	0.51	–	0.13
	PO_4-P	–	1.34	0.83	–	1.01	0.59
7	NO_3-N	1.16	–	0.97	0.91	–	0.86
	PO_4-P	–	1.65	0.98	–	1.36	0.86
8	NO_3-N	0.77	–	0.62	1.01	–	8.92
	PO_4-P	–	0.81	0.69	–	2.08	3.26
9	NO_3-N	1.40	–	1.01	1.00	–	0.09
	PO_4-P	–	1.12	0.85	–	0.69	0.23

TABLE 3. Mean Chlorophyll *a* Values (mg/m^2 ± SD) for Control and Nutrient Treatments From Periphytometers Retrieved After 15 Days in Shaded and Sunny Conditions at Nine Blackwater Stream Sites in the Southern Coastal Plain of Georgia Between April and June 2004.

	Shade*				Sun			
Site	**C****	**N**	**P**	**N + P**	**C**	**N**	**P**	**N + P**
1	**1.47** (0.45) **c**	**2.09** (0.52) **c**	**4.45** (0.65) **b**	**14.52** (3.15) **a**	**2.18** (0.35) **f**	**2.32** (0.27) **f**	**6.47** (0.75) **e**	**17.42** (1.27) **d**
2	**3.29** (1.76) **a**	**2.01** (1.14) **a**	**2.85** (1.47) **a**	**2.14** (1.30) **a**	**7.17** (2.88) **de**	**7.22** (4.39) **de**	**10.74** (4.85) **d**	**5.22** (2.11) **e**
3	**1.64** (0.49) **a**	**1.85** (0.45) **a**	**2.16** (1.50) **a**	**1.69** (0.36) **a**	**35.38** (3.52) **d**	**36.44** (3.80) **d**	**34.24** (3.89) **d**	**36.22** (4.53) **d**
4	**2.22** (0.59) **a**	**2.95** (0.99) **a**	**2.43** (0.74) **a**	**3.16** (0.80) **a**	**18.69** (3.15) **e**	**22.10** (2.78) **de**	**23.17** (2.32) **d**	**23.63** (2.31) **d**
5	**2.68** (0.76) **a**	**2.83** (0.75) **a**	**3.55** (1.12) **a**	**3.03** (0.84) **a**	**18.49** (4.64) **e**	**19.48** (4.11) **e**	**22.92** (3.53) **e**	**40.11** (3.33) **d**
6	**1.38** (0.31) **b**	**2.05** (0.46) **ab**	**1.50** (0.42) **b**	**4.08** (2.00) **a**	**6.01** (1.48) **f**	**10.79** (3.30) **e**	**6.60** (1.59) **f**	**28.10** (6.53) **d**
7	**4.75** (1.45) **a**	**3.88** (1.86) **a**	**4.57** (1.86) **a**	**3.87** (0.59) **a**	**18.83** (5.19) **d**	**14.07** (1.63) **e**	**20.09** (1.93) **d**	**19.09** (3.81) **d**
8	**0.34** (0.09) **b**	**0.32** (0.06) **b**	**0.44** (0.09) **a**	**0.36** (0.06) **ab**	**11.10** (5.00) **e**	**14.76** (6.37) **e**	**15.77** (5.48) **e**	**50.02** (11.52) **d**
9	**0.48** (0.18) **a**	**0.42** (0.14) **a**	**0.53** (0.20) **a**	**0.40** (0.09) **a**	**5.53** (1.59) **e**	**6.66** (3.86) **e**	**55.38** (8.27) **d**	**69.28** (19.34) **d**

*Treatment means were compared for individual sites only, within shaded and sunny conditions. Different letters within each category (shade or sun) at each site represent treatment means that are significantly different (Tukey's test, $p < 0.05$).

**Treatment groups: C, control; N, NO_3-N; P, PO_4-P; and N + P, NO_3-N + PO_4-P.

treatments from all sites indicated that residual concentrations were not a significant predictor of chlorophyll *a* values for periphytometers deployed in the sun ($p > 0.05$). For P and N + P treatments in the shade, however, there was a significant relationship between residual nutrient concentrations and chlorophyll *a* values ($p < 0.05$).

DISCUSSION

Light Limitation

Our results strongly suggest that periphyton standing crop is primarily light limited in heavily shaded coastal plain streams subjected to different land uses. Light conditions clearly affected chlorophyll *a* production: chlorophyll *a* levels for both controls and nutrient treatments were significantly lower in canopy-shaded conditions ($p < 0.05$; Figures 4a-4i). Nutrient concentrations can affect primary productivity but nutrient limitation of algal biomass generally occurs only if light conditions are favorable (Mosisch *et al.*, 1999); algal biomass in some streams can therefore be primarily limited by light and secondarily limited by nutrients (e.g., Lowe *et al.*, 1986; Rosemond, 1994). Experiments at both Site 1 and Site 6 further demonstrated the relative importance of light. Periphytometers in the shade at Site 1 (incomplete canopy cover because it was early Spring) and Site 6 (trees were cut during the experiment) were unintentionally exposed to higher light levels and resultant chlorophyll *a* values had similar patterns to data obtained from periphytometers in the sun (Table 2; Figures 4a and 4f).

Light-limiting effects of the blackwater itself may also inhibit periphyton growth in coastal plain streams. Riparian canopy cover can intercept up to 95% of the incident solar radiation in narrow stream channels and, as light penetrates the water, high DOC concentrations and suspended solids can scatter and absorb light (Hill, 1996). DOC concentrations in low-gradient blackwater streams can approach 50 mg/l, while concentrations >3 mg/l are rarely found in higher gradient, nonblackwater southeastern streams (Smock and Gilinsky, 1992). Our results suggest that the lack of algal response to nutrients in sunny conditions at Sites 2 and 3 (Table 3; Figures 4b and 4c) may be because of high DOC levels (41-52 mg DOC/l; Table 2). Site 4 also exhibited high DOC levels (48-63 mg DOC/l) but there was a slight nutrient (P and N + P treatments) response (Figure 4d).

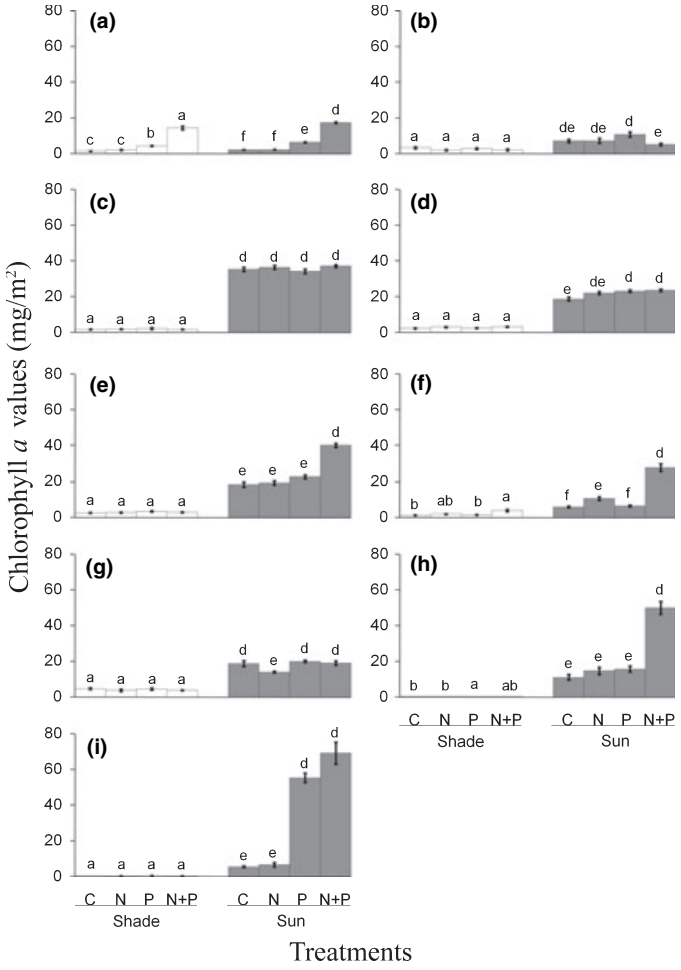

FIGURE 4. Summary Chlorophyll *a* Data for Nine Blackwater Stream Sites in the Southern Coastal Plain of Georgia; Different Letters Within Each Category (shade or sun) Represent Treatment Means That Are Significantly Different (Tukey's test, $p < 0.05$) and Error Bars Represent Standard Error. (a) Site 1 – Broxton Rocks (Broxton), (b) Site 2 – Suwannoochee Creek (Fargo), (c) Site 3 – Suwannoochee Creek (DuPont), (d) Site 4 – Suwannoochee Creek (Fruitland), (e) Site 5 – Five Mile Creek (Weber), (f) Site 6 – Little Satilla Creek (Odum), (g) Site 7 – Little Satilla Creek (Screven), (h) Site 8 – Little River (Station B, Tifton), (i) Site 9 – Little River (Station N, Tifton).

Nutrient Limitation

Periphyton standing crops (chlorophyll *a* concentrations) reported here are within the range of values previously reported in the literature for nutrient-diffusing substrata (NDS) experiments. Initial studies using the Matlock periphytometer technique (i.e., Matlock *et al.*, 1998, 1999a,b) reported mean chlorophyll *a* values between 2.1 and 62.2 mg/m^2 after two weeks. Tank and Dodds (2003) used glass fiber filters, 60 ml plastic containers and agar-nutrient solutions to investigate nutrient-limited algal growth in 10 streams across the U.S. and obtained chlorophyll *a*

values between 1 and 132 mg/m^2 after three weeks. In Canada, Corkum (1996) compared algal growth in forested and agricultural rivers using agar nutrient solutions as well and after five-six weeks, chlorophyll *a* values ranged from 2 to 95 mg/m^2.

Periphytometer results indicated that ambient stream nutrient concentrations limited algal standing crop in sunny conditions at six locations: Sites 1, 4, 5, 6, 8, and 9 (Table 1; Figures 4a, 4d-4f, 4h, and 4i). In terms of nutrient limitation, we classified algal growth for these sites as (1) P limited, (2) primarily P limited and secondarily N limited, (3) primarily N limited and secondarily P limited, or (4) colimited by both nutrients (Table 1). For example, algal growth at Site 9 was classified as P limited because chlorophyll *a* values for P and N + P treatments were not significantly different ($p > 0.05$) but both were greater than the control and N treatment ($p < 0.01$; Table 3; Figure 4i). However, Site 1 was primarily P limited and secondarily N limited because chlorophyll *a* values for the P treatment were significantly greater than the control and N treatment ($p < 0.01$), while the N + P treatment produced the highest chlorophyll *a* values ($p < 0.01$; Table 3; Figure 4a). Sites 5 and 8 were colimited by both nutrients because the control, N and P treatments were not significantly different ($p > 0.05$) but all were significantly less than chlorophyll *a* values for the N + P treatment ($p < 0.01$; Table 3; Figures 4e and 4h).

Nutrient limitation in sunny conditions can also be understood in terms of the Lotic Ecosystem Trophic Status Index (LETSI) (Matlock *et al.*, 1999b). The LETSI compares baseline primary productivity (i.e., mean chlorophyll *a* values for controls) to the maximum potential productivity (MPP) (i.e., mean chlorophyll *a* values for N + P treatments) in streams. Theoretical LETSI ratios (0-1) are dependent upon stream nutrient concentrations during sampling periods (15 days). When stream nutrient concentrations meet or exceed saturation levels for algal growth, LETSI ratios are equal to 1.0 because these streams are at MPP. Dissolved inorganic nitrogen (DIN: $NO_3 + NH_4$) concentrations below 100 μg/l may limit algal growth but streams with values above 400 μg/l are unlikely to be N limited (Horne and Goldman, 1994). For SRP, several studies have reported saturation threshold levels between 3 and 25 μg/l (Horner *et al.*, 1983; Bothwell, 1985; Rosemond *et al.*, 2002). LETSI ratios above 1.0 may indicate the presence of opportunistic algal species or high system variability (Matlock *et al.*, 1999b).

N-LETSI and P-LETSI compare mean chlorophyll *a* values for N and P treatments, respectively, to MPP (N + P treatments) and these ratios can indicate potential limiting nutrients in streams. For example, the P-LETSI ratio for Site 9 (0.80) suggests that P is the limiting nutrient because the P treatment accounts for 80% of MPP (Table 5). Site 5 (N-LETSI: 0.49; P-LETSI: 0.57) and Site 8 (N-LETSI: 0.30; P-LETSI: 0.32) suggest that N and P are simultaneously limiting periphyton biomass.

TABLE 5. Lotic Ecosystem Trophic Status Indices (LETSI) Comparing the Ratios of Mean Chlorophyll *a* Values (mg/m^2) for Control, N, and P Treatments to the Maximum Potential Productivity (mean chlorophyll *a* values for N + P treatments) in Sunny Conditions at Nine Blackwater Stream Sites in the Southern Coastal Plain of Georgia Between April and June 2004.

Site	LETSI	N-LETSI	P-LETSI
1	0.13	0.13	0.37
2	1.37	1.38	2.06
3	0.98	1.01	0.95
4	0.79	0.94	0.98
5	0.46	0.49	0.57
6	0.21	0.38	0.23
7	0.99	0.74	1.05
8	0.22	0.30	0.32
9	0.08	0.10	0.80

For all sites except Site 6, stream DIN: SRP molar ratios on periphytometer deployment and retrieval dates suggested P limited algal growth (Table 1). N:P ratios in healthy algal tissues are 16:1 (Redfield, 1958) and in streams, DIN: SRP molar ratios below 16:1 should theoretically lead to N limitation of algal growth. However, N limitation actually occurs anywhere from 10-30:1 (Allan, 1995). Nutrient ratios for Site 6, the only site that was primarily N limited, were between 23:1 and 27:1. The combined N + P treatment at Site 6 produced the greatest periphyton biomass and this is consistent with findings from other NDS experiments. Tank and Dodds (2003) reviewed several NDS studies and found that colimitation, or primary limitation by one nutrient and secondary limitation by another, was reported most frequently (41% of experiments). Multiple species algal communities may be limited by different nutrients because of species-specific nutrient requirements. Francoeur (2001), for example, conducted a meta-analysis of lotic nutrient amendment experiments and found that stimulation of algal communities by simultaneous additions of nutrients (e.g., N *vs*. P) was common.

Periphyton standing crop at Sites 2, 3, and 7 were not nutrient limited in sunny conditions (Table 3; Figures 4b, 4c, and 4g). Sites 2 and 3 were located in a relatively undisturbed, forested basin, while Site 7 was located in a moderately disturbed basin. Lack of nutrient treatment effects at Site 7 may reflect a saturated nutrient environment, given relatively high nutrient (NO_3-N, NH_4-N, and PO_4-P) levels (Table 1) and a LETSI ratio of 0.99 (Table 5). Particularly high

chlorophyll *a* values (35 mg/m^2) for the control treatment in the sun at Site 3 (Table 3) suggest that the periphyton community here was also nutrient saturated (LETSI: 0.98), although nutrient levels were not nearly as high as those observed at Site 7.

The stream water level dropped during the experiment at Site 2 and unfortunately, the channel substrate shifted the intended position of the apparatus; instead of being flat on the water surface, the periphytometer was at an angle. This was only observed on the retrieval date but the periphytometer may have been in that position for the majority of the experiment and the angle may have been more severe as well. Both the nutrient-diffusion rate (i.e., from 20 ml vials) and the subsequent periphyton response may have been affected by the periphytometer position. Therefore, periphytometer results and LETSI ratios for Site 2 may not reflect actual stream conditions.

Periphyton biomass for Site 4 was P limited in the sun (Figure 4d) as stream DIN: SRP molar ratios (55-219) suggested P limited algal growth and mean chlorophyll *a* values for P (23.20 mg/m^2) and N + P (23.60 mg/m^2) treatments were significantly greater ($p < 0.01$) than controls (18.70 mg/m^2). However, the LETSI ratio indicated that the stream was at 79% of MPP (Table 5) and this could explain the slight nutrient response at Site 4.

CONCLUSIONS

Utility of Periphytometer Technique and Residual Concentrations in Nutrient Reservoirs

The modification of the Matlock *et al.* (1998) periphytometer presented here – with a secondary frame, 20 ml nutrient reservoirs, membrane filters and glass fiber filters (Figures 2 and 3) – is an effective technique to examine nutrient limitation of algal standing crop in small streams. The 10 treatment replicates on each periphytometer produced more quantitative data than the original design (six replicates) and thus the technique can be a good comparative tool across multiple sites. For deployed periphytometers, 20 ml vials function as point sources of nutrients that assess (1) whether algal growth in a stream is nutrient-limited and (2) what nutrient(s) is/are limiting.

Morin and Cattaneo (1992) analyzed several periphyton field studies and found that respective sampling designs used would only detect significant differences ($\alpha = 0.05$) in periphyton productivity if nutrient treatment means differed by at least a factor of two. Periphytometer results from Sites 1, 5, 6, 8, and 9 generally supported this analysis. Throughout the entire study, however, the low degree of chlorophyll *a* variability between replicates within a given treatment (Table 3) suggested that smaller significant differences could be detected and this was indeed evident at both Sites 4 and 7 (Figures 4d and 4g).

Differences in residual concentrations (Table 4) among nutrient-diffusing scintillation vials may have been caused by membrane porosity differences and leaks between the edge of the membrane and the bottle lid. The extent and potential influence of this variability is unclear. It is unlikely that stream nutrient concentrations affected residual concentrations in the vials, since the diffusion gradient was from the vials to the surrounding water. Flow rates may have influenced relative residual concentrations because higher current velocities would induce greater nutrient-diffusion rates. For Sites 8 and 9, greater residual concentrations in treatment vials corresponded to lower current velocities (Tables 2 and 4). Flow rates may therefore be negatively correlated to residual concentrations but, without streamflow measurements at all sites, it is not possible to accurately assess this relationship. Excessive periphyton growth on glass fiber filters could also potentially reduce nutrient-diffusion rates from nutrient reservoirs. However, linear regressions of chlorophyll *a* values and residual concentrations from periphytometers deployed in the sun revealed that there was not a significant relationship between these two variables ($p > 0.05$). In the shade, residual nutrient concentrations for P and N + P treatments were a significant predictor of chlorophyll *a* values ($p < 0.05$). Except for the N + P treatment at Site 1, the relatively low chlorophyll *a* values obtained in the shade (Table 3; Figures 4a-4i) likely influenced these results.

Implications for Georgia Coastal Plain Streams

Multiple factors reduce the possibility of nuisance algal growth within blackwater streams draining Georgia's coastal plain. Heterotrophic competition for nutrients may occur because adjacent wetlands contribute dissolved organic material that promotes bacterial abundance in pristine blackwater systems (Edwards and Meyer, 1987; Meyer, 1990). Mallin *et al.* (2004) investigated nutrient loading in blackwater streams and found that nutrient additions stimulated both photosynthetic and heterotrophic activity, increasing the biochemical oxygen demand. Algal chlorophyll *a* levels between 100 and 200 mg/m^2 have been reported to represent nuisance conditions because dense algal growth can have a significant effect on DO by addition of oxygen through photosynthesis during the day and consumption of oxygen

through respiration at night (Horner *et al.*, 1983; Welch *et al.*, 1988; Dodds *et al.*, 1997). Chlorophyll *a* levels in our experiments (especially on the controls) were well below potential nuisance thresholds (Table 3, Figures 4a-4i). While this suggests that algae in our study streams would not necessarily affect DO deficits, we may have observed greater algal standing crops if we had run our experiment for longer than 15 days.

Several lines of evidence indicate that prevailing conditions were not conducive to optimal algal growth in our study streams, however. First, all of our sites had extensive riparian swamp forests and were heavily canopy-shaded with no visibly dense algal growth on natural substrata. Second, even in relatively atypical sunny conditions (light gaps), algal growth was found to be nutrient-limited in six streams (Figures 4a, 4d-4f, 4h, and 4i). Results from highly disturbed stream sites at Sites 8 and 9 also suggest that seasonally low flows in southeastern blackwater streams may be unfavorable for benthic algae. Both sites were located in nutrient-replete agricultural areas where critical factors, such as light conditions, not nutrient deficiencies, would be expected to limit algal growth. However, our experiments showed that algal growth was significantly nutrient limited (Table 3; Figures 4h and 4i). It is possible that minimal stream flow at these sites reduced the delivery rate of nutrients throughout the watershed. Both Sites 8 and 9 had extremely low DO concentrations (0.35-2.19 mg/l), yet the actual nutrient environment for benthic algae was poor. Even in blackwater streams with relatively high nutrient inputs from surrounding land uses, low-flow conditions that are prevalent during summer can alter overall nutrient resources available to benthic algae.

In summary, algal periphyton productivity in streams draining Georgia's coastal plain appears to be primarily light limited as a result of forested riparian zones and elevated DOC concentrations. Additionally, in light gaps and/or open areas receiving sunlight within these streams, algal periphyton growth can be secondarily nutrient limited (most commonly by P and/or N + P). Chlorophyll *a* values from our study streams were well below nuisance levels (100-200 mg/m^2) even for nutrient-enriched treatments in sunny, nutrient-poor conditions and this may be attributed to natural blackwater characteristics. Finally, the statistical discriminating power of our periphytometer technique modified from Matlock *et al.* (1998) allows significant differences in algal standing crop to be accurately detected by decreasing the variability within treatment replicates, which could potentially increase the utility of nutrient enrichment studies in small streams by providing a clearer indication of treatment effects.

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