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NONPHOSPHORUS LIPIDS IN PERIPHYTON REFLECT AVAILABLE NUTRIENTS IN THE FLORIDA EVERGLADES, USA[1]

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Algal and plant production of nonphosphorus lipids in place of phospholipids is a physiological response to low phosphorus (P) availability. This response has been shown in culture and in marine plankton studies, but examples from freshwater algae remain minimal. Herein, we analyzed the nutrient contents and lipid composition of periphyton communities across the Florida Everglades ecosystem. We hypothesized that in phosphate-poor areas, periphyton in high- and low-sulfate waters would vary the proportion of sulfolipids (SLs) and betaine lipids (BLs), respectively. In phosphate-enriched areas, periphyton would produce more phospholipids (PLs). We observed that at low-P sites, PLs were a minor lipid component. In cyanobacteria-dominated periphyton where sulfate was abundant, BLs were only slightly more abundant than SLs. However, in the low-P, low-sulfate area, periphyton were comprised to a greater degree green algae and diatoms, and BLs represented the majority of the total lipids. Even in a P-rich area, PLs were a small component of periphyton lipid profiles. Despite the phosphorus limitations of the Everglades, periphyton can develop tremendous biomass. Our results suggest a physiological response by periphyton to oligotrophic conditions whereby periphyton increase abundances of nonphosphorus lipids and have reduced proportions of PLs.

Key index words: **betaine lipid; everglades; oligotrophic; periphyton; phospholipid; sulfolipid**

Abbreviations: **BL, betaine lipid; DGCC, diacylglyceryl carboxyhydroxymethylcholine; DGDG, digalactosyldiacylglycerol; DGTA, diacylglyceryl hydroxymethyl-trimethyl-β-alanine; DGTS, diacylglyceryl trimethylhomoserine; ENP, Everglades National Park; GL, glycolipid; IP-DAG, intact polar diacylglycerolipid; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; SL, sulfolipid; SQDG, sulfoquinovosyldiacylglycerol; WCA, water conservation area**

P availability is a determinant of algal production and growth rate in freshwater and marine ecosystems (Sterner and Elser 2002, Hill and Fanta 2008). However, in wetlands, greater algal biomass and areal production may be observed under low nutrient (oligotrophic) conditions as a result of complex feedback mechanisms between biotic and abiotic factors (e.g., emergent macrophyte shading, mat dissolution, species shifts) (Grimshaw et al. 1997, Gaiser et al. 2011, Hagerthey et al. 2011). Algae cope with low total P (TP) concentrations by utilization of organic phosphorus via enzymatic processes (Newman et al. 2003), rapid uptake rates (Scinto and Reddy 2003), and stoichiometrically imbalanced growth (Sterner and Elser 2002).

A physiological modification that algae use is reduced production of PLs, in favor of nonphosphorus lipids (Frentzen 2004, Sato 2004, Van Mooy et al. 2009). Nonphosphorus lipids can compensate for reduced PL contents during low P availability, which in principle ensures a greater P supply for continued cell growth (Benning et al. 1993, Yu and Benning 2003, Van Mooy et al. 2009). This physiological plasticity has been documented in monocultures of higher plants (Essigmann et al. 1998, Härtel and Benning 2000), green algae (Sato et al. 2000a, b), cyanobacteria (Awai et al. 2007, Van Mooy et al. 2009), and anoxygenic photosynthetic bacteria (Benning et al. 1995, Benning 1998, Van Mooy et al. 2009). Observation of nonphosphorus lipid production in natural environments has been limited to marine (Van Mooy et al. 2006, 2009, Schubotz et al. 2009) and freshwater (Ertefai et al. 2008) phytoplankton, and marine algal mats (Bühring et al. 2009). The type and abundance of alternative

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lipids produced may differ for eukaryotic algae and cyanobacteria (Van Mooy et al. 2009).

Sulfur (S) is an essential plant macronutrient (e.g., for amino acids, lipids, vitamins), but S limitation is rarely observed in aquatic environments (Dahl et al. 2008). SLs are a natural component of cyanobacterial, algal, and plant chloroplast, and thylakoid membranes and envelopes along with glycolipids (GLs) and PLs (Benson et al. 1959, Sato 2004). The SL's anionic charge enables it to be substituted for the chloroplast PL phosphatidylglycerol (PG), sparing P for cell growth (Sato et al. 1995, Yu and Benning 2003). An additional lipid class, the zwitterionic nitrogen-containing BLs, can be produced in excess relative to phosphatidylcholine (PC) under P-limiting conditions (Benning et al. 1995, Van Mooy et al. 2009). Betaine lipids appear to be widely distributed in higher algae and lower plants (Eichenberger 1993, Kunzler and Eichenberger 1997, Rozentsvet et al. 2000), but so far observations have been limited in photosynthetic bacteria (Benning et al. 1995) and cyanobacteria (Řezanka et al. 2003).

This study documents *in situ* SL and BL contents in intact periphyton assemblages in an oligotrophic wetland ecosystem, the Florida Everglades, USA (Fig. 1). The Everglades provides a variety of P and sulfate gradients for quantifying alternative lipid abundances in response to nutrient availability. We hypothesized that in P-limited areas of the Everglades, PLs would constitute a small component of the total lipid pool, and nonphosphorus lipids would be prominent. The abundance of SLs and BLs would be dependent on sulfate availability and the relative proportions of cyanobacterial and eukaryotic algal groups in periphyton. Cyanobacteria-dominated periphyton in sulfate-rich areas would have greater proportions of SLs relative to PLs and BLs. Conversely, at an area where the periphyton had a greater eukaryotic algal component (e.g., diatoms and green algae), and both P and sulfate were limited in availability, periphyton would have greater proportions of BLs relative to both PLs and SLs. At a site that had increased P concentrations, periphyton would have greater PL content, reducing the relative abundance and proportion of alternative lipids compared to oligotrophic areas.

MATERIALS AND METHODS

Study sites. The Everglades is an oligotrophic (Total P < 0.01 mg · L^{-1}) ecosystem that nonetheless supports substantial (more than 1 kg ash-free dry weight [AFDW] •m^{-2} common) periphyton biomass (Gaiser et al. 2011, Hagerthey et al. 2011). Due to compartmentalization and water management, water chemistry varies significantly across the Everglades (Table 1), with an effect on periphyton assemblage structure (Gaiser et al. 2011, Hagerthey et al. 2011). From the South Florida Water Management District's DBHydro database (http://www.sfwmd.gov/portal/page/portal/xweb%20environmental%20monitoring/dbhydro%20application), we downloaded water-quality data collected for long-term monitoring and assessment of the ecosystem (e.g., Newman and Hagerthey 2011) or associated with an active management and monitoring project (Cattail Habitat Improvement Project [CHIP], Sklar et al. 2009).

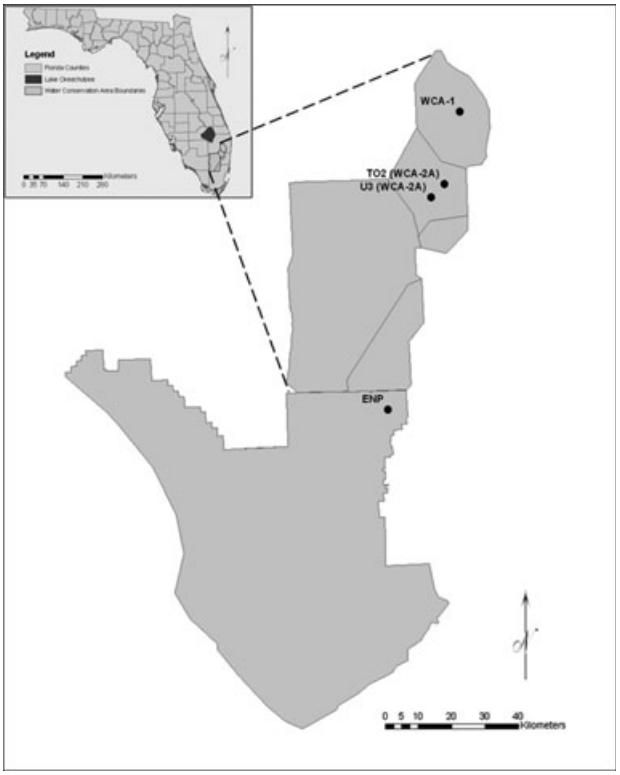

FIG. 1. Map of the Everglades and periphyton sampling sites within the water conservations areas (WCAs) and Everglades National Park (ENP).

TABLE 1. Water-quality parameters (mean ± SE) for monitoring sites at or near collection points of this study.

Area (site)	Conductivity (μ.s · cm^{-1})	SO_4^{2-} (mg · L^{-1})	TP (ug · L^{-1})	TKN (mg · L^{-1})
WCA-1 (Lox8)[a]	115 ± 4	0.4 ± 0.2	9 ± 1	1.4 ± 0.03
WCA-2A (TO2)[b]	770 ± 210	24 ± 15	20 ± 10	1.8 ± 0.6
WCA-2A (U3)[a]	840 ± 19	26 ± 4	5 ± 1	1.9 ± 0.06
ENP (NE1)[a]	527 ± 14	5 ± 1	7 ± 1	1.3 ± 0.04

[a]South Florida Water Management District database (DBHydro) from 2000 to 2011.
[b]Cattail Habitat Improvement Project, from July 25, 2006, to September 15, 2009.
ENP, Everglades National Park; TKN, total Kjeldahl nitrogen; TP, total phosphorus; WCA, water conservation area.

To examine lipid differences, we collected epiphytic periphyton within 1 m^2 quadrats from four study sites ($n = 3$ plots per site) in three regions of the Everglades with contrasting P and sulfate concentrations (Fig. 1, Table 1). The first site sampled was in the interior of WCA-1 (Fig. 1), the last remnant ombrotrophic region, containing trace amounts of P and sulfate (0.009 $mg \cdot L^{-1}$ and <0.4 $mg \cdot L^{-1}$ [detection limits], respectively) (Table 1). Periphyton was a loosely consolidated matrix comprised of desmids, diatoms, filamentous green algae, and cyanobacteria associated with *Utricularia purpurea* with a species composition distinctive within the Everglades (Gaiser et al. 2011, Hagerthey et al. 2011).

The next two sites sampled were in WCA-2A, a minerotrophic, sulfate-rich (26 $mg \cdot L^{-1}$) area that suffers P enrichment downstream from discharge structures, but P concentrations decline along a gradient moving toward to the interior (McCormick et al. 1996) (Fig. 1). The second site was P impacted (TO2-WCA-2A) with TP concentrations up to 0.05 $mg \cdot L^{-1}$ (mean 0.02 $mg \cdot L^{-1}$); the third site is oligotrophic (U3-WCA-2A), with average TP of 0.005 $mg \cdot L^{-1}$ (Table 1). The fourth site sampled was in Everglades National Park (ENP) (Fig. 1), a minerotrophic low TP (0.007 $mg \cdot L^{-1}$) and sulfate (5 $mg \cdot L^{-1}$) area. Dissolved N is typically plentiful across all study sites (total Kjeldahl nitrogen [TKN] > 1 $mg \cdot L^{-1}$) (Table 1). Thick calcareous periphyton of similar species compositions growing as epiphytic ''sweaters'' on the stems of *Eleocharis* and *Cladium* was collected at U3 and the ENP. Periphyton collected from the P-impacted TO2 was a gelatinous matrix associated with *Chara*, the algal species assemblage reflective of eutrophication (Gaiser et al. 2011).

Subsamples (20–40 g wet weight) of periphyton were dried at 60°C for dry weights (DW) and ashed for 1 h at 350°C for AFDW. Remaining periphyton (0.5–1 kg) was frozen at −80°C and lyophilized to dryness in a FreeZone 6 L console freeze dry system (Labconco, Kansas City, MO, USA). Subsamples (1–10 g DW) were shipped to the University of Florida for nutrient analysis, Florida Atlantic University for pigment analysis, and Woods Hole Oceanographic Institution for lipid composition determination. Data were expressed as content (mass $\cdot g^{-1}$ DW) or concentration (mass $\cdot m^{-2}$).

Nutrient analysis. Periphyton samples were analyzed for total nutrient contents of: carbon (TC), organic carbon (TOC), nitrogen (TN), phosphorus (TP), and sulfur (TS). Total carbon and TN were determined using a Carlo-Erba NA 1500 CNS analyzer (Haak-Buchler Instruments, Saddlebrook, NJ, USA). TP was determined after the procedure of Anderson (1976) followed by automated colorimetric determination (Method 365.1, U.S. Environmental Protection Agency 1993). Total sulfur was determined using a Costech 4112 elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) by combustion at 1,000°C in the presence of reduced copper and vanadium pentoxide catalysts with oxygen and helium carrier gases.

Pigment analysis. Periphyton total chl *a* content was determined using the method of Hagerthey et al. (2006). Briefly, ~100 mg of freeze-dried periphyton material was extracted in methanol/acetone/N,N-dimethylformamide/water (30:30:30:1 by vol.). After centrifugation and filtration of the extract, chl *a* and its derivatives were separated using two-dimensional reverse phase HPLC (RP-HPLC) using either: (i) a Thermo Separation Products (San Jose, CA, USA) Spectra-System AS3000 HPLC equipped with a P4000 quaternary pump and a UV1000 detector or (ii) a Thermo Separation Products model 4100 quaternary pump and a Waters 990 dual photodiode array detector (330–800 nm) (Waters Corp. Milford, MA, USA). Data acquisition employed Windows Peak Simple™ (SRI Instruments, Torrance, CA, USA) or Waters-990 software, respectively. Both systems were equipped with a Rheodyne Model 7120 injector (Rheodyne, Rohnert Park, CA, USA) with a 100 μL loop, and a 3.9–150 mm Waters Nova-Pak C18 RP column. Chromatograms were developed using a ternary gradient ([1] 0.5 M ammonium acetate in methanol/water, 85:15; [2] 90% acetonitrile; [3] 90% acetonitrile/ethyl acetate, 30:70) in linear changes. The injectate was prepared using 1.0 mL of filtered extract plus 0.125 mL of ion-pairing solution. Approximately 250 pmol $\cdot mL^{-1}$ of Cu-mesoporphyrin-IX dimethylester was used as an internal standard for development of a procedural correction factor (Hagerthey et al. 2006, J. W. Louda pers. comm.). Pigment quantification was based on the Beer–Lambert relationship where peaks were integrated ($mV \cdot min^{-1}$) and converted to quantities (~pigment response factors).

Contribution of different phototroph groups to the total chl *a* content was determined using a linear regression of pigments representative of cyanobacteria (zea and echinenone), green algae (chl *b*), diatoms (fucoxanthin), dinoflagellates (peridinin), and cryptophytes (alloxanthin) (Hagerthey et al. 2006 and references therein), with a modification to the chl *b* multiplier from 3.2 to 2.5 (J. W. Louda pers. comm.).

Lipid analysis. Lipids were extracted from dry samples using a modified Bligh and Dyer (1959) method, and the intact polar diacylglycerolipid (IP-DAG) composition of the extract was analyzed by using HPLC-mass spectrometry (MS) as described by Van Mooy et al. (2009). Briefly, ≈100 mg subsamples were placed in 15 mL glass centrifuge tubes. Next, 2 mL methanol (MeOH), 1 mL of dichloromethane (DCM), and 0.8 mL of PBS were added to the tubes, with 1.15 μg of the recovery standard 2,4 dinitrophenyl-phosphatidylethanolamine (DNP-PE). Tubes were capped with argon, vortexed rigorously for 1 min, sonicated for 15 min, and allowed to stand overnight at −20°C. Next, 1 mL of DCM and 1 mL of PBS were added and the tubes centrifuged for 10 min at 500*g*. The lower (DCM) phase, composed of the total lipid extract (TLE), was recovered and transferred to a 2 mL HPLC vial. All glassware were combusted at 450°C prior to use.

IP-DAGs in the TLEs were separated using normal phase solvents and a LiChrospher diol column (4 × 150 mm; 5 μm size) (Grace, Deerfield, IL, USA), and analyzed on a triple quadrupole mass spectrometer via an electrospray ionization source. Specific HPLC and MS conditions are described by Sturt et al. (2004) and Popendorf et al. (2011), respectively. External standard curves were constructed as described by Van Mooy and Fredricks (2010) using triplicate analyses of 20 μL aliquots of the mixed standards at five concentrations. The IP-DAGs in the sample TLEs were quantified against these standard curves, and then normalized to the recovery of DNP-PE. Phosphatidylglycerol (PG), phosphatidylethanolamine (PE), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG) were identified and quantified based on their diagnostic retention times and ''neutral fragments'' detected by MS. Phosphatidylcholine (PC), diacylglyceryl trimethylhomoserine (DGTS), diacylglyceryl hydroxymethyl-trimethyl-β-alanine (DGTA), and diacylglyceryl carboxyhydroxymethylcholine (DGCC) were identified and quantified based on their diagnostic retention time and product ions detected by MS. Reported BL content is based on DGTS only, as DGTA and DGCC were absent or were a small component of the samples.

Statistical analysis. To relate biochemical data to the organic components of periphyton, periphyton nutrients (TP, TS, and TN), chl *a*, and lipid contents were normalized to organic carbon (OC) content. This was especially important for periphyton from U3 and ENP where inorganic C can represent upwards of 50% of the total carbon content (as $CaCO_3$) (Hagerthey et al. 2011). Although phosphorus may be bound inorganically to $CaCO_3$ within periphyton, the relative proportion is generally small (<15%) in comparison to organically bound P (Noe et al. 2003, Scinto and Reddy 2003). Data were

$Log_{10}(n + 1)$ transformed to normalize where appropriate, and analyzed using one-way analysis of variance (ANOVA) ($\alpha = 0.05$). If significant, Tukey's HSD was applied to determine pair-wise differences between sites. Linear and nonlinear regression were used to assess the relationships among chl *a*, lipid, and nutrient contents. Data were analyzed with JMP v. 9 (SAS Corp., Cary, NC, USA) and Systat v. 11 (Chicago, IL, USA).

RESULTS

Periphyton biomass. Periphyton was abundant at all sites, but standing stocks differed across the system (Table 2). Biomass was greatest at TO2 (36.5 ± 7.7 g OC · m^{-2}) (mean ± SE), whereas WCA-1 had the lowest periphyton biomass (10.1 ± 1.6 g · OC m^{-2}) (Table 2). Chl *a* content at TO2 (7,622.9 ± 745.5 μg · g^{-1} OC) was more than double compared to all other sites ($P < 0.05$) (Table 2). Based on the pigment analysis, cyanobacteria dominated all periphyton types, but abundances relative to green algae and diatoms differed among WCA-1 (cyanobacteria 55%, green algae 29%, and diatoms 15%), TO2 (cyanobacteria 98%, green algae 2%), U3 (cyanobacteria 86%, diatoms 14%), and ENP (cyanobacteria 95%, green algae <1%, and diatoms 4%).

Periphyton nutrient content. Nutrient content and stoichiometric ratios of periphyton reflected both S and P availability. Corresponding with the highest water column P concentrations, TP content in periphyton from TO2 (2.3 ± 0.3 g · kg^{-1} OC) was significantly greater than that in other sites (Table 2). TP content in periphyton from WCA-1 (1.5 ± 0.1 g · kg^{-1} OC) was significantly greater than that of oligotrophic sites U3 and ENP. TS content of periphyton at U3 (50.4 ± 0.9 g · kg^{-1} OC), the site with the highest water column sulfate, was significantly greater than that in other sites (Table 2). Lowest TS content was observed in WCA-1 (14.9 ± 0.4 g · kg^{-1} OC) where water column sulfate concentrations were lowest. Periphyton TS content declined exponentially relative to TP content ($r^2 = 0.25$) (Fig. 2A).

The molar ratio of OC:P of all periphyton ranged from 1,100:1 to 4,500:1, while OC:N ratios were more constrained (7–12:1) (Table 2). The OC:S ratio was significantly lower in periphyton from U3 (53:1), followed by the ENP and TO2 (109:1), while WCA-1 (180:1) had a significantly higher ratio (Table 2). The S:P ratios were significantly lower for periphyton in WCA-1 and at TO2; U3 (83:1) had the largest ratio among periphyton.

Membrane lipid composition. Among periphyton analyzed, total detected IP-DAGs did not significantly differ, but the distribution of lipid classes between sites significantly varied (Table 3). Total phospholipid contents were significantly greater at nutrient-impacted TO2 (3.2 ± 1.3 μmols · g^{-1} OC) relative to U3 and WCA-1 (Table 3). Total PL contents were similar between periphyton at the three other oligotrophic sites ($P > 0.05$). PLs were an overall small component of the membrane lipids measured, representing between 1 and 4 mol% of the total detected lipids. At all sites, PC was the most abundant PL, followed by PG and PE (Table 3). Total PL content increased with periphyton TP content ($r^2 = 0.46$, $P < 0.05$) (Fig. 2B).

The abundance and proportion of SLs was similar across minerotrophic sites (TO2, U3, and ENP) (17–33%) (Table 3). SL content in WCA-1 (5.4 ± 0.5 μmols · g^{-1} OC) was significantly lower than that in all other sites, and represented 8% of the total detected lipids (Table 3). No significant relationship between SL content and the TS content of periphyton was observed ($r^2 = 0.16$, $P = 0.20$). For periphyton at oligotrophic sites, the abundance of SLs declined exponentially with increasing periphyton TP content ($r^2 = 0.92$), whereas at the P- and sulfate-rich TO2 site, both SLs and PLs were abundant (Fig. 3). Differential presence of SLs in thylakoid membranes was reflected

TABLE 2. Biomass and nutrient contents (mean ± SE, $n = 3$) of periphyton. Nutrient ratios based on molar masses. Analysis of variance *F*-statistics and *P*-values given; values with the same letter are not significantly different as determined by Tukey's HSD post hoc analysis.

Metric	Units	WCA-1	T02 (WCA-2A)	U3 (WCA-2A)	ENP	ANOVA $F_{3,8}$, *P*-value
Biomass	g OC·m^{-2}	10.1 ± 1.6^{b}	36.5 ± 7.7^{a}	26.6 ± 2.8^{ab}	17.1 ± 2.8^{ab}	6.73, < 0.05
Chl	μg·g^{-1}·OC	3,373.4 ± 344.1^{b}	7,622.9 ± 745.5^{a}	3,116.7 ± 163.4^{b}	1,977.4 ± 442.4^{b}	27.34, < 0.001
TC	g·kg^{-1}	376.3 ± 9.2^{a}	291.4 ± 5.5^{b}	254.0 ± 3.6^{C}	250.1 ± 3.4^{C}	322.84, < 0.001
TOC	g·kg^{-1}	328.8 ± 12.3^{a}	186.8 ± 7.2^{b}	143.4 ± 5.0^{C}	124.0 ± 4.0^{d}	427.64, < 0.001
TN	g·kg^{-1} OC	107.6 ± 1.9^{b}	144.4 ± 7.4^{a}	91.0 ± 2.2^{C}	113.0 ± 1.7^{b}	29.72, < 0.001
TP	g·kg^{-1} OC	1.5 ± 0.1^{b}	2.3 ± 0.3^{a}	0.6 ± 0.2^{C}	0.6 ± 0.1^{c}	104.82, < 0.001
TS	g·kg^{-1} OC	14.9 ± 0.4^{C}	24.7 ± 1.8^{b}	50.4 ± 0.9^{a}	24.8 ± 1.0^{b}	135.46, < 0.001
OC:N		11 ± 0^{b}	8 ± 1^{c}	13 ± 1^{a}	10 ± 0^{b}	41.85, < 0.001
OC:P		1,693 ± 89^{b}	1,128 ± 192^{c}	4,382 ± 305^{a}	4,531 ± 604^{a}	104.81, < 0.001
OC: S		180 ± 8^{a}	109 ± 12^{b}	53 ± 2^{c}	109 ± 8^{b}	112.83, < 0.001
N:P		156 ± 2^{b}	141 ± 7^{b}	341 ± 2^{c}	438 ± 2^{a}	60.82, < 0.001
S:P		9 ± 1^{d}	10 ± 1^{d}	83 ± 8^{a}	42 ± 5^{bc}	403.36, < 0.001

ENP, Everglades National Park; OC, organic carbon; TC, total carbon; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TS, total sulfur; WCA, water conservation area.

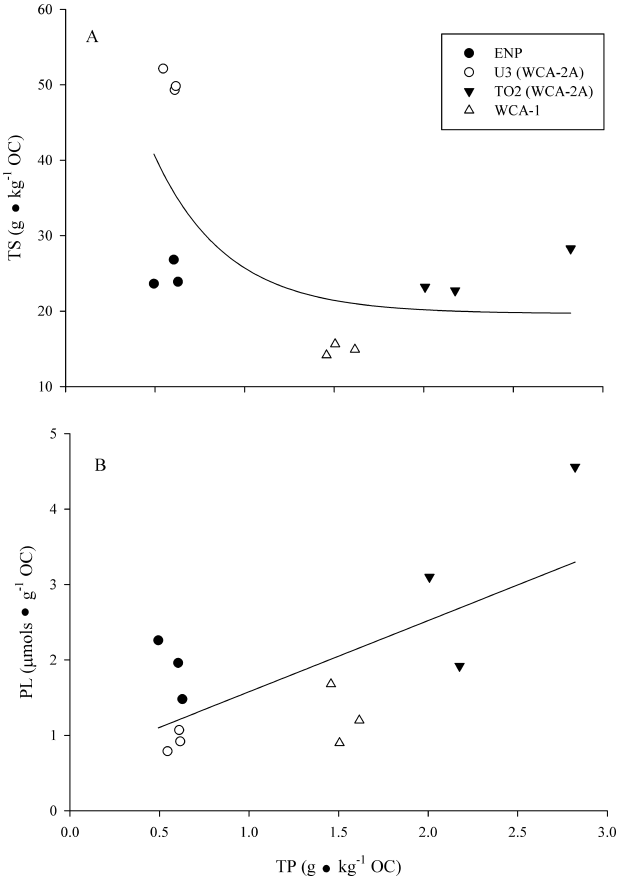

FIG. 2. Periphyton TP content ($g \cdot kg^{-1}$ OC) relative to (A) periphyton TS content ($g \cdot kg^{-1}$ OC), $y = 19.67 + 71.67e^{(-2.47x)}$, $r^2 = 0.25$; and (B) periphyton PL content ($\mu mols \cdot kg^{-1}$ OC), $y = 0.63 + 0.94x$, $r^2 = 0.46$. ENP, Everglades National Park; OC, organic carbon; PL, phospholipid; TP, total phosphorus; TS, total sulfur; WCA, water conservation area.

in the SL:chl *a* ratios. The lowest ratio of SL:chl *a* ($16 \times 10^{-4} \pm 2 \times 10^{-4}$) was observed in WCA-1 (Table 3), the site with lowest water column sulfate. The SL:chl *a* ratio was significantly greater for epiphyton at ENP ($128 \times 10^{-4} \pm 41 \times 10^{-4}$) (Table 3). Neither SL ($r^2 = 0.19$, $P = 0.16$) nor PL ($r^2 = 0.28$, $P = 0.08$) content was significantly related to chl *a* content.

BLs were the most abundant lipid class in all periphyton, representing 35–77 mol% of the total detected lipids (Table 3). Greatest BL content was observed for periphyton in WCA-1 (53.2 ± 7.5 $\mu mols \cdot g^{-1}$ OC) but was only significantly greater than that of periphyton in ENP (23.0 ± 0.9 $\mu mols \cdot g^{-1}$ OC) (Table 3). A positive, marginally nonsignificant relationship was observed between BL and TP content of periphyton ($r^2 = 0.27$, $P = 0.08$) (Fig. 4A), while BL content was negatively related to SL content ($r^2 = 0.33$, $P = 0.052$) (Fig. 4B).

Glycolipid content was greatest at TO2 (≈30 $\mu mols \cdot g^{-1}$ OC) and lowest in WCA-1 (≈9 $\mu mols \cdot g^{-1}$ OC) (Table 3). MGDG was more abundant than DGDG for periphyton at minerotrophic sites, but in WCA-1, DGDG was more abundant (Table 3). The proportion of GLs was similar between sites in WCA-2A and the ENP (20–30 mol%) but was reduced for periphyton in WCA-1 to ≈13 mol% of the total detected lipids (Table 3).

DISCUSSION

The Florida Everglades are hydrologically compartmentalized, with areas of distinct water chemistries as a result of natural and managed flows. Periphyton assemblage structure and biomass differs between areas (bog, poor and rich fen) of the Everglades, but despite typically low P availability, periphyton biomass is high (Hagerthey et al. 2011). We observed varying abundances of nonphosphorus sulfolipids and betaine lipids between periphyton types and with sulfate concentration, although phospholipids were a minor component in periphyton from all sites.

TABLE 3. Lipid contents (mean ± SE, $n = 3$) and ratios for periphyton. Analysis of variance *F*-statistics and *P*-values given; values with the same letter are not significantly different as determined by Tukey's HSD post hoc analysis.

Metric	Units	WCA-1	TO2 (WCA-2A)	U3 (WCA-2A)	ENP	ANOVA $F_{3,8}$, *P*-value
Total IP-DAG	$\mu mol \cdot g^{-1}$ OC	68.5 ± 9.6	100.1 ± 10.7	74.8 ± 4.3	66.8 ± 6.1	3.61, 0.065
PL-PG	$\mu mol \cdot g^{-1}$ OC	0.3 ± 0.0^{b}	0.7 ± 0.1^{a}	0.3 ± 0.0^{b}	0.6 ± 0.1^{ab}	11.58, <0.005
PE	$\mu mol \cdot g^{-1}$ OC	0.4 ± 0.1^{ab}	1.2 ± 0.4^{b}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	6.23, <0.05
PC	$\mu mol \cdot g^{-1}$ OC	0.6 ± 0.1^{a}	1.2 ± 0.3^{a}	0.5 ± 0.0^{a}	1.0 ± 0.1^{a}	4.36, <0.05
SL	$\mu mol \cdot g^{-1}$ OC	5.4 ± 0.5	26.9 ± 3.1^{a}	21.4 ± 1.3^{a}	21.9 ± 2.8^{a}	18.43, <0.001
BL	$\mu mol \cdot g^{-1}$ OC	53.2 ± 7.5^{a}	39.9 ± 8.5^{ab}	35.7 ± 2.0^{ab}	23.0 ± 0.9^{b}	4.63, <0.05
GL-MGDG	$\mu mol \cdot g^{-1}$ OC	4.1 ± 0.5^{a}	19.4 ± 2.6^{b}	10.7 ± 0.7^{a}	10.1 ± 1.3^{ab}	18.03, <0.001
DGDG	$\mu mol \cdot g^{-1}$ OC	4.6 ± 0.9^{b}	10.6 ± 1.5^{a}	6.2 ± 1.0^{ab}	9.9 ± 1.0^{a}	8.16, <0.01
SL: PG		21 ± 1^{c}	40 ± 10^{bc}	83 ± 4^{a}	40 ± 2^{b}	23.60, <0.0001
SL: PL		4 ± 1^{c}	10 ± 3^{bc}	23 ± 1^{a}	12 ± 0^{ab}	22.31, <0.001
BL: PC		92 ± 9^{a}	33 ± 1^{bc}	73 ± 5^{ab}	23 ± 2^{c}	39.82, <0.01
BL: PL		43 ± 4^{a}	13 ± 1^{b}	39 ± 3^{a}	12 ± 1^{b}	51.89, <0.001
BL: SL		10 ± 1^{b}	2 ± 1^{a}	2 ± 0^{a}	1 ± 0^{a}	124.94, <0.0001
SL: Chl ($\times 10^{-4}$)		16 ± 2^{c}	35 ± 0^{b}	69 ± 7^{ab}	128 ± 41^{a}	25.11, <0.001

BL, betaine lipid; DGDG, digalactosyldiacylglycerol; ENP, Everglades National Park; GL, glycolipid; IP-DAG, intact polar diacylglycerolipid; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; SL, sulfolipid; WCA, water conservation area.

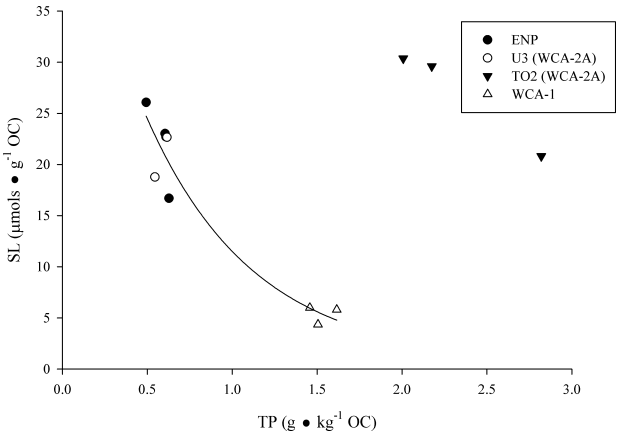

FIG. 3. Periphyton TP content (g · kg^{-1} OC) relative to SL content (μmols · kg^{-1} OC). Negative exponential line ($y = 0.67 + 52.29e^{(-1.57x)}$, $r^2 = 0.92$) does not include the P-impacted site TO2. ENP, Everglades National Park; OC, organic carbon; TP, total phosphorus; SL, sulfolipid; WCA, water conservation area.

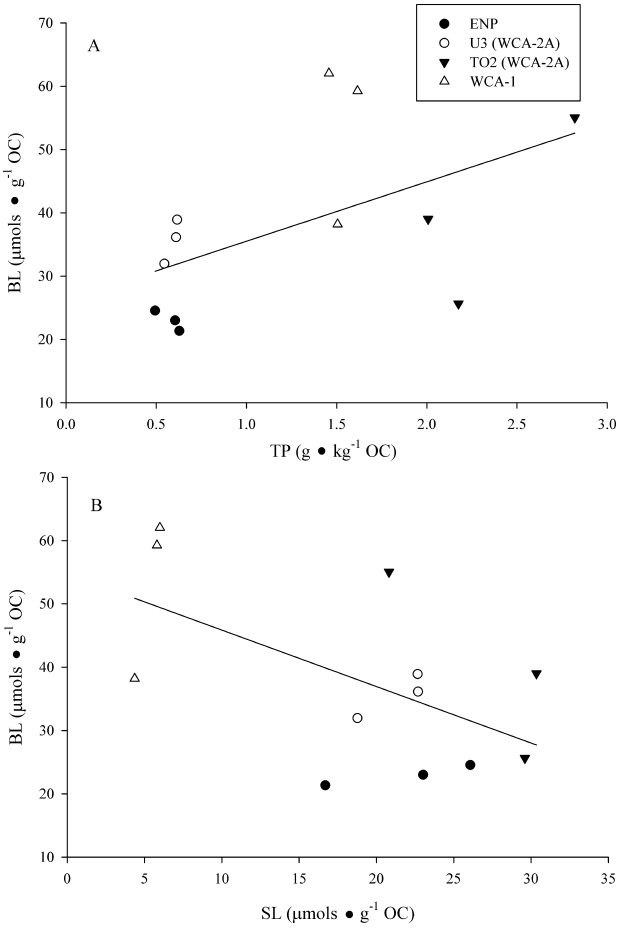

FIG. 4. (A) Periphyton TP content (g · kg^{-1} OC) relative to BL content (μmols ·kg^{-1} OC), $y = 26.13 + 9.38x$, $r^2 = 0.27$; and (B) Periphyton SL content (μmols · kg^{-1} OC) relative to BL content (μmols · kg^{-1} OC), $y = 54.78 - 0.89x$, $r^2 = 0.33$. BL, betaine lipid; ENP, Everglades National Park; OC, organic carbon; SL, sulfolipid; TP, total phosphorus; WCA, water conservation area.

Stoichiometric imbalance in response to P limitation is well documented in the Everglades (Scinto and Reddy 2003). Ratios of C:P and N:P reflect P limitation, and we also found periphyton S:P ratios in excess of the suggested Redfield ratio of 1.3:1. In addition, in WCA-1 periphyton OC:S ratios (180:1) were greater than a Redfield ratio of 95:1 (Ho et al. 2003), suggesting a potential S limitation. Cultures of *Dunaliella salina* were sulfate limited at concentrations near 0.5 mg · L^{-1} (Giordano et al. 2000), typical levels in WCA-1. Periphyton in WCA-1 had elevated TP contents relative to the other oligotrophic sites (U3 and ENP), while simultaneously having the smallest proportion of phospholipid-bound P (2.5%) relative to all other periphyton (3%–11%). In response to S and P limitation, algae may accumulate P as polyphosphate bodies (Lawry and Jensen 1979, Giordano et al. 2000), increase betaine lipid production (Van Mooy et al. 2009), and reduce SL production (Sugimoto et al. 2007).

Betaine lipids were the most abundant lipid class measured for all periphyton, and in WCA-1 represented more than 75% of the total measured lipids. BLs have been found in photosynthetic bacteria (Benning et al. 1995, Benning 1998), eukaryotic phytoplankton (Van Mooy et al. 2009), and lower and higher plants (Eichenberger 1993, Kunzler and Eichenberger 1997, Rozentsvet et al. 2000). As per our hypothesis, abundant BLs were expected for periphyton in WCA-1 as assemblages comprised to a greater degree of eukaryotic algae, in addition to the area having reduced sulfate availability. However, the prominent BL component in the cyanobacteria-dominated periphyton from WCA-2A and ENP was contrary to our initial hypothesis and is significant as these lipids are absent from freshwater (Wada and Murata 1998) and marine (Van Mooy et al. 2006, 2009) planktonic cyanobacteria. To date, identification of betaine lipids in cyanobacteria has been limited to primitive cyanobacterial species (Řezanka et al. 2003).

Bühring et al. (2009) noted varying abundances of BLs (10% to >20% of the total detected lipids) in marine mat layers composed of diatoms, cyanobacteria, and phototrophic sulfur bacteria, but they did not specifically attribute BLs to cyanobacteria. Wider distribution among bacteria has been suggested by Schubotz et al. (2009) given BL abundances (25%–45%) in anoxic regions of the Black Sea. Although it is unlikely that the observed BL contents could be solely derived from eukaryotic algae, production in cyanobacteria remains to be confirmed. Among the three BL classes measured, DGTA was absent from periphyton in WCA-1 and the ENP but was abundant (3–5 μmols · g^{-1} OC or 3%–6% of the total lipids) in periphyton at U3 and TO2 (data not shown). DGTA has been identified as the most abundant BL in eukaryotic algae of the South Pacific (Van Mooy and Fredricks 2010). The

absence in periphyton from WCA-1 but presence in WCA-2A is intriguing. Although this is likely due to species assemblage differences between regions, speculation of a reason is beyond the data available.

The PLs PC and PE are also typically not observed in cyanobacteria (Wada and Murata 1998); thus, the small contents observed are likely derived from the eukaryotic components in the periphyton matrix. Presence and abundance of BLs, PC, and PE in the photosynthetic bacterium *Rhodobacter sphaeroides* suggests an early evolutionary origin (Benning et al. 1993) and capacity to respond to P limitation (Benning et al. 1995). PC was the most abundant PL in Everglades periphyton, consistent with observations of other photoautotrophs (Sato and Furuya 1985, Jouhet et al. 2003, Yu and Benning 2003, Sanina et al. 2004) but was an overall small lipid component (<2%). Ratios of BL:PC were similar to, or in excess of, P-limited phytoplankton (0.9–28) (Van Mooy et al. 2009, Van Mooy and Fredricks 2010) and various green alga species (0.3–160) (Sato and Furuya 1985).

Our hypothesis regarding sulfolipids was confirmed as relative abundances were greatest for periphyton at U3 and ENP where sulfate was abundant and phosphorus was limiting. Conversely, in S- and P-limited WCA-1 the proportion of SLs (8%) and sulfolipid-S (1%) was significantly lower than all other periphyton. SL abundances in WCA-2A and the ENP were similar to cultures of *R. sphaeroides* (17%–40%) (Benning et al. 1995, Benning 1998), phytoplankton from the North Pacific (~25%) (Van Mooy et al. 2006), and cyanobacterial isolates (22%–32%) (Řezanka et al. 2003). Although there is no absolute necessity for SLs in the function of thylakoid and chloroplast membranes (Sato 2004 and references therein), growth impairment and reduced photosynthetic capacity have been observed in photoautotrophs unable to produce SLs in response to low P availability (Benning et al. 1993, Güler et al. 1996, Yu et al. 2002).

Under P-limiting conditions, PG, a prominent thylakoid component, is reduced in abundance corresponding with increased SL content (Güler et al. 1996, Sato et al. 2000b). Here, PG tended to be one of the lowest contributing lipids, resulting in SL:PG ratios of 20–80. An indicator of lipid substitution, SL:PG ratios can range from 4 to >350 for P-limited algae (Van Mooy et al. 2009). However, this index suffers from the influence of PG from organisms other than algae (Sanina et al. 2004, Van Mooy et al. 2006, 2009), and comparisons for all the lipid ratios between freshwater and marine systems (the bulk of research to date) are made herein with a bit of caution, given the differences in autotrophic species, ambient water chemistries, and evolutionary pressures.

At the P-impacted site (TO2 in WCA-2A), we observed a distinct periphyton assemblage and biochemistry. While OC:P ratios (>1,100) still indicate a degree of P limitation, periphyton at TO2 had lower proportions of SLs and greater PL abundances relative to oligotrophic sites U3 and ENP. However, contrary to our expectations, despite TP contents approximately five times greater in periphyton from TO2 relative to U3 and ENP, PL contents remain within a similar range. This increase in PL content relative to alternative lipids being less than expected may be a reflection of continued P limitation as evidenced in the OC:P ratio. The SL:PG and SL:PL ratios at TO2 differed significantly relative to U3 (both within WCA-2A), but not relative to ENP. This is potentially related to periphyton producing different amounts of chl *a*. Chl contents for periphyton in ENP were ~60% that of U3 and 25% of TO2, corresponding with SL:chl *a* ratios 2–4× greater in the ENP. Correlations have been observed between chl *a* and SL contents (Benning et al. 2008, Van Mooy and Fredricks 2010), but in this study, that relationship was not evident. One potential factor is the differences in assemblage structure observed (i.e., relative proportions of cyanobacteria and diatoms), resulting in thylakoids differently modified with SLs and PG. Variable use of PLs relative to alternative lipids between periphyton could factor into the observed variability in PL contents between periphyton of similar TP contents.

In addition to modulating SL and PG ratios in thylakoid membranes, autotrophs have been shown to increase MGDG and DGDG contents in response to P limitation (Andersson et al. 2003, Sato 2004, Cruz-Ramirez et al. 2006, Awai et al. 2007). GLs are commonly a significant component of chl membranes, representing upward of 75% of the total lipids (Mendiola-Morgenthaler et al. 1985, Eichenberger 1993, Härtel et al. 2000, Sato 2004). Proportions of GLs at oligotrophic sites in WCA-2A and ENP were similar to P-impacted TO2, and lowest in WCA-1. Thus, periphyton appeared to limit up-regulation of GLs in response to P limitation. The apparent noncompensatory role of GLs combined with a potential limitation to the degree of SL substation for PG in thylakoids (Sato et al. 2000a) provide an explanation for the observed significant BL contents observed in this study.

CONCLUSIONS

This work has shown the physiological trade-offs by periphyton in environments of differing P and S availability. We also expand on the overall understanding of periphyton sulfate utilization and role in the S cycle. Relative to average water column sulfate, SL contents increase quickly between periphyton in WCA-1 and ENP, but SL contents level off despite continued sulfate enrichment between ENP and WCA-2A. Maximal uptake rates of sulfate have been reported at concentrations of 9 $mg \cdot L^{-1}$ or lower (C. Gilmour and W. Orem, unpubl. data),

levels experienced in ENP and exceeded in WCA-2A. Cultures of *Chlamydomonas reinhardtii* were considered S-replete in medium containing >40 mg · L^{-1} sulfate (Sato et al. 2000b). In the interior of WCA-1, average sulfate concentrations remain well below 9 mg · L^{-1} as inputs are primarily through rainfall, thus likely contributing to the reduced SL and TS contents (Giordano et al. 2000, Sugimoto et al. 2007). Due to potential limitations in compensatory SL production, we found betaine lipids to be abundant and widespread in periphyton across the Everglades. Expanding research in marine systems is finding significant BL abundances within complex and diverse algal and microbial communities where P is limiting (e.g., Bühring et al. 2009, Van Mooy et al. 2009). The inverse relationship between two nonphosphorus lipids indicates differential utilization as an adaptive strategy, but additional research into their distribution and role(s) in freshwater autotrophs is needed.

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