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COMPOSITION OF EXTRACELLULAR POLYMERIC SUBSTANCES FROM PERIPHYTON ASSEMBLAGES IN THE FLORIDA EVERGLADES[1]

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In wetland habitats, periphyton is a common component of open-water areas with species assemblage determined by local water quality. Extracellular polymeric substances (EPS) secreted by algae and bacteria give structure to periphyton, and differences in EPS chemistry affect the functional roles of these polymers. The Florida Everglades provide a unique opportunity to study compositional differences of EPS from distinctive algal assemblages that characterize areas of differing water chemistry. Water conservation area (WCA)-1 is a soft-water impoundment; periphyton was loosely associated with *Utricularia* stems and amorphous in structure, with a diverse desmid and diatom assemblage, and varying cyanobacterial abundance. Extracellular polymers were abundant and were loosely cell-associated sheaths and slime layers in addition to tightly cell-associated capsules. The EPS were complex heteropolysaccharides with significant saccharide residues of glucose, xylose, arabinose, and fucose. Carboxylic acids were also prominent, while ester sulfates and proteins were small components. Structured, cohesive cyanobacteria-dominated periphyton was observed in WCA-2A, a minerotrophic impoundment, and filaments were heavily encrusted with calcium carbonate and detrital matter. EPS were primarily cell-associated sheaths, and polymer residues were dominated by glucose, xylose, fucose, and galactose, with uronic acids also a significant component of the polymers. Principal components analysis revealed that periphyton community assemblage determined the monosaccharide composition of EPS, which ultimately determines a range of biogeochemical processes within the periphyton.

Key index words: **cyanobacteria; desmids; EPS; minerotrophy; periphyton; polysaccharides**

Abbreviations: **ANOVA, analysis of variance; EPS, extracellular polymeric substances; IAP, ion activity product; PCA, principal components analysis; SI, saturation index; VPSEM, variable pressure scanning electron microscopy; WCA, water conservation area**

In aquatic habitats, phototrophs and heterotrophic prokaryotes often exist in complex cohesive communities referred to as periphyton or biofilms. The cohesive properties, and indeed the bulk of the periphyton biomass, are due to the matrix of biopolymers produced by constituent microorganisms and are referred to as EPS (Decho 1990, Hoagland et al. 1993, Wingender et al. 1999). EPS are primarily composed of polysaccharides but may also contain significant amounts of proteins and lipids (Decho 1990, Wingender et al. 1999). Complex heteropolysaccharides have been observed for EPS isolated from cyanobacteria (Bertocchi et al. 1990, Nicolaus et al. 1999, Barberousse et al. 2006), desmids (Domozych et al. 2005, Kiemle et al. 2007), and diatoms (Hoagland et al. 1993, Wustman et al. 1997, Underwood and Paterson 2003) in field and culture investigations. Organisms secreting EPS utilize these polymers for a variety of functions (i.e., adhesion, cohesion, motility), but it also represents a significant carbon source for heterotrophic components of the periphyton (Decho 1990, Middelburg et al. 2000, Decho et al. 2005, Bellinger et al. 2009).

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A fundamental question is whether saccharide composition of EPS is similar among diverse algal assemblages. While numerous algal and cyanobacterial EPS have been investigated from unialgal cultures and for whole biofilms, studies tend to only compare composition of EPS within a group. For example, Barberousse et al. (2006) isolated 12 green algal and 11 cyanobacterial strains from biofilms and analyzed two EPS fractions, with only cursory statements about saccharide similarities and differences among and between the two algal groups. Studies such as Underwood and Paterson (2003) and Bellinger et al. (2005) have compared the relative abundance of monosaccharides composing EPS fractions between diatom species and diatom-dominated biofilms using multivariate analysis and reported compositional similarities between extracts. However, for a cyanobacterial mat, greater EPS content and compositional variability relative to the diatom-dominated biofilms were observed (Bellinger et al. 2005).

In this study, we tested the hypothesis that distinct EPS biochemistries are associated with a periphyton type, determined by the prominent algal groups present (i.e., cohesive cyanobacterial mats vs. loose desmid and diatom conglomerations). The effects of nutrient enrichment on freshwater wetlands and their corresponding algal communities are well known (Swift and Nicholas 1987, Browder et al. 1994, Rejmánková and Komárková 2005, Gaiser

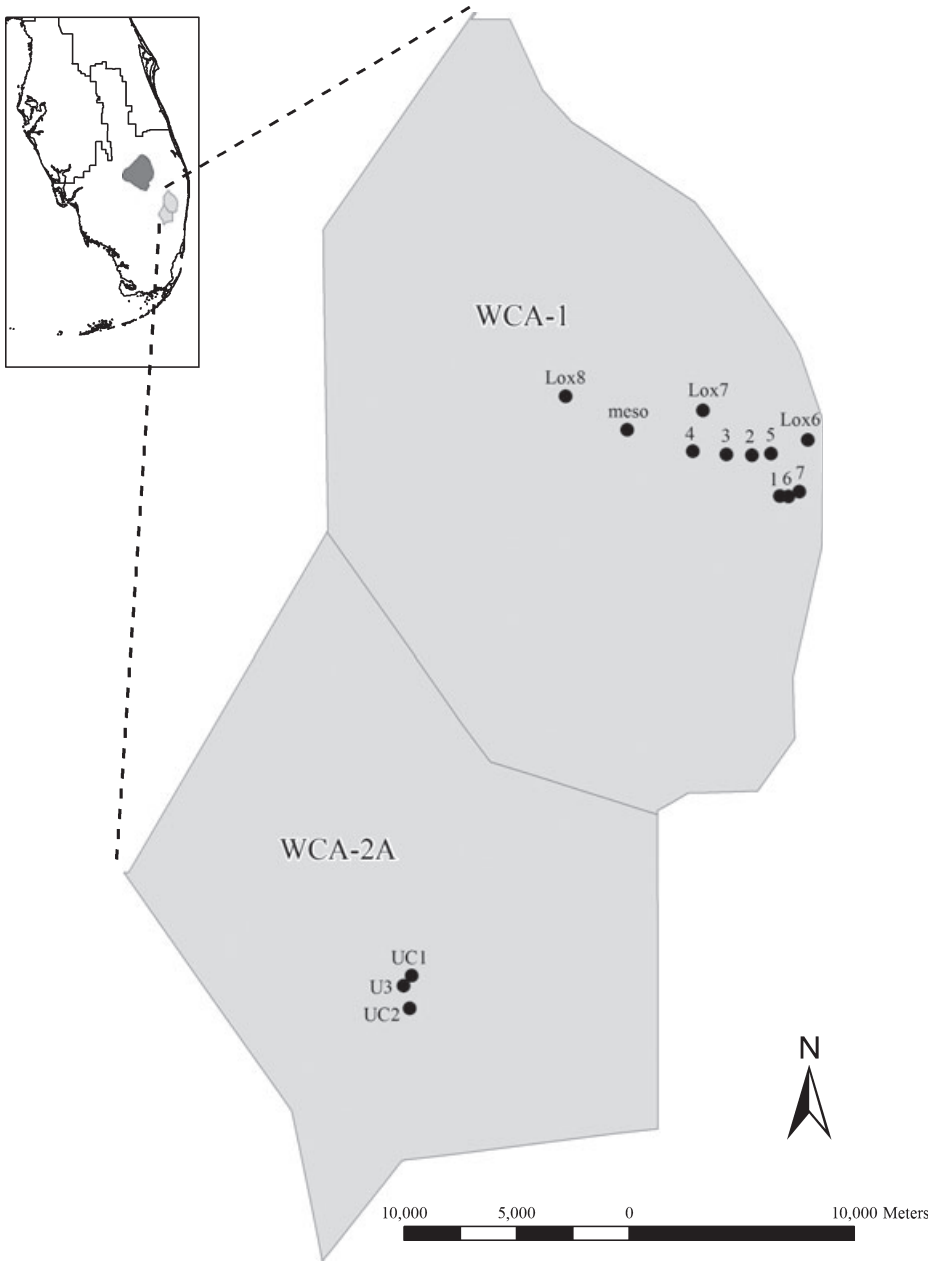

FIG. 1. Map of water-quality monitoring sites (LOX6, 7, and 8 in WCA-1, and U3 in WCA-2A) and sites of periphyton collection for EPS analysis (sites 1–7 and mesocosm [meso] in WCA-1, and sites UC1 and UC2 in WCA-2A).

et al. 2006), but increasingly, minerotrophy is being studied as significantly impacting wetlands, independent of nutrient effects (S. E. Hagerthey, S. Newman, and A. Gottlieb, unpublished data). Desmids (Streptophyta, Virdiplantae) have been determined to be very sensitive to high mineral content and, therefore, typify slightly acidic, mineral-poor conditions (Woelkerling 1976, Watanabe et al. 2000). In high mineral, oligotrophic waters, cyanobacteria can dominate the algal community, developing rigid, cohesive mats capable of precipitating calcium carbonate (Browder et al. 1994, Rejmánková and Komárková 2005). Diatoms are found in all water-quality conditions, autecological optima being species specific (Kelly et al. 2001, Potapova et al. 2004).

The Florida Everglades provide the opportunity to study periphyton communities that are indicative of local water chemistries. The northern Everglades have been compartmentalized into water conservation areas (WCA) (Fig. 1), and large differences in water chemistry are found (Table 1) (Swift and Nicholas 1987). In the ombrotrophic impoundment WCA-1, amorphous desmid-rich communities occur at interior sites, while more cyanobacteria-dominated communities are observed near the canals; in the minerotrophic interior of WCA-2A, cohesive cyanobacterial mats are prominent. Here, periphyton matrices were visualized using SEM, and the biochemical makeup of EPS was examined. Relative abundances of neutral sugars derived from EPS isolated from each periphyton type were compared using multivariate analysis to elucidate similarities and differences between the periphyton assemblages.

MATERIALS AND METHODS

Study sites. Periphyton was collected in WCA-1 and WCA-2A, located in the northern Everglades, at sites of relatively low water-column phosphorus concentration but variable ion concentrations (Fig. 1, Table 1). Water-quality data were downloaded from the South Florida Water Management District data portal DBHYDRO. Data are collected as a part of long-term monitoring of marsh health (McCormick et al. 1998, Harwell et al. 2008). In WCA-1, nonquantitative grab samples of periphyton were sampled from eight open-water sloughs of varying specific conductivity on 3 April 2007 (Fig. 1). Seven of these sites were randomly chosen, while the most interior site, referred to as the mesocosm site (ME), was established as an area of long-term research and study (Newman et al. 2001). Periphyton was loosely associated with *Utricularia purpurea* but is typically referred to as metaphyton. At a few sites, periphyton was epiphytic on *Eleocharis* or associated with the benthos (epipelon). In WCA-2A, periphyton was collected from interior sites UC1 and UC2 on 16 and 17 January 2007 (Fig. 1). Periphyton was distinguished as epiphyton (thick ''sweaters'' on *Eleocharis* sp. stems), metaphyton, and epipelon. Floating metaphyton results from epiphyton dislodged from *Eleocharis* stems, or epipelon becoming too buoyant and floating up. Samples were kept on ice in the field until processing. In the lab, subsamples of periphyton were frozen (−80°C) and lyophilized (Labconco freeze-drier, Kansas City, MO, USA) for EPS extraction and analysis.

TABLE 1. Water-quality data (average and standard deviations given) from 2000 to 2007 for monitoring sites in WCA-1 ($n = 70$) and WCA-2A (U3) ($n = 81$) (see Fig. 1 for site locations).

Area	Site	Conductivity ($\mu s \cdot cm^{-1}$)	Concentration ($mg \cdot L^{-1}$)							
			Na^+	Cl^+	Alk	CO_3^{2-}	SO_4^{2-}	TP	Mg^{2+}	Ca^{2+}	SI
WCA-1	Lox6	261.2 ± 102.5	29.6 ± 14.8	44.7 ± 23.0	70. 8 ± 40.3	96. 2 ± 30.8	7.6 ± 14.3	0.007 ± 0.003	6.1 ± 3.5	23. 3 ± 12.7	−0.1
	Lox7	96.4 ± 12.4	14.5 ± 4.2	25.1 ± 8. 3	15.0 ± 6.2	26. 6 ± 4.7	0.2 ± 0.2	0.01 ± 0.004	2.0 ± 0.5	7.0 ± 1.9	−1.0
	Lox8	106.2 ± 37.2	13.8 ± 4.1	23.9 ± 8.1	10.5 ± 3. 7	21.2 ± 2.9	0.4 ± 0.2	0.009 ± 0.004	1.9 ± 0.5	5.9 ± 1.7	−1.1
WCA-2A	U3	1009.4 ± 215.6	82.9 ± 21.3	121.2 ± 33.7	224.7 ± 44.1	186.9 ± 33.7	46.2 ± 20.2	0.008 ± 0.004	26.3 ± 6.1	65.3 ± 14.8	0.9

Sites represent proxy water-quality conditions to the sampled locations. SI refers to the saturation index of the water for calcium carbonate precipitation.

EPS extraction. Periphyton (0.5–1.0 g dry weight [dwt]) was sequentially extracted for EPS. First, periphyton was rinsed three times with 95% ethanol to defat and remove chl (Wustman et al. 1997). To isolate the loosely cell-associated slime EPS (Bertocchi et al. 1990), periphyton was treated with 20 mL of deionized water (dH_2O) at room temperature for 1 h and centrifuged (Eppendorf 5810R; Hamburg, Germany) at 1,200*g* for 15 min, and the supernatant was removed. Extraction with dH_2O was repeated, and supernatants were pooled, yielding a water soluble (WS) fraction (Hirst et al. 2003). Periphyton was then treated with 0.1 M EDTA at room temperature for 1 h to extract cell wall and sheath EPS (Decho 1990, Underwood et al. 1995, Decho et al. 2005). Periphyton was centrifuged at 1,200*g* for 15 min, and the supernatant was removed. The EDTA extraction was repeated, and the supernatants were pooled, giving an EDTA-soluble (EDTA) fraction. The EDTA fraction was dialyzed against 0.5 M imidazole over a 24 h period with four changes. Both EDTA and WS fractions were then dialyzed against dH_2O for 24–36 h, with changes every couple of hours, in dialysis tubing with a molecular weight (MW) cutoff of 8–10 kDa. Dialyzed samples were lyophilized for analysis.

The sequential fractionation protocol is an operational process that tries to mitigate the continuum in which polymers naturally exist. Carryover of polymers is possible during the extraction process; in addition, extraction may also isolate intracellular polysaccharides. For example, while extraction with dH_2O has been shown effective at isolating loosely cell-associated polymers, it may also remove intracellular storage glucans such as chrysolaminaran (Chiovitti et al. 2004, but see de Brouwer and Stal 2004).

EPS analysis. Total carbohydrate content of EPS was determined using the phenol–sulfuric acid assay with glucose as the standard (Underwood et al. 1995, Wustman et al. 1997). Uronic acid content was measured with the carbazole assay using galacturonic acid as the standard (Wustman et al. 1997). Proteins were quantified using the BioRad assay (BioRad, Hercules, CA, USA). Content is expressed as a percent of the total EPS fraction [weight:weight EPS (w:w)].

Monosaccharide composition of EPS fractions was determined following Wustman et al. (1997). Briefly, polysaccharides were hydrolyzed using 2 M trifluoroacetic acid for 3 h at 121°C, reduced, and acetylated, yielding alditol acetates for analysis by gas chromatography–mass spectrometry (GC–MS) (Finnigan-MAT magnum ion trap GC–MS; Thermo-Finnigan, Austin, TX, USA). Inositol was used as an internal standard, and identification of alditol acetates was based on comparison of mass spectra with a database of known sugars and comparison of retention times relative to a nine-sugar standard solution run with the samples. Total neutral sugar content is expressed as a unitless ratio normalized to the total amount of periphyton extracted ($g \cdot g^{-1}$ dwt periphyton; Tolhurst et al. 2005). Analysis of the WS fraction at WCA-1 site 4 and mesocosm could not be performed due to sample loss.

Variable pressure scanning electron microscopy (VPSEM). VPSEM was employed for gathering ''snapshots'' of the periphyton communities. Freshly collected periphyton was shipped overnight to Skidmore College for imaging. Periphyton was excised and immediately placed onto 2 cm diameter circles of Magna Nylon Filter (Fisher Scientific, Pittsburg, PA, USA). Periphyton was plunged into liquid nitrogen (LN_2) and attached to an LN_2-cooled cryostub (JEOL, Peabody, MA, USA). The cryostub was loaded into a JEOL 6480 VPSEM, and images were obtained under 40 Pascal (Pa) vacuum pressure, 10 kV accelerating voltage, backscattered electron detection, and 60 spot size (Domozych et al. 2007).

Statistical analysis. Within a WCA, relative abundances of individual neutral sugars were compared using one-way analysis of variance (ANOVA). Only two to four replicates of each sample were available for analysis; however, results are presented to show within-region variability. Post hoc analysis using Tukey's HSD indicated pair-wise differences in saccharide relative abundances. Relative abundance data were arcsin transformed prior to analysis (Zar 1999).

Principal components analysis (PCA). PCA analysis elucidated differences in composition of EPS between periphyton types from WCA-1 and WCA-2A. Relative abundances of saccharides for all sites were analyzed for variance-covariance to determine significant saccharide components for an extract and how sites grouped along saccharide vectors (Bellinger et al. 2005). Data were analyzed using PC-ORD (v. 4; Gleneden Beach, OR, USA).

RESULTS

VPSEM of periphyton communities. Common algal species from WCA-1 and WCA-2A have been described in detail elsewhere (Swift and Nicholas 1987, McCormick and O'Dell 1996), and here we only refer to the prominent groups observed in the VPSEM images. In WCA-1, periphyton was loosely conglomerated within an amorphous, gelatinous matrix associated with *Utricularia* stems and leaves. The diversity of algae within assemblages and abundance of EPS were evident (Fig. 2). Panels A and B in Figure 2 are representative of periphyton indicative of the interior of the marsh (sites 4 and 3), while panels C and D are from sites 1 and 6 nearer the canal. The latter sites are more impacted by canal water intrusion (Table 1) and have a reduced desmid abundance and greater cyanobacterial biomass. Extracellular polymers were evident as strands (relicts of dehydration), sheets, and sheaths associated with cells (Fig. 2, A and B).

In WCA-2A, periphyton was present in three different forms: epipelon (Fig. 3A), epiphyton ''sweaters'' on the stems of *Eleocharis* (Fig. 3B), and metaphyton (Fig. 3, C and D). Sheaths and sheets of EPS were observed, with deposition of calcium carbonate and detritus clearly visible on cyanobacterial filaments (Fig. 3). However, differing amounts of calcium carbonate appeared to be deposited within each periphyton type; metaphyton and epipelon were heavily encrusted with calcium carbonate (Fig. 3, A, C, and D), whereas in the epiphyton, noncalcified filaments were more prevalent (Fig. 3B).

EPS biochemistry. From periphyton in WCA-1, neutral sugar content for the WS fraction was in the range of $2.6–11.8 \times 10^{-3}$, and the EDTA fraction was in the range of $2.6–18.7 \times 10^{-3}$. In the periphyton from WCA-2A, neutral sugar content of the WS fraction was in the range of $0.7–3.0 \times 10^{-3}$, and the EDTA fraction was in the range of $2.1–26.2 \times 10^{-3}$. Relative abundances of proteins and uronic acids differed between periphyton types (Table 2). Both fractions for periphyton from WCA-1 contained more proteins relative to that found in WCA-2A. Uronic acid content of the WS

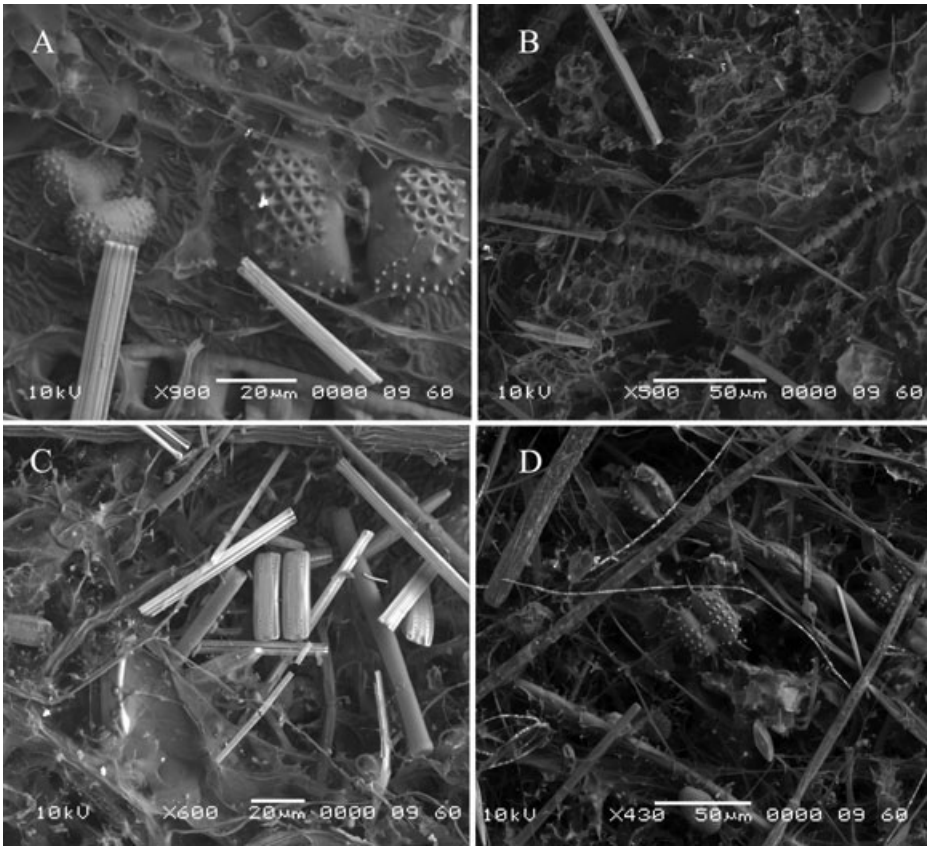

FIG. 2. VPSEM images of periphyton from sites in WCA-1 moving from the interior (A) toward the canal (D). Epiphyton associated with *Utricularia* from (A) site 4, (B) site 3, and (C) site 1, and (D) epiphyton associated with *Eleocharis* from site 6. VPSEM, variable pressure scanning electron microscopy.

fraction from cyanobacterial-dominated periphyton was, on average, greater than desmid-dominated, but for the EDTA fraction, the opposite was observed (Table 2).

The WS fraction isolated from periphyton in WCA-1 was carbohydrate rich (9%–36%), with uronic acid content typically <7% of the total fraction weight (Table 2). Protein content was typically between 2% and 4% (Table 2). Seven neutral sugars were identified: glucose, galactose, mannose, xylose, arabinose, fucose, and rhamnose (Table 3). Glucose was the prominent saccharide residue (35%–65%), followed by galactose, fucose, mannose, and xylose. Arabinose and rhamnose were variable in relative abundance (Table 3). Relative abundances of sugars varied for glucose, galactose, mannose, and fucose between sites within WCA-1 ($P < 0.05$) (Table 3). The general pattern was for significant differences between periphyton from interior sites and those nearest the canal (pair-wise $P < 0.05$), and overlap at intermediate sites with those near the canal and interior ($P > 0.05$).

The EDTA fraction generally had a large carbohydrate component (7%–46%) and low protein content (<2%); uronic acid content ranged from 2% to 25% (Table 2). Saccharide residues of the EDTA fraction were dominated by glucose, but relative abundances were lower than that of the WS fraction. Galactose, xylose, mannose, and fucose were the next most abundant saccharides (Table 3). Rhamnose and arabinose generally were a greater proportion of the EDTA fraction relative to the WS fraction. Relative abundances of all monosaccharides varied between periphyton ($P < 0.05$), with significant differences between canal and interior sites (pair-wise $P < 0.05$); periphyton from intermediate sites (2, 3, and 5) was not significantly different from either extreme ($P > 0.05$) (Table 3).

The WS fraction for the cyanobacterial communities in WCA-2A was rich in carbohydrates (20%–30%) and had a significant uronic acid content (7%–17%) (Table 2). Protein content and sulfate content were generally very low or not detected. Glucose was the prominent saccharide residue of the polymers, comprising between 53% and 69% of the total neutral sugars detected (Table 4). Neutral sugar relative abundances were generally similar between periphyton types collected in WCA-2A; only xylose and rhamnose differed ($P < 0.001$ for each) (Table 4).

The EDTA fraction had slightly lower carbohydrate content (7%–19%), but a greater uronic acid content relative to the WS fraction (Table 2). Sulfate content and protein content were again a small portion (<1%). The monosaccharide composition was primarily glucose (33%–42%), though xylose and fucose also made up a significant component (combined 30%–37% of the total detected sugars) (Table 4). Galactose was followed by mannose and

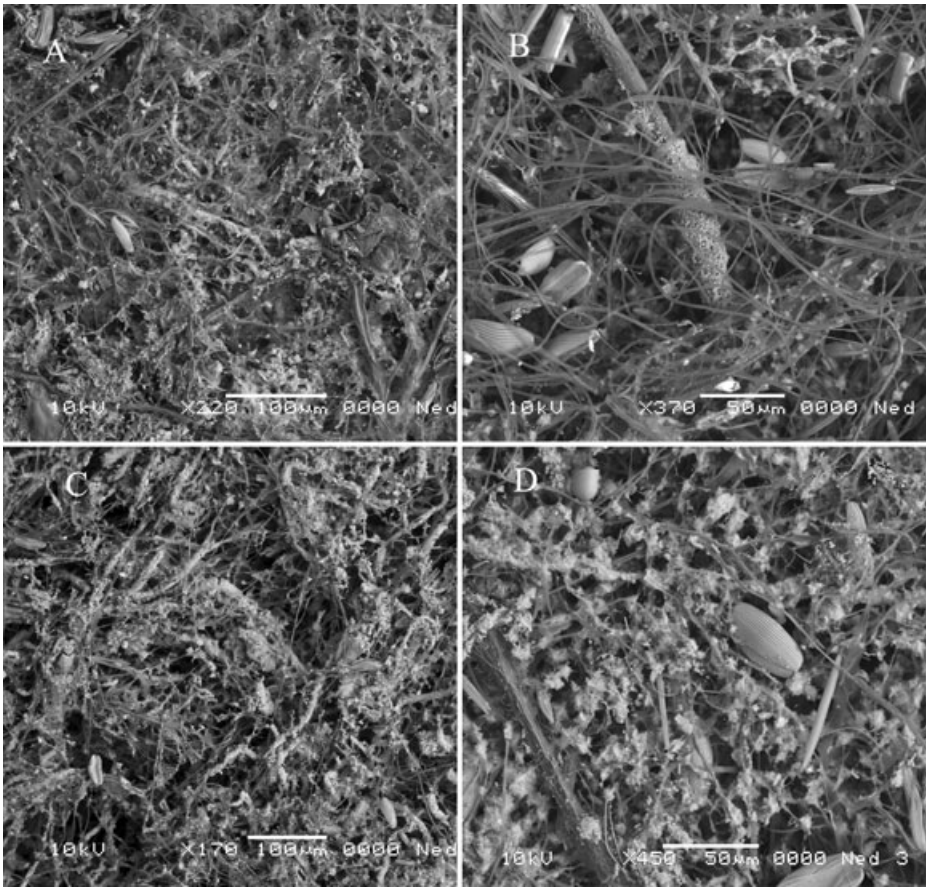

FIG. 3. VPSEM pictures of periphyton mats from site UC2 in WCA-2A. (A) Epipelon, (B) epiphyton, and (C) and D) metaphyton. VPSEM, variable pressure scanning electron microscopy.

rhamnose in abundance. Glucose relative abundances were not significantly different between periphyton types ($F = 1.5147$, $P = 0.2098$) (Table 4). However, abundances of all other saccharides were significantly different ($P < 0.05$) (Table 4). No clear patterns for periphyton type were evident, though epipelon was generally more dissimilar from metaphyton.

PCA analysis of EPS monosaccharide composition. PCA of monosaccharide composition for each EPS fraction resulted in distinct groupings, delineated by periphyton type and local water chemistry. Differences in monosaccharide composition for the periphyton types between WCAs overshadowed those within an area. For the WS fraction, the first axis described 88% of the variance, and the second axis nearly 8%. All periphyton from WCA-2A grouped together farthest out on the glucose vector, indicating the significance of this saccharide in the fraction (Fig. 4A). The periphyton from sites nearest the canal in WCA-1 was similar to the cyanobacterial-dominated communities of WCA-2A, glucose and mannose being significant vector determinants. Grouping farthest from the cyanobacterial mats were periphyton from the interior of WCA-1. These communities were most influenced by galactose, xylose, and fucose (Fig. 4A).

The first and second PCA axes for the EDTA fraction described 49% and 25% of the variability, respectively. Periphyton types had a similar pattern as the WS fraction (i.e., reflecting local water chemistry). Saccharide composition for periphyton from WCA-2A clustered within the glucose, xylose, and fucose vectors (Fig. 4B), and periphyton from WCA-1 nearest to the canal also grouped near them. The periphyton from the interior of WCA-1 was most influenced by arabinose and galactose and clustered farthest from all other periphyton types (Fig. 4B).

DISCUSSION

A significant amount of EPS is produced within Everglades' periphyton and represents a critical structural determinant in community organization. Carbohydrates were the prominent component of the EPS fractions. Neutral sugar content normalized per gram of periphyton extracted indicated a significant mass, typically in excess for periphyton from intertidal mudflats ($<1–10 \times 10^{-3}$) (Underwood and Paterson 1993, de Brouwer and Stal 2001, Hanlon et al. 2006), lakes ($\sim 1 \times 10^{-3}$) (Hirst et al. 2003), and rivers ($2–6 \times 10^{-3}$) (Spears et al. 2008). The EPS content in freshwater periphyton tends to be greater relative to estuarine and marine algal mats

TABLE 2. Content (% w:w) of organic components of extracellular polymeric substances (EPS) fractions extracted from periphyton in the Everglades.

Area	Site	Sample type	Fraction	Uronic acid (%)	Sulfate (%)	Carbohydrate (%)	Protein (%)	Fraction	Uronic acid (%)	Sulfate (%)	Carbohydrate (%)	Protein (%)
WCA-1	1	Metaphyton	WS	3.2	0.9	22.4 ± 0.1	2.4	EDTA	21.0	0.2	22.4 ± 0.1	1.5
	2	Epiphyton	WS	3.7	*	19.6 ± 0.1	*	EDTA	9.1	*	10.8 ± 0.1	1.8
	3	Metaphyton	WS	4.3	0	19.6 ± 0.1	*	EDTA	1.9	0.2	10.8 ± 0.1	1.4
	4	Metaphyton	WS	20.0	*	36.2 ± 0.1	2.3	EDTA	20.8	0.6	36.2 ± 0.1	1.6
	ME	Metaphyton	WS	15.4	0.1	–	2.9	EDTA	21.7	*	14.5 ± 0.1	1.7
	5	Metaphyton	WS	7.4	0.8	8.6 ± 0.1	2.3	EDTA	8.4	0	8.6 ± 0.1	1.7
	6	Epiphyton	WS	4.1	0.3	20.5 ± 0.1	2.9	EDTA	21.3	1.5	39.0 ± 0.5	2.2
	7	Floc/metaphyton	WS	2.8	1.5	17.4 ± 0.1	3.4	EDTA	7.7	*	17.4 ± 0.1	2.5
	7	Metaphyton	WS	5.7	0.5	24.7 ± 0.1	3.0	EDTA	25.1	0.6	26.6 ± 0.1	1.8
WCA-2A	UC1-1	Epipelon	WS	16.6 ± 1.1	0.4	43.8 ± 0.1	2.1	EDTA	12.1 ± 1.6	0.6	17.5 ± 0.3	0.1
	UC1-4	Epipelon	WS	14.1 ± 3.0	–	43.6 ± 0.1	*	EDTA	6.7 ± 1.4	–	7.6 ± 0.1	0.2
	UC1-1	Metaphyton	WS	6.2 ± 1.6	0.4	20.7 ± 0.1	*	EDTA	2.3 ± 1.4	0.6	7.0 ± 0.1	0.2
	UC1-2	Epiphyton	WS	7.8 ± 1.5	*	22.8 ± 0.1	1.9	EDTA	17.7 ± 4.2	0.2 ± 0.1	16.7 ± 0.2	0.3
	UC1-3	Epipelon	WS	12.2 ± 2.9	*	21.6 ± 0.1	3.4	EDTA	14.7 ± 2.9	0.2 ± 0.1	19.6 ± 0.1	0.5
	UC2-1	Metaphyton	WS	15.7 ± 4.2	*	31.4 ± 0.3	*	EDTA	11.4 ± 2.0	0.1 ± 0.1	19.6 ± 0.1	0.4
	UC2-1	Epiphyton	WS	–	–	–	–	EDTA	14.8 ± 1.1	0.1 ± 0.1	18.6 ± 0.1	0.4
	UC2-2	Epipelon	WS	7.0 ± 1.9	*	15.9 ± 0.1	*	EDTA	8.9 ± 2.2	*	7.5 ± 0.1	0.2

WS, water soluble; –, sample not analyzed; *, results were below detection limits.
Number of samples analyzed varied for each metric; standard deviations given when $n \geq 2$.

(de Brouwer and Stal 2001, Hirst et al. 2003, Hanlon et al. 2006, Spears et al. 2008), and studies have shown the environmental significance of these polymers in the latter systems (e.g., sediment stabilization; Underwood and Paterson 1993). This study shows the tremendous EPS content potential in periphyton from a wetland ecosystem. Periphyton biomass at the oligotrophic sites exceeded that found in most aquatic ecosystems (i.e., upward of 1,000 g ash-free dwt $\cdot$ m^{-2} has been observed in WCA-2A) (Browder et al. 1994, McCormick et al. 1998), suggesting that EPS is an overall significant carbon pool in the greater Everglades.

Production of EPS is typically greater when a nutrient is limiting (N or P) (Decho 1990, Coesel 1994, Underwood and Paterson 2003); severe P limitation (<10 $\mu g \cdot L^{-1}$) occurs throughout most areas in the Everglades, and the sites sampled here specifically (Table 1). The importance of low-phosphorus conditions on algal species composition and, more importantly, the development and maintenance of the cohesive cyanobacterial mats are well described (Swift and Nicholas 1987, Gaiser et al. 2004, 2006). Once water-column total phosphorus concentrations exceed 12–15 $\mu g \cdot L^{-1}$, the cohesive mats (e.g., in WCA-2A) will disintegrate. Numerous hypotheses have been put forth, but the role of EPS (i.e., production, composition) in maintaining mat structure remains to be thoroughly tested. Between the two marshes studied here, loading rates of phosphorus are known to differ, so while observed water-column phosphorus concentrations were low, periphyton in WCA-1 may have greater exposure to phosphorus (Gaiser et al. 2006), impacting EPS production. The increased light experienced by periphyton in the shallow Everglades also enhances photosynthesis (Grimshaw et al. 1997, McCormick et al. 1998), and in nutrient-depleted conditions, it has been shown that there is an increased need for a photosynthetic overflow mechanism in the form of EPS production to protect the photosystems from damage (Stal 2003, Otero and Vincenzini 2004).

Ionic strength of the water plays an important role in the functioning of EPS (i.e., the ability to cross-link and form cation bridges). In intertidal systems, salinity of water increases floc formation and sediment stability through the cross-linking action of EPS (Perkins et al. 2004, Spears et al. 2008). In the Everglades, thick epipelic mats in WCA-2A result in significant stabilization of sediments (S. E. Hagerthey and K. Black, unpublished data) and will only become dislodged if they become too buoyant from photosynthetic activity. In contrast, in WCA-1, algal assemblages developed as loosely consolidated desmid- and diatom-dominated communities. Though the EPS was rich in uronic acids, polymers from periphyton in WCA-1 were unable to form the rigid matrices observed in WCA-2A, presumably due to reduced divalent cation concentrations of the water (Table 1).

TABLE 3. Relative abundance of saccharide residues for periphyton from WCA-1. Mean and standard deviations given.

				Monosaccharides (%)							
Area	Site	Sample type	Fraction	Glc	Gal	Man	Xyl	Ara	Fuc	Rha	*n*
WCA-1	1	Metaphyton	WS	50.3 ± 2.7^{bc}	11.4 ± 1.7^{bcd}	6.1 ± 0.3^{bcd}	5.5 ± 0.5	10.9 ± 0.1^{a}	7.9 ± 0.8^{b}	7.9 ± 0.9	2
	2	Epiphyton	WS	46.2 ± 3.9^{bcd}	16.2 ± 2.1^{ab}	10.0 ± 0.9^{ab}	4.3 ± 1.8	8.6 ± 0.9^{ab}	8.4 ± 0.6^{b}	6.3 ± 0.7	2
	3	Metaphyton	WS	40.2 ± 2.2^{cd}	18.1 ± 0.6^{a}	11.7 ± 0.2^{a}	5.5 ± 0.1	11.4 ± 2.1^{a}	7.6 ± 0.8^{b}	7.9 ± 0.1	2
	4	Metaphyton	WS	–	–	–	–	–	–	–	
	ME	Metaphyton	WS	–	–	–	–	–	–	–	
	5	Metaphyton	WS	34.7 ± 0.3^{cd}	10.8 ± 1.3^{abc}	7.5 ± 0.6^{abcd}	9.1 ± 2.9	5.5 ± 1.3^{bc}	25.8 ± 3.5^{a}	6.8 ± 0.3	2
	6	Epiphyton	WS	54.8 ± 3.6^{ab}	7.4 ± 2.8^{c}	4.5 ± 2.0^{d}	4.9 ± 3.0	4.0 ± 1.1^{cd}	19.8 ± 4.2^{a}	4.6 ± 1.9	2
	7	Floc/metaphyton	WS	49.4 ± 0.5^{bc}	10.2 ± 0.7^{bc}	8.6 ± 0.8^{abc}	5.5 ± 0.6	12.2 ± 0.5^{a}	6.0 ± 0.4^{b}	8.1 ± 0.7	2
	7	Metaphyton	WS	65.1 ± 6.0^{a}	8.0 ± 1.3^{c}	4.6 ± 0.7^{cd}	5.5 ± 1.0	2.0 ± 0.0^{d}	9.4 ± 1.8^{b}	5.5 ± 1.1	2
$F_{0.05,6,7}$ = 3.866	*F*-value			17.520	9.544	11.835	1.299	31.151	24.221	3.765	
	P-value			0.0007	0.0044	0.0023	0.3685	0.0001	0.0002	0.0532	
	1	Metaphyton	EDTA	19.9 ± 0.2^{c}	16.8 ± 1.3^{bc}	11.3 ± 0.1^{abc}	11.1 ± 0.1^{abcd}	9.5 ± 1.0^{ab}	13.0 ± 1.4^{abc}	18.4 ± 0.8^{a}	2
	2	Epiphyton	EDTA	30.3 ± 1.3^{abc}	19.6 ± 0.8^{ab}	15.1 ± 0.1^{a}	8.9 ± 0.7^{cd}	13.5 ± 3.4^{a}	5.7 ± 0.3^{bc}	6.9 ± 0.2^{bc}	2
	3	Metaphyton	EDTA	26.5 ± 0.8^{bc}	22.1 ± 0.9^{a}	10.4 ± 0.1^{abc}	7.0 ± 0.7^{d}	12.7 ± 1.5^{a}	9.7 ± 0.7^{abc}	10.4 ± 0.4^{b}	2
	4	Metaphyton	EDTA	33.4 ± 1.3^{ab}	12.5 ± 1.5^{e}	7.8 ± 2.7^{c}	10.4 ± 3.1^{cd}	4.6 ± 0.8^{bc}	24.3 ± 2.0^{a}	6.9 ± 0.4^{bc}	3
	ME	Metaphyton	EDTA	42.1 ± 6.8^{a}	12.6 ± 1.1^{de}	8.5 ± 1.0^{bc}	10.1 ± 5.6^{cd}	10.6 ± 2.4^{a}	10.2 ± 4.6^{abc}	5.9 ± 3.1^{bc}	2
	5	Floc/metaphyton	EDTA	30.2 ± 0.3^{abc}	17.8 ± 1.2^{b}	12.4 ± 0.5^{ab}	11.1 ± 1.2^{bcd}	3.1 ± 0.1^{cd}	18.1 ± 4.7^{ab}	6.6 ± 0.7^{bc}	3
	6	Epiphyton	EDTA	22.6 ± 3.1^{c}	17.0 ± 1.1^{bc}	13.2 ± 0.4^{a}	18.8 ± 1.8^{ab}	1.7 ± 0.3^{cd}	7.0 ± 4.2^{c}	19.8 ± 1.9^{a}	3
	7	Floc/metaphyton	EDTA	34.4 ± 1.1^{ab}	13.9 ± 0.9^{cde}	8.7 ± 0.3^{bc}	17.3 ± 1.8^{abc}	8.4 ± 3.6^{ab}	13.3 ± 1.4^{abc}	3.9 ± 0.3^{c}	3
	7	Metaphyton	EDTA	26.5 ± 3.8^{bc}	16.0 ± 0.7^{bcd}	11.2 ± 0.5^{abc}	19.4 ± 1.9^{a}	1.6 ± 0.8^{d}	6.9 ± 2.5^{bc}	18.4 ± 1.9^{a}	2
$F_{0.05,8,13}$ = 2.767	*F*-value			8.795	18.469	8.229	8.242	25.830	6.414	41.775	
	P-value			0.0004	<0.0001	0.0005	0.0005	<0.0001	0.0017	<0.0001	

Glc, glucose; Gal, galactose; Man, mannose; Xyl, xylose; Ara, arabinose; Fuc, fucose; Rha, rhamnose; WS, water soluble; –, sample not analyzed.
The *F*- and *P*-values for across-site saccharide comparisons are reported; pair-wise differences between saccharides indicated by different letters ($P < 0.05$).

TABLE 4. Relative abundance of saccharide residues for periphyton from WCA-2A. Mean and standard deviations given.

				Monosaccharides (%)							
Area	Site	Sample type	Fraction	Glc	Gal	Man	Xyl	Ara	Fuc	Rha	*n*
WCA-2A	UC1-1	Epipelon	WS	62.1 ± 3.5	11.1 ± 1.4	9.9 ± 2.7	5.7 ± 0.5^{ab}	3.2 ± 1.8	3.7 ± 0.7	4.4 ± 0.7^{b}	4
	UC1-4	Epipelon	WS	47.5 ± 3.0	12.9 ± 0.6	12.2 ± 0.4	7.1 ± 0.6^{a}	4.2 ± 0.6	7.2 ± 0.3	8.8 ± 0.4^{a}	4
	UC1-1	Metaphyton	WS	69.0 ± 4.5	9.8 ± 2.3	5.2 ± 0.7	4.4 ± 0.6^{bcd}	1.8 ± 0.1	3.8 ± 0.2	5.9 ± 0.4^{ab}	2
	UC1-2	Epiphyton	WS	67.7 ± 3.3	9.8 ± 0.7	5.8 ± 0.6	5.4 ± 0.6^{abc}	3.1 ± 2.0	4.1 ± 0.5	4.2 ± 1.0^{b}	2
	UC1-3	Epipelon	WS	53.5 ± 2.5	14.4 ± 0.2	10.3 ± 0.9	6.2 ± 0.9^{ab}	2.2 ± 0.4	5.0 ± 0.9	8.3 ± 1.3^{a}	2
	UC2-1	Metaphyton	WS	68.8 ± 19.1	7.7 ± 4.6	8.8 ± 7.9	2.4 ± 1.1^{d}	2.5 ± 1.9	5.5 ± 4.4	4.2 ± 1.4^{b}	2
	UC2-1	Epiphyton	WS	–	–	–	–	–	–	–	
	UC2-2	Epipelon	WS	60.8 ± 19.5	11.5 ± 6.9	10.2 ± 7.0	3.3 ± 0.2^{cd}	2.7 ± 1.7	5.9 ± 2.3	5.5 ± 2.0^{ab}	2
$F_{0.05,6,11}$ = 3.095	*F*-value			2.341	1.156	1.428	12.840	1.058	2.016	9.400	
	P-value			0.1052	0.3942	0.2876	0.0002	0.4414	0.1486	0.0008	
	UC1-1	Epipelon	EDTA	40.2 ± 8.9	20.8 ± 4.3^{a}	12.7 ± 2.0^{a}	12.7 ± 0.9^{b}	1.7 ± 1.1^{ab}	7.2 ± 0.7^{c}	2.8 ± 0.7^{c}	4
	UC1-4	Epipelon	EDTA	34.0 ± 0.8	13.4 ± 0.6^{bc}	11.9 ± 0.1^{ab}	20.2 ± 0.2^{a}	3.1 ± 0.1^{a}	11.3 ± 0.2^{bc}	6.1 ± 0.4^{b}	4
	UC1-1	Metaphyton	EDTA	33.3 ± 8.5	13.8 ± 2.5^{bc}	8.5 ± 1.9^{bcd}	17.0 ± 2.5^{ab}	2.3 ± 0.6^{ab}	14.9 ± 1.8^{ab}	9.5 ± 1.7^{a}	4
	UC1-2	Epiphyton	EDTA	35.9 ± 2.4	13.9 ± 1.4^{b}	6.5 ± 0.4^{d}	20.6 ± 2.1^{a}	1.4 ± 0.2^{ab}	16.2 ± 0.6^{ab}	5.5 ± 0.3^{b}	4
	UC1-3	Epipelon	EDTA	38.0 ± 3.4	11.4 ± 1.4^{bc}	8.1 ± 1.5^{bcd}	17.2 ± 1.4^{ab}	1.8 ± 0.2^{ab}	16.6 ± 1.1^{ab}	6.8 ± 0.7^{b}	4
	UC2-1	Metaphyton	EDTA	36.7 ± 8.1	9.4 ± 1.1^{c}	8.1 ± 3.1^{cd}	16.9 ± 6.3^{ab}	1.0 ± 0.7^{b}	20.9 ± 8.2^{a}	7.0 ± 0.9^{b}	4
	UC2-1	Epiphyton	EDTA	41.9 ± 3.4	12.8 ± 1.1^{bc}	7.1 ± 1.1^{d}	15.7 ± 1.9^{ab}	1.7 ± 0.3^{ab}	14.2 ± 1.0^{ab}	6.4 ± 0.9^{b}	4
	UC2-2	Epipelon	EDTA	34.2 ± 1.6	14.0 ± 1.2^{b}	10.8 ± 0.7^{abc}	17.0 ± 1.4^{ab}	1.8 ± 0.5^{ab}	15.0 ± 0.9^{ab}	7.1 ± 0.2^{b}	4
$F_{0.05,7,24}$ = 2.423	*F*-value			1.515	9.781	8.377	2.821	2.990	9.660	24.590	
	P-value			0.2098	<0.0001	<0.0001	0.0271	0.021	<0.0001	<0.0001	

Glc, glucose; Gal, galactose; Man, mannose; Xyl, xylose; Ara, arabinose; Fuc, fucose; Rha, rhamnose; WS, water soluble; –, sample not analyzed.
The *F*- and *P*- values for across-site saccharide comparisons reported; pair-wise differences between saccharides indicated by different letters ($P < 0.05$).

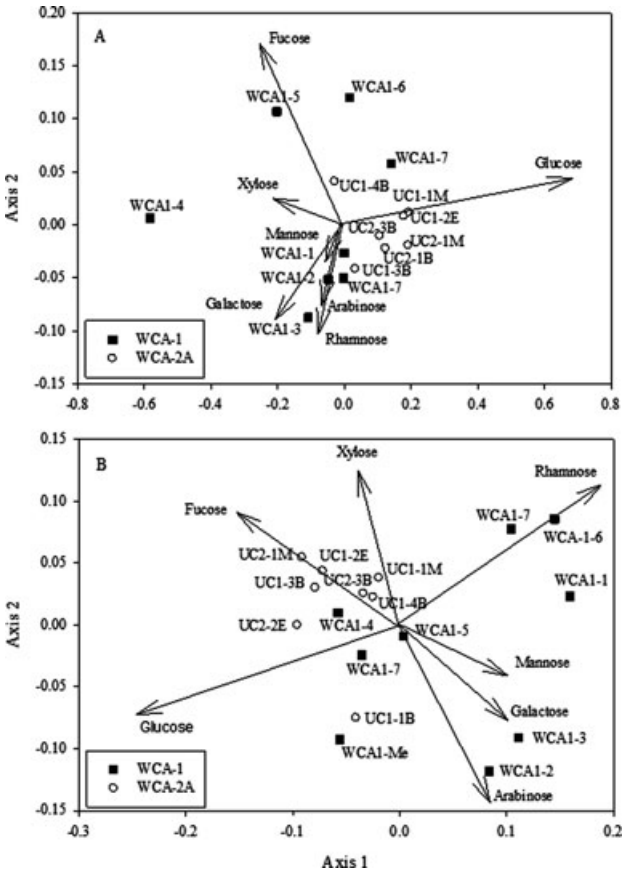

FIG. 4. Principal components analysis (PCA) of monosaccharide composition of the (A) water soluble (WS) fraction and (B) EDTA fraction, from all sites sampled. Vectors indicate the most significant saccharide components of the fraction for all sites.

Amorphous desmid-rich communities. Desmid EPS has been found in the form of gels, mucilage, and slimes that encapsulate cells or are secreted for motility (Domozych et al. 1993, 2005, Coesel 1994). In addition to cell-associated EPS (i.e., EDTA fraction), desmids also produce complex cell-wall polysaccharides, similar to higher plants (Domozych et al. 2007). Results indicate that EPS for desmid-rich periphyton in a subtropical, ombrotrophic marsh are similar to polymers for desmids isolated from temperate bogs. Glucose, galactose, fucose, xylose, and arabinose were significant neutral sugars, and uronic acid residues were also prominent in the EPS fractions. The saccharides galactose, fucose, xylose, and glucuronic acid are typically the primary components of desmid EPS (Domozych et al. 1993, 2005, Giroldo et al. 2005, Kiemle et al. 2007), and glucose and arabinose content may increase in abundance through time in desmid cultures (Domozych et al. 2005). Arabinose has also been observed to be a potentially significant component of the EPS isolated from a number of desmid species in culture (Kiemle et al. 2007). Compositional data about EPS for desmid-dominated periphyton in general, and desmid species in particular, are significantly lacking relative to diatoms and cyanobacteria, but are being increasingly studied due to the abundance of desmids in ombrotrophic bogs and wetlands (Kiemle et al. 2007, Domozych and Domozych 2008).

Cohesive cyanobacterial mats. Cyanobacteria produce EPS, which range from loosely bound polymers (i.e., capsules and slime EPS) to tightly cell-bound sheaths and cell walls (Bertocchi et al. 1990). The abundances of the saccharides identified in EPS from the cyanobacterial mats in the Everglades correspond with results from cyanobacterial stromatolites, other freshwater ecosystems, and EPS extracted from cultured cyanobacterial species (Bertocchi et al. 1990, Kawaguchi and Decho 2000). Glucose may be a significant component of cyanobacterial sheaths, but cell-wall polymers are also rich in fucose, galactose, and xylose (Bertocchi et al. 1990). In the EDTA fraction, glucose, xylose, fucose, and galactose were prominent saccharides (Table 4), while polymers extracted from *Oscillatoria* within stromatolites had increased proportions of glucose, galactose, xylose, and fucose saccharide residues (Kawaguchi and Decho 2000). These small differences may be due to the more diverse algal assemblage present in the periphyton analyzed here contributing to the EPS matrix. Extracellular polymers isolated from cyanobacteria native to Polynesian microbial mats were proteoglycans with greatest molar ratios of glucose, xylose, fucose, and galactose for all species (Richert et al. 2005). Glucose- and xylose-rich polysaccharides were observed by Nicolaus et al. (1999) for the Oscillatoriales and Nostocacae, and EPS produced by *Nostoc commune* studied from field and culture samples contained polysaccharides rich in glucose, xylose, and galactose, with significant amounts of uronic acids from cultured cells (Huang et al. 1998, Brüll et al. 2000).

Imaging of periphyton from WCA-2A revealed the extent of biotic and abiotic material associated with the EPS matrix (Fig. 3). Isolation of cross-linked polymers associated with the sheaths and cell walls using EDTA gave insight into the biochemical composition of polymers serving as the template in calcium carbonate deposition. The extracellular sheaths, capsules, and cell walls of cyanobacteria have been associated with calcium carbonate deposition (Pentecost and Riding 1986, Merz 1992, Schultzelam et al. 1992, Kawaguchi and Decho 2002a), though EPS may also inhibit precipitation through binding of calcium ions by carboxylated sugars, thus preventing crystal growth (Arp et al. 1999, Riding 2000, Kawaguchi and Decho 2002b, Dupraz et al. 2004). Images taken here confirm the former; sheaths of cyanobacterial filaments were completely encrusted with calcium carbonate (Fig. 3, A, C, and D). The plausibility of calcium carbonate precipitation was determined by calculating the saturation index (SI) value. The SI quantifies the ion activity

product (IAP) of $Ca^{2+}$and CO_3^{2-} relative to the solubility product (K_{sp}) of $CaCO_3$ (SI = $\log([Ca^{2+}] \times [CO_3^{2-}])/K_{sp}$) (Thompson and Ferris 1990, Merz-Preiß and Riding 1999). Water chemistry analysis verified that interior sites of WCA-2A had an SI conducive for calcium carbonate precipitation (SI > 0.8), whereas waters in WCA-1 did not (SI < 0) (Table 1).

Within all the periphyton types analyzed, diatoms were abundant, especially in WCA-2A and near the canals in WCA-1 (Swift and Nicholas 1987). Marine, estuarine, and freshwater diatoms have been shown to be prolific producers of EPS in the form of sheaths, tubes, stalks, pads, and mucilage for attachment and motility, or during nutrient-starved conditions (Decho 1990, Hoagland et al. 1993). Abundant saccharides in capsular or sheath polymers include glucose, fucose, galactose, and xylose, with varying amounts of glucuronic and galacturonic acid residues (Hoagland et al. 1993, Underwood and Paterson 2003). To date, there has been minimal study of freshwater diatom EPS (Wustman et al. 1997, Giroldo et al. 2003), with more research on marine planktonic (e.g., DeAngelis et al. 1993, Thornton 2002, Skoog et al. 2008) and estuarine benthic diatom EPS (e.g., de Brouwer et al. 2003, Underwood and Paterson 2003, Bellinger et al. 2005). While stalks and sheaths were not abundantly evident in the images of this study, diatoms are likely making a contribution to the overall periphyton EPS matrix.

CONCLUSIONS

This study has provided an initial first view of the complex EPS associated with the diverse periphyton assemblages in the Florida Everglades. The periphyton community organization was a function of the local water-quality conditions and resulted in distinct EPS biochemistries. The composition of EPS fractions isolated from the distinct periphyton communities in WCA-1 and WCA-2A was indicative of the prominent desmid and cyanobacterial algal assemblages, respectively. Polysaccharides found in the extracellular matrices of photosynthetic organisms (e.g., EPS of cyanobacteria and eukaryotic algae, cells walls of green plants) represent the most abundant and complex biopolymers found on the planet. The study of algal EPS is only in an infancy stage, and preliminary data suggest exceptional monomer diversity and linkage complexity. It will only be when more sophisticated studies are performed to elucidate EPS glycomics that we will be able to understand the myriad structural and functional aspects of EPS in ecosystem dynamics. In the Florida Everglades, WCA-1 is the last remnant ombrotrophic marsh. However, water management resulting in increased discharge of ion-rich water would likely result in a change in water chemistry and algal species composition (S. E. Hagerthey, S. Newman, and A. Gottlieb, unpublished data). It is also likely that the loss of the soft-water algal communities in WCA-1 would have biological effects (e.g., carbon availability and quality to heterotrophs) and ecological effects (e.g., nutrient and mineral adsorption, sediment stabilization). Further studies into the structure of EPS are needed to understand the biogeochemical roles of EPS within periphyton and how changes and losses may affect other ecosystem processes.

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