Characterization of suspended particles in Everglades wetlands

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Abstract

We report the concentration, phosphorus (P) and nitrogen (N) content, and size and chemical fractionation of fine suspended particles (0.2–100 μm) and colloids (3 kilodalton [kDa]–0.1 μm) in the surface water of Everglades wetlands along regional and P-enrichment gradients. Total suspended sediment concentrations ranged from 0.7 to 2.7 mg L⁻¹. Total particulate P concentrations increased from 0.05 μmol L⁻¹ to 0.31 μmol L⁻¹ along the P-enrichment gradient. Particles contained from 20% to 43% of total P but <12% of total N in surface water. Dissolved (<0.2 μm) organic N contained about 90% of total N, with the 3–100-kDa colloidal size class containing the most N of any size class. The 0.45–2.7-μm size fraction held the most particulate P at all sites, whereas particulate N was most abundant in the 2.7–10-μm size class at most sites. Standard chemical fractionation of particles identified acid-hydrolyzable P as the most abundant species of particulate P, with little reactive or refractory organic P. Sequential chemical extraction revealed that about 65% of total particulate P was microbial, while about 25% was associated with humic and fulvic organic matter. The size and chemical fractionation information suggested that P-rich particles mostly consisted of suspended bacteria. Suspended particles in Everglades wetlands were small in size and had low concentrations, yet they stored a large proportion of surface-water P in intermediate reactive forms, but they held little N.

Suspended particulate matter plays a large role in the cycling and transport of energy and nutrients in aquatic ecosystems. In particular, phosphorus (P) transport and reactivity are strongly associated with suspended sediment in streams and rivers (Froelich 1988; Meybeck 1982; Fox 1993). Particulate and colloidal P fractions are especially important for the transport of P from agricultural fields to streams (Heathwaite et al. 2000). A large proportion of P exported in streams is also associated with fine particulate organic matter (<1 mm; Meyer and Likens 1979). Thus, nutrient spiralling models for streams and rivers incorporate the differential transport and biogeochemistry of particulate and dissolved nutrients (Webster and Patten 1979). In lakes, suspended particles and colloids (Lean 1973) and small bacterioplankton (Currie and Kalff 1984) are also very important to P cycling. Furthermore, suspended particles quickly incorporate inorganic P in estuarine waters (Correll et al. 1975).

Wetlands efficiently retain suspended particles due to their reduction of surface-water velocities (Boto and Patrick 1978; Leonard and Luther 1995; Kadlec 1999). The deposition of suspended sediment results in a large flux of nutrients, particularly P, from the water column to wetland soil (Johnston 1991). In fact, wetlands created or managed to improve water quality rely on particle retention to increase nutrient retention (Kadlec and Knight 1996). Suspended particles in surface water also dominate short-term P cycling in wetland ecosystems (Richardson and Marshall 1986; Noe et al. 2003). Wetlands can differentially transport solutes compared to particles, which makes it important to distinguish the fate of particulate and dissolved nutrients (Jordan et al. 1999). However, despite their importance, surprisingly little information is available on the biogeochemical characteristics of suspended particles in wetlands.

The Everglades, a naturally oligotrophic and P-limited wetland landscape, has a long history of anthropogenic P enrichment and resulting ecosystem changes (Davis 1994; Noe et al. 2001; McCormick et al. 2002). Similar but less impacted ecosystems are found elsewhere in the Caribbean basin (Rejmáková et al. 1996). Decades of increased P loading from agricultural and urban runoff have created large regions with increased P content, mostly in the upstream, northern Everglades close to the canal sources of P (Reddy et al. 1998). Although many studies have documented ecosystem responses to P enrichment (Gaiser et al. 2005), present understanding of the processes that influence downstream P transport is poor. A goal of the Comprehensive Everglades Restoration Program is to increase sheet-flow velocity through the hydroscape (http://www.evergladesplan.org). However, current models of P transport in the Everglades do not include suspended particulate P (e.g., Walker 1995; Fitz and Sklar 1999). Given the different transport characteristics of particles compared to solutes in the Everglades (Saiers et al. 2003; Harvey et al. 2005), information on particle biogeochemical

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characteristics is needed to improve predictions of the effects of increased sheet-flow velocities on contaminant movement through the hydroscape, as well as the restoration of natural topographic heterogeneity (Science Coordination Team 2003).

Limited information currently exists on suspended particles in the Florida Everglades. The Everglades differs from most other flowing wetland and aquatic ecosystems by having low-turbidity surface water without terrigenous mineral sediment. Despite the appearance of relatively clear water, suspended particles have been shown to dominate $^{32}$PO$_4$ uptake in the surface water of Everglades wetlands (Noe et al. 2003). Limited size-distribution information exists for Everglades particles, but 0.2–1-μm particles incorporated the most $^{32}$PO$_4$ in laboratory incubations of Everglades surface water (Jones 2001). A high proportion of P in canals draining agricultural fields upstream of the Everglades is contained in the particulate fraction (Stuck et al. 2002). In addition, particulate P (>0.4 μm) mineralized faster than colloidal (0.05–0.4 μm) or dissolved P (<0.05 μm; Pant et al. 2002) and was largely microbial (Farve et al. 2004) in treatment wetlands at the northern edge of the Everglades. Potential sources of suspended particles in Everglades wetlands include planktonic organisms, resuspended benthic flocculent organic matter (detrital or algal), organic matter or calcium carbonate shed from periphyton, or fragmented macrophyte tissues. Particulate nutrient concentrations can be estimated indirectly by the difference between total and total dissolved concentrations, which have been measured by many Everglades monitoring and research studies. However, estimation of particulate nutrient concentrations by difference can generate large error due to sampling and analytical bias and error propagation, especially in an oligotrophic ecosystem, such as the Everglades, where P concentrations are near detection limits. Additional information on the biogeochemical characteristics of suspended particles in the Everglades is necessary to better understand nutrient cycling and transport in this wetland landscape and other wetlands. Therefore, our research objectives were to quantify the concentration of suspended particles and their associated nutrients across regional and P-enrichment gradients, compare direct to indirect particle sampling methods, characterize the chemical forms of particulate nutrients, and identify the potential role of suspended particles in ecosystem biogeochemistry in Everglades wetlands.

Methods

**Study sites**—We sampled seven sites across the Everglades, spanning the longitudinal-flow gradient of the landscape as well as an anthropogenic P-enrichment gradient (Fig. 1). The sites ranged from periphyton-dominated sloughs with deeper water to macrophyte-dominated marshes with shallower water (Table 1). Four oligotrophic sites were sampled in the interiors of the large Everglades regions, from north to south: Loxahatchee National Wildlife Refuge (WCA-1), Water Conservation Area 2A (WCA-2A), Water Conservation Area 3A (WCA-3A), and Shark River Slough in Everglades National Park. A slough at the long-term South Florida Water Management District (SFWMD) site, Lox8, was sampled in the oligotrophic interior of WCA-1 (Table 1). The U.S. Geological Survey (USGS) Ridge and Slough site WCA-3A-5, which includes adjacent oligotrophic slough (3AS Slough) and saw grass (*Cladium jamaicense*; 3AS Cladium) habitats, was sampled in the interior of WCA-3A. The slough sampling site in oligotrophic Shark River Slough was upstream of a long-term Florida International University site, Flume A (SrsA). Another SFWMD slough site, U3, was sampled in the interior of WCA-2A. Site U3 is considered to be an oligotrophic end of a P-enrichment gradient in WCA-2A. A highly P-enriched *Typha domingensis* site, SFWMD site F1, was sampled at the upstream end of WCA-2A near the canal that delivers P-rich waters. A moderately P-enriched *C. jamaicense* and *T. domingensis* site, SFWMD site F4, was sampled near the downstream limit of *T. domingensis* dominance in WCA-2A.

**Particle sampling methods**—Measuring particulate nutrients directly, by filtering a large volume of water and then digesting the filter with accumulated particles, may provide several methodological benefits compared to measuring indirectly, by subsampling a small volume of unfiltered surface water and calculating the difference between unfiltered and filtered subsamples. Potential sources of error for indirect methods include sampling and analytical bias. Unfiltered water samples used in indirect analyses often have low volume when digested for nutrient analysis (10 mL in this study) compared to direct analysis of particles (~1000 mL filtered in this study). The smaller volume likely results in lower precision due to the variable capture of particles in the smaller samples. In addition, the filtration of a large volume of water and subsequent digestion of the filters with accumulated particles concentrates particulate nutrients. The concentration of particulate nutrients avoids analytical error associated with P concentrations close to the detection limit. Finally, calculation of particulate nutrient concentrations by difference generates error propagation. These cumulative errors could result in an unbiased but much more variable estimate of particulate nutrient concentrations from indirect compared to direct sampling. Indirect methods are widely used, and therefore two sets of water samples were collected from each location to identify differences in the estimation of particulate nutrient concentrations due to sampling particles directly or indirectly, with additional samples taken to conduct size and chemical speciation analyses. Surface water was collected in an identical way for both the direct and indirect particle samples.

**Sample collection and analyses**—All sites were visited in daylight from 18 to 20 November 2004, with the exception of the 3A5 sites, which were sampled on 08 November and 08 December 2005. No precipitation or high-velocity winds were observed during sampling. Ongoing research has documented little temporal variation in concentrations of suspended particles, particulate P, or particulate N during
periods of surface-water inundation at the 3A5 Slough or 3A5 Cladium sites (Noe unpubl. data).

The sites sampled in 2004 (Lox8, F1, F4, U3, and SrsA) were approached from downstream by airboat, the airboat engine was turned off, and the final ~10 m to a representative patch of marsh was traveled by slowly poling the airboat upstream. We observed no changes in suspended sediment concentrations at the sampling locations as the result of airboat movement. Water samples were collected by a peristaltic pump with tubing fitted with a 100-μm

Table 1. Site characteristics.

<table>
<thead>
<tr>
<th>Site</th>
<th>Region</th>
<th>Surface-water total P (μmol L⁻¹)</th>
<th>Water depth to floc (cm)</th>
<th>Dominant macrophyte species</th>
<th>Total macrophyte biomass (g m⁻²)</th>
<th>Periphyton type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lox8</td>
<td>WCA-1</td>
<td>0.34</td>
<td>32</td>
<td><em>Nymphaea odorata</em></td>
<td>191</td>
<td>Noncalcareous epiphytic</td>
</tr>
<tr>
<td>F1</td>
<td>WCA-2A</td>
<td>1.10</td>
<td>22</td>
<td><em>Typha domingensis</em></td>
<td>1,564</td>
<td>None</td>
</tr>
<tr>
<td>F4</td>
<td>WCA-2A</td>
<td>0.45</td>
<td>44</td>
<td><em>Cladium jamaicense</em>,</td>
<td>2,174</td>
<td>None</td>
</tr>
<tr>
<td>U3</td>
<td>WCA-2A</td>
<td>0.28</td>
<td>68</td>
<td><em>Typha domingensis</em></td>
<td>138</td>
<td>Calcareous benthic, epiphytic</td>
</tr>
<tr>
<td>3A5 Slough</td>
<td>WCA-3A</td>
<td>0.40</td>
<td>68</td>
<td><em>Eleocharis elongata</em>,</td>
<td>549</td>
<td>Calcareous epiphytic, floating</td>
</tr>
<tr>
<td>3A5 Cladium</td>
<td>WCA-3A</td>
<td>0.43</td>
<td>48</td>
<td><em>Nymphaea odorata</em>,</td>
<td>926</td>
<td>None</td>
</tr>
<tr>
<td>SrsA</td>
<td>Everglades National Park</td>
<td>0.26</td>
<td>61</td>
<td><em>Eleocharis celulosa</em></td>
<td>115</td>
<td>Calcareous floating, epiphytic</td>
</tr>
</tbody>
</table>

Fig. 1. False-color satellite image of the Everglades and south Florida showing the locations of the sampling sites and individual photos of each sampling site. Generalized flow direction is shown as an arrow on the satellite image. See Table 1 for the site labels.
Nitex prefilter (Sefar America) and routed through a stable sampling boom. Particle generation and resuspension due to sampling were avoided by locating the sampling end of the boom above a portion of the water column that was relatively devoid of periphyton or submerged vegetation and then gently inserting the boom vertically into the water column until the end of the peristaltic tubing was at the desired depth. The middle of the water column was sampled at two locations at each site (1.5–2 m apart), with the exception of SrsA, 3A5 Slough, and 3A5 Cladium, where multiple depths were sampled at one location. The middle of the water column was defined as the midpoint between the air or the bottom of any floating periphyton or Utricularia spp. and the top of the flocculent detrital organic matter (floc) at the bottom of the water column. In addition, samples were taken from 5 cm below the periphyton/Utricularia spp. layer and from 5 cm above the floc layer at SrsA. Water-column depths at each site during the time of sampling are provided in Table 1.

Water was pumped at a slow rate (~200 mL min⁻¹) to avoid particle entrainment from submersed surfaces, and sample collection began 5 min after the initiation of pumping. The first set of samples was collected to measure particulate matter (>0.2 μm) directly, the second set was collected to fractionate total and dissolved (<0.2 μm) surface-water nutrients in order to measure particulate nutrients indirectly (by difference) and quantify nutrient speciation, and the third set was collected to measure small colloidal (3 kilodalton [kDa]–0.1 μm) and ultradissolved (<3 kDa) size fractions. All materials contacting samples were acid-washed (5% concentrated HCl solution) prior to sampling. For the first set, two replicate 1-L subsamples were collected in low-density polyethylene (LDPE) bottles, chilled in the field, and filtered the following day. Each subsample was sequentially filtered through 10-μm (Pall polyporeplene), 2.7-μm (Whatman GF/D), 0.45-μm (Pall polyporeplene), and 0.2-μm (Pall polypropylene) filters (all 47-mm diameter) by vacuum filtration. A preliminary analysis indicated that polyporeplene and glass fiber filters had the lowest P and N concentrations when digested. The volume of filtrate from each size filter was measured. Each filter was stored separately in a petri dish, the final filtrate (<0.2 μm) was collected in a LDPE bottle, and then filters and filtrate were frozen. For the second set, additional 60-mL field-filtered (0.2-μm Pall polyethylysulfone) and unfiltered subsamples were collected in LDPE bottles, chilled in the field, and then frozen. Delaying filtration by one day did not influence the concentrations of P between the direct and indirect samples: dissolved reactive P (paired t-test, n = 17, p = 0.78), dissolved organic P (p = 0.90), and total dissolved P (p = 0.57) did not differ between the field- and laboratory-filtered filtrates. The third set was filtered (0.45-μm Durapore polyvinylidene fluoride) in the field into polycarbonate bottles, chilled, and then frozen. These samples were then processed by tangential-flow ultrafiltration (0.1-μm, 100-kDa, and 3-kDa polysulfone filters) and permeates from each filter size were collected in poly-carbonate bottles and frozen.

The 3A5 Slough and 3A5 Cladium sites sampled in 2005 were accessed by a permanent platform and boardwalk. Surface water was sampled similar to the 2004 sampling, with the exception that no samples were collected for ultrafiltration of the small colloidal size fractions, but additional particle sampling was conducted. Samples at both 3A5 sites were collected from the middle of the water column, 10 cm below the periphyton/Utricularia spp. layer or water surface, and 10 cm above the floc layer. Samples were collected with a peristaltic pump (~70 mL min⁻¹) from each site and depth. Single, 1-L samples for direct particle analysis collected on 8 November 2005 were sequentially filtered as in 2004. Paired unfiltered and field-filtered samples were also collected for N and P speciation analyses. Finally, duplicate 500-mL samples for particle analysis collected on 08 December 2005 were each filtered only with a 0.2-μm filter in a single step in order to conduct sequential chemical extraction of P fractions in captured particles. These filters were then stored at 4°C.

Analysis—The mass and elemental compositions of suspended particles were directly analyzed from the material collected on filters. The size distribution and concentrations of suspended sediment mass were determined by drying and weighing each filter before and after filtration. Total particulate P and N were measured directly by digesting one set of replicate filters and their accumulated particles with potassium persulfate in an autoclave (Patton and Kryskalla 2003). The resulting orthophosphate and nitrate were measured colorimetrically using an Astoria-Pacific segmented flow autoanalyzer. Digestions included water and filter blanks and glycerophosphate and glycine checks to validate complete digestion efficiency. Total particulate Ca, Fe, and Al were also measured directly by digesting the second set of replicate filters with their accumulated particles collected in November 2004. Filters with particles and filter blanks were digested with HNO₃, HCl, and HF acids using microwave-assisted digestion (Bettinelli et al. 1989). Ca, Fe, and Al concentrations in the resulting digest solutions were measured by inductively coupled plasma optical-emission spectroscopy.

Phosphorus and N speciation in unfiltered and field-filtered (0.2 μm) surface-water samples was also measured. Particulate (total – dissolved) and dissolved forms of reactive, acid-hydrolyzable, and organic P were measured, as were particulate and dissolved organic N, ammonium, nitrate, and nitrite. Total particulate P and N were measured indirectly as the difference in total P and N between unfiltered and filtered water samples. Total P and N concentrations in unfiltered and filtered samples, using 10-mL subsamples, were measured by persulfate digestion. Acid-hydrolyzable P concentrations in unfiltered and filtered samples were measured by weak acid digestion, using a variant of American Public Health Association (APHA) Method 4500-P (Eaton et al. 1995) that used a final concentration of 0.11 mol L⁻¹ H₂SO₄ and heating in an autoclave at 121°C for 30 min, followed by measurement of orthophosphate concentrations. Acid-hydrolysis digested inorganic condensed phosphates (Eaton et al. 1995) and some labile organic phosphorus (Maher and Woo 1998) into orthophosphate. Tests indicated that glycerophosphate was not digested by this acid-hydrolysis method.
Finally, reactive P concentrations in unfiltered and filtered samples were measured as orthophosphate. Nitrite, nitrate, ammonium, and orthophosphate concentrations were measured colorimetrically using an Astoria-Pacific segmented flow autoanalyzer. The acid-hydrolyzable P (AHP) fractions were calculated as the difference between acid-
hydrolyzable and reactive P. The organic P fractions were calculated as the difference between total P and AHP + reactive P. Minimum detection limits for orthophosphate, acid-hydrolyzable P, total (T) P, TN, nitrite, nitrate, and ammonium were 0.012 µmol L⁻¹, 0.027 µmol L⁻¹, 0.168 µmol L⁻¹, 3.056 µmol L⁻¹, 0.061 µmol L⁻¹, 0.240 µmol L⁻¹, and 0.329 µmol L⁻¹, respectively.

Total dissolved P (TDP) and total dissolved N (TDN) concentrations in the ultrafiltered permeates were also measured by persulfate digestion. Phosphorus and N concentrations in each colloidal size fraction were calculated as the difference in TDP or TDN between sequential size classes. Concentration factors for the permeates were low (1 to 1.1); therefore, we report uncorrected concentrations in the permeates.

The forms of P held in suspended particles were also determined by sequential chemical extraction of 0.2-µm filters with their accumulated particles that were collected from the 3A5 Slough and 3A5 Cladium sites in December 2005. We used a modification of the Hedley et al. (1982) P extraction method, which has been applied to Everglades soils previously (Qualls and Richardson 1995): the labile and microbial fractions were extracted together simultaneously to increase the replication of the sequential fractions. Each field-moist filter was placed in a 15-mL centrifuge tube and then sequentially extracted for 16 h at 200 rpm on an orbital shaker table with 5 mL of extract solution. The first extractant was 0.5 mol L⁻¹ NaHCO₃, following chloroform treatment. This extract was then filtered and analyzed for TP using persulfate digestion in order to estimate the microbial + labile P fraction. Microbial + labile P concentrations were corrected to account for the incomplete extraction (43.3%; Qualls and Richardson 1995) of microbial P using this method (Walbridge 1991). The second extractant was 0.1 mol L⁻¹ NaOH. This extract was then filtered and analyzed for reactive P and for TP using persulfate digestion. The reactive P estimated the Fe + Al P fraction, and organic P (TP – reactive P) estimated the humic + fulvic P fraction.

The third extractant was 1.0 mol L⁻¹ HCl, which was filtered and analyzed for reactive P to estimate the Ca P fraction. Finally, the filter was digested using persulfate digestion and analyzed for TP to estimate the refractory organic P fraction. To estimate the extractability of particulate P and the accuracy of the microbial P correction, the concentrations of P in each fraction were summed to estimate total particulate P and then compared to additional filters with accumulated particles that were analyzed for total particulate P directly.

Results

Total suspended sediment (TSS; >0.2 µm) concentrations were generally low across the Everglades (<3 mg L⁻¹; mean = 1.5 mg L⁻¹; Table 2). TSS concentrations and the percent of water-column P held in particles (%PP) were greatest in Lox8 and lowest in SrsA. TSS and %PP were positively associated across the Everglades (Pearson product-moment correlation of log-log transformed data: r = 0.92, p = 0.003, n = 7), as were TSS and %PN (r = 0.83, p = 0.02). %PP and %PN, however, were unrelated (r = 0.54, p = 0.21). The particulate fraction was a large proportion of total surface-water P (%PP) across the Everglades, ranging from 20% to 43% (mean = 31%). However, total particulate N was a small fraction of total N (%PN), ranging from 2 to 11% (mean = 6%). There were not consistent depth trends in the measured variables at the SrsA and 3A5 sites; data from each of these sites are reported as averages across the three sampled depths.

Table 2. Summary of total suspended sediment concentrations, total particulate (>0.2 µm) phosphorus (P) and nitrogen (N) concentrations and ratios, and the percent of total surface-water P and N in the particulate fraction (particulate/total) at each site. Mean ± one SE; n=2 locations per site (Lox8, F1, F4, and U3) or n=3 depths (5A Cladium, 5A Slough, and SrsA).

<table>
<thead>
<tr>
<th>Site</th>
<th>Total suspended sediment (mg L⁻¹)</th>
<th>Total particulate P (µmol L⁻¹)</th>
<th>Total particulate N (µmol L⁻¹)</th>
<th>Percent particulate P</th>
<th>Percent particulate N</th>
<th>Particulate N: P (molar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lox8</td>
<td>2.71 ± 0.09</td>
<td>0.19 ± 0.01</td>
<td>6.8 ± 0.2</td>
<td>43 ± 2</td>
<td>7 ± 0</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>F1</td>
<td>0.85 ± 0.12</td>
<td>0.31 ± 0.02</td>
<td>4.8 ± 0.7</td>
<td>25 ± 2</td>
<td>3 ± 0</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>F4</td>
<td>1.19 ± 0.41</td>
<td>0.18 ± 0.00</td>
<td>3.2 ± 0.1</td>
<td>38 ± 0</td>
<td>2 ± 0</td>
<td>18 ± 0</td>
</tr>
<tr>
<td>U3</td>
<td>0.81 ± 0.11</td>
<td>0.10 ± 0.01</td>
<td>3.7 ± 0.2</td>
<td>27 ± 0</td>
<td>2 ± 0</td>
<td>38 ± 0</td>
</tr>
<tr>
<td>3A5 Slough</td>
<td>1.90 ± 0.27</td>
<td>0.09 ± 0.01</td>
<td>6.5 ± 0.5</td>
<td>31 ± 3</td>
<td>10 ± 1</td>
<td>69 ± 1</td>
</tr>
<tr>
<td>3A5 Cladium</td>
<td>2.15 ± 0.30</td>
<td>0.11 ± 0.01</td>
<td>7.0 ± 0.3</td>
<td>33 ± 3</td>
<td>11 ± 1</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>SrsA</td>
<td>0.69 ± 0.14</td>
<td>0.05 ± 0.00</td>
<td>3.0 ± 0.2</td>
<td>29 ± 2</td>
<td>3 ± 0</td>
<td>65 ± 1</td>
</tr>
</tbody>
</table>
Suspended particle masses were relatively evenly distributed among the size classes at many sites (Fig. 2). However, small particles dominated at Lox8 and the 3A5 Slough and 3A5 Cladium sites, where most particle mass was associated with particles smaller than 2.7 \( \mu m \). These are the same sites with the highest TSS concentrations (Table 2). Particle mass was also slightly greater in the 10–100– \( \mu m \) size class compared to the 2.7–10– \( \mu m \) size class at several sites.

Particulate P concentrations were always greatest in the 0.45–2.7– \( \mu m \) size class, and least in the largest and smallest size classes (Fig. 3). The 10–100– \( \mu m \), 2.7–10– \( \mu m \), 0.45–2.7– \( \mu m \), and 0.2–0.45– \( \mu m \) particle size classes comprised a mean 4\%, 21\%, 73\%, and 3\% of total particulate P, respectively, across all sites. Colloidal (3 kDa–0.1 \( \mu m \)) P concentrations were similar to P concentrations in the various particulate size classes, and total colloidal P ranged from 11% to 28% (mean = 19%) of total P among sites. The distribution of particulate, colloidal, and ultrafiltered dissolved (<3 kDa) P size fractions was mostly similar across the Everglades, including along the P-enrichment gradient in WCA-2A. The 10–100– \( \mu m \) particulate P size class, however, did decrease from the northern to southern Everglades. In addition, the 3–100-kDa colloidal size class was enriched with P at the most P-enriched site, F1.

The size distribution of surface-water N was similar among sites across the Everglades (Fig. 3). The largest concentrations of particulate N were associated with either the 2.7–10– \( \mu m \) or 0.45–2.7– \( \mu m \) size classes. The smaller colloidal size fractions dominated total surface-water N. The 3–100-kDa and 100 kDa–0.1– \( \mu m \) size classes together held from 44% to 71% (mean = 54%) of total N in surface waters across the Everglades. The 3–100 kDa fraction, in particular, held the most N among the particulate and colloidal size classes at all sites. The ultrafiltered dissolved N size fraction (<3 kDa) was the largest size class at two of the oligotrophic sites, U3 and SrsA.

The different size distributions of N and P were also evident in differences in the N:P ratios among the particulate and colloidal size fractions. The ratio of N:P generally decreased from the two largest particle fractions to the 0.45–2.7– \( \mu m \) particulate size fraction, which had the lowest particulate N:P ratios at all sites, and increased again in the 0.2–0.45– \( \mu m \) size class (Fig. 4). The 0.45–2.7– \( \mu m \) particles at the enriched sites in WCA-2A had N:P ratios of 10 at F1 and 11 at F4. Otherwise, particulate N:P ratios were well above the Redfield ratio, often well above 100 at the oligotrophic sites. The colloidal size fractions had the highest N:P ratios compared to particles at all sites. Among the colloidal size classes, N:P increased in the smaller size class.

The mean proportion of total particulate Fe in each size class was negatively correlated with the mean proportion of total particulate P in each size class across the sites (Pearson product-moment correlation: \( r = -0.65, p = 0.002, n = 20 \)). However, the proportions of total particulate Ca (\( r = -0.14, p = 0.56 \)) and Al (\( r = -0.31, p = 0.19 \)) in each size class were not correlated with the proportion of total particulate P. In contrast, the size distribution of total particulate N was positively correlated with the size distributions of total particulate Ca (\( r = 0.47, p = 0.04 \)) but not Fe (\( r = -0.24, p = 0.30 \)) or Al (\( r = 0.37, p = 0.10 \))

\( N \) and \( P \) speciation—Dissolved (<0.2 \( \mu m \)) organic N made up more than 90% of total surface-water N at all sites (Fig. 5). Dissolved organic N concentrations were roughly 1.5× greater in WCA-2A, irrespective of location along the P-enrichment gradient, than the other regions of the Everglades. Dissolved inorganic N and particulate N species were minor everywhere, each contributing <5% of total N. Ammonium dominated the small dissolved inorganic N pool.
Fig. 3. Phosphorus and nitrogen concentrations in particulate (left bars, μm), colloidal (center bars, μm to kDa), and ultrafiltered dissolved (right bars, kDa) size classes at sites across the Everglades. Colloidal size classes were not sampled at 3A5 Slough and 3A5 Cladium sites. P<dl = phosphorus below detection limit. Mean ± one SE; n = 2 locations (Lox8, F1, F4, and U3) or 3 depths (3A5 Cladium, 3A5 Slough, and SrsA).
All P species, with the exception of dissolved reactive P (DRP), were greatest at the P-enriched, *T. domingensis* marsh site F1 in WCA-2A (Fig. 5). Particulate acid-hydrolyzable P was the largest of the three particulate P chemical species at every site except 3A5 Slough. Particulate organic P was also an important form of particulate P at the P-enriched and oligotrophic sites in WCA-2A and at the 3A5 sites. Particulate reactive P concentrations were similar throughout the Everglades and always comprised a small proportion of TPP.

Dissolved organic P (DOP) was the dominant dissolved P species at most sites (Fig. 5). Dissolved organic P concentrations were greater at the enriched F1 site, but otherwise were similar across the Everglades. Dissolved acid-hydrolyzable P concentrations increased with P enrichment in WCA-2A and were low at oligotrophic sites. Dissolved reactive P concentrations were low, <0.1 μmol L⁻¹, throughout the Everglades, including the P-enriched sites.

The sequential chemical extraction directly identified the forms of P in suspended particles. Microbial and labile P dominated the suspended particles at the 3A5 Slough and
Cladium sites (Fig. 6). Microbial and labile P held on average 65% of all the P contained in suspended particles. Humic and fulvic P held on average 24% of total particulate P. Calcium-associated P held on average 9% of particle P. Finally, Fe- and Al-associated P and refractory organic P were both unimportant. The forms of particulate P were very similar across the different depths of the water column and the two habitat types. In addition, the sum of all of the P fractions measured by sequential extraction was similar to total particulate P measured directly on another set of filters, suggesting that sequential extraction accounted for all the P in suspended particles and that the microbial P correction was valid.

**Direct versus indirect measurement**—Measurement of particulate nutrient concentrations directly was much more accurate than indirect measurements (by difference between total and total dissolved). Mean differences (direct – indirect) for PP and PN were 0.02 and 1.34 μmol L⁻¹, respectively, and did not significantly differ from zero (paired t-test, n = 17; PP, p = 0.14, PN, p = 0.21), suggesting that there was no major bias. However, total PP concentrations measured indirectly frequently differed by up to 50% compared to direct measurements; total PN concentrations often differed by 100% (Fig. 7).

**Discussion**

This study found that suspended particles in Everglades wetlands: (1) held a large proportion of total P in the surface waters of the Everglades; (2) were not abundant; (3) were small; (4) had most of their P associated with intermediately labile microbial biomass compared to reactive and refractory P; (5) became more enriched with P in conjunction with P enrichment; (6) showed little relation between N and P characteristics; and (7) were better measured by direct capture and analysis rather than indirect methods using calculations of the difference between unfiltered and filtered samples. Suspended particles have also been shown to quickly cycle P in Everglades surface water (Noe et al. 2003). The importance of suspended particles to P dynamics in Everglades wetlands is not surprising given the role of particulate P in other aquatic ecosystems (Lean 1973; Correll et al. 1975; Meyer and Likens 1979; Fox 1993). The findings of this study should be relevant to other wetland and shallow aquatic ecosystems, especially oligotrophic and P-limited wetlands with sheet flow or low terrigenic mineral inputs. The most comparable wetlands to the Everglades may be found elsewhere in the Caribbean basin, such as in Belize (Rejmánková et al. 1996).

The composition of the suspended particles has implications for the identification of particle sources, reactivity, and transport. Multiple lines of evidence suggest that suspended, unattached bacteria (bacterioplankton) are the dominant source of P-rich particles in the Everglades. The most abundant size class of particulate P was 0.45–2.7 μm, similar to the findings of Jones (2001). This size class is most likely composed of bacteria (Currie and Kalff 1984). In addition, acid-hydrolyzable P dominated the P fractions in particles. As previously mentioned, CaCO₃-P was likely measured in the more labile molybdate-reactive P pool, leaving microbial P as the likely dominant source of acid-hydrolyzable P. Finally, the sequential chemical extraction revealed that most particulate P was associated with microbial biomass. Therefore, suspended and unattached bacteria are likely the dominant P-bearing particles in Everglades wetlands.

It is also possible to eliminate other potential sources for the dominant P-bearing particles. First, the suspended particles were likely not algae or fungi, because the
dominant size class of particulate P (\(<2.7\,\mu m\)) was too small to include phytoplankton or mycoplankton cells (Sieburth et al. 1978). In addition, phytoplankton productivity is undetectable in the Everglades, where periphyton productivity is high (McCormick et al. 1998). However, phytoplankton has been shown to be an important component of particulate P in enriched-treatment wetlands to the north of the Everglades (Farve et al. 2004). Fungi and yeast can also dominate particulate P cycling in other wetlands (Richardson and Marshall 1986). Second, mineral sediment was not important. Terrigenous silt and clay sediment is not present in the Everglades watershed (Noe et al. 2001). In addition, particulate Fe was negatively associated with particulate P in the current study. Sequential chemical extraction of particles also indicated that particulate P was not associated with Fe and Al. Third, refractory organic detritus was not a major component of suspended particles. Refractory organic P was a very small component of total particulate P in the sequential chemical extraction. The near-Redfield N:P ratio in the 0.45–2.7–\(\mu m\) particles also suggested that these fine particles were not likely fresh macrophyte detritus because Everglades macrophytes have much higher ratios (Noe et al. 2001). Fourth, particulate P was not associated with CaCO\(_3\) produced by periphyton. The size classes of particles that were rich in P were not associated with particulate Ca. The low concentrations of particulate reactive P also decrease the likelihood of CaCO\(_3\) being the source of particles, because it is likely that the low pH during molybdate-reactive P measurement would dissolve CaCO\(_3\) or desorb P from CaCO\(_3\) particles. The sequential chemical extraction also revealed that CaCO\(_3\) held small amounts of particulate P. It should be noted that suspended sediment for that analysis was collected in December and that CaCO\(_3\) may be more important earlier in the wet season when periphyton productivity may be higher. Most initial uptake of orthophosphate by Everglades periphyton is associated with CaCO\(_3\) precipitated by the periphyton mats, but most of this P is eventually cycled into periphyton organic matter (Noe et al. 2003). To summarize, phytoplankton, fungi, yeast, refractory organic detritus, and allogenic and autogenic mineral sediment were not likely important sources of particulate P in Everglades wetlands.

In addition to the N present in the bacteria, N-rich particles likely consisted of detrital organic matter and associated CaCO\(_3\) that were sloughed from periphyton mats (periphyton rain). Particulate N was often more abundant in larger size classes than particulate P, suggesting a source other than suspended bacteria. In addition, particulate N was positively correlated with particulate Ca. Furthermore, the periphyton mats of the Everglades have high N fixation rates (Inglett et al. 2004). It is likely that the various forms of periphyton biofilms and mats in the water column of Everglades wetlands, including floating metaphyton and attached epiphyton, are the source of the larger suspended particles that were relatively N-rich and were often a large proportion of particle mass. The different characteristics of particulate N and P in these waters present an interesting dichotomy in ecosystem nutrient biogeochemistry.

The suspended particles in Everglades wetlands were likely biogeochemically reactive. Death of the bacteria cells would result in the release of labile organic and inorganic P and N. Again, the dominance of acid-hydrolyzable P in particles suggested that most P in particles was more bioavailable than refractory organic P but less bioavailable than molybdate-reactive P. In addition, the N:P ratios of suspended particles were near the Redfield ratio, especially smaller particles (that held the most P) and particles in P-enriched sites. This balance of the N and P content of particles suggests that particles important to P cycling and transport are labile. Furthermore, Pant et al. (2002) found that particulate P in Everglades treatment wetlands had high mineralization rates. The predominance of humic +

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**Fig. 7.** (A) Particulate nitrogen (PN) and (B) particulate phosphorus (PP) concentrations estimated by direct versus indirect measurement. Each symbol is a location (2 per site; Lox8, F1, F4, and U3) or depth (3 per site; 3A5 Cladium, 3A5 Slough, and SrsA) from sites across the Everglades.
fulvic organic forms of P over refractory organic P in the sequential chemical extraction of particles suggested that particulate N was also labile.

Phosphorus in the small colloidal size range (3 kDa–0.1 μm) constituted a small but consistent proportion of total P in the surface water of Everglades wetlands. In contrast, small colloidal N dominated surface-water N content. Across the Everglades, the 3–100 kDa size fraction had the highest N concentration and N:P ratios compared to other size classes. The colloidal size classes may contain an abundance of proteinaceous material bound to humic substances (Steinberg and Muenster 1985). In fact, Everglades marshes produce dissolved (<0.7 μm) protein (Lu et al. 2003). Phosphorus-reactive colloids have been shown to be important to P cycling in lakes (Lean 1973) and P transport from agricultural fields to aquatic ecosystems (Heathwaite et al. 2005). We also observed elevated concentrations of 3–100 kDa colloidal P at enriched sites downstream from agricultural P loading. Colloidal P can include mineral-sorbed P (Al, Fe, and Ca) (Turner et al. 2004), humic-metal-phosphate complexes (Hens and Merckx 2001), and extracellular products of microbial metabolism (Paepl and Lean 1976). Colloids can also form suspended particles by self-organizing into 0.4–0.8 μm aggregates (Kerner et al. 2003). In the Everglades, Wang et al. (2002) found that dissolved organic matter smaller than 1 kDa is younger and more labile than colloidal dissolved organic matter (1 kDa–0.2 μm). In addition, colloidal organic P (0.05–0.4 μm) mineralized at a slower rate than particulate organic P (>0.4 μm) in an Everglades treatment wetland (Pant et al. 2002). Although little is known about colloids in the Everglades, their importance in other aquatic ecosystems suggests that more research is needed.

The fractionation of P in the surface water of wetlands has important implications for predictions of downstream P transport (Kadlec 2003). In the Everglades, the large mass of anthropogenic P stored in the upstream, northern Everglades is rapidly cycled from soils to the water column through the production of aboveground T. domingensis biomass (Noe and Childers in press). Leaching of P from dead plant tissues to microbes provides a quantitatively large flux (Davis et al. 2006) that could eventually result in the production of suspended particles. Hydrologic tracer studies have shown that suspended particles have very different transport characteristics than solutes in the Everglades wetlands water column (Saiers et al. 2003; Harvey et al. 2005). Nearly all P associated with small particles (0.2–1 μm) passed through soil columns containing Everglades peat during saturated-flow experiments, whereas nearly all particles larger than 1 μm were retained (Jones and Amador 1992). The mobility of small, P-rich particles through porous media (peat or periphyton and vegetation) could explain their relative abundance compared to larger particles in this study. Furthermore, it has been shown that particulate P had intermediate retention rates compared to dissolved reactive P or dissolved organic P in a south Florida wetland (Davis 1981). However, existing models of P transport in Everglades wetlands do not account for the differential transport of solutes versus particles (e.g., Walker 1995; Fitz and Sklar 1999). Thus, the new information on particulate and colloidal P concentrations generated by this study can facilitate improved modeling of the effects of hydrology on ecosystem nutrient cycling and transport.

References


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