Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, U.S.A.

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SUMMARY

1. We wanted to determine if changes in algae in the Everglades were due to increased phosphorus (P) loading. Epiphytic algae, water chemistry, and surface sediment chemistry were characterized from 32 sloughs along a P gradient in the Everglades and changes in the algal assemblages along the P gradient were compared with those along an experimental P gradient of in situ mesocosms. The sloughs are the wettest open water habitats characterized by floating and submerged aquatic plants in the Everglades.

2. Algal species composition was much more sensitive to P concentration than algal biomass. The diatom species variance among sloughs, captured by 1st ordination axis, was more highly correlated with total P (TP) in surface sediments ($r = -0.79$), than soluble reactive P (SRP) ($r = -0.08$) and TP ($r = -0.48$) in the water column. Algal biomass (µg chl a cm$^{-2}$) was not significantly correlated with P (SRP: $r = 0.22$, TP: $r = 0.19$, sediment TP: $r = 0.07$) along the P gradient in the Everglades. Cluster analysis classified diatom species assemblages in 32 sloughs into three groups (TWIN I, II, III), which corresponded to three zones along the P gradient. Dominant diatom species shifted from *Mastogloia smithii* (40.3%), *Cymbella scotica* (22.3%), and *Fragilaria synegrotesca* (21.8%) in TWIN I to *Nitzschia amphibia* (22.4%) and *C. microcephala* (12.4%) in TWIN III. TP in surface sediments and TP in epiphyton assemblages increased 4- and 5-fold from TWIN I to TWIN III, respectively.

3. Patterns in epiphytic assemblages along the experimental P gradient in the mesocosms were very similar to those along the Everglades P gradient. Shannon diversity indices and species richness significantly increased along both P gradients. TN : TP ratio in epiphyton assemblages significantly decreased as sediment TP increased along both P gradient. Ordination analysis showed that diatom assemblages in the impacted zone (TWIN III) were ordinated closely to the assemblages from the highest P treatments in the mesocosms. The assemblages from the less impacted zone (TWIN I) were ordinated closely to the assemblages from controls in the mesocosms.

4. Concurrence between results of our survey and experiments suggest that changes in epiphytic assemblages along the P gradient in the Everglades are caused by increases in P concentrations.

Keywords: periphyton, phosphorus gradient, mesocosms, wetlands

Introduction

Multiple lines of evidence may be necessary to identify causes of biotic community changes in ecosystems in which many environmental factors
have been altered by humans. Field observations along environmental gradients can show changes in biotic communities from one state to another (Gosz, 1992). These changes in state are often manifestations of a complex interplay of multiple factors in time and space (O’Neill et al., 1986). Probable causes for such changes can be inferred from the gradient studies and then tested with experimental manipulation. Research that combines experimental and field gradient approaches is important for reliable complementary information that can test ecological models and solve applied ecological problems (Daehler & Strong, 1996).

Loss of calcareous periphyton assemblages in the Everglades has been a great concern to ecologists and resource management (Browder et al., 1994; McCormick et al., 1996). Calcareous periphyton assemblages are an essential part of sloughs and southern marl-forming marshes, two important habitats in the Everglades ecosystem due to their high biodiversity and primary production. Periphyton contribute significantly in primary production, detrital processes, and nutrient cycling in sloughs (Browder et al., 1994).

Changes in phosphorus (P) loading along with other factors (e.g. hydrology) have been related to the loss of calcareous periphyton assemblages in the Everglades. Several studies suggest that increasing P loading in the Everglades, a historically P-limited system, may be one of the major causes to changes in periphyton assemblages (Grimshaw et al., 1993; Raschke, 1993; McCormick et al., 1996). It is still, however, difficult to relate the causes of calcareous periphyton changes in the Everglades to P loading. Dynamic exchanges of P between particulate and soluble forms in the water column, sediments, macrophytes and algae make it difficult to quantitatively estimate P availability, and thus impacts of P loading on algal assemblages in shallow aquatic ecosystems such as the Everglades (Bradford & Peters, 1987; Carlton & Wetzel, 1988; Rattray et al., 1991; Pakulski, 1992). Habitat complexity in wetlands further increases difficulties in assessing the effects of P loading on algae because algal responses are often habitat-specific (Gabor et al., 1994; Campeau et al., 1994). Murkin et al. (1994) reported that epipelon had no responses to P additions while plankton and epiphyton had immediate responses and metaphyton had delayed responses. Furthermore, not all signs of algal responses appear at the onset of perturbation. For example, changes in algal species composition may be more sensitive than biomass in response to lake eutrophication (Schindler, 1987).

In this study, we wanted to determine if changes in algae in the Everglades were due to increased P loading. First, we characterized changes in epiphytic algal assemblages (species composition, biomass, calcareous deposition, chemical composition, and species diversity) along the P gradient in the Everglades. We then assessed whether patterns observed along the Everglades P gradient could be corroborated experimentally by manipulating P conditions in mesocosms. Epiphytic algae on Eleocharis were characterized in 32 sloughs located along the P gradient in the Everglades and in 12 in situ mesocosms with different PO4-P dosing rates. Specifically, we hypothesized that (1) changes in epiphytic algal assemblages with experimental manipulation of P loading in the mesocosms would be the same as the changes in algal assemblages along the Everglades P gradient if increased P loading is the major cause for the algal changes in the Everglades; (2) changes in algal species composition would be more sensitive than biomass in response to P loading if ecological redundancy is important in algal species assemblages (Gitay et al., 1996), and (3) TP in the water column or sediments would be more highly correlated to changes in algal assemblages than water column soluble reactive P (SRP) in both mesocosms and over broad area of the Everglades.

**Methods**

**P gradient in the Everglades**

The north–south P gradient in Water Conservation Area (WCA)-2A of the Everglades has been documented by several studies (Craft & Richardson, 1993; Reddy et al., 1993; Urban et al., 1993). WCA-2A, a 547-km² diked marsh with a mosaic of sawgrass (Cladium jamaicense Crantz) prairies and open water sloughs, receives inflows of agricultural runoff for decades. Agricultural runoff carried by canal systems converges into the WCA-2A through inflow structures.
(gates) located along the north levee. Therefore the gates function as point sources for P which produces a north–south gradient in P concentrations that is ≈ 12 km long. Urban et al. (1993) showed a negative logarithmic relationship between water column TP concentrations and distance from the gates. A similar TP gradient in sediments was also evident (Reddy et al., 1993).

**P gradients in mesocosms**

The experimental P gradients were established in mesocosms during November 1992 at two sites in minimally impacted sloughs located in southern WCA-2A (site one: 26°15’00’’ N, 80°23’14’’W, site two: 26°14’57’’ N, 80°23’20’’W). The two sites are ≈ 100 m apart. Six mesocosms, 2 × 8 m channels, were constructed or delineated at each site. At each site, five mesocosms were enclosed on three sides by PVC sheets with the downstream end open. One of these enclosed mesocosms was not enriched and served as a control (CW). An open and unenriched ‘mesocosm’ was delineated and used as an unwalled control (CU) to assess possible ‘wall effects’.

Four mesocosms at each site were continuously dosed with Na$_2$HPO$_4$, which resulted in average PO$_4$-P concentrations of 20, 39, 57, and 126 μg L$^{-1}$ over two year period. These P concentrations were chosen to cover the P range along the P gradient in WCA-2A (Craft & Richardson, 1993). Slough water was mixed with concentrated P solutions and pumped into mixing tanks located at the head of each channel. A concentrated solution of Na$_2$HPO$_4$ from a holding tank in each mesocosm was pumped into a T-junction on the influent side of each water pump by a peristaltic pump when slough water was pumped into mixing tanks. Water drained continuously at a rate of about 1.9 L min$^{-1}$ from the mixing tanks into a distribution pipe and then into a perforated head box. A T-shaped distribution tube and a perforated PVC plate in the mixing tank was used to distribute the flow into the channel evenly in the vertical and horizontal directions. The perforated plate covered the entire cross section of the channel and water trickled into the channel through the perforations. The mixing tanks were refilled when water drained down to a level corresponding to 45% of the 379 L capacity. The flow gauges for each tank were calibrated twice a month.

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**Sampling designs**

Epiphytic algae were sampled in 32 sloughs along the north–south P gradient in WCA-2A from 6 to 8 April 1995. We sampled epiphytic algae because they may be more sensitive to changes in P loading in wetlands than those of other algal assemblages. Epiphytic algae acquire P leached from their host plants which transport P from sediments, a major sink for P in the Everglades (Craft & Richardson, 1993), to shoots and leaves (Rattray et al., 1991; Pakulski, 1992). Approximately 50% of P that eelgrass takes up from sediments is transported to the water via the plants (McRoy et al., 1972). The growth of epiphytic assemblages may therefore reflect P availability in sediments more closely than in the water column. Our sampled P gradient only covered the southern end of the entire north–south P gradient (approximately between 6 and 10 km from the north levee). We only sampled the low P region of the P gradient because we wanted to restrict sampling to sloughs with Eleocharis cellulosa Torrey, a common macrophyte in WCA-2A sloughs, and thereby to reduce the variance associated with algal–host interactions, which have been reported in oligotrophic lakes (Phillips et al., 1978). E. cellulosa is replaced by other species as P levels increase further (personal observation). In addition, we wanted to concentrate our sampling effort on the area where calcareous epiphyton changed to non-calcareous epiphyton.

Epiphytic algae, water chemistry and sediment chemistry were characterized from several transects along the P gradient to assess spatial variation within the sampled area. A composite sample of algae in Eleocharis was collected in each slough. A minimum of ten Eleocharis stems (10 cm long) were collected to account for spatial variation within each slough. Sections of the Eleocharis stems below the water surface were cut and placed in Whirl-pak bags.

An epiphytic sample was collected in each mesocosm at the two dosing facility sites ($N = 12$) in June 1994. Eleocharis stems were only collected at locations in the mesocosms that were 2 m from the mixing tank and 0.5 m from each wall to avoid potential disturbance caused by proximity to the mixing tanks and shading or other wall effects. A sediment core was taken using a plastic coring device (diameter: 2.75 cm, length: 60 cm) in each sampled slough and mesocosm at the same location where epiphyton
were collected. The top 5 cm of surface sediments were extruded vertically in the field for analyzing sediment chemistry.

Filtered (pore size: 0.45 μm) and unfiltered water samples were collected for nutrient analyses in acid-rinsed centrifuge tubes (50 mL) along the Everglades P gradient. Water samples in each mesocosm were sampled every two weeks for SRP analysis at the same location where epiphyton were collected. Samples for total P (TP), total dissolved P (TDP), total nitrogen (TN), total dissolved nitrogen (TDN), nitrate (NO₃-N), and ammonium (NH₄-N) were collected once every month. All samples were placed in coolers with ice in the field. Water chemistry samples were analyzed within 48 h. Other samples were stored frozen in the lab until analysis. The sampling locations in each slough were recorded using a global positioning system.

Chemistry analysis

Nutrient concentrations were determined using standard methods (APHA, 1992). SRP was analyzed using the ammonium molybdate-ascorbic acid method (APHA, 1992). NH₄-N was measured by the autoanalyzer phenate method (APHA, 1992). NO₃/NO₂-N was analyzed by the autoanalyzer diazotization method (APHA, 1992). TP was determined by persulphate digestion of the sample and measuring PO₄-P in the digests. TN was determined by persulphate digestion of the sample and measuring NO₃-N in the digests. Sediment TP was determined by digesting 100 mg of sediments in nitric and perchloric acid (Sommers & Nelson, 1972) and measuring PO₄-P in the digests using a TRAACS 800 autoanalyzer (Branen-Luebbe Inc., Elmsford, NY). Sediment TC and TN were measured in 10–15 mg samples using a Perkin-Elmer 2400 CHNS analyzer (Perkin-Elmer, Norwalk, CN). Pine needles, National Bureau of Standards (NBS) no. 1575 for TP, was digested and measured for PO₄-P using the same method. Buffalo river sediment (NBS no. 2704) and peach leaves (NBS no. 1547) were employed as standards for TC and TN, respectively. The measured values for these NBS standards were within 4% of the actual values.

Epiphyton analysis

Epiphyton were scraped from the surface of Eleocharis stems with a soft toothbrush and rinsed with distilled water. Substrate area was calculated with measurements of stem length, stem diameter, and a formula for surface area of a cylinder. The resulting epiphyton suspension was homogenized with a hand-held, variable-speed blender and then was adjusted to a standard volume. Subsamples were taken for assay of chlorophyll a (chl a), ash free dry mass (AFDM) and ash weight, nutrient contents in epiphyton assemblages (TP and TN), and cell enumeration. Chl a was assayed spectrophotometrically with standard procedures (APHA, 1992). AFDM and ash weight were also assayed with standard procedures (APHA, 1992) except that epiphyton samples were dried at 100 °C for 72 h instead of 24 h. Epiphyton nutrient contents (TP and TN) were analyzed with the same procedures as TP and TN in water.

At least 500 algal cells with protoplasm (both diatoms and non-diatom algae) were counted in wet mounts at 1000× magnification to determine algal densities (cells cm⁻²). Wet mounts were prepared by spreading separate subsamples of 0.02 mL of the epiphyton suspension, preserved with 2% M₃ fixative (APHA, 1992), on both ends of a microscopic slide. Each aliquot was covered with a coverglass and sealed by applying fingernail polish that overlapped the coverglass edge and the microscope slide. When dry, the fingernail polish prevented evaporation of the sample. This procedure enables identification and enumeration of non-diatom algae, especially small blue-green algae, at 1000× magnification using an immersion oil lens. Taxonomy for non-diatom algae mainly followed Prescott (1962), Drouet & Baily (1956), and Drouet (1968). For small blue-green coccoids (e.g. Aphanothece), cell numbers in each colony were estimated, but a colony was counted as one unit for purposes of tallying 500 cells in a count. For thin filamentous blue-green algae (e.g. Schizothrix), in which cross-walls are often difficult to detect, a 10-μm length of trichomes was counted as one unit. Diatoms with protoplasm were counted but not identified in wet mounts.

Diatom frustules were cleaned with concentrated sulphuric acid and potassium dichromate on a hot plate. After numerous rinses with distilled water, cleaned diatom frustules were mounted in HYRAX (Patrick & Reimer, 1966). A minimum of 500 diatom valves was counted at 1000× magnification. Patrick & Reimer (1966, 1975) and Kramer & Lange-Bertalot (1986, 1988, 1991a, 1991b) were used as primary references for diatom taxonomy.
Data analysis

Gradient analysis. If P is one of major regulatory factors of algal species distribution, we expected a high correlation between changes in algal assemblages and P concentrations along the P gradient. Detrended correspondence analysis (DCA), a multivariate ordination technique, was used to summarize changes of diatom assemblage structure (Hill, 1973). Changes in diatom assemblage structure were then related to environmental variables using correlation analysis. Ordination sample scores for the first two ordination axes, representing species dissimilarity among samples, were correlated to measured environmental variables. DCA was performed using CANOCO v. 3.1 (ter Braak, 1987, 1990).

Zonation along the gradient. If algal distribution patterns were discrete along the P gradient, unique characteristics of algal assemblages should be identifiable. After the environmental gradient that was most correlated to changes in diatom assemblage structure was identified in DCA and correlation analysis, zonation along the gradient was assessed using two indicator species analysis (TWINSPAN), a technique of cluster analysis (Hill et al., 1975). Canonical discriminant function analysis (CDFA) was used to identify which environmental variables had the best discriminating power among TWINSPAN zones (SAS, 1985).

Comparing changes in diatom assemblages along observed and experimental P gradients. Our goal was to assess whether patterns observed along the P gradient in the Everglades could be corroborated experimentally by manipulating P conditions in mesocosms. We expected that a correspondence between changes in diatom assemblages would occur along both P gradients in the mesocosms and Everglades. DCA axes were constructed based on 12 samples in the mesocosms. Then 32 Everglades slough samples were superimposed onto the DCA ordination diagram based on species similarity between the mesocosm and Everglades P gradient samples (ter Braak, 1987).

Results

P gradients

Sediment TP had a greater range and coefficient of variance among sampled sloughs along the Everglades P gradient than water column TP, TDP, and SRP. Sediment TP ranged from 0.27 to 2.05 mg g⁻¹ dry weight among sites. Water column TP ranged from 8.5 to 38.5 µg L⁻¹ and SRP ranged from 1.1 to 5.4 µg L⁻¹. The coefficient of variance for sediment TP (66%) was higher than the other fractions of P in the water column (TDP, 29%; SRP, 34%; and TP, 39%). Sediment TP was positively correlated with TP in the water column (r = 0.44, P = 0.01) but not with SRP (r = 0.09, P = 0.64) (Fig. 1a).

In the mesocosms, SRP in the water column had a
greater range and coefficient of variance than along the Everglades P gradient. Average SRP in the water column over a two year period ranged from 5.8 to 45 μg L⁻¹. Average water column TP ranged from 9.5 to 66.4 μg L⁻¹ (Fig. 1b). Sediment TP, representing almost two-year accumulations of P in the mesocosms, ranged from 0.19 to 0.87 mg g⁻¹ dry weight (Fig. 1c). The coefficient of variance for SRP (91%) was higher than sediment TP (58%) and water column TP (60%). SRP in the water column was positively correlated with TP in the water column ($r = 0.69$, $P = 0.01$) and sediment TP ($r = 0.87$, $P = 0.0001$). In the mesocosms, the TN : TP ratio was also negatively correlated with TP ($r = -0.78$, $P = 0.01$) and sediment TP ($r = -0.65$, $P = 0.03$). Both AFDM and ash weight (as percentage of AFDM) declined along the Everglades P gradient while algal biomass (μg chl a cm⁻²) was not significantly correlated with P concentrations (SRP: $r = 0.22$, TP: $r = 0.19$, sediment TP: $r = 0.07$) (Fig. 3). A similarly weak correlation between algal biomass and P concentrations was also observed in the mesocosms (SRP: $r = -0.17$, TP: $r = 0.34$, sediment TP: $r = 0.25$).

Changes in algal composition at the functional group level were also evident along both P gradients (Fig. 4). The proportion of coccoid blue-green algae (e.g. Oscillatoriaceae) increased significantly along both the Everglades and mesocosm P gradients (Fig. 4, Table 1). In mesocosms but not along the Everglades

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mesocosms ($n = 12$)</th>
<th>WCA-2A ($n = 32$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRP: TP: Sed TP</td>
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<tr>
<td>% of biovolume</td>
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<tr>
<td>BGC</td>
<td>0.12 ± 0.84†</td>
<td>0.21 ± 0.33</td>
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<tr>
<td>BGF</td>
<td>0.36 ± 0.44</td>
<td>0.43 ± 0.24</td>
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<tr>
<td>BGFH</td>
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<td>0.60* ± 0.06</td>
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<td>Diatoms</td>
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<td>-0.59 ± 0.21</td>
</tr>
<tr>
<td>Biovolume</td>
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<tr>
<td>BGC</td>
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<td>0.11 ± 0.08</td>
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<td>BGF</td>
<td>-0.11 ± 0.15</td>
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<tr>
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<td>-0.32 ± 0.12</td>
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<td>AFDM</td>
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<tr>
<td>Ash weight</td>
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<td>Chl a</td>
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<tr>
<td>Biovolume</td>
<td>0.03 ± 0.13</td>
<td>0.59 ± 0.07</td>
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<tr>
<td>N : P ratio</td>
<td>-0.66* ± 0.78*</td>
<td>-0.65* ± 0.09</td>
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<tr>
<td>Shannon diversity</td>
<td>0.53 ± 0.66*</td>
<td>0.44 ± 0.22</td>
</tr>
<tr>
<td>Species richness</td>
<td>0.59 ± 0.84†</td>
<td>0.61* ± 0.23</td>
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Fig. 2 Relationship between TN : TP ratio and phosphorus. (A) The Everglades P gradient (B) The mesocosms. The numbers at the top of the Fig. 2 (b) show sediment TP (mg g⁻¹ dry weight) for each mesocosm. CU: unwalled control; CW: walled control.
P gradient, the proportion of filamentous blue-green algae with heterocysts increased significantly in response to P while the diatom proportion decreased (Fig. 4, Table 1). However, the absolute biomass of both filamentous blue-green algae with heterocysts (*Scytonema hoffmanii* Ag. ex Born. et Flah., > 95%) and diatoms (measured as biovolume, \( \text{mm}^3 \text{cm}^{-2} \)) declined significantly as sediment TP increased along the Everglades P gradient but not in the mesocosms (Table 1).

Algal species variance among sites was high, suggesting high species dissimilarity among sites and sensitivity of algae species to changing P conditions. With all algae included in DCA analyses, the 1st two ordination axes explained 28% (relative abundance), 25.5% (relative abundance weighted by cell volume), and 29.1% (absolute abundance) of variance in algal species data along the Everglades P gradient (Table 2). On the other hand, 51.2% of variance in diatom species distribution along the Everglades P gradient was explained by the 1st two ordination axes (Table 2). Each remaining axis explained less than 5% of species variance. Thus the 1st two axes captured the majority of interpretable diatom species variance and the 1st axis alone explained \( \approx 38.6\% \) of species variance in diatom species distribution. Having one ordination axis explained such a high proportion of species variance suggested diatom species were distributed in a systematic way in association with a relatively simple ecological gradient. Because a large amount of diatom species variance could be explained by two ordination axes and because diatom taxonomy is well-known, we used diatoms for further analyses. Blue-green algae, highly clumped as colonies in their distribution, required higher cell counts than we used to characterize their population abundance as accurately as diatoms.

Changes in diatom assemblages along ordination axes were highly correlated with surface sediment TP along the Everglades P gradient. Correlation analysis

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**Fig. 3** Relationship between epiphyton biomass (AFDM) and phosphorus (A) ash weight as percentage of AFDM (B) and surface sediment TP along the Everglades P gradient.

**Fig. 4** Shifts of major algal functional groups along the P gradients. (A) The WCA-2A P gradient (B) The mesocosms. BGFH: filamentous and heterocystous blue-green algae, BGF: non-heterocystous filamentous blue-green algae, BGC: coccoid blue-green algae. The numbers in Fig. 4 (b) show sediment TP (mg g\(^{-1}\) dry weight) for each channel in the mesocosms. CU: unwalled control, CW: walled control.

of the 1st two DCA axes (DCA sample scores) and measured environmental variables showed that the 1st DCA axis was highly correlated with surface sediment TP ($r = -0.79$), $\text{NH}_4$-N in the water column ($r = -0.59$), and TP in the water column ($r = 0.48$) (Table 3). The 2nd DCA axis was only correlated with water column TP ($r = 0.4$).

In the mesocosms, the 1st two DCA axes captured $\approx 45.2\%$ of the variance in diatom species distribution (Table 2). The 1st axis alone explained $31\%$ of variance. Correlation analysis of the 1st two DCA axes and measured chemistry variables showed the 1st DCA axis was highly correlated with TP in the water column ($r = 0.89$). The 2nd axis was positively correlated with TDP ($r = 0.86$) and SRP ($r = 0.81$) (Table 3).

TWINSPAN cluster analysis separated all sites along the Everglades P gradient into three groups (MANOVA, Wilks’ lambda $F_{18,42} = 2.27$, $P = 0.03$) (Fig. 5). Three zones were categorized as the less impacted (TWIN I), transitional (TWIN II), and impacted (TWIN III) zones. Spatially, TWINSPAN classification in general corresponded with the distance of the sites from the north levee (e.g. point-source of P) (Fig. 6).

Species replacement from TWIN I to TWIN III was evident in diatom assemblages (Fig. 7a). Diatom assemblages in TWIN I were dominated by $\textit{Mastogloia smithii}$ (40.3%), $\textit{Cymbella scotica}$ Wm. Smith (22.3%), and $\textit{Fragilaria synegrotesca}$ Lange-Bertalot (21.8%). Dominance of these three species was replaced by $\textit{Nitzschia amphibia}$ Grunow (22.4%) and $\textit{C. microcephala}$ Grunow (12.4%) in TWIN III, with significant changes in both relative and absolute abundance between TWIN I and III ($P < 0.05$).

Table 2 Results of Detrended correspondence analysis (DCA)

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<th>Data type</th>
<th>Ordination axis</th>
<th>Eigenvalues</th>
<th>% of variance explained</th>
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<td>WCA-2A gradient ($n = 32$)</td>
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<td>All algae, as proportions of total counts</td>
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<td>All algae, as proportions of total biovolume</td>
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<td></td>
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<td></td>
<td>II 0.50</td>
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<td>Diatoms only, as proportions of total counts</td>
<td>I 0.46</td>
<td>38.6</td>
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<td></td>
<td>II 0.15</td>
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<td>Mesocosm ($n = 12$)</td>
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<td>Diatoms only, as proportions of total counts</td>
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<td></td>
<td>II 0.26</td>
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Table 3 Pearson correlation coefficients between DCA sample scores (1st 2 axes) and log-transformed chemical variables. SRP: soluble reactive phosphorus, TN: total nitrogen, TP: total phosphorus, TC: total carbon. (*0.001 < $P < 0.05$, †$P < 0.001$)

<table>
<thead>
<tr>
<th>Variables</th>
<th>WCA-2A ($n = 32$)</th>
<th>Mesocosm ($n = 12$)</th>
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<td>DCA II</td>
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<td>Water chemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtered TN</td>
<td>0.43*</td>
<td>0.25</td>
</tr>
<tr>
<td>FilteredTP</td>
<td>-0.28</td>
<td>0.25</td>
</tr>
<tr>
<td>$\text{NH}_4$</td>
<td>0.59*</td>
<td>-0.19</td>
</tr>
<tr>
<td>$\text{NO}_3$</td>
<td>-0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>$\text{SiO}_2$</td>
<td>0.29</td>
<td>-0.20</td>
</tr>
<tr>
<td>SRP</td>
<td>-0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>UnfilteredTN</td>
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<td>-0.01</td>
</tr>
<tr>
<td>UnfilteredTP</td>
<td>-0.48*</td>
<td>-0.40*</td>
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</table>

| Sediment chemistry |        |        |        |        |
| TC                | -0.45* | 0.15   |        |        |
| TN                | -0.41* | 0.03   |        |        |
| TP                | -0.79**| -0.15  | -0.51  | 0.29   |

Fig. 5 Plot of two canonical axes showing three clusters based on TWINSPAN of diatom species composition.

similar trend in dominant diatom species shift was also observed along the P gradient in the mesocosms (Fig. 7b). Both *Amphora lineolata* Ehrenberg and *F. synegrotesca* decreased as P increased while *N. amphibia* increased in the mesocosm channels.

Causes for changes in diatom assemblages among three zones may be related to the environmental variables which have best discriminant power among three zones. Nine of 16 abiotic and biotic variables were significantly different among three zones (Table 4). Nutrients in surface sediments (TP, TN, and TC) and in the water column (TP, TDN, and NH₄-N) in TWIN III were significantly higher than in TWIN I (Table 4). TP in surface sediments increased 4-fold from TWIN I to TWIN III. It is noticeable that SRP was not significantly different among the three zones.

Concordant with the elevation in nutrient levels, physico-chemical structure of epiphytic assemblages also changed from TWINSpan I to TWINSpan III. Epiphyton biomass as AFDM decreased from an average of 3.34 mg cm⁻² in TWIN I to 0.62 mg cm⁻² in TWIN III (Table 4, Fig. 3a). Ash weight, a measure of CaCO₃ in epiphyton assemblages, also decreased from 2.14 mg cm⁻² in TWIN I to 0.34 mg cm⁻² in TWIN III (Table 4). In contrast, P concentration in epiphyton assemblages was 5-fold higher in TWIN III than in TWIN I (Table 4).

The pattern of diatom zonation along the Everglades P gradient generally corresponded with that of the mesocosms (Fig. 8). Based on species similarity, the gradient samples were ordinated from TWIN I to TWIN III in the DCA diagram defined by the mesocosm samples. The samples from the impacted group (TWIN III) were ordinated closely to the samples from the 57 and 126 µg L⁻¹ mesocosms while the samples from the less impacted group (TWIN I) were ordinated closely to the controls. TWIN II sites closely followed the middle level P treatments in the mesocosms. Changes in species composition with increasing P dosing varied between the two dosing sites (Fig. 8). Changes in species composition along the Everglades P gradient matched better with those at site one. At site two, species changed drastically between the controls and P enriched mesocosms as indicated by distances.
among the samples along the 1st DCA ordination axis. The changes in species composition between P enriched treatments then shifted to a direction along the 2nd ordination axis.

### Discussion

**Changes in epiphyton structure along the P gradients**

Epiphyton biomass (AFDM) decreased with P elevation, which is unusual in a P-limited ecosystem like the Everglades. The Everglades is a historically P limited system with precipitation as the major source of P (Belanger et al., 1989; Davis, 1994). P limitation of algal growth is indicated by extremely high TN : TP ratios in epiphyton assemblages (Vymazal & Richardson, 1995; this study), stimulation by P in algal growth bioassays (McCormick et al., 1996), and low species diversity. Relaxation of P limitation should initially increase algal biomass and primary production as for algae in lakes and streams (e.g. Schindler, 1974; Bothwell, 1989).

Changes in P loading may have complex effects on epiphyton in the Everglades, probably affecting heterotrophic microbial biomass, detritus, and Ca content more than algal biomass. Increased water column TP or sediment TP concentrations were negatively correlated with AFDM and ash weight of epiphyton assemblages, but not chl a in our study. Since AFDM measures biomass of all microbes and detritus in epiphyton, our
High P concentrations can also inhibit the rate of CaCO3 crystallization for shell growth of the marine bivalve and freshwater gastropod (Bernhardt et al., 1985). These inhibitory effects on CaCO3 deposition in periphyton have a significant effect on habitat loss for other components of the Everglades periphyton, especially the heterotrophic components such as bacteria and fungi.

Changes in algal species composition were much more sensitive and predictable than algal biomass (e.g. chl a) in response to P loading. Native dominant diatoms species such as F. synegrotesca were replaced by a set of eutrophic species along the P gradients in both the Everglades and mesocosms. Blue-green algae with heterocysts, mostly Scytonema hoffmanii, and other common native diatom species decreased as P increased along both the Everglades P gradient, but became dominant again in two high P treatments. A much more profound increase in Scytonema was observed in the 126 μg L⁻¹ mesocosms than along the Everglades P gradient, though this group was not abundant in the controls. Increases in Scytonema and other blue-green algae with heterocysts in the 126 μg L⁻¹ mesocosms may indicate nitrogen limitation caused by constant P dosing. The sensitivity of algal species composition relative to biomass in response to P loading may be largely due to ecological redundancy in algal assemblages. Species composition or relative abundance of species may change while overall measures of ecological function such as biomass may remain constant or near constant at the early stages of P loading (Gitay et al., 1996). Algal species assemblages, especially diatoms, have great potentials to indicate P conditions in the Everglades where P concentrations, especially SRP, vary temporally.

Changes along the P gradients: mesocosms vs. survey
Relating results of small-scale experiments to largescale patterns observed in nature requires comparisons of important ecological processes at both scales. It has been argued that most processes, such as resource subsidy of productivity as well as stress due to biotic and abiotic factors, can be important processes at both scales; however, processes such as dispersal and speciation of algae do not operate, respectively, at small spatial and temporal scales (Stevenson, 1996). Results from mesocosm experiments should be applicable to assessing causes of ecological changes in natural systems if most of the natural large-scale processes also operate in mesocosms.

Changes in epiphyton assemblages along the Everglades and mesocosms P gradients were similar at several levels of biological organization in epiphyton assemblages. At the community level, Shannon diversity indices and species richness increased as P increased along both P gradients, which also was observed for macro-invertebrates (Rader & Richardson, 1992). The complex relations between biomass and indicators of P availability were similar along both Everglades and mesocosm P gradients. TN:TP ratios of epiphyton assemblages decreased as P increased along both P gradients. At the functional group level, the proportion of coccoid blue-green algae significantly increased along both P gradients. The proportion of filamentous blue-green algae with heterocysts also increased significantly in responses to P in the mesocosms while the diatom proportions decreased. A similar trend was observed along the Everglades P gradient. At the population level, dominant native diatom species were replaced by eutrophic species along both P gradients. For example, F. synegrotesca, abundant in both mesocosm controls and TWIN I group of the Everglades P gradient, decreased along both P gradients. Meanwhile, N. amphibia, a eutrophic species, increased with P loading along both P gradients. Even with all diatom species used for comparison, changes in diatom assemblages along the Everglades P gradient still generally corresponded with those of the meso-
cosms. For example, species composition of diatom assemblages in the impacted zone (TWIN III) along the Everglades P gradient were ordinated closely to assemblages from the highest P treatments in mesocosms. The assemblages from the less impacted zone (TWIN I) along the Everglades P gradient were ordinated closely to the assemblages from controls in mesocosms. The 20 and 39 μg L⁻¹ mesocosms of site one were close to the TWIN II sites. Our data show that many of the large-scale patterns in epiphyton responses to P elevation observed in spatially and temporally heterogeneous Everglades were reproduced in the mesocosm studies.

Discrepancies between the mesocosms and Everglades P gradient were noticeable in some cases. Such discrepancies may be largely due to the difference between observed and experimental P gradients in habitat complexity, the spatial and temporal scales of important ecological processes, and the frequency and magnitude of environmental changes.

The degree of habitat complexity caused by macrophytes and floating periphyton mats differed substantially between the Everglades P gradient and mesocosms. Extensive, often patchy coverage of both macrophytes, especially broad-leaf water lilies, and floating periphyton mats can exert significant confounding effects on light environments and thus algae and nutrient interaction (Canfield et al., 1983; Goldsborough & Robinson, 1996). Benthic algae failed to respond to nutrient enrichment while significant responses to nutrients were observed in unshaded treatments in a California stream (Hill & Knight, 1988). Light limitation was also one of the reasons for habitat-specific responses to nutrient enrichment in a Canadian wetland study (Murkin et al., 1994). In their study, epipelon showed no responses to nutrient additions in a nutrient limited wetland though epiphyton and phytoplankton both had immediate responses. Epiphytic algae were primarily sampled in Eleocharis dominated sloughs along the Everglades P gradient when there was no or little floating periphyton mats. In the mesocosms, however, floating periphyton mats covered an average of 76% of the channel areas in the controls. Water lilies were absent in three of the six mesocosms at site one but abundant at site two. Confounding effects caused by habitat heterogeneity may account for the lack of a significant response of algal biomass to P and the discrepancy in changes of diatom species composition between the two dosing sites as noted early.

Different temporal scales in the mesocosms and Everglades P gradients may also contribute to discrepancies between changes in species composition along the observed and experimental P gradients. The P gradient in WCA-2A has been there for about 30 years. P loading and corresponding algal species changes have occurred for a longer time with respect to the algal dispersal rates that are usually high, and short generation times. Rates of habitat and assemblage change may have slowed after 30 years along the P gradient such that communities have adapted to P related alteration in habitat conditions. Questions about similar adaptive states in the mesocosms remain largely open and need further evaluation.

The frequency and magnitude of P supply also differed between ‘natural’ and experimental P gradients. P in WCA-2A is often supplied as irregular pulses. In contrast, relatively constant P supply was maintained in the mesocosms. Patchiness of limiting nutrients can be an important factor affecting algal community structure and species succession. Turpin & Harrison (1979) reported that phytoplankton assemblages were dominated by different species when the same amount of the limiting nutrient (NH₄-N) was supplied as continual addition, eight additions per day, and one addition per day. Different species may well be able to optimize uptake of limiting nutrients under a particular nutrient supply or light regime. However, mesocosm experiments reproduced many changes that occur in a studied ecosystem. Results from mesocosm experiments provide useful corroboration of cause-effect relationships for correlative patterns between P and algae in the Everglades.

Assessing P availability in wetlands

Chemical structure of the Everglades and mesocosm P gradients was substantially different. Along the Everglades P gradient, sediment TP varied most and was highly correlated with water column TP but not SRP. In the mesocosms, SRP in the water column varied most. However, SRP, water column TP, and sediment TP were all highly correlated. Despite the major differences in chemical structure of the P gradients in the Everglades and mesocosms, similar biological effects were evident with increasing sediment TP along the Everglades P gradient and along the experimental P gradients in the mesocosms.

SRP, a commonly used assessor of P availability to algae, was poorly correlated with sediment TP, an integrated measure of P conditions over time, along the Everglades P gradients. The poor correlation between SRP and sediment TP could be due to the effects of temporal variation in biotic activities on SRP and one-time sampling of SRP along the Everglades gradient. Daily measurement of SRP in our mesocosms shows that SRP in the 126 μg L⁻¹ mesocosms can be as low as the ambient level during the late afternoon despite the fact that SRP is continuously pumped into the mesocosms and higher at night (P. Vaithyanathan, unpublished data). Rapid loss of dissolved P from the water column was also reported in other P dosing studies in wetlands (Murkins et al., 1994; McCormick et al., 1996). Active uptake of SRP by algae and bacteria may be responsible for such diurnal temporal variations and low values during the day.

Due to the dynamic nature of SRP, it may be necessary to use a reliable surrogate to indicate the P availability that affect algae in wetlands. Our data suggest that surface sediment P concentrations are more highly correlated with the P conditions that affect epiphyton on Eleocharis in WCA-2A than other chemical measures of P. Surface sediments in sloughs are often composed of flocculent material with a cover of 5–10 cm of viable epiphyton (personal observation). Algal biomass in surface sediments was the highest among several microhabitats in sloughs (R. J. Stevenson, unpublished data). Algae and bacteria in sediments can act as a significant sink for P, especially in P limited systems such as the Everglades (Craft & Richardson, 1993). The surface sediments may be able to assimilate increases of P and dampen temporal fluctuation of SRP in the water column until the sediments are saturated by P (Richardson et al., 1997). Surface sediment P and changes in algal assemblage composition, especially diatom assemblage composition, may be valuable methods for characterizing trophic status in wetlands.

Acknowledgments

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References

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