Microbial Indicators of Eutrophication in Everglades Wetlands

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Nutrient loading has been implicated as a major cause of ecological changes in Everglades wetlands. The main objective of this study was to assess changes in microbial indicators in response to nutrient loading across the Everglades landscape. Soil chemical, physical, and microbial properties were measured for nutrient-impacted and oligotrophic sites within Water Conservation Area (WCA)-1, WCA-2a, WCA-3a, and Taylor Slough of Everglades National Park. Impacts of nutrient loading were most evident by the development of gradients in floc and soil total P from peripheral to interior areas of all wetlands following the paths of surface water flow. Floc was more responsive to nutrient loading than the underlying soil. The sensitive indicators of eutrophication were floc and soil total P, microbial biomass P, and mineralized P. Total P averaged 185 and 140% greater for nutrient-impacted than oligotrophic areas for floc and soil (0–3 cm), respectively. Microbial biomass P averaged 97 and 52% higher at nutrient-impacted than oligotrophic areas for floc and soil, respectively. Mineralized P was the most sensitive indicator of eutrophication, being 689 and 135% higher at nutrient-impacted than oligotrophic areas for floc and soil, respectively. Microbial indicators in WCA-3a and Taylor Slough were more responsive to nutrient loading than in WCA-1 and WCA-2a, which received higher P loads. Delineation of nutrient-impacted and oligotrophic areas in Everglades wetlands may serve as a baseline to assess future impacts of eutrophication. The consistent pattern in the response of microbial processes to nutrient loading across the range of conditions in different Everglades wetlands demonstrates their suitability as sensitive indicators of eutrophication.

Abbreviations: EAA, Everglades Agricultural Area; SOD, soil oxygen demand; WCA, Water Conservation Area.

The Florida Everglades wetlands developed as nutrient-poor and supported vegetation adapted to these conditions (Davis, 1991). In the past century, the Everglades was drained and separated into hydrologic units where water movement and storage were regulated, including the Everglades Agricultural Area (EAA), WCA-1, WCA-2a, WCA-3a, and Taylor Slough of the Everglades National Park. The compartmentalization of the Everglades into distinct hydrologic units altered the traditional water flow through the landscape, modifying the hydroperiod and increasing nutrient loading (Childers et al., 2003). Nutrient runoff from agricultural soils in the EAA and altered hydrologic conditions have altered Everglades wetlands by increasing soil nutrient levels, particularly P, which promoted shifts in vegetation patterns (Davis, 1991; Childers et al., 2003). The ecosystem change most evident is the replacement of *Cladium*-slough communities by *Typha* in northern Everglades wetlands (Craft and Richardson, 1993b; Miao and Sklar, 1998; Childers et al., 2003).

The impacts of anthropogenic nutrient loading to the Everglades is well documented in the distribution of floodwater and soil total P from nutrient-impacted peripheral areas of wetlands extending into the oligotrophic interior (Craft and Richardson, 1993a,b; DeBusk et al., 1994; Newman et al., 1997; Reddy et al., 1998). Water flow through the Everglades often proceeds through the water conservation areas and Taylor Slough and eventually into Florida Bay. Thus, WCA-1, WCA-2a, WCA-3a, and Taylor Slough have been exposed to different levels of nutrient enrichment (Childers et al., 2003). The exposure of wetlands across the Everglades landscape to variable nutrient loading provided an opportunity to assess the response of soil and microbial properties. Soils of different wetlands exhibit variable responses to nutrient loading, thus measurements of microbial activity may be used as indicators of a change in trophic status or environmental conditions (McCormick et al., 1996; Corstanje et al., 2007). Microbial properties have responded to nutrient loading in specific Everglades wetlands (Amador and Jones, 1993; Wright and Reddy, 2001b; Corstanje et al., 2007) but may also be dependent on soil chemical properties and vegetation patterns (Newman et al., 2001; Wright and Reddy, 2001a).

An understanding of the impacts of nutrient loading on biogeochemical processes is important because organic matter decomposition and nutrient cycling depend on the chemical and physical composition of soil, microbial activity, and nutrient availability. The addition of limiting nutrients to ecosystems enhances the productivity of vegetation and stimulates microbial processes. Soil microbial properties may be more sensitive or respond more readily to increased nutrient levels than vegetation, however, as the response of vegetation to nutrient loading is slower than that of biogeochemical indicators (McCormick and O'Dell, 1996; Noe et al., 2002). The objectives of this study were therefore to assess the impacts of long-term nutrient loading on changes in microbial

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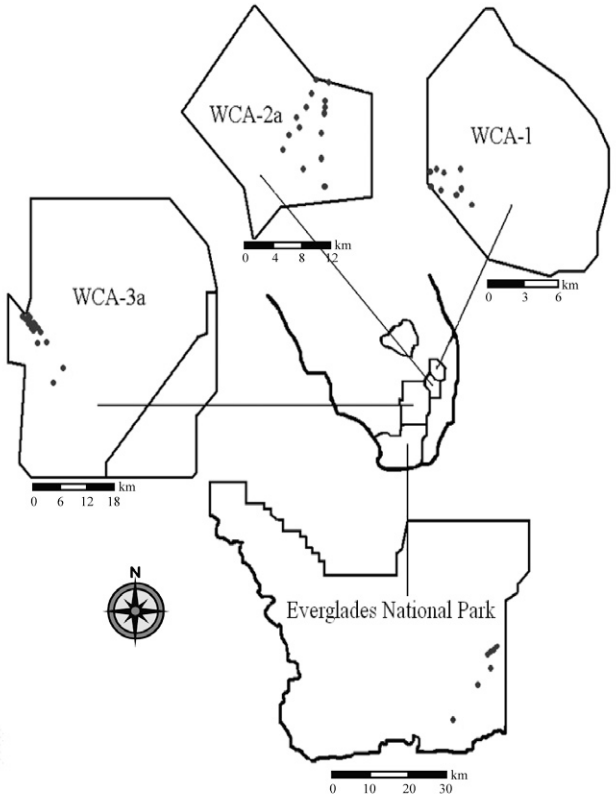

Fig. 1. The location of wetlands in the Florida Everglades and sampling sites in Water Conservation Area (WCA)-1, WCA-2a, WCA-3a, and Taylor Slough.

processes and to identify sensitive microbial indicators of eutrophication for diverse wetlands across the Everglades landscape.

MATERIALS AND METHODS

Site Description

Floc and soil (0–3 cm) samples were collected along nutrient enrichment gradients located in four wetlands of the Florida Everglades: WCA-1, WCA-2a, WCA-3a, and Taylor Slough of the Everglades National Park (Fig. 1). The location of sampling sites within wetlands is presented in Table 1. Water Conservation Area 1 encompasses 59,000 ha of the northern Everglades. Rainfall is a major water input, and areas adjacent to water-inflow points have elevated soil total P levels. *Typha* comprises the vegetation in nutrient-impacted areas while *Cladium*, open sloughs, and tree islands are common in oligotrophic, interior areas. Two transects, encompassing a range of soil total P concentrations, were sampled in the southwestern section of WCA-1 from the S-6 water pump station extending into the oligotrophic interior.

Water Conservation Area 2a (54,700 ha) receives drainage water from WCA-1 in addition to discharge water from the EAA. The nutrient-impacted areas of WCA-2a are much broader and extend much farther into the interior compared with WCA-1, thus nutrient impacts on vegetation community structure are more evident in WCA-2a (Miao and Sklar, 1998). Samples were taken along transects extending from the S10-C water inflow structure to the interior of the wetland, encompassing *Typha*-dominated areas near the inflow and *Cladium* and periphyton communities in the interior.

Water Conservation Area 3a (233,000 ha) receives drainage water from northern wetlands, particularly WCA-2a and the Big Cypress National Preserve through the L-28 gap, but the hydrology is primarily rainfall driven. Tree islands and *Cladium* prairies comprise the vegetation and are interspersed with sloughs, as this wetland is characterized by a ridge and slough landscape oriented in the direction of water flow. Sampling sites were selected up to 15.5 km from the L-28 gap and encompassed a wide range of soil total P concentrations.

Taylor Slough (40,900 ha), a broad shallow basin located in Everglades National Park, serves as a conduit from northern uplands to Florida Bay and receives water from WCA-3a through the L31W and C-111 canals. Vegetation includes *Cladium* marshes in northern areas and *Rhizophora* in southern areas near the coast. Sampling sites were selected along two gradients encompassing a range of soil total P from the C-111 canal to the interior of the slough.

Soil Sampling and Analysis

Triplicate soil samples were collected at each site using a 10-cm-diam. corer. The top 0 to 3 cm was collected along with the overlying floc, which consisted of suspended sediments and periphyton above the soil surface. All samples were stored at 4°C until analysis. Water Conservation Area 1 was sampled in October 1998, WCA-2a in September 1998, WCA-3a in November 2000, and Taylor Slough in December 2000.

Total P was determined using the ashing method (Anderson, 1976) followed by colorimetric analysis (Kuo, 1996). Microbial biomass C was determined by fumigation-extraction with an efficiency factor of 0.37 (Vance et al., 1987; Sparling et al., 1990). Microbial biomass N was determined by fumigation-extraction (Brookes et al., 1985) using an efficiency factor of 0.54. Microbial biomass P was calculated as the difference between the total P of 0.5 mol L^{-1} $NaHCO_3$ extracts of chloroform-fumigated and unfumigated samples (Ivanoff et al., 1998). Mineralized N and P were measured for 5 g of soil incubated for 10 d under N_2, followed by extraction with 0.5 mol L^{-1} K_2SO_4 (for N) and 1.0 mol L^{-1} HCl (for P) and subtraction of initial N and P concentrations (Corstanje et al., 2007).

Anaerobic CO_2 production was measured by incubating 5 g of soil under N_2 in 120-mL glass bottles with vials containing 3 mL of 0.5 mol L^{-1} NaOH at 30°C. Vials containing NaOH were removed and capped at 2-d intervals until 10 d. For

Table 1. Sampling sites in Water Conservation Area (WCA)-1, WCA-2a, WCA-3a, and Taylor Slough with distance from the primary water-inflow point for each wetland.

WCA-1 (*n* = 27)		WCA-2a (*n* = 39)		WCA-3a (*n* = 36)		Taylor Slough (*n* = 36)	
Site	Distance	Site	Distance	Site	Distance	Site	Distance
	km		km		km		km
X1	0.5	E1	2.3	E-05	0.6	E-05	0.3
X2	1.3	E2	3.3	E-10	1.5	E-1	1.0
X3	2.2	E3	4.2	E-15	2.4	E-15	1.7
X4	4.4	E4	7.0	E-20	3.0	W-05	0.4
Y4	3.2	E5	10.1	E-40	6.3	W-1	1.1
Z1	0.3	F1	1.8	W-05	0.7	W-15	1.7
Z2	1.1	F2	3.8	W-10	1.2	2–3	2.3
Z3	2.2	F3	5.6	W-15	2.2	2–4	2.5
Z4	3.1	F4	6.8	W-20	2.9	3–3	6.5
		F5	8.2	W-40	5.8	3–4	6.5
		U1	14.5	N-meso	12.9	N-meso	12.0
		U2	12.6	S-meso	15.5	S-meso	24.1
		U3	10.8				

analysis, 1.0 mL of 3 mol L^{-1} HCl was added to enclosed vials and the resulting headspace CO_2 quantified by gas chromatography (Shimadzu GC-8A, thermal conductivity detector at 25°C, Porapak N column at 20°C). Anaerobic CO_2 production rates were calculated as the slope of the regression of cumulative CO_2 production during the 10-d period. Aerobic CO_2 production was measured using the same method but with incubation under 21% O_2. Carbon dioxide production was linear during the 10-d incubation period.

Methane production rates were measured by incubating 5 g of soil in 60-mL glass serum bottles under N_2 at 30°C for 10 d. Aliquots of headspace were analyzed every 2 d for CH_4 using a Shimadzu GC-8A with a flame ionization detector (160°C) and a Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 110°C. Methane production rates were calculated as the slope of the linear regression of cumulative CH_4 production during 10 d.

Soil O_2 demand (SOD) was determined by measuring dissolved O_2 depletion in soil at 20°C. Approximately 5 g of soil was added to 60-mL bottles followed by addition of O_2–saturated water to volume. Dissolved O_2 contents of slurries were measured using a YSI Model 58 O_2 meter (Yellow Springs Instrument Co., Yellow Springs, OH). Soil O_2 demand was calculated as the difference in dissolved O_2 between the initial O_2–saturated slurry and concentrations after a 1-d incubation.

Sampling sites along nutrient gradients in each wetland were grouped for statistical comparisons into nutrient-impacted and oligotrophic areas. Delineation between areas designated as nutrient-impacted and oligotrophic within the respective wetlands were based on significant differences in floc total P concentrations as a function of distance from primary water-inflow points using a one-way ANOVA model at $P < 0.05$. Interior areas of wetlands typically exhibit the lowest total P concentrations relative to peripheral areas near water-inflow points, and as such are often deemed oligotrophic (DeBusk et al., 1994; Childers et al., 2003; White and Reddy, 2003; Wright and Reddy, 2008). Thus, sites showing significantly higher floc total P concentrations than oligotrophic interior areas were impacted by nutrients. For the wetlands in this study, abrupt and statistically significant changes in floc total P for sites along gradients were evident, which facilitated delineation of eutrophic and oligotrophic areas. A three-way ANOVA model was used to determine the main effects of wetland, site (nutrient-impacted and oligotrophic), and soil depth (floc and 0–3-cm soil). Treatment comparisons were based on Fisher's LSD at $P < 0.05$ (CoHort v. 6.2, CoStat Statistics Software, Monterey, CA). Relationships between various biogeochemical indicators were investigated using Pearson's correlation coefficients at $P < 0.05$.

RESULTS AND DISCUSSION

Floc and Soil Total Phosphorus

Samples taken along transects encompassed a wide range of total P concentrations, which decreased from nutrient-impacted areas in the wetland periphery to oligotrophic areas in the interior (Fig. 1). The nutrient gradients generally corresponded to patterns of water flow through the wetlands, such that nutrient impacts were most evident near water-inflow points and decreased with increasing distance from the inflow. Sampling sites were statistically differentiated, based on floc total P concentrations, into nutrient-impacted and oligotrophic areas. The decreases in floc and soil total P with increasing distance from major water-inflow points for the four wetlands are shown in Fig. 2. Even though current soil P concentrations and loadings may differ from the levels measured in this study, the relationships between soil chemical properties and microbial indicators should be consistent.

The delineation between nutrient-impacted and oligotrophic areas was abrupt for all wetlands except for Taylor Slough (Fig. 2). The impacts of nutrient loading in WCA-1 extended up to 0.5 km from the water-inflow structure. Sites farther than 0.5 km from the inflow point did not differ in total P, but all had significantly lower levels than sites <0.5 km. The effects of nutrient loading extended much farther into the interior of WCA-2a, with nutrient-impacted sites up to 7 km from the S10-C water-inflow structure. The nutrient-impacted areas of WCA-3a extended 3 km from the L-28 gap. Due to lower total P levels and less exposure to P loads, Taylor Slough exhibited greater spatial variability, making the delineation of the nutrient-impacted and oligotrophic areas somewhat more difficult. Taylor Slough floc total P was significantly highest at sites from 0.4 to 12 km of the C-111 canal. The site 24 km from the inflow had the lowest total P along the gradient and thus was designated as the oligotrophic, reference area. Osborne et al. (2005) demonstrated that the most interior areas of Taylor Slough farthest from water-inflow points indeed exhibited low P levels similar to those observed in our study.

Background total P levels in oligotrophic areas varied considerably among the wetlands. Floc total P ranged from 205 to 619 mg P kg^{-1} and soil total P from 200 to 553 mg P kg^{-1} (Tables 2 and 3). Total P averaged 185 and 140% greater for nutrient-impacted than oligotrophic areas for floc and soil, respectively. For nutrient-impacted floc, WCA-1 and WCA-3a had higher total P

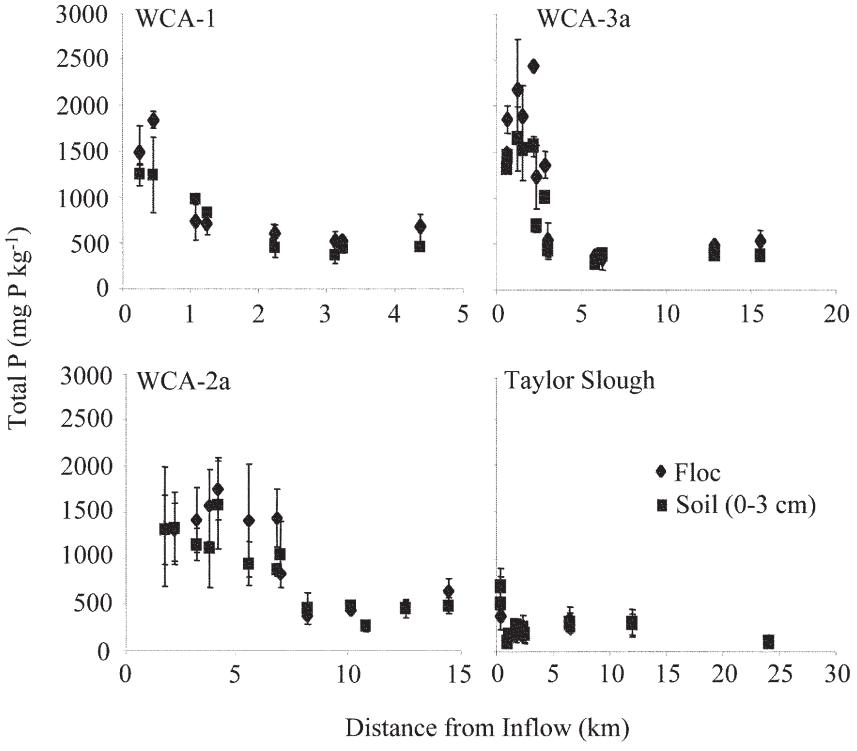

Fig. 2. Floc and soil total P concentrations in the water conservation areas (WCAs) and Taylor Slough as a function of distance from primary water-inflow points.

Table 2. Properties of floc and soil from nutrient-impacted and oligotrophic areas of Water Conservation Area (WCA)-1 and WCA-2a.

Parameter	WCA-1		WCA-2a	
	Impacted	Oligotrophic	Impacted	Oligotrophic
	Floc			
Total P, mg P kg^{-1}	1670	619*	1410	497 *
Microbial biomass C, g C kg^{-1}	27	49*	14	23 NS†
Microbial biomass N, mg N kg^{-1}	4700	6240 NS	1830	2600 NS
Microbial biomass P, mg P kg^{-1}	662	401 NS	331	160*
Soil O_2 demand, mg kg^{-1} h^{-1}	145	82*	124	159 NS
Aerobic CO_2 production, mg C kg^{-1} h^{-1}	51	51NS	13	15 NS
Anaerobic CO_2 production, mg C kg^{-1} h^{-1}	65	152*	16	16 NS
N mineralized at 10 d, mg N kg^{-1} d^{-1}	346	378 NS	78	92 NS
P mineralized at 10 d, mg P kg^{-1} d^{-1}	45	11*	14	6*
	Soil (0–3 cm)			
Total P, mg P kg^{-1}	1220	553*	1140	519*
Microbial biomass C, g C kg^{-1}	14	10 NS	6	8 NS
Microbial biomass N, mg N kg^{-1}	2030	1320 NS	824	776 NS
Microbial biomass P, mg P kg^{-1}	237	225 NS	146	140 NS
Soil O_2 demand, mg kg^{-1} h^{-1}	39	53 NS	56	43 NS
Aerobic CO_2 production, mg C kg^{-1} h^{-1}	60	26 NS	28	21 NS
Anaerobic CO_2 production, mg C kg^{-1} h^{-1}	27	18 NS	32	18*
N mineralized at 10 d, mg N kg^{-1} d^{-1}	158	122 NS	65	49 NS
P mineralized at 10 d, mg P kg^{-1} d^{-1}	24	15 NS	10	6*

* Significant difference between sites at $P < 0.05$,
† NS = not significant.

than WCA-2a and Taylor Slough. At 0 to 3 cm, Taylor Slough had the lowest total P of all nutrient-impacted areas. Taylor Slough soils were predominantly marl, while soils of the other wetlands were dominated by peat. Hydrologic conditions and water levels fluctuate considerably in Taylor Slough, which may influence nutrient loads from surface waters (Sutula et al., 2001). Possible low-level nutrient loading may have occurred in Taylor Slough because this wetland is a conduit for water flow from the northern water conservation areas into Florida Bay; however, atmospheric P deposition was reportedly greater than P loading from surface water inflow in this wetland (Sutula et al., 2001). Since Taylor Slough did not directly receive water from the EAA, but rather water that was previously filtered through the water conservation areas, it historically received lower nutrient loading and thus exhibited lower soil P levels than the water conservation areas.

The response of biogeochemical properties to nutrient loading was typically greater for floc than for the underlying soil. Floc consisted of suspended sediments of algae, periphyton, or particulate organic matter present on the soil surface or suspended in the water column (McCormick et al., 1996). Soil at nutrient-impacted sites represented accumulated organic matter derived from decomposed periphyton and plant residues exposed to nutrient loading, thus soil P often approximated levels of floc P. Total P was higher for floc than soil for nutrient-impacted areas of the four wetlands but not for the oligotrophic areas. Periphyton and floc often exhibit greater sensitivity to changes in environmental conditions than the underlying soil (Craft and Richardson, 1993b; McCormick et al., 1996; Wright and Reddy, 2001a), and the response of microbial properties to P loading is often greater in surface detrital material than the underlying soil (Newman et al., 2001).

Microbial Biomass

Microbial biomass C exhibited a mixed response to P loading, tending to be higher in oligotrophic than nutrient-impacted floc and soil (Tables 2 and 3). Water Conservation Area 3 and Taylor Slough soils had higher biomass C for nutrient-impacted than oligotrophic areas. Microbial biomass is often not directly related to P loading in wetlands but rather related to a complex assortment of soil properties (Corstanje et al., 2007). Microbial biomass C was significantly higher for floc than soil for all wetlands. Differences in microbial biomass C between depths depended on nutrient status, as biomass C was 189% higher for floc than soil at nutrient-impacted areas but 489% higher for floc than soil at oligotrophic areas. Nutrient loading probably enhanced the biomass C of nutrient-impacted soils relative to oligotrophic soils. Since the floc layer was exposed to greater nutrient loads than the underlying soil, floc at oligotrophic sites often had lower P levels and microbial biomass relative to floc at nutrient-impacted sites. Biomass C was highest for WCA-1 and WCA-3a and lowest for WCA-2a and Taylor Slough.

Table 3. Properties of floc and soil from nutrient-impacted and oligotrophic areas of Water Conservation Area 3a (WCA-3a) and Taylor Slough. Significant differences between sites were noted by * ($P < 0.05$) and NS.

Parameter	WCA-3a		Taylor Slough	
	Impacted	Oligotrophic	Impacted	Oligotrophic
	Floc			
Total P, mg P kg^{-1}	1720	452*	258	205*
Microbial biomass C, g C kg^{-1}	45	72*	15	21 NS†
Microbial biomass N, mg N kg^{-1}	4180	3330 NS	1060	1050 NS
Microbial biomass P, mg P kg^{-1}	508	169*	69	68 NS
Soil O_2 demand, mg kg^{-1} h^{-1}	181	195 NS	32	23*
CH_4 production, mg C kg^{-1} d^{-1}	710	454*	133	216 NS
N mineralized at 10 d, mg N kg^{-1} d^{-1}	383	257*	122	101 NS
P mineralized at 10 d, mg P kg^{-1} d^{-1}	144	8*	10	2*
	Soil (0–3 cm)			
Total P, mg P kg^{-1}	1280	371*	306	200*
Microbial biomass C, g C kg^{-1}	8	5*	7	5*
Microbial biomass N, mg N kg^{-1}	1140	326*	460	298*
Microbial biomass P, mg P kg^{-1}	277	58*	61	52 NS
Soil O_2 demand, mg kg^{-1} h^{-1}	98	84 NS	43	22*
CH_4 production, mg C kg^{-1} d^{-1}	258	165*	158	57*
N mineralized at 10 d, mg N kg^{-1} d^{-1}	75	27*	26	10*
P mineralized at 10 d, mg P kg^{-1} d^{-1}	24	4*	3	1*

* Significant difference between sites at $P < 0.05$.
†NS = not significant.

The response of microbial biomass N to nutrient loading was similar to biomass C. The only significant difference between nutrient-impacted and oligotrophic soils occurred for WCA-3a and Taylor Slough. Microbial biomass N was highest in WCA-1 and WCA-3a and lowest in WCA-2a and Taylor Slough. Moreover, the two wetlands that had the least extensive nutrient-impacted area, WCA-1 (0.5 km) and WCA-3a (3 km), had the highest biomass C and N while WCA-2a (7 km) and Taylor Slough (12 km) had the lowest. Overall, microbial biomass C and N were not responsive to P loading and not sensitive indicators of eutrophication. Since extractable organic C levels in Everglades wetlands are generally high (Corstanje et al., 2007), N limitations to microbial activity may have developed in nutrient-impacted areas. In fact, N limitations to organic matter decomposition were observed in nutrient-impacted areas of WCA-2a (White and Reddy, 2003). Sites within short distances of the inflow in WCA-1 and WCA-3a may have been exposed to higher N loading, which increased microbial biomass C and N relative to other wetlands. Microbial biomass C was significantly correlated with biomass N and P, but biomass C and N were seldom related to total P (Table 4).

Table 4. Significant correlation coefficients ($P < 0.05$) between total P and microbial indicators for floc and soil in Water Conservation Area (WCA)-1, WCA-2a, WCA-3a, and Taylor Slough ($n = 138$ for each depth).

Indicator†	Total P	MBC	MBN	MBP	SOD	Aer. CO_2	Anaer. CO_2	CH_4	PMN
	Floc								
MBC	NS‡								
MBN	NS	0.64							
MBP	0.75	0.37	0.61						
SOD	0.43	0.64	0.58	0.36					
Aer. CO_2	NS	0.63	0.66	NS	0.62				
Anaer. CO_2	0.50	0.48	0.57	NS	NS	NS			
CH_4	0.66	NS	0.63	0.60	0.58	NS	NS		
PMN	NS	0.66	0.74	0.64	0.53	0.51	0.81	0.59	
PMP	0.59	NS	NS	0.64	NS	NS	NS	0.41	0.51
	Soil (0–3 cm)								
MBC	NS								
MBN	0.49	0.82							
MBP	0.58	0.41	0.61						
SOD	0.51	NS	NS	NS					
Aer. CO_2	NS	0.45	0.41	NS	NS				
Anaer. CO_2	0.39	0.65	0.54	NS	0.93	0.81			
CH_4	0.56	NS	0.61	0.54	0.43	NS	NS		
PMN	NS	0.71	0.80	0.41	0.53	0.39	0.65	0.81	
PMP	0.47	0.46	0.53	0.76	0.47	0.53	0.74	0.50	0.56

† MBC, MBN, MBP = microbial biomass C, N, and P, respectively; SOD = soil O_2 demand; Aer. CO_2 = aerobic CO_2 production; Anaer. CO_2 = anaerobic CO2 production; PMN, PMP = potentially mineralizable N and P, respectively

‡ NS = not significant.

Microbial biomass P was somewhat more responsive to nutrient loading than biomass C and N, being higher for nutrient-impacted than oligotrophic areas for WCA-2a floc and WCA-3a floc and soil. Whereas biomass C and N averaged 63 and 12% higher, respectively, in oligotrophic than nutrient-impacted areas, biomass P was 97% higher in nutrient-impacted areas. In another study, microbial biomass P responded directly to P loading to oligotrophic soils in Everglades wetlands (Qualls and Richardson, 2000). Microbial biomass P decreased with depth for all wetlands except Taylor Slough, which was the wetland with the lowest biomass P and lowest total P levels. Similar to microbial biomass C and N, wetlands with the smallest nutrient-impacted geographic area had the highest biomass P. Patterns of microbial biomass P were similar to total P (Table 4).

Heterotrophic Microbial Activity

Measurements of heterotrophic microbial activity included SOD, CO_2, and CH_4 production. Whereas nutrient loading effects on microbial biomass were primarily observed in wetlands with the least nutrient-impacted areas (WCA-1 and WCA-3a), significant differences in heterotrophic microbial activity between nutrient-impacted and oligotrophic areas were most readily observed in WCA-3a and Taylor Slough (Tables 2 and 3). Other studies showed that heterotrophic microbial activity is often enhanced by P loading (Craft and Richardson, 1993b; Amador and Jones, 1997; Wright and Reddy, 2008).

Soil O_2 demand was generally greater in nutrient-impacted than oligotrophic areas, as nutrient loading to historically nutrient-poor ecosystems enhances heterotrophic microbial activity. High C/P ratios at oligotrophic sites may limit organic matter decomposition (Amador and Jones, 1997), but external P loading removed this limitation to heterotrophic microorganisms at nutrient-impacted sites and probably induced changes in the quantity and quality of vegetation and periphyton substrates (McCormick et al., 1996; DeBusk and Reddy, 1998; Wright and Reddy, 2008), which explains lower SOD in oligotrophic areas. Soil O_2 demand was highest in WCA-3a and lowest in Taylor Slough, and significantly higher in floc than soil for all wetlands except Taylor Slough. Organic matter decomposition in these wetlands is dominated by anaerobic pathways such as denitrification, SO_4 reduction, and methanogenesis (White and Reddy, 2003; Wright and Reddy, 2001a). Soils in Everglades wetlands are flooded for most of the year but often experience dry periods when the soil surface is exposed to the atmosphere (Childers et al., 2003), which supports aerobic decomposition. Aerobic CO_2 production rates were not related to soil P concentrations and did not differ between soil depths but were correlated with microbial biomass (Table 4). Rates were higher in WCA-1 than WCA-2a, however, for both floc and soil.

Anaerobic CO_2 production rates showed a mixed response to nutrient enrichment and were higher in WCA-1 than WCA-2a for floc but not soil, and decreased with depth in WCA-1 but not WCA-2. Anaerobic CO_2 production was significantly related to microbial biomass C and total P. Methane production was higher for WCA-3a than Taylor Slough for all depths, and decreased with depth for nutrient-impacted and oligotrophic areas of WCA-3a and the oligotrophic area of Taylor Slough. Of all measurements

of heterotrophic microbial activity, CH_4 production was the most sensitive to nutrient enrichment, being higher for nutrient-impacted than oligotrophic floc and soil from WCA-3a and soil from Taylor Slough. Methane production was significantly correlated with total P and microbial biomass N and P (Table 4).

Mineralized Nitrogen and Phosphorus

Mineralized N was higher for nutrient-impacted than oligotrophic floc and soil for WCA-3a and Taylor Slough (Tables 2 and 3). For both depths, mineralized N was highest for WCA-1 and WCA-3a, and rates were three and four times higher for floc than soil for nutrient-impacted and oligotrophic areas, respectively. Mineralized N was significantly related to all microbial parameters but not to total P (Table 4).

The most responsive microbial indicator to nutrient enrichment was mineralizable P. Overall, mineralized P was 689% greater for nutrient-impacted than oligotrophic floc, and 135% greater for nutrient-impacted than oligotrophic soil. The difference between nutrient-impacted and oligotrophic areas in wetlands was greater for mineralized P than other microbial indicators, suggesting that mineralized P was the most suitable microbial indicator of nutrient loading. A similar result was observed in WCA-2a, where patterns of potentially mineralizable P but not mineralizable N reflected the soil P status (Corstanje et al., 2007). Mineralized P of nutrient-impacted floc was the highest in WCA-3a, followed by WCA-1, then WCA-2a and Taylor Slough. No differences in mineralized P for oligotrophic floc occurred between wetlands, however, except for Taylor Slough having the lowest rate. Mineralized P was significantly higher for nutrient-impacted floc than soil, but for oligotrophic sites there were no differences between depths for any wetland, presumably due to the lack of significant differences in total P between depths. Mineralized P was significantly related with total P and microbial biomass P (Table 4).

Microbial Indicators of Eutrophication

Phosphorus-related parameters were the most sensitive microbial indicators of nutrient enrichment. Microbial C and N indicators were more related to organic matter cycling and decomposition than to nutrient loading. In WCA-2a, biogeochemical properties were related to soil C and N cycles and were not as sensitive to nutrient loading as P-related indicators (Corstanje et al., 2007). Heterotrophic microbial activity, including SOD and CO_2 production, were more related to microbial biomass and extractable nutrient concentrations than nutrient loading (Corstanje et al., 2007; Wright and Reddy, 2001a), hence their generally poor response to nutrient loading.

Everglades wetlands developed under P-limited conditions, thus the addition of P to the wetlands significantly altered biogeochemical processes and vegetation patterns. The northern wetlands received higher P loading than the southern wetlands such as Taylor Slough (Childers et al., 2003). Consequently, microbial indicators were significantly enhanced by P loading in the floc and soil of Taylor Slough. Therefore, P loading into the initially low-P soils of Taylor Slough provoked a more significant response in microbial indicators such that the impacts of nutrient loading on microbial indicators appeared dependent at least in part on the background P level of the wetlands. Because of its calcareous nature, the soil in Taylor Slough may have coprecipitated or adsorbed P to $CaCO_3$, rendering it unavailable to microorganisms. Thus, due to the probable P limitation, external P loading would have a dramatic effect on the enhancement of microbial activity and lead to a greater response per unit of P loading than wetlands having higher background P levels.

The limited geographic extent of nutrient enrichment in WCA-1 and WCA-3a may be hydrologically driven, as low surface-water inputs coupled with slow water flow rates may have increased loads in the peripheral areas of these wetlands, which led to elevated nutrient levels relative to the oligotrophic interior. High primary productivity in nutrient-impacted areas of the wetlands resulted in high organic matter accumulation rates (Miao and Sklar, 1998), which supported high levels of microbial activity. Likewise, accumulation of nutrients other than P, such as N in nutrient-impacted areas (DeBusk et al., 1994; Bruland et al., 2006), may have stimulated microbial biomass, heterotrophic microbial activity, and organic matter decomposition to a greater extent for WCA-1 and WCA-3a than WCA-2a and Taylor Slough. The extent of nutrient loading into these two former wetlands was less than WCA-2a and Taylor Slough (Fig. 2), indicating potentially greater N accumulation in P-impacted areas closest to water-inflow points. These factors may explain the higher levels of microbial biomass and heterotrophic activity for WCA-1 and WCA-3a than WCA-2a and Taylor Slough. Nutrient-impacted areas of WCA-1 and WCA-3a were also more frequently inundated (David, 1996) than the other wetlands, which further enhanced organic matter accumulation rates in addition to altering vegetation patterns and microbial processes (Reddy et al., 1993; Childers et al., 2003).

The floc consisted of benthic periphyton while the soil consisted primarily of consolidated and decomposed plant matter. Surface floc sediments were more sensitive to P loading than the underlying 0- to 3-cm soil (Tables 2 and 3), as the higher substrate quality of floc enhanced microbial biomass and heterotrophic microbial activity compared with the underlying soil (DeBusk and Reddy, 1998). Most microbial indicators including microbial biomass and heterotrophic microbial activity were the highest in floc and decreased to the 0- to 3-cm soil depth. Similar decreases in microbial activity with depth were reported in Everglades soils (Newman et al., 2001; Wright and Reddy, 2001b). Thus, the response of various biogeochemical indicators to nutrient loading varied with soil depth.

CONCLUSIONS

Phosphorus loading led to the development of distinct gradients in floc and soil total P from sites of water inflow extending into the interior of Everglades wetlands. The geographic extent of nutrient loading differed among wetlands, as microbial indicators exhibited a greater response to nutrient enrichment in southern wetlands, such as Taylor Slough, receiving low P loading. The most sensitive indicators of eutrophication were floc and soil total P, microbial biomass P, and mineralized P. Other microbial indicators were probably related to soil chemical properties and the indirect effects of nutrient loading on vegetation patterns and organic matter cycling. This study illustrated the effectiveness of using microbial indicators in assessing the response of ecosystems to changing soil and environmental conditions for four distinct wetlands in the Everglades landscape. Due to the diversity of the wetlands tested and their varied exposure to historical P loading,

the reaction of microbial indicators to changes in nutrient enrichment may be used to assess potential future effects of P loading for these four wetlands having variable soil P content.

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