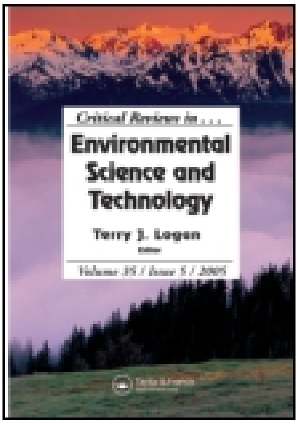

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Microbial Ecology and Everglades Restoration

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Microbial Ecology and Everglades Restoration

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Much of the activity proposed for Everglades restoration is associated with processes either controlled by or impacting microbial activities. The authors summarize some recent studies related to restoration objectives conducted in a range of Everglades environments, including marsh and tree island soils, and periphyton assemblages. These studies include research related to the development of restoration performance measures based on nutrient status, analysis of controls on organic matter decomposition that may have lead to the development of soil microtopography responsible for water flow paths, microbial drivers of methane production, and analysis of the architecture of periphyton mats and their potential use in nutrient removal treatment strategies. The authors highlight the complexity inherent in microbial control of biogeochemistry, as well as the multitude of approaches that are needed to explain these interactions. Compared to larger ecosystem attributes such as vegetation community structure, the structure and function of microbial communities have remained elusive, and significantly more research into this area is essential to ensure that restoration goals are accomplished.

KEYWORDS: Everglades restoration, wetlands microbial ecology

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The Everglades is composed of a complex landscape of ridges and sloughs, marshes and tree islands. Each environmental compartment harbors unique microbial communities that are integral to the development and functioning of the greater Everglades ecosystem, and should be considered when devising strategies for restoration. Much of the present and proposed efforts toward restoring the Everglades are focused on water quality and its impacts on an array of ecosystem characteristics ranging from the accumulation of peat and internal nutrient cycling to mercury toxicity. Central to these issues are the specific roles played by microbial communities in the decomposition of organic matter coupled with the biogeochemical cycling of phosphorus, sulfur, and mercury, and the complex interactions between diverse functional groups of microorganisms, and the environmental impacts on the structures and activities of microbial communities. Characterization of the compositions and activities of microbial communities in the Everglades provides important information regarding fundamental processes impacted by anthropogenic activities, as well as information required for the development of restoration strategies and evaluation of progress toward restoration.

A variety of approaches are presently being applied to the analysis of microbial communities in the Everglades, each of which provides an important perspective on the present state of the Everglades and of the impacts, both direct and indirect, of phosphorus (P) enrichment. Phosphorus is the main factor limiting primary productivity in the Everglades, such that P enrichment in the northern Everglades resulting from runoff from the Everglades Agricultural Area (EAA) yielded dramatic shifts in vegetation type and density, as well as changes in the composition and activities of the resident microbial communities (Castro et al., 2004, 2005; Chauhan and Ogram, 2006a, 2006b).

This review highlights recent studies conducted throughout the greater Everglades ecosystem, most of which focus on the impacts of P enrichment on microbial community structure and activity, either directly, as in the case of phosphatase enzyme activities, or indirectly, as in the response of communities to increased carbon input resulting from P enrichment. Most of these studies are based in the P-gradient observed in Water Conservation Area 2A (WCA-2A); runoff of P into areas adjacent to the EAA into WCA-2A produced a gradient in P-concentrations in soil and water, providing a very convenient system for studying the response of the Everglades to P enrichment and to restoration.

CONTROLS ON ORGANIC MATTER ACCUMULATION AND DECOMPOSITION

Of particular interest to restoration efforts is characterization of the factors leading to the historic ridge and slough landscape and the impacts of nutrient enrichment on the sustainability of this landscape. Vegetation community composition and productivity, along with the rates of plant matter decomposition, influence peat accumulation, which in turn alters the topography of the Everglades. Characterization of the factors controlling plant growth and mechanisms leading to the accumulation and decomposition of organic matter in different regions of the Everglades is therefore essential.

One of the key mechanisms most organisms employ to obtain P under low P conditions, such as those characteristic of much of the Everglades, is to access P in organic carbon pools via production of phosphatase enzymes (Cembella et al., 1983). Both constitutive and inducible phosphatases are present, and phosphatase activity (PA) is strongly inversely related to P concentrations. Several studies in the Everglades have demonstrated that PA is reduced in response to elevated P concentrations in the water, periphyton (Newman et al., 2003), soil (Penton and Newman, 2007; Wright and Reddy, 2001), and plants (Kuhn et al., 2002). For example, Wright and Reddy (2001) observed a significant increase in PA in WCA-2A at stations 7–8 km from inflow from the EAA (Figure 1). This zone is considered to be the transition point between P-enriched and P-unenriched areas of WCA-2A (Sklar et al., 2005), where biological community shifts due to increasing P concentrations are observed. As confirmation of the link between PA and P concentrations, Newman et al. (2003) demonstrated that within 3 weeks of the addition of high P loads to enclosed in situ mesocosms, periphyton PA was reduced two- to fourfold, from 0.42 and 0.70 nmol cm^{-2} min^{-1} to 0.11 to 0.29 nmol cm^{-2} min^{-1}, suggesting that PA may be used as an early warning indicator of P enrichment.

Enzyme activity is considered a rate limiting step in decomposition. Thus, most studies examining enzymatic controls on decomposition in P-limited areas of the Everglades have emphasized the role of PA (Newman et al., 2003; Penton and Newman, 2007, 2008). While still oligotrophic,

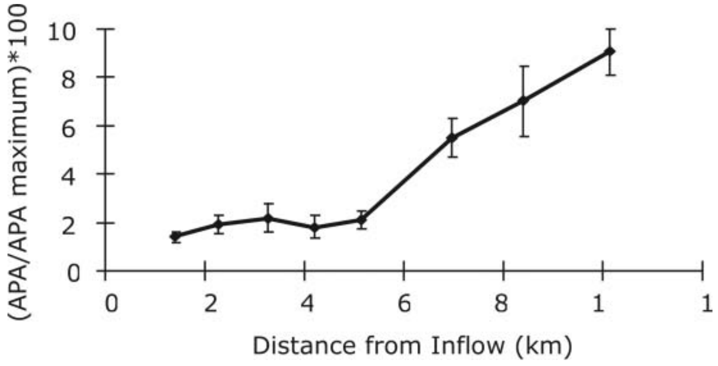

FIGURE 1. Phosphatase activity measured in detritus along the nutrient gradient in WCA-2A. Values expressed as a percent of maximum activity for each of 4 sampling periods. *M* ± 1 *SE*. Adapted from Wright and Reddy (2001).

P concentrations in the northern and central Everglades are generally higher than those in Everglades National Park (ENP; McCormick et al., 2002). Concomitantly, PA in benthic floc and soils in Taylor Slough of the ENP were recently shown to be significantly higher in sites not enriched in P compared to the water conservation areas further north (Penton and Newman, 2007). For example, benthic PA collected from P-unenriched sites averaged 11.2, 16.2, 18.1, and 61.3 μmol methylumbelliferone (MUF) g^{-1} ash free dry mass h^{-1}, for WCA-2A and the adjacent Water Conservation Areas WCA-1A and WCA-3A, and Taylor Slough, respectively (Penton, 2004). Most studies to date on P cycling have focused on the turnover of monoester P; however, a recent NMR study highlighted relatively high levels of diesters in some regions (Turner and Newman, 2005), prompting addition of phosphodiesterase to the enzymes measured to follow P dynamics in the Everglades and similar systems.

Control of organic matter decomposition in Everglades peatlands is key to restoring topography and associated water flow patterns in the anthropogenically impacted regions. Numerous studies have measured decomposition rates of above ground plant material (Chimney and Pietro, 2006; Davis, 1991; DeBusk and Reddy, 2005; Newman et al., 2001; Qualls and Richardson, 2000, 2008; Troxler and Childers, 2008). Further investigation of enzyme activities can be used to identify key processes that are driving decomposition at a more mechanistic level, thus providing a greater understanding of how restoration strategies may influence this process. Enzymatic decomposition is both a function of litter quality as well as nutrient limitation (Güsewell and Freeman, 2005; Rejmánková and Houdková, 2006). Therefore, in an effort to understand organic matter turnover, enzymes that indicate litter quality as well as nutrient status have been evaluated in the Everglades, including enzyme suites associated with the breakdown of cellulose (cellobiohydrase, β-glucosidase), nitrogen availability (aminopeptidase, protease), sulfur cycling (aryl sulfatase), lignin degradation (phenol oxidase, peroxidase) and dehydrogenase, an indicator of total microbial oxidative capacity (Corstanje et al., 2007; Newman et al., 2003; Penton and Newman, 2007, 2008; Wright and Reddy, 2001). Phenol oxidase is an enzyme of particular importance due to its critical role in wetland C storage (Freeman et al., 2001) and appears to be a key driver controlling decomposition of ridge and slough habitats in the Everglades (Penton and Newman, 2008).

Because P limitation is the primary factor limiting decomposition in the Everglades, it is not surprising that most enzyme suites measured in the Everglades showed no distinct inverse relationship with distance from nutrient inputs (i.e., P enrichment gradient), similar to that observed for PA. However, glucosidase activities were twofold higher in enriched and transitional sites in WCA-2A (60 and 59 μg MUF g^{-1} h^{-1}, respectively) than at an unenriched site (30 μg MUF g^{-1} h^{-1}; Corstanje et al., 2007). Enzyme activities are generally highest in the detrital and floc layers at the soil surface and decrease with depth (Penton and Newman, 2007; Wright and Reddy, 2001).

Ratios between C, N, and P acquiring enzymes have been used to highlight the relative importance of different nutrients and the changes in the response of the microbial community (Sinsabaugh and Moorhead, 1994). Individually, relative benthic and soil N and P limitations (suggested by the ratios of cellulose degrading enzyme activities to those related to nitrogen availability, Ec/En, and the ratios of cellulose degrading enzyme activities to phosphatase activities, Ec/Ep, respectively) indicated conditions supportive of decomposition processes in the low-P sawgrass sites (Figure 2; Penton and Newman, 2008). However, relative carbon controls, observed as Ec/Eox (the ratio of cellulose degrading enzyme activities to lignin degrading enzyme activities), predicted lower decomposition rates than the slough. All ratios indicate higher decomposition rates under P-enriched conditions. Thus, the ratio of hydrolytic and oxidative enzymes (Figure 2), in combination with soil chemistry, suggests that Everglades slough habitats have higher quality detritus and higher enzyme activities, which in association with lower C inputs,

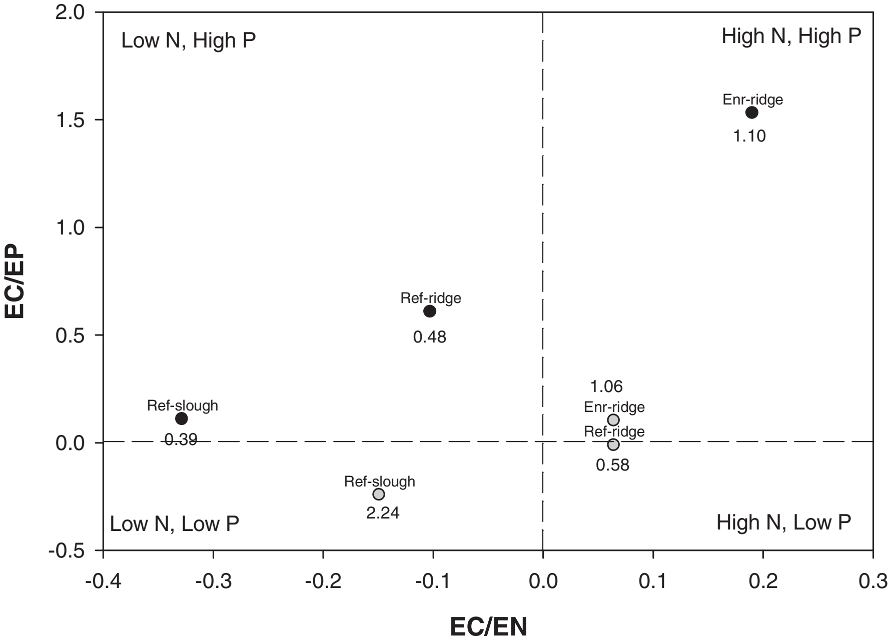

FIGURE 2. Benthic and soil microbial N and P resource allocation in terms of C mineralization at sawgrass ridge and slough habitats. The y axis represents apparent P limitation, and the x axis represents apparent N limitation on C mineralization. Values are log transformed for comparison. Numerical values reflect Ec/Eox, the ratio of cellulose degrading to lignin degrading enzymes, the apparent lignin control on C mineralization. Grey circles = benthic values, black circles = soil values. Labels are nutrient and habitat designations; Enr = enriched, Ref = reference. Adapted from Penton and Newman (2008).

results in increased decomposition potential and therefore less peat accretion than the adjacent sawgrass ridge (Penton and Newman, 2008). In addition, sawgrass peat mineralization was more influenced by lignin than was the slough. This analysis of the activities of selected suites of enzymes therefore provides support for differential decomposition being a controlling factor in the formation and sustainability of the ridge and slough microtopography.

THE MICROBIAL ECOLOGY OF METHANOGENESIS IN THE EVERGLADES

Decomposition of organic matter in the Everglades, as in most wetland soils, is conducted by a web of functional groups of microorganisms composed of species that may respond rapidly to changes in environmental conditions. The composition of guilds, or assemblages, of microorganisms participating in a given function such as fermentation, methanogenesis, and sulfate reduction, is determined by various environmental factors, including quality and quantity of electron donors and acceptors, and concentrations of nutrients. The physiologies of members of the guild, in turn, determine the pathway through which biogeochemical cycles proceed. Changes in the structure of these guilds reflect changes in the environment and may result in changes in the specific function of the guilds. Characterization of the individual microbial groups responsible for these processes will likely aid in the development of sensitive indicators of nutrient status in the Everglades, and thorough characterization of the flow of carbon and electrons through these trophic levels will provide needed insight into the fundamental mechanisms by which eutrophication affected ecosystem processes in the Everglades. Of particular interest are the pathways leading to production of methane, both because of the importance of methane as greenhouse gas and its role as an indicator of ecosystem function.

Linkages between rates of ecosystem productivity and methane emission have been clearly demonstrated across a variety of wetlands (Bellasario et al., 1999; Chanton et al., 1995; Chasar et al., 2000; Whiting and Chanton, 1993), including the Everglades. Increased methanogenesis rates are typically observed in nutrient impacted wetlands, and natural wetlands account for over 20% of global methane produced. CH_4 is 20-fold more potent as a greenhouse gas than CO_2, such that a detailed understanding of the pathways leading to methanogenesis and how eutrophication impacts these pathways will contribute to refinement of models of CH_4 production and emission from the Everglades.

In addition to impacting the quantity of substrates that fuel methanogenesis, nutrient enrichment can influence the quantity of methane produced by affecting the methane production pathways (Hines et al., 2001, 2008). On a global basis, the majority of terrestrial methane is thought to arise directly from acetate via acetotrophic methanogenesis. Stoichiometric conversion of carbohydrates to acetate and H_2 via primary fermentation (fermentation of sugars to fermentation of fatty acids and alcohols) and secondary fermentation (fermentation of fermentation products such as fatty acids and alcohols) can only supply sufficient H_2 to reduce CO_2 to CH_4 (hydrogenotrophic methanogenesis) to account for one third of the total methane produced. Thus, it has generally been observed that acetate fermentation (acetotrophic methanogenesis) accounts for approximately 70% of the methane produced in terrestrial environments (Conrad, 1999). A few notable exceptions to this paradigm have recently been described, including the Everglades, where the majority of methane is produced via the hydrogenotrophic route (Castro et al., 2004, 2005; Chauhan et al., 2004) in both P-enriched and unenriched soils.

Methanogenesis rates are much higher in P-enriched and transition regions than in low-nutrient soils, and sequence analysis of 16S rRNA gene clone libraries constructed from samples taken from these regions revealed differences in composition and activities of methanogenic assemblages. Methanogens from P-enriched and transition regions were almost exclusively composed of hydrogenotrophic methanogens, with approximately a 10,000-fold greater population of most probable numbers of hydrogenotrophs than of acetotrophs (Castro et al., 2004, 2005; Chauhan et al., 2004). Significantly, principal components analyses (PCA) of the diversity of a gene characteristic of methanogens, *mcrA,* taken monthly over 1 year indicate significantly different species distributions of methanogens in each of the three soils (Figure 3; Castro et al., 2005). The dominant methanogens in all soils are hydrogenotrophs, and the individual phylotypes of methanogens were strongly selected by nutrient status of the soil. It is likely that methanogen species were selected in the different soils by differences in H_2 concentrations. This selection strongly suggests that a rapid and sensitive indicator of nutrient status based on rapid characterization of methanogenic species may be developed. In addition, these data strongly suggest that H_2, not acetate, is the dominant electron donor in these soils, which would impact a range of microbial processes, including sulfate reduction. The sources of H_2 for methane are not completely known at this time, although much of the H_2 is likely supplied by secondary fermenters, also known as syntrophs.

Syntrophs are responsible for the conversion of volatile fatty acids (VFAs) to acetate, CO_2, and H_2, which in turn are consumed by methanogens and sulfate reducing bacteria. As was observed with methanogens, syntrophs vary with nutrient status of the soil, and the specific distribution of these species provides important information regarding the effects of P enrichment on the flow of carbon and electrons in the Everglades. To investigate the fate of carbon in soils, syntrophs capable of metabolizing specific VFAs were characterized at three sites along the nutrient gradient in WCA-2A by stable isotope probing using ^{13}C-propionate and ^{13}C-butyrate (Chauhan and

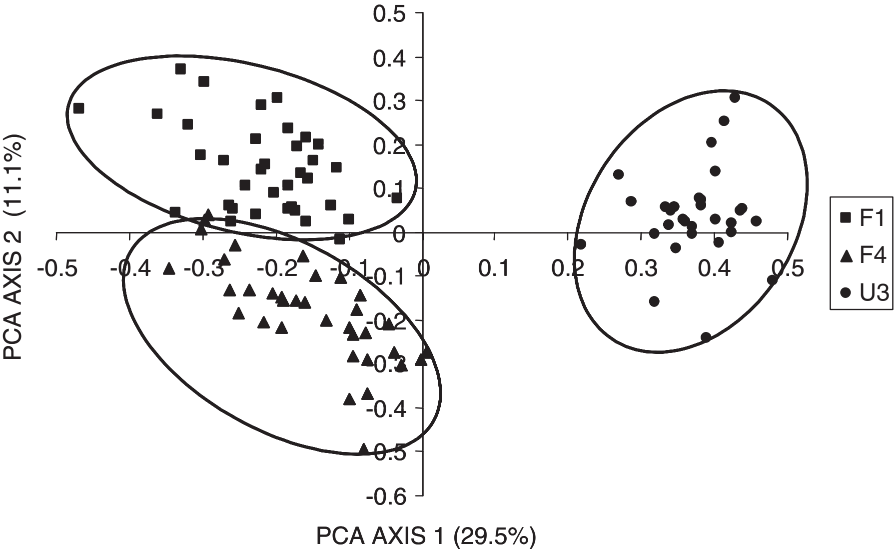

FIGURE 3. Principal components analysis ordering generated from T-RFLP profiles for *mcrA* of eutrophic (F1), transition (F4) and oligotrophic (U3) soils. Figure reprinted with permission from Castro et al. (2005).

Ogram, 2006a). For propionate-amended microcosms (Figure 4), 16S rRNA gene clone libraries from P-enriched and transition sites were dominated by sequences related to previously described propionate oxidizers, such as *Pelotomaculum* spp. and *Syntrophobacter* spp. Significant representation was also observed for sequences related to *Smithella propionica*, which dismutates propionate to butyrate. Sequences of dominant phylotypes from oligotrophic samples did not cluster with known syntrophs but with sulfate-reducing prokaryotes (SRP) and *Pelobacter* spp. In butyrate-amended microcosms, sequences clustering with *Syntrophospora* spp. and *Syntrophomonas* spp. dominated eutrophic microcosms, and sequences related to *Pelospora* dominated the transition microcosm. Sequences related to *Pelospora* spp. and SRP dominated clone libraries from oligotrophic microcosms. Sequences from diverse bacterial phyla and primary fermenters were also present in most libraries. Archaeal sequences from eutrophic microcosms included sequences characteristic of *Methanomicrobiaceae*, *Methanospirillaceae*, and *Methanosaetaceae*. Low-P microcosms were dominated by acetotrophs, including sequences related to *Methanosarcina*, suggesting accumulation of acetate. Stable isotope studies using ^{13}C-acetate indicated that most acetate is consumed by syntrophs in nutrient impacted soils, and by methanogens and sulfate reducers in unimpacted soils (Chauhan and Ogram, 2006a, 2006b).

The significant differences observed in microbial community structure and function as a response to nutrient enrichment in these soils provides

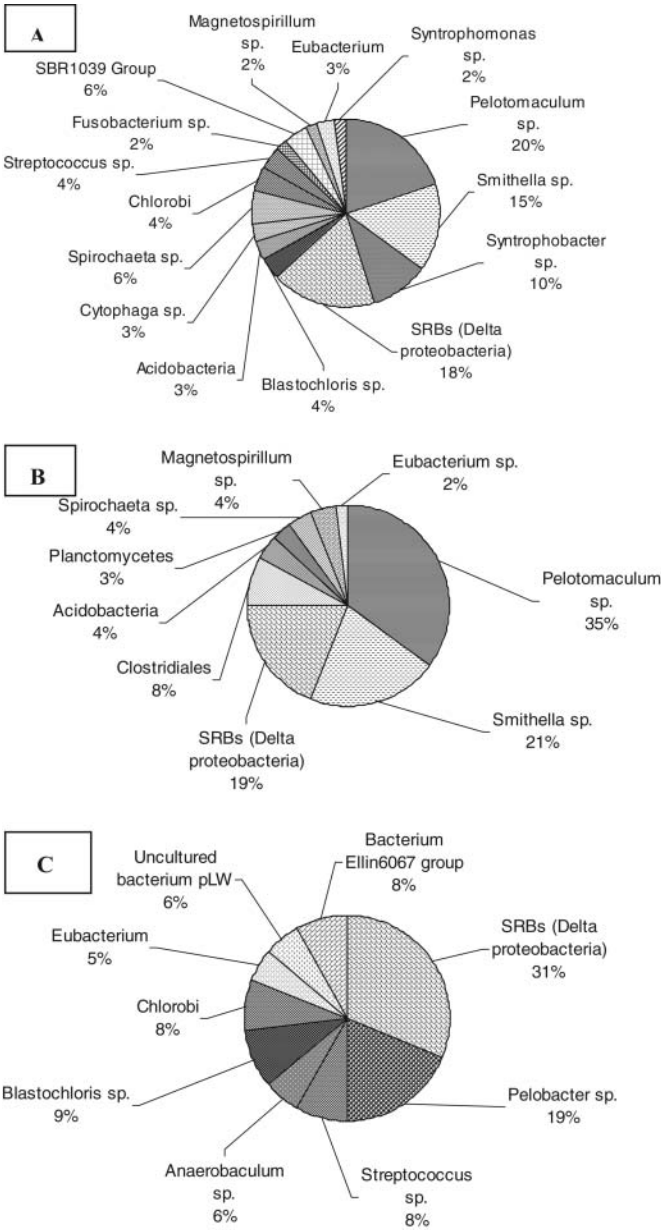

FIGURE 4. Distribution of bacterial clones in [^{13}C]-DNA fractions from microcosms spiked with [^{13}C]-propionate. A, F1 (eutrophic); B, F4 (transition); and C, U3 (oligotrophic) microcosms. Adapted from Chauhan and Ogram (2006a).

information on the impacts of nutrient enrichment on methanogenic pathways, and may lead to the development of sensitive indicators of ecosystem response to nutrients and provide a valuable benchmark for evaluation of restoration.

MICROBIAL ECOLOGY OF EVERGLADES PERIPHYTON

Periphyton is composed of complex communities of microbiota (including algae, fungi, bacteria, animals, inorganic and organic detritus) attached to substrata (Wetzel, 1983). As is discussed at length subsequently, periphyton can serve as important regulators of water chemistry, and hence impact a range of ecosystem processes. Everglades periphyton is typically dominated by cyanobacterial filaments of *Schizothrix* sp. and *Scytonema* sp. (Gleason and Spackman, 1974). These mats occur in association with the benthos (benthic) or with emergent macrophytes (epiphytic periphyton; so called *sweaters*), such as *Typha* and *Cladium*, and submersed macrophytes such as *Utricularia*. These mats range in size from thin films (ca. 1–2 mm) to well-developed, thick (ca. 1–4 cm) mats, and can detach from the substrata via buoyancy from trapped gases and form floating periphyton mats at the water surface. In thick cyanobacterial mats, vertical gradients of light, oxygen, pH, nutrients and microbial metabolic products may exist (Jørgensen, 1983; Stal et al., 1985), resulting in stratification of communities within the mat.

Periphyton provides a variety of important functions in the Everglades, including serving as a dominant contributor to primary production (Ewe et al., 2006) and as the base of aquatic food webs (Campeau, 1994; Murkin, 1989). Water chemistry and nutrient cycling are also affected by a range of periphyton functions, such as oxygen release and consumption (Belanger, 1989), nutrient uptake and storage (McCormick and Stevenson, 1998; Wetzel, 1996), and chemical precipitation of calcium carbonate (Gleason and Spackman, 1974). In low-P systems such as the Everglades, periphyton maintains low P levels in the water column through a number of mechanisms, including acting as a barrier to nutrient release from the soil and by taking up significant amounts of soluble reactive P.

The ability of periphyton to control water P concentrations has sparked interest in its use for removal of elevated P from nutrient impacted sites in the Everglades. In recent pilot-scale studies of periphyton-dominated constructed wetlands in South Florida, approximately half of the inflowing total P was removed from water (Dodds, 2003). Furthermore, the use of periphyton mats for water treatment (termed periphyton-based storm water treatment area, PSTA) is presently being used in some sections of the Everglades (Bays et al., 2001).

The potential of periphyton to act as an sensitive indicator for the trophic status of the Everglades has been highlighted by several studies that have characterized changes in periphyton structure, function, and nutrient status with changes in water quality, hydroperiod, and water saturation with calcium carbonate (Browder et al., 1981; Browder et al., 1982; Browder et al., 1994; Childers et al., 2002; Gaiser et al., 2009; Gaiser et al., 2004; Gleason and Spackman, 1974; Gottlieb et al., 2006; McCormick and Stevenson, 1998; Van

Meter-Kasanhof, 1973). These features are further accentuated by its wide distribution in the pristine Everglades.

Further refinement of periphyton composition as an indicator of nutrient impact might incorporate a greater understanding of the mechanisms underlying the observed composition shifts with nutrient status. Although numerous studies have analyzed the composition of periphyton mats, relatively little is known of the relationships between periphyton structure and function. Few studies have attempted to link the composition of periphyton with function and of the potential effects of P enrichment on the functional ecology of these mats. Jasrotia and Ogram (2007) investigated the distribution of *nifH* gene floating periphyton mats along the nutrient gradient in the Everglades. They reported relative dominance of heterocystous cyanobacteria in nutrient enriched areas when compared to low-nutrient sites. Their data also suggested higher Type I methanotrophs in the nutrient enriched areas and Type II methnaotrophs it the low-nutrient areas. In a study by Inglett et al. (2004), they investigated the potential of nitrogen fixation in periphyton communities (floating and benthic floc), soil, water, and detrital plant material. In the oligotrophic Everglades system, they reported highest rates in floating mats followed by benthic floc, soils, water and the detritus, in that order. Other studies have focused on the ecology of benthic mats by examining the potential linkage between the fermentation nd the methanogenesis in the oligotrophic areas of the Everglades (Uz et al., 2007). A preliminary isotope tracer study focused on linking the microbial structure and function of the floating mats in the WCA-2A region of the Everglades. The microbial structure, as elucidated by phospholipid fatty acid analysis, indicated increased δ^{13}C in lipid biomarkers belonging to cyanobacteria in the presence of daylight. (PLFA; Inglett et al., 2006). A considerable amount of work remains to be done to fully link microbial structural and functional ecology within these mats.

Characterization of the particular architecture of periphyton mats, especially with regard to the physical distribution of controls on P transformation in the mat, is of great importance to understanding the mechanisms through which these mats respond to nutrients. Toward this end, Sharma et al. (2005) attempted to define contributions of various functional groups within a P-limited Everglades periphyton mat toward production of phosphatase, and proposed a model for microbial interactions within the mat. The model illustrates that the cooperative interaction within the microbial groups is due to the dependence of heterotrophs on cyanobacteria for the photosynthetically fixed C and for cyanobacterial dependence on the heterotrophs for the required supply of inorganic P. The heterotrophs were shown to be chief producers of phosphatase. Briefly, the fluorescent stains Enzyme Labeled Fluorescence (ELF-97) and 4,6-diamidino-2-phenylindole (DAPI) were used to stain thin sections of floating mats taken from the oligotrophic area of the Everglades. The microscopic examination of ELF-stained periphyton mat sections revealed that the majority of phosphatase activity (PA) was localized in the middle and lower sections of the mat. Lack of PA in the upper regions of periphyton mats was attributed to the absence of phosphatase producing organisms.

Of note from this study was the absence of observable PA in aggregated chlorophyll-containing filaments towards the interior of the clusters. Nonphotosynthetic organisms on the aggregate exterior, most likely heterotrophic bacteria, exhibited the PA and appeared to be the dominant producers of phosphatase in the mat. This hypothesis was further supported by the observed PA on both the outer sheath of intact cyanobacterial cells and the remnant sheaths of dead filaments. They proposed a cooperative interaction between the algae and bacteria wherein the former provide the photosynthetically fixed C whereas the latter increase the levels of bioavailable P. More information is required to definitively document the role of the heterotrophs in cyanobacterial mat phosphatase production. For this reason, the eventual fate and ecological importance of phosphatase produced by heterotrophic bacteria within such mat communities represents an exciting and another potentially important area of new research.

MICROBIAL ECOLOGY OF EVERGLADES TREE ISLANDS

Because of the sensitivity of tree islands to both flooding and drought conditions, and because of their importance to the ecology of the region, tree islands are considered to be key indicators of the health of the Everglades ecosystem. They also act as sinks and sources for nutrients in the ecosystem and may play an important role in regulating nutrient dynamics in the greater Everglades ecosystem. Many studies have focused on the macroecology of tree islands; however, relatively few studies have focused on the microbial ecology of these unique environments. Fundamental research on the microbial ecology of tree islands would yield basic information on the function of these islands in biogeochemical cycling of the Everglades and would likely provide sensitive indicators of ecosystem health.

Initial studies on the microbial ecology have yielded significant information on the functional diversity of microbial communities on three tree islands (Black Hammock, Satin Leaf, Gumbo Limbo) in Shark Slough of the Everglades National Park. The three tree islands are found on woody peat or limestone outcrops in an area of freshwater calcite mud (marl), or peat. Four tree-dominated plant community types constitute tree island vegetation: bayhead, tropical hardwood hammock, bayhead swamp forests, and willow head. These forest types differ markedly in their vegetation and soil type. These plant communities differ significantly with respect to percent total carbon, pH, moisture content, and microbial counts, but no significant difference was observed with respect to% total P,% total nitrogen, microbial respiration, and extracelluar enzyme activities. Biogeochemical analysis of tree island soils indicated that hammocks have lower percent total carbon than bayheads and swamps.

Microbial diversity in tree islands has been assessed by Length Heterogeneity-Polymerase Chain Reaction (LH-PCR; Ritchie et al., 2000; Suzuki et al., 1998), which distinguishes different phylotypes based on natural variation in the lengths of 16S ribosomal RNA gene sequences. Shannon-Weiner diversity indices of LH-PCR profiles of surface soils indicated that microbial communities in hammocks harbor greater species richness and are more diverse than bayheads and swamps, although bayhead communities exhibited a greater eveneness in all tree islands studied (Table 1). LH-PCR fragments of 342–361 bp (corresponding with gammaproteobacteria) dominated in soils with lower moisture contents, whereas the relative abundance of fragments 314–321 bp (corresponding with gammaproteobacteria) increased with moisture content. This analysis strongly suggests that hammock soils are dominated by beta-, gamma-, and deltaproteobacteria, Flexibacter-Bacteriodes-Cytophaga, and Actinobacteria, and that bayheads and swamps are dominated by alphaproteobacteria and cyanobacteria.

DNA sequences of colony forming units (CFU) from the various soils grown on different solid media indicated that *Bradyrhizobium* sp. and *Xanthomonas* sp. (members of the alphaproteobacteria) represented the greatest numbers cultivated on relatively nutrient poor R2A media, and that *Stenotrophomonas* sp. (gammaproteobacteria) were the dominant CFU on

TABLE 1. Diversity and evenness indices of LH-PCR in tree islands soils. S, H, Hmax, and E are parameters derived from Shannon-Wiener diversity equation (Shannon and Weaver, 1949)

Sample	Ecosystem	Richness (S)	Diversity (H)	Hmax	Evenness (E)
Black Hammock					
Surface	Hammock	26	3.22	2.90	0.88
	Bayhead	23	2.92	3.09	0.93
	Swamp	24	3.02	3.18	0.92
Subsurface	Hammock	25	2.85	3.14	0.88
	Bayhead	23	2.89	3.14	0.92
	Swamp	24	2.73	3.17	0.86
Satin Leaf					
Surface	Hammock	23	2.82	3.14	0.90
	Bayhead	23	2.80	3.14	0.90
	Swamp	22	2.38	3.09	0.77
Subsurface	Hammock	21	2.13	3.04	0.70
	Bayhead	20	2.55	2.99	0.85
Gumbo Limbo					
Surface	Hammock	28	3.13	3.33	0.93
	Bayhead	24	3.12	3.18	0.98
Subsurface	Hammock	25	3.04	3.22	0.94
	Bayhead	24	2.92	3.18	0.92

SCA, a relatively high-nutrient medium. This suggests that alphaproteobacteria in these soils may be selected by nutrient poor growth conditions, while gammaproteobacteria are selected by more nutrient rich conditions. These results are in good agreement with the cultivation-independent LH-PCR data, and provide insight into mechanisms driving diversity and species selection in the different soils.

In low-carbon soils, the dominant community structure is shaped by competitive interactions within communities and is referred to as a competitive diversity pattern (Zhou et al., 2002). In this study, the hammocks with low total carbon contents can be considered to exhibit a competitive diversity pattern. In contrast, the diversity pattern observed in the relatively high-carbon bayheads and swamps are characterized by a noncompetitive diversity pattern. The noncompetitive diversity pattern observed in the relatively high carbon content soils may be due to (a) superabundant resources (if sufficient resources are available, there is no competition and communities evolve towards high diversity), (b) resource heterogeneity (if resources are available in many different forms, populations can avoid competition by niche separation), and (c) spatial isolation (if the habitat is subdivided into many pockets of resources, populations can avoid competition by physical isolation).

Studies linking microbial community structure to nutrient status on tree islands provide essential information on the fundamental mechanisms through which these islands function and may serve as sensitive indicators of the nutrient status and environmental quality of tree islands during restoration. In addition, information of this type may result in a greater understanding of the role of tree islands in the functioning of the greater Everglades ecosystem as a whole.

RELEVANCE TO RESTORATION AND FUTURE DIRECTIONS

Restoration objectives include removal of P from water and altering present water flow patterns to approximate historic flows (U.S. Army Corp of Engineers and South Florida Water Management District, 1999). The processes central to these issues are underlain by the specific activities of microbial communities in soil and periphyton, such that characterization of the structure and activities of microbial communities involved in phosphorus cycling and organic matter decomposition aid in development of a range of management strategies. Much of the work summarized in this review falls into three broad categories: (a) development and application of microbial parameters for use as rapid and sensitive indicators of the nutrient status of the Everglades, which could be used to evaluate the progress and trajectory toward restoration and would aid in establishment of restoration endpoints; (b) applied research into application of microbial ecological principles to directly address restoration goals, such as use of periphyton to lower available P concentrations; and (c) characterization of fundamental microbial processes that control the biogeochemical cycles impacted by nutrient enrichment, thereby leading to deeper understanding of the responses and sensitivities to nutrient impacts of the different environmental compartments that make up the greater Everglades ecosystem.

Further work is needed in all three areas. Characterization of the fundamental mechanisms through which microbial communities respond to nutrient impacts will allow further refinement of both indicators of nutrient impact and management strategies for reconstruction of historic flow patterns in the Everglades. For example, phosphatase expression in periphyton mats provides an attractive indicator of water P concentrations, but much is unknown regarding the fundamental mechanisms that control phosphatase expression. The work initiated by Sharma et al. (2005) points toward fundamental gaps in knowledge regarding phosphatase expression, including questions related to identification of organisms responsible for production of phosphatase and interactions between microbial groups within the mat that control phosphatase expression. If known, these processes might be utilized to better control phosphatase expression in treatment wetlands.

Further characterization of fundamental pathways of organic matter decomposition is needed for a number of reasons. Microbial pathways are responsible for differential decomposition and accumulation rates that form and maintain sloughs and ridges. Organic matter decomposition drives many processes in anaerobic soils such as those in the Everglades, including formation of greenhouse gases such as methane, and sulfate reduction leading to mercury methylation, and yet the microbial groups responsible for these processes and the specific factors that control their activities are poorly understood and may not be easily predicted. Organic matter decomposition also drives so-called internal eutrophication processes, or the release of phosphorus and sulfate from soils by microbial processes that are poorly understood.

Continued investigation into the fundamental relationships between microbial community activities and shifting nutrient patterns likely to develop during restoration will provide greater ability to predict shifts in biogeochemical cycles, thereby increasing options for efficient management.

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