Biogeochemistry **67:** 213–233, 2004.

Periphyton chemistry and nitrogenase activity in a northern Everglades ecosystem

P.W. INGLETT[1,*], K.R. REDDY[1] and P.V. MCCORMICK[2]
[1]*Wetland Biogeochemistry Laboratory, Department of Soil and Water Science, University of Florida, Gainesville, Florida 32611-0510, USA;* [2]*Loxahatchee National Wildlife Refuge, Boynton Beach, FL 33437-9741, USA; *Author for correspondence (e-mail: paul_mccormick@nps.gov)*

Received 9 October 2001; accepted in revised form 9 October 2002

Abstract. Cyanobacterial periphyton communities are a dominant feature of oligotrophic Everglades marshes, however, little is known regarding the biogeochemical aspects of this ecosystem component. This study was undertaken to investigate the potential for N_2 fixation in the periphyton communities of a hydrologically-controlled portion of the northern Everglades marsh (Water Conservation Area 2A, WCA-2A). The objectives of this research were to characterize the temporal patterns of nutrient composition and N_2 fixation of the natural WCA-2A periphyton communities and to compare fixation rates of periphyton with those of other ecosystem components in both natural and nutrient-impacted WCA-2A areas. In general, N_2 fixation (measured by the acetylene reduction (AR) method) of natural WCA-2A periphyton was enhanced under light conditions showing a nitrogenase pattern characteristic of autotrophic cyanobacteria. Winter (November–March) rates of AR expressed per gram organic carbon (gOC) ranged from 147–240 nmol C_2H_2 g OC^{-1} h^{-1}, while summer rates were elevated with an observed peak of 1148 nmol C_2H_2 g OC^{-1} h^{-1} in July 1998. This translates into an estimated yearly contribution of approximately 10 g N m^{-2} to an unimpacted WCA-2A slough ecosystem. Nitrogenase activity did not correlate seasonally with nutrients (Ca, Mg, Fe, N, P, Mn), but closely followed measured N stable isotopic ratios ($\delta^{15}N$) in floating periphyton. In oligotrophic marsh areas, AR (on a weight basis) decreased in the order floating periphyton > benthic periphyton floc > soil > water > detrital plant biomass, while highest AR rates were observed for detrital biomass in areas impacted by agricultural discharges.

Introduction

With biomass estimates reaching 2500 g AFDM m^{-2}, floating periphyton mats (metaphyton) are a dominant feature of open-water slough areas of the Florida Everglades (McCormick et al. 1998). As described by Gleason and Spackman (1974), cyanobacterial filaments of the genera *Scytonema* and *Schizothrix* form the basis of the Everglades periphyton mat serving as a matrix for the growth of other algae and microorganisms. Photosynthetic activity of the mat organisms locally influences pH leading to the precipitation of calcite ($CaCO_3$) within the mat structure (Gleason and Spackman 1974). As such, the periphyton mats of the natural Everglades are similar to other calcifying cyanobacterial mat communities (Cohen and Rosenberg 1989; Rejmánková and Komárková 2000).

The dominance of cyanobacteria in Everglades periphyton raises an important question concerning nutrient limitation within the Everglades ecosystem. Fre-

quently, cyanobacteria are found to dominate aquatic ecosystems under conditions of nitrogen (N) limitation or when levels of other potentially limiting elements (e.g., phosphorus (P)) are in excess relative to N (Paerl 1990). This observation is commonly explained by the ability of many cyanobacteria to convert atmospheric N_2 gas into the biologically available ammonium (NH_4^+) form via the nitrogenase enzyme complex (N_2 fixation). General theory suggests that these N_2-fixing cyanobacteria will dominate at low ambient total N to total P (TN:TP) weight ratios (< 30) due to the ability to access the potentially unlimited atmospheric N supply (Smith 1983). The oligotrophic Everglades, however, is a profoundly P-limited ecosystem with typical water column TP levels below 10 μg P L^{-1} and water column TN:TP in excess of 250 (Table 1), (McCormick et al. 1996). Under such conditions, N_2 fixation would seemingly offer no benefit compared to the apparent need for P.

By contrast, in more eutrophic Everglades regions (e.g., in areas receiving discharges of agricultural drainage), P inputs have led to increasing N limitation of the system as evidenced by a lowered water column N:P (TN:TP < 30) and algal nutrient limitation assays (Table 1), (Swift and Nicholas 1987; McCormick et al. 1996). In these more eutrophic marsh areas, periphytic mats are much less abundant, lack a visible calcareous structure, and are comprised of algal assemblages dominated by cyanobacteria, (e.g., *Lyngbya* sp. and *Oscillatoria* sp.) and filamentous greens (e.g., *Spirogyra* sp. and *Mougeotia* sp.) (Swift and Nicholas 1987; McCormick et al. 1996). Several studies have indicated P enrichment to be a primary cause of the mat breakdown (e.g., Flora et al. (1988) and Hall and Rice (1990), Craft et al. (1995), McCormick and O'Dell (1996)) and the shift toward pollution-tolerant taxa (Flora et al. 1988; Hall and Rice 1990; McCormick and O'Dell 1996).

The presence of cyanobacteria (and other diazotrophic microorganisms) indicates the potential for nitrogenase activity within the oligotrophic Everglades periphytonperiphyton mats. No comprehensive studies exist, however, to document the potential and significance of N_2 fixation within this prominent component of the Everglades ecosystem (Browder et al. 1994). Additionally, the lowered N:P coupled with the presence of cyanobacteria in the periphyton of nutrient-impacted marsh areas could lead to enhanced rates of N_2 fixation in the periphyton near the points of agricultural drainage discharges. As such, periphyton nitrogenase activity could serve as an indicator of nutrient impacts (in particular those of P) to the Everglades ecosystem. For these reasons, the following study was conducted to characterize the N_2 fixation process of the periphyton mat communities of a Florida Everglades ecosystem.

The primary objective of this study was to document the potential for nitrogenase activity in the more natural (i.e., unimpacted) Everglades system. Because nutrient chemistry has been shown as a key variable defining both the activity and type (e.g., structure and species composition) of Everglades periphyton (Swift and Nicholas 1987; Browder et al. 1994), we wanted to assess nitrogenase activity in relation to the seasonal cycles of periphyton nutrient composition. Additionally, N stable isotopes are becoming a popular methodology to indicate N_2 fixation. For

Table 1. Comparison of selected water quality variables in agricultural drainage (Canal) and marsh surface water at enriched (Periphery) and oligotrophic (Interior) slough sites along the WCA-2A eutrophication gradient. Data summarized from McCormick et al. (1996).

Variable	Units	Canal	Periphery	Interior
Conductivity	$\mu S\ cm^{-1}$	1041	1093	885
Alkalinity	$mg\ L^{-1}$	277	280	222
Dissolved Organic Carbon (DOC)†	$mg\ L^{-1}$	40	43	40
Total Nitrogen (TN)	$mg\ N\ L^{-1}$	2.6	3.0	2.2
Ammonium (NH_4^+)	$\mu g\ N\ L^{-1}$	296	70	59
Nitrate ($N0_3^-$)	$\mu g\ N\ L^{-1}$	241	252	11
Total Phosphorus (TP)	$\mu g\ P\ L^{-1}$	104	116	8
Soluble Reactive Phosphorus (SRP)	$\mu g\ P\ L^{-1}$	48	38	5
Total Dissolved Calcium (Ca)	$mg\ L^{-1}$	91	85	70
Total Dissolved Magnesium (Mg)	$mg\ L^{-1}$	28	29	25
Total Dissolved Potassium (K)	$mg\ L^{-1}$	7.4	7.4	6.9
Total Dissolved Iron (Fe)	$\mu g\ L^{-1}$	44	38	21

† Dissolved fractions were obtained following filtration through a 0.45 μm membrane filter.

this reason, we analyzed the N stable isotopic composition of the Everglades periphyton to determine its potential as an indicator of seasonal nitrogenase activity.

Materials and methods

Site description

During the last century, the Florida Everglades has been fragmented through the construction of a system of dikes, levees, and canals, into distinct hydrologic units known as Water Conservation Areas. Of these units, Water Conservation Area 2A (WCA-2A) is a 547 km^2 diked marsh adjacent to other conservation areas (WCA1, WCA-2B, and WCA-3) and north of the Everglades National Park (Figure 1). WCA-2A receives periodic inputs of agricultural drainage water from the Hillsborough Canal via spillways along the northeastern perimeter. The overall management goal of WCA-2A is water storage during the wet season (roughly May–October) and water supply during the dry season (November–April) (Davis and Ogden 1994). The result of the drainage discharges has been the creation of a nutrient enrichment gradient of the surface water and periphyton (McCormick et al. 1996), marsh porewaters (Vaithiyanathan and Richardson 1997), dominant macrophytes (Koch and Reddy 1992), and soil (Craft and Richardson 1993; DeBusk et al. 1994; Reddy et al. 1998) with high nutrient levels nearer the spillways and unenriched, background levels in the interior of WCA-2A (Table 1). Along with the changes in nutrient levels, the discharges have favored the development of extensive stands of cattail (*Typha domingensis* Pers.) in impacted areas replacing the native Everglades marshes (dominated by sawgrass (*Cladium jamaicense* Crantz)) and openwater sloughs (dominated by periphyton mats, *Utricularia purpurea* Walt., and *Nymphaea odorata* Ait.).

Field sampling

Seasonal patterns of floating periphyton mat N_2 fixation, chemistry, and N stable isotopic composition were determined using periphyton mat samples collected from an interior WCA-2A slough site (mesocosm site, Figure 1). This area was established and is maintained as an experimental mesocosm site by the South Florida Water Management District, and is characteristic of the oligotrophic interior marsh areas (Table 1), (McCormick et al. 1996). At this site, 1.8 m^2-areas of undisturbed slough have been designated as open control (i.e., non-enclosed) plots. Grab samples of floating mat material were collected from these control plots on the following dates: March 18, May 4, July 6, September 8, November 9, 1998 and January 11, 1999. In August, 1998 and February, 1999, samples of four other ecosystem components (floodwater, benthic periphyton ″floc″ material, soil, and detrital plant biomass) were collected for comparison of nitrogenase activity with that of floating periphyton. Sampling of these WCA-2A ecosystem components was conducted at

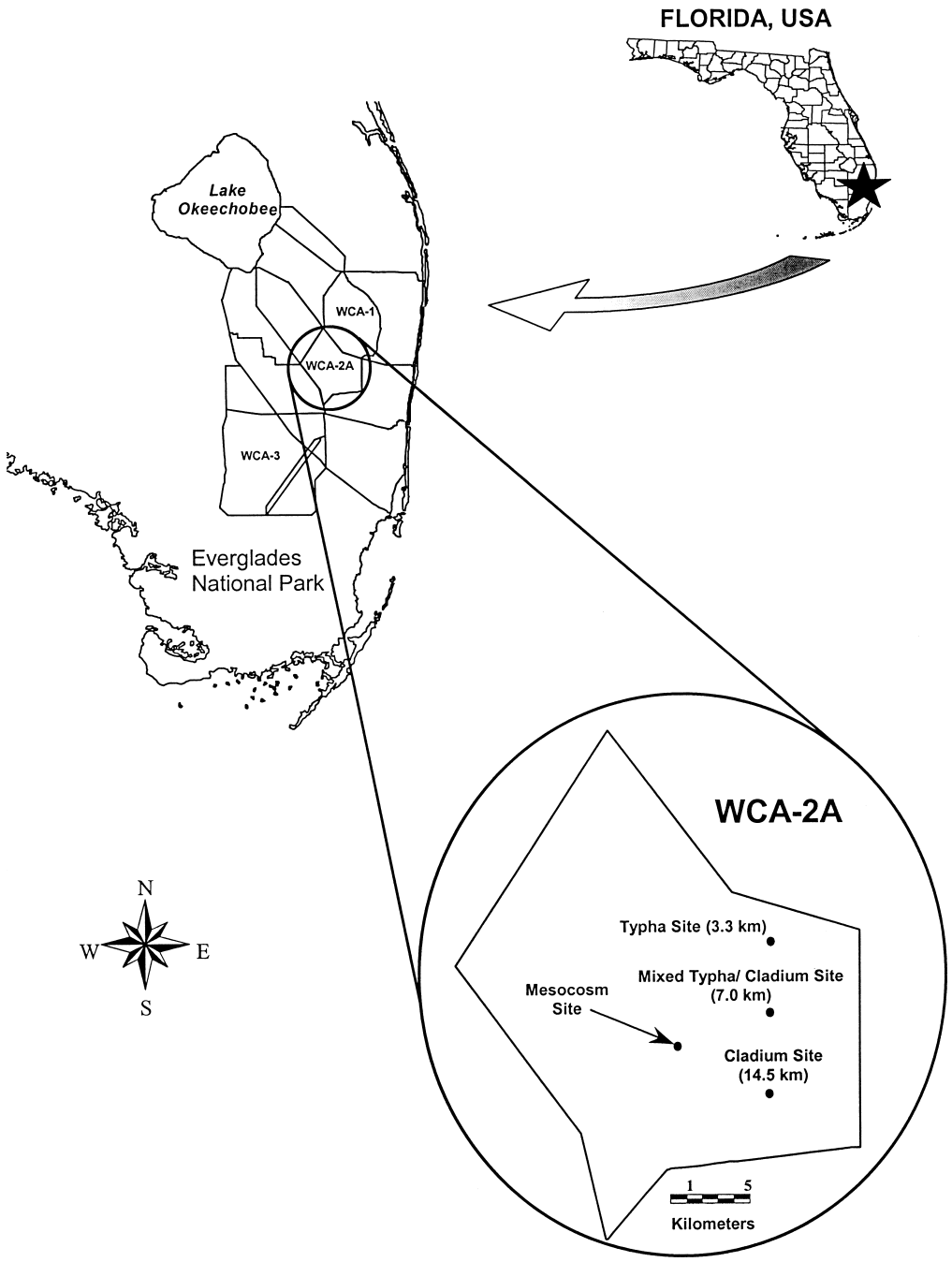

Figure 1. Location of Water Conservation Area 2A (WCA-2A) in relation to other South Florida features and sampling locations used in this study.

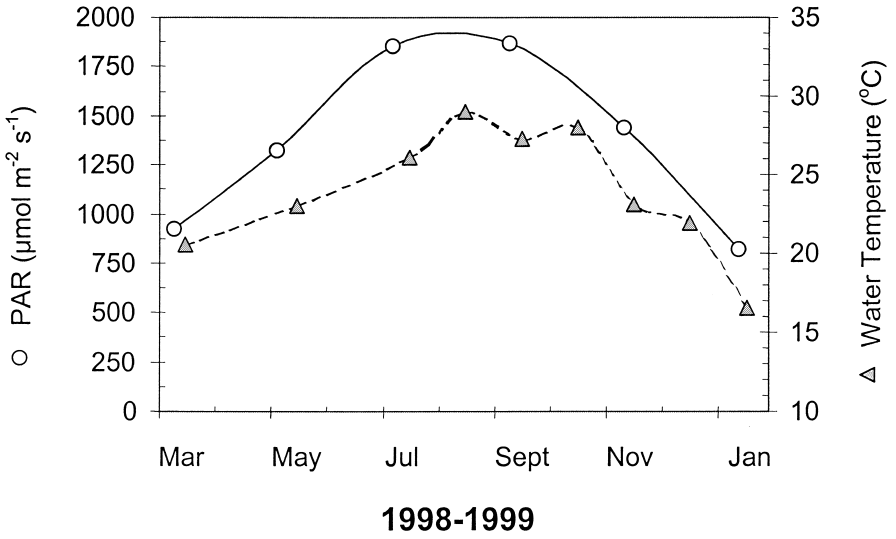

Figure 2. Light intensity (PAR measured at time of sampling) and average monthly temperature (measured independently) observed during the year of this study. Points represent the mean of three time interval averages (light intensity) or the average of all obtained values (temperature).

four WCA-2A transect stations representing the range of plant communities and nutrient conditions along the gradient: 1) an unimpacted interior *Cladium* community (14.5 km), 2) an intermediate *Cladium* plant community (7.0 km), 3) an intermediate *Typha* community (also 7.0 km), and 4) a *Typha* dominated plant community near the inflow (3.3 km) (Figure 1). At these stations, three replicate floating periphyton grab samples were collected at the edge of the nearest macrophyte stand. Three replicate samples of surficial soil and associated floc material were collected within the macrophyte stand using 6 cm diameter PVC tubes. Replicate, unfiltered water samples were obtained by filling three brown, 125-mL, polyethylene bottles. Bulk standing, detrital plant samples were collected from the appropriate plant community by cutting randomly selected submerged dead plant leaves and stems and storing the pieces in a container filled with unfiltered site water. Due to the large distances between sites, helicopter sampling was employed to allow rapid processing of collected ecosystem components (< 3 h storage).

Analytical methods

Nitrogenase activity

The measurement of nitrogenase activity (potential N_2 fixation) was conducted using an adapted version of the acetylene (C_2H_2) reduction (AR) assay for microbial mat N_2 fixation by Stal (1988). Benthic core samples were separated into surficial periphyton floc material and soil fractions. Benthic periphyton floc material is here defined as the material which could be poured from the inverted core tube and was primarily an aqueous phase consisting of unconsolidated benthic periphyton, soil,

and detrital materials. Benthic floc, soil (collected from the upper 2 cm of core sample), periphyton, water and plants were subsampled and placed into 50-mL, Kimax™, screw-capped culture tubes. Approximate sample sizes in tubes were 3–5 g wet weight for periphyton mat, soil, and plant materials, while ~10 mL and 20 mL were used for floc and water, respectively.

To allow insertion of syringe needles, the tubes were equipped with an open-top cap containing a teflon-lined, silicone septa (0.120″ thick). Acetylene gas (6 mL) (generated by adding distilled deionized water to calcium carbide chips inside an evacuated serum bottle) was injected into each sample tube to initiate the AR incubation. Incubation blanks were prepared by injecting acetylene into replicate tubes containing no sample. Tubes were then inverted and placed into tube racks, and held in place using a wire screen (1-cm square mesh openings). The resulting assembly floated under its own buoyancy maintaining the samples approximately 1 cm below the water surface. All incubations were conducted at approximately midday on any given sampling date. Temperature regulation during the incubation was then accomplished by floating the tube/rack assemblies in marsh water (collected at ambient temperature) contained in coolers either open to ambient light levels (Light Incubation) or closed to prevent light transmission (Dark Incubation). Three blanks with added acetylene were included in each Light or Dark incubation. Soil samples were incubated only under dark conditions. During the incubation, photosynthetic irradiance was measured using a LI- 1000 datalogger attached to a LI-193SA spherical quantum sensor (LI-COR, Lincoln, NE) secured just below the water surface.

Following incubation (~2 h), headspace samples (5 mL) of each tube were obtained following vigorous shaking (~5 s) of tube contents to equilibrate aqueous and headspace gas phases. Gas samples were stored in evacuated, 3.5-mL serum vials sealed with gray, butyl-rubber stoppers and aluminum crimp seals. All gas samples were analyzed for ethylene within 36 hr of collection using a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detector (110 °C). Separation of gases was accomplished at 80 °C using a 6-foot, Poropak-N column (Supelco, Bellefonte, PA). Standards (prepared using pure (99.5%) ethylene) and a pre-mixed standard concentration gas (Scott Specialty Gases, Inc., Plumsteadville, PA) were used to calibrate the measurement. Rates of ethylene production (acetylene reduction) were calculated using both headspace and aqueous phase ethylene concentrations (determined using tabulated solubility constants), corrected for blank ethylene concentration, and expressed as molar quantities of ethylene produced per hour of incubation (nmol C_2H_4 h^{-1}). AR values were used to estimate actual rates of N_2 fixation using a theoretical conversion ratio of 3 moles of C_2H_2 reduced to 1 mole of N_2 fixed (Howarth et al. 1988).

Chemical and isotopic analysis

Soil and biomass samples used for the AR assay were dried (70 °C), weighed, and ground using a ball mill for analysis of chemical parameters. Total N (TN) and total carbon (TC) were measured simultaneously using a Carlo-Erba NA-1500 CNS elemental analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P and metals were determined by inductively coupled argon plasma emission spectroscopy

(Thermo Jarrell Ash ICAP 61E; Franklin, MA) following nitric-perchloric acid digestion (Kuo 1996). Total inorganic carbon (TIC) was measured on periphyton samples using an acid dissolution/pressure calcimeter method for total carbonate (Loeppert and Suarez 1995). Total organic carbon (TOC) was then determined indirectly as the difference between TC and TIC.

Ratios of N stable isotopes ($^{15}N/^{14}N$) were determined on dried, ground periphyton samples using a Finnigan MAT Delta Plus isotopic ratio mass spectrometer (Finnigan Corp., San Jose, CA). Sample ratios were expressed as permil (‰) differences from the standard isotopic ratio of atmospheric N_2 (0.3663%) using delta notation (δ) as follows:

$$\delta^{15}N_{sample} = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Statistical analysis

All statistical analyses were performed using Statgraphics® Plus, Version 3.1 (Manugistics, Inc., Rockville, MD). Seasonal patterns of WCA-2A periphyton mat AR, Light:Dark AR, isotopic composition, as well as changes in chemistry of unenriched mesocosm mat samples were tested using a one-way ANOVA model. Where applicable, values for ANOVA tests were log transformed to account for variance inequalities.

Results and discussion

Seasonal characterization

One way to characterize the ecophysiology of the WCA-2A periphyton is to follow the seasonal changes in the mat chemistry. A seasonal trend was observed in all variables (TIC, TOC, Ca, Mg, N, P, and Fe) except K and Mn (Figure 3). This seasonality is most likely the result of changes due to increased calcification during the dry season months (roughly December–May) as shown in the trends of TIC and Ca. Lowest TIC (43 g kg^{-1}) occurred in September and corresponded to the seasonal low in periphyton Ca (167 g kg^{-1}). Coincidental with this carbonate minimum were seasonal maxima in TOC (220 g kg^{-1}), Fe (348 mg kg^{-1}), TN (14 g kg^{-1}), and TP (112 mg kg^{-1}). Following the wet season peak, concentrations of these nutrients declined, presumably as a result of increased calcification or through senescence and leaching.

The seasonal TP values observed for these unimpacted periphyton mats were low, ranging from 29–112 mg kg^{-1}, but occur within ranges reported for similar Everglades periphyton by Vymazal (1995) (30–454 mg kg^{-1}) and Swift (1984) (60–490 mg kg^{-1}). Total N varied almost two-fold from 8.4 g kg^{-1} in March to 14.0 g kg^{-1} in September. A similar trend for floating WCA-2A periphyton was noted by Vymazal and Richardson (1995), though the TN concentration range of

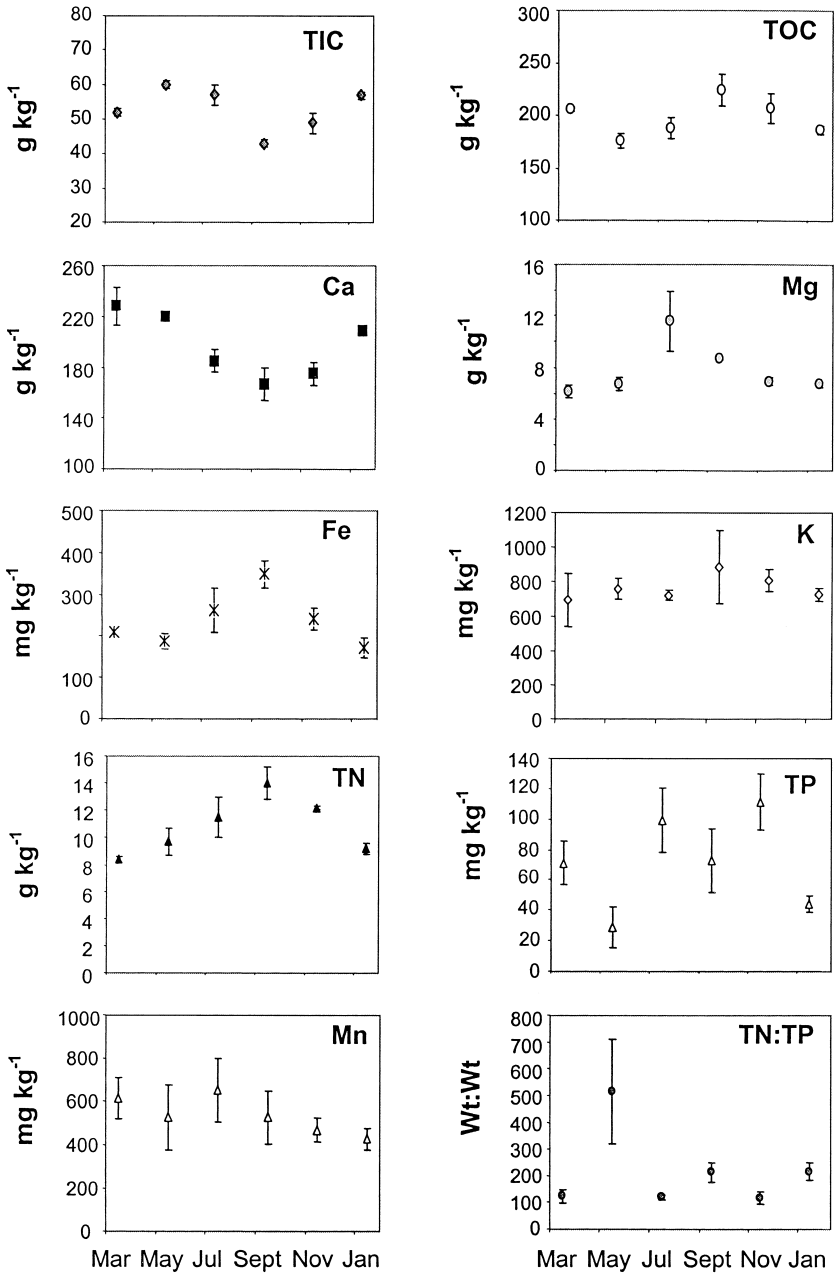

Figure 3. Seasonal patterns of oligotrophic WCA-2A periphyton nutrient composition. Points represent the means (±1 SE) of samples (n = 3) collected from the open control WCA-2A mesocosm plots. Displayed periphyton nutrient parameters are inorganic carbon (TIC), organic carbon (TOC), Calcium (Ca), Magnesium (Mg), Iron (Fe), and Potassium (K), Total Nitrogen (TN), Manganese (Mn), Total Phosphorus (TP), and TN:TP (wt:wt).

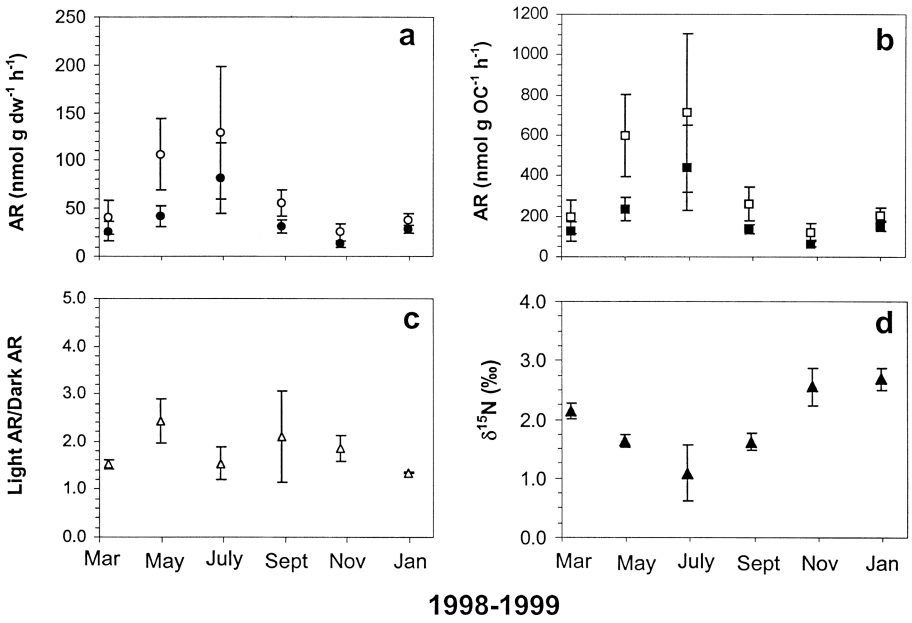

Figure 4. Seasonal patterns of Acetylene Reduction (AR) under light conditions expressed on (a) dry weight basis, (b) an organic carbon basis, (c) the ratio of Light AR:Dark AR, and (d) nitrogen stable isotopic composition ($\delta^{15}N$) for oligotrophic WCA-2A floating periphyton mats. Points represent the means (±1 SE) of samples (n = 3) collected from the open control WCA-2A mesocosm plots.

that study was wider ($\sim$6–23 g kg^{-1}). Despite the fluctuations in both TN and TP, the weight ratio of TN to TP showed little seasonality throughout this study. Primarily, values ranged from 117 to 216 except during May where exceptionally high TN:TP values (mean 515) were recorded. Such high values are typical of unenriched WCA-2A sloughs, where TN:TP values in excess of 100 have frequently been observed (Swift 1984; Vymazal and Richardson 1995).

Like the trends in floating mat nutrients, AR under both light and dark conditions seemed to follow a seasonal pattern with a peak value of 129 nmol g DW^{-1} h^{-1} in July (Figure 4). Following the July peak, AR of the floating mat dropped dramatically in September and continued the decline to the observed minimum in November (25 nmol g DW^{-1} h^{-1}). The results of the one-way ANOVA using log-transformed data, however, do not support the presence of a significant trend (P = 0.081). Expressing these results on a per gram organic carbon (g OC) basis (123–712 nmol g OC^{-1} h^{-1}) had little effect (Figure 4), indicating that the observed temporal pattern in AR is not likely the result of weight changes of the inorganic periphyton fraction.

One source of evidence to substantiate a seasonal fixation pattern could be the seasonal trend of WCA-2A periphyton N stable isotopic composition (Figure 4). Several studies have used natural abundance levels of ^{15}N (as $\delta^{15}N$) to reflect algal fixation of atmospheric N ($\delta^{15}N$ = 0.0‰) (Gu and Alexander 1993; Yamamuro et

al. 1995; France et al. 1998). One advantage of this approach is that the isotopic signature of one sample should reflect the overall daily fixation rate without requiring numerous measurements to accurately describe a diel fixation cycle. Therefore, in seasonal studies such as this, the isotopic approach may serve as an effective assay to qualitatively describe relative N_2 fixation patterns. In this study, N isotopic composition had a significant seasonal pattern ($P < 0.01$) with a recorded maximum enrichment of the heavier isotope in January ($\delta^{15}N = 2.7‰$) and a seasonal minimum in July ($\delta^{15}N = 1.1‰$) (Figure 4). Because this ^{15}N depletion also corresponds with the seasonal peak in nitrogenase activity, we feel this represents the time of maximum incorporation of atmospheric N, which by definition has a $\delta^{15}N = 0‰$. This fact combined with the apparent high sensitivity and precision of the measurements indicates that N stable isotopes may be a very useful tool (especially in combination with other enzyme assays) in studies of N cycling in wetlands.

In general, the $\delta^{15}N$ values observed in this study are typical of average natural abundance ratios of most N_2-fixing freshwater algae (summarized by France et al. (1998)) but are lower than values recorded for WCA-2A periphyton at an unimpacted site near to that of the present study (Kendall et al. 2001). In their study, Kendall et al. (2001) observed a strong negative correlation between periphyton N isotopic composition and water depth. In contrast, the seasonal trend in isotopic composition of this current study revealed a strong positive correlation with water depth (data not shown) with the period of shallow water depth (June–August) showing the lowest $\delta^{15}N$ values. We suggest that this summer depletion is a reflection of increased nitrogenase activity in the summer, however, Kendall et al. (2001) suggested that the increased $\delta^{15}N$ values of their study were likely the result of periphyton uptake of isotopically-enriched DIN in the water column. It is unfortunate, but no acetylene reduction data were obtained in that study, so it is impossible to compare their isotopic data with corresponding measures of nitrogenase activity. Likewise, this current study failed to assess the relationship of periphyton isotopic composition with that of watercolumn DIN. For this reason, any conclusions regarding these isotopic data are precluded by further study.

It is interesting to note that the seasonal peak in AR and the seasonal depletion in ^{15}N do not coincide with those of most of the periphyton nutrients (Figures 3 and 4). Rather the observed peak in nitrogenase activity occurs seasonally with the highest Mg concentration and the approximate peak in periphyton TN:TP (Figures 3 and 4). Also, the abrupt decline in AR in September is puzzling and could indicate a suppression of the nitrogenase activity of the mat community. One cause for such a suppression could be high levels of inorganic N in the water column or seasonally low DIN:DlP ratios. Analysis of surface water monitoring data collected by the South Florida Water Management District, however, revealed that the maximum nitrogenase activity observed in this study corresponded with the highest seasonal watercolumn DIN (105 μg N L^{-1}) and DIN:DIP ratios (11.7 on weight basis). Therefore it is unlikely that external N sources were responsible for the sharp decline in nitrogenase activity in September.

Alternatively, the September decline could represent a suppression of biological activity by extreme solar intensities and temperatures present at the marsh water

Table 2. Values for selected water quality variables observed at the WCA-2A Mesocosm Site during the time of seasonal sampling in this study (March 1998–January 1999). Data were summarized from unpublished monitoring data collected by the South Florida Water Management District.

Variable	Units	Mean	Maximum	Minimum
Conductivity	μS cm^{-1}	967	1187	618
Alkalinity	mg L^{-1}	237	296	141
Dissolved Organic Carbon (DOC)†	mg L^{-1}	36	47	23
Total Nitrogen (TN)	mg N L^{-1}	2.3	3.0	1.5
Ammonium (NH_4^+)	μg N L^{-1}	38	90	19
Nitrate (NO_3^-)	μg N L^{-1}	8	20	4
Total Phosphorus (TP)	μg P L^{-1}	15	73	6
Soluble Reactive Phosphorus (PO_4^{3-})	μg P L^{-1}	8	15	4
Total Dissolved Calcium (Ca)	mg L^{-1}	69	92	39
Total Dissolved Magnesium (Mg)	mg L^{-1}	26	32	14
Total Dissolved Potassium (K)	mg L^{-1}	6.6	9.4	3.2
Total Dissolved Iron (Fe)	μg L^{-1}	10	26	6

† Dissolved fractions were obtained following filtration through a 0.45 μm membrane filter.

surface. Flotation of the benthic communities at the water surface during summer months exposes the periphyton to potentially damaging levels of solar radiation, often in excess of 2800 μmol m^{-2} s^{-1} PAR (Grimshaw et al. 1997), and summer temperatures > 35 °C (Rader and Richardson 1992). Though not observed at the times of sampling (Figure 2), it is quite possible that such conditions were present during the summer of this study. Transient exposure to high temperatures has been shown to inhibit N_2 fixation in cyanobacteria by the inactivation of oxygen protective mechanisms of nitrogenase (Gallon et al. 1993). Grimshaw et al. (1997) give support for this hypothesis with their conclusion that in WCA-2A submerged periphyton photosynthesis is light saturated at even at PAR levels 40% of that at the water surface. Also, the seasonal peak in periphyton gross primary production rates (measured using O_2 evolution) has been observed as early as late spring (May) at unimpacted WCA-2A sites (McCormick et al. 1997). Low photosynthetic and nitrogenase activity in September may also be evidenced by the loss of the calcareous nature of the mat as seen in the sharp decline in September of TIC (4.3% dw) and Ca (167 g kg^{-1}).

Ecosystem characterization

The comparison of nitrogenase activity of the WCA-2A ecosystem components demonstrates the relative importance of periphyton mat N_2 fixation as a source of fixed N to the Everglades ecosystem (Table 3). It is important to note that the basis used for these measurements differs between material types (e.g., water (per mL), floating periphyton (per g DW), etc.). Also, no direct estimates of areal biomass of each of the components were made during this study, therefore it is not possible to compare areal rates of N_2 fixation for the ecosystem components. They do, how-

Table 3. Nitrogenase activities (measured using the acetylene reduction assay) for variousWCA-2A ecosystem components during the August 1998 and February 1999 transect sampling events. All measurements conducted under ambient light conditions except for soil which was incubated in the dark. Unless noted, values represent the mean (standard error) of three replicate measurements.

Distance (km)	Community	Ecosystem Component				
		Periphyton	Water	Detritus	Floc	Soil
		Acetylene Reduction (nmol C_2H_4 g^{-1} h^{-1})‡				
August 1998						
3.3	Slough	116.2 (14.9)	0.03 (0.00)			
	Typha			53.9 (13.4)	8.3 (2.7)	2.0†
7.0	Slough	115.4 (10.1)	0.03 (0.00)			
	Cladium			9.6 (2.5)	–	–
	Typha			23.6 (7.5)	7.0 (1.4)	1.0 (0.2)
14.5	Slough	64.9 (14.5)	0.04 (0.00)			
	Caldium			15.0 (4.5)	6.6 (1.9)	1.6 (0.1)
February 1999						
3.3	Slough	90.0 (27.2)	0.02 (0.00)			
	Typha			212.3 (63.8)	7.7 (1.9)	0.07 (0.43)
7.0	Slough	10.3 (4.1)	0.01 (0.00)			
	Cladium			4.1 (0.7)	4.8 (1.4)	BDL*
	Typha			31.0 (4.4)	24.7 (9.5)	BDL
14.5	Slough	70.0 (13.2)	0.01 (0.00)			
	Cladium			26.7 (11.8)	4.3 (0.5)	0.15 (0.10)

†n = 1; ‡AR values expressed per gram of component material. Periphyton, soil, benthic floc, and plant detrital materials expressed per gram dry weight. 1 g water refers to 1 mL.; *Below Detection Limit.

ever, offer a means of assessing the potential N_2 fixation associated with different ecosystem components. With this in mind, AR measurements at the three transect sites reveal that at a given site, periphyton mat N_2 fixation is potentially much higher than the fixation of the water, benthic floc material, soil, or detrital plant ecosystem components. In general, fixation potential was observed to decrease in the order: floating periphyton mat > plant detritus > benthic floc > soil > water. This trend was largely consistent regardless of transect location or season with the only exceptions of the low AR of the floating periphyton at the 7 km station and the high AR of the 3.3 km detritus during February 1999 (Table 3).

The low AR rates of WCA-2A soil were anticipated due to the high concentrations of porewater NH_4^+ (Koch et al. 1994). However, low rates of ethylene production could also be the result of the activity of methane oxidizing bacteria. These bacterial populations are known to reduce low molecular weight carbon sources (e.g., C_2H_4), and thus, may lead to underestimates of nitrogenase activity as measured using the AR assay (Flett et al. 1975). This source of error could also explain the unexpectedly low AR rates encountered in the benthic periphyton floc. Presumably, this precursor to the floating mat would also be an active N_2-fixer, however none of the benthic samples obtained in this study were characteristic of the extensive periphytic growths on benthic slough surfaces described by others (e.g., Brow-

der et al. (1994)). Rather, the benthic materials assayed were primarily detrital with little presence of a periphytic mat. Thus, the low observed rates could be the result of NH_4^+ inhibition as in the soil, or more likely, the result of sampling and AR assay procedures in this study which disrupted both microsites and chemical/metabolic gradients present in the benthic materials (Paerl 1990).

Based on the assumption that light and dark AR rates measured in this study approximate the daily maximum and nightly fixation rates, respectively, the average diel N_2 fixation rate for the unimpacted WCA-2A floating periphyton mat is the average of both the light and dark values. This calculation yields a yearly range of 93–577 nmol g OC^{-1} h^{-1} which converted to a dry mass basis yields the range 19–105 nmol g dw^{-1} h^{-1}. Using reported values of ash content (Scinto 1997) and wet and dry season values of periphyton biomass for oligotrophic WCA-2A slough sites (570–957 g AFDM m^{-2}, McCormick et al. (1998)), this range can be expressed on an areal basis to yield a seasonal range of 23–213 μmol m^{-2} h^{-1}.

Using the theoretical ratio of 3 moles of C_2H_2 reduced per mole of N_2 fixed, it is possible to estimate the amount of N_2 fixed for a given AR rate. This ratio has proven reasonable for other cyanobacterial mats (reviewed by Howarth et al. (1988)) and yields estimated seasonal rates of unimpacted WCA-2A slough N_2 fixation ranging from 0.21–2 mg N $m^{-2}h^{-1}$ or 1.8–18 g N m^{-2} yr^{-1}, with a mean annual fixation rate of 9.7 g N m^{-2} yr^{-1}. It is important to note that the biomass of all periphyton forms (benthic, epiphytic, and floating mat) have been included in this calculation with the assumption that their nitrogenase activity approximates that measured for the floating mat. This assumption potentially results in an overestimate, but is reasonable considering the previously mentioned uncertainty of the benthic floc material measurements. Of this annual 9.7 g N fixed by all slough periphyton, we calculate that between 8% and 30% may be contributed by floating mats (based on dry/wet season standing-crop biomass as reported by McCormick et al. (1998)).

Nitrogen accumulation rates have been estimated at 7–10 g N m^{-2} yr^{-1} for unimpacted WCA-2A soils (Reddy et al. 1993). The fact that the estimate for N_2 fixation of the current study (9.7 g N m^{-2} yr^{-1}) falls within this reported range gives some support for the validity of the calculation. However, because the total N fixed within the system will be even higher when considering the nitrogenase activity of other ecosystem components (i.e., water, soil, plant detritus etc.), and N_2 fixation is only one of several other potential N inputs (e.g., DIN uptake, particulate sedimentation), then only a fraction of the gross N contributed through N_2 fixation is actually being retained within the system. This occurrence is quite understandable given the dynamic nature of the Everglades wetland system where several N export/loss processes occur (e.g., denitrification, organic/inorganic N flux, etc.). But it also raises the question of the ultimate fate and ecological importance of the periphyton fixed N. Therefore, while the estimated gross fixation rate of 9.7 g N m^{-2} yr^{-1} is significant, the exact net contribution of N to the marsh via the periphyton N_2 fixation pathway remains uncertain.

In comparison with other ecosystems, the N_2 fixation rates of unimpacted WCA-2A sloughs (9.7 g N m^{-2} yr^{-1}) are higher than similar estimates from other

freshwater marshes (0.01–6.0 g N m^{-2} yr^{-1}), peat bogs (0.05–2.1 g N m^{-2} yr^{-1}), cypress swamps (0.4–2.8 g N m^{-2} yr^{-1}), and within the ranges reported for coastal salt marshes (0.2–15 g N m^{-2} yr^{-1}), and cyanobacterial mats (1.3–76 g N m^{-2} yr^{-1}) (summarized by Howarth et al. (1988)). Measurements of Bahamanian calcifying marine cyanobacterial mats dominated by *Schizothrix* sp. and *Calothrix* sp. show a range of AR rates very similar to those observed in this study (28–561 μmol m^{-2} h^{-1}) (Pinckney et al. 1995). More recent studies of cyanobacterial mats of Belize show fixation rates only slightly lower than those estimated in this study (50–175 μmol m^{-2} h^{-1}) (Rejmánková and Komárková 2000). Thus, it appears that the N_2 fixation rates of WCA-2A floating periphyton mats are typical of calcareous cyanobacterial mats of other tropical, oligotrophic environments.

Organismal characterization

Analysis of the calculated ratios of Light:Dark (L:D) AR may offer some explanation for the observed patterns of WCA-2A AR rates. The response of nitrogenase activity to extremes in light intensity can give a strong indication of the metabolic nature of the diazotrophic organisms present in a microbial community (Fay 1992). In general, fixation rates of organisms with photosynthetically-driven nitrogenase systems (e.g., heterocystous cyanobacteria) are stimulated by light, while other organisms show decreased nitrogenase activity in the presence of photosynthetically-derived oxygen. This latter group of organisms includes heterotrophic bacteria (e.g. *Azotobacter* sp.) or cyanobacteria lacking the structural (i.e., heterocysts) protection of nitrogenase from oxygen. Complicating this issue are microbial assemblages (periphyton or microbial mats) composed of mixtures of nitrogenase types and displaying a variety of diel N_2 fixation patterns and responses to light (Bebout et al. 1993; Zehr et al. 1995; Stal 1995; Bergman et al. 1997). Therefore, L:D AR is presented here only as a means of comparing different N_2 fixing communities of WCA-2A.

Comparisons of AR rates under light and dark conditions demonstrated the light enhancement of unimpacted WCA-2A periphyton N_2 fixation (Table 4). High values of AR were typically observed for floating periphyton and standing plant detritus, whereas ratios closer to 1 were usually observed for benthic floc and water. These results suggest that heterotrophic organisms are largely responsible for the pattern of N_2 fixation in the benthic material, while the predominant N_2 fixation occurring in the periphyton is phototrophic in nature and likely the result of heterocystous cyanobacteria. This conclusion is supported by previous WCA-2A studies (Gleason and Spackman 1974; Swift and Nicholas 1987; McCormick and O'Dell 1996) listing the heterocystous cyanobacterium *Scytonema hoffmannii* as a significant component of the periphyton of oligotrophic areas. Results similar to these were also found for stromatolithic marine microbial mats dominated by *Schizothrix gracilis* where light AR rates greatly exceeded dark rates in incubations similar to those in this study (Pinckney et al. 1995).

The high August L:D AR of the 3.3 km site can also be explained based on species composition. Non-heterocystous cyanobacteria (e.g., *Oscillatoria* and *Lyng-*

Table 4. : Ratios of light acetylene reduction (AR) to dark AR for WCA-2A ecosystem components measured during the August 1998 and February 1999 sampling events. All values represent the mean (standard error) of three replicate observations.

		Ecosystem Component			
		Periphyton	Water	Detritus	Floc
Distance (km)	Community	Light AR: Dark AR			
August 1998					
3.3 km	Slough	10.1 (2.7)	1.4 (0.1)		
	Typha			6.8 (2.6)	1.7 (0.5)
7.0 km	Slough	13.1 (8.5)	0.9 (0.1)		
	Cladium			*0.5 (0.2)*	–
	Typha			*2.6 (1.2)*	*0.7 (0.2)*
14.5 km	Slough	*5.9 (1.5)*	1.2 (0.1)		
	Caldium			1.7 (0.4)	1.3 (0.3)
February 1999					
3.3 km	Slough	0.8 (0.3)	1.4 (0.2)		
	Typha			*4.6 (0.9)*	*0.8 (0.1)*
7.0 km	Slough	0.2 (0.1)	1.2 (0.3)		
	Cladium			1.6 (0.1)	0.8 (0.3)
	Typha			1.5 (0.3)	0.9 (0.4)
14.5 km	Slough	3.3 (0.5)	0.8 (0.1)		
	Cladium			3.0 (1.5)	1.1 (0.4)

bya sp.) have been observed to dominate these eutrophic assemblages (McCormick and O'Dell 1996). Typically, the nitrogenase activity of these microbial communities is enhanced under dark conditions where photosynthetic O_2 production is at a minimum (e.g., Paerl et al. (1989) and Diaz et al. (1990)). However, Paerl et al. (1991) observed simultaneous photosynthetic O_2 production and N_2 fixation in axenic cultures of the non-heterocystous, *Lyngbya aestuarii*, though nitrogenase activity was much lower under these conditions than in similar dark incubations. In that study, microautoradiography revealed that photosynthetic and N_2 fixation activities were spatially separated along individual filaments in a pattern similar to heterocystous cyanobacteria. Other studies have found a predominance of daytime N_2 fixation in periphyton mats of both *Oscillatoria* and *Lyngbya* sp. (reviewed by Bergman et al. (1997)). An additional enhancement of phototrophic N_2 fixation could occur through heterotrophic consumption of photosynthetically-produced O_2 (Paerl 1990). Measurements of diel oxygen production at eutrophic WCA-2A transect stations do reveal an increase in community heterotrophy as compared to reference sites (Belanger et al. 1989; McCormick et al. 1997). Increased heterotrophic oxygen consumption may scavenge available O_2, and thus, enhance N_2 fixation rates of non-heterocystous cyanobacteria in enriched areas of WCA-2A.

Available evidence does not explain the light enhancement of August N_2 fixation at the 7.0 km site. The observed August AR and L:D AR pattern is not consistent with the dominant filamentous green (e.g., *Spirogyra* and *Mougeotia* sp.)

composition documented by previous studies (McCormick and O'Dell 1996). Filamentous green algae are incapable of N_2 fixation; therefore, the AR pattern observed in this study is likely the result of increased N_2 fixation by a cyanobacterial component or may represent a significant bacterial component of the periphyton at this site. The high L:D AR is most indicative of fixation by heterocystous cyanobacteria which may have increased in abundance since the sampling by McCormick et al. in 1994. However, it is also possible that heterotrophic bacteria may be symbiotically fixing N in association with the photosynthetic activity of the green algal periphyton component (Paerl 1990; Steppe et al. 1996; Paerl et al. 1996). In this regard, the results of this study may indicate a further degradation in the species composition of these intermediate periphyton communities (favoring cyanobacterial dominance) or an increased significance of bacterially-mediated N_2 fixation.

Based on differences between the August and February L:D AR, there also appears to be a seasonal variation in the diazotrophic component of the WCA-2A periphyton at both the 3.3 and 7.0 km sites (Table 4). High light AR rates were observed in August, while dark AR predominated in February. This change in light enhancement indicates a shift in the predominant N fixing population of the periphyton from autotrophic in August to more heterotrophic fixation in February. This occurrence could be due to sampling error but is more likely the result of seasonal changes in the species composition of the periphyton communities at these sites where dry season (winter months) senescence of the photosynthetic component is more pronounced in the impacted periphyton assemblages than in the unimpacted periphyton of the WCA-2A interior.

Conclusions

Compared with other ecosystem components, the floating periphyton mat plays a significant role in the N cycle of WCA-2A through its ability to fix atmospheric N_2. The estimated contribution of 9.7 g N m^{-2} y^{-1} to WCA-2A sloughs ranks among the highest rates recorded for aquatic and wetland ecosystems and is similar to rates estimated for other cyanobacterial mat communities. In general, the natural floating periphyton of WCA-2A is similar to marine microbial mat communities of other oligotrophic, calcareous environments. The floating periphyton of WCA-2A are, however, characteristically dominated by heterocystous cyanobacteria. These cyanobacteria give the mat its calcareous structure as well as its characteristic light enhancement of nitrogenase activity.

An apparent seasonal pattern exists in the periphyton of unimpacted areas of WCA-2A areas. Nutrient composition shows a strong relationship between the degree of mat calcification (Ca, Mg, TIC) and the concentration of other periphyton nutrients (TOC, N, P, Fe) as well as in the N stable isotopic composition ($\delta^{15}N$) of the mat material. Maximum mat calcification occurred during the dry season (November–April) and was accompanied by minimum values for TOC, N, P, and Fe.

These trends were reversed during the wet season (May–October). Independent of the pattern of mat calcification and nutrient composition is the peak of periphyton nitrogenase activity in July. Overall, the pattern of N_2 fixation (via AR) corresponded well with the seasonal minimum of $\delta^{15}N$ in the floating mat and, thus, supports the use of natural abundance levels of N stable isotopes to assess nitrogenase activity. The precise explanation of the lack of correlation between the seasonal patterns of nitrogenase activity and nutrient composition is uncertain, however, one hypothesis to explain the seasonal offset is the suppression of summer periphyton activity resulting from high irradiances and temperatures encountered by the mat material floating at the WCA-2A marsh water surface.

Like the floating mats of unimpacted sites, the eutrophic WCA-2A periphyton are characterized by high ratios of Light:Dark AR indicative of phototrophically driven N_2 fixation. In the unimpacted areas, high Light:Dark AR is likely the result of heterocystous cyanobacteria (e.g., *Scytonema* sp.), while no clear explanation exists for the nitrogenase pattern observed for the more eutrophic periphyton types. Despite the enhancement of nitrogenase activity, dense macrophyte cover (and subsequent shading) and reduced periphyton biomass relative to unimpacted marsh sloughs diminishes the importance of this process as a potential input of N in the eutrophic marsh zones. However, increased rates of AR were noted for the standing detritus of *Typha* in the near inflow zones, possibly indicating an increased significance of detrital-associated N_2 fixation in the highly impacted marsh zones.

Questions to be addressed with future research concern a more accurate description of the N_2 fixation of the WCA-2A periphyton mat including a more conclusive identification of the organisms responsible for the observed N_2 fixation pattern and rates. This could be accomplished by characterizing the seasonal effects of a complete diel cycle and through the possible use of molecular probe techniques to identify and quantify the various types of nitrogenase enzyme present. A more accurate assessment of the N_2 fixation associated with the various WCA-2A ecosystem components is also essential to better quantify the relative contribution of each in the WCA-2A N cycle. This would include improved incubation methods (i.e., for the benthic floc material), coupling of weight-based measurements to ecosystem measurements allowing areal-based comparisons, and the calibration of the AR assay (using $^{15}N_2$ incorporation) to determine the actual amount of N being fixed by ecosystem components.

Thus far, the bulk of Everglades research has focused on the importance of P as a limiting nutrient; however, the results of this and previous (e.g., McCormick et al. (1996)) research has shown the increasing importance of N as a limiting nutrient in the P-enriched areas near the inflows. This study highlights the significance of N_2 fixation as a mechanism to overcome potential N limitation of biotic growth and decomposition processes in highly impacted areas of WCA-2A. This study also provides an example of the use of periphyton as an indicator of nutrient impacts to wetland ecosystems. In a similar manner, the enhancement of periphyton nitrogenase activity near the WCA-2A inflows illustrates a potential use of this relatively simple enzyme assay to gauge the impacts of agricultural nutrient loading (Reddy et al. 1999).

Acknowledgements

Florida Agricultural Experiment Station Journal Series No. R-08389. This research was funded in part by the South Florida Water Management District (SFWMD). The authors acknowledge the assistance of Dr. E. D' Angelo, University of Florida (now at University of Kentucky) for initial methods development and to the SFWMD Personnel (J. Backus, C. Kennedy, M. O'Dell) for field sampling assistance, and Y. Wang (Wetland Biogeochemistry Laboratory) for assistance in analysis. Critical reviews by Drs. A. Ogram, D. Sylvia, and three anonymous reviewers greatly improved the quality of the manuscript.

References

Bebout B.M., Fitzpatrick M.W. and Paerl H.W. 1993. Identification of the sources of energy for nitrogen fixation and the physiological characterization of nitrogen-fixing members of a marine microbial mat community. Appl. Environ. Microbiol. 59: 1495–1503.

Belanger T.V., Scheidt D.J. and Platko J.R. II 1989. Effects of nutrient enrichment on the Florida Everglades. Lake and Reservoir Management 5: 101–111.

Bergman B., Gallon J.R., Rai A.N. and Stal L.J. 1997. N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol. Rev. 19: 139–185.

Borchardt M.A. 1996. Nutrients. In: Bothwell R.J., Stevenson M.L. and Lowe R.L. (eds), Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Browder J.A., Gleason P.J. and Swift D.R. 1994. Periphyton in the Everglades: spatial variation, environmental correlates, and ecological implications. In: Davis S.M. and Ogden J.C. (eds), Everglades: The Ecosystem and its Restoration. St. Lucie Press, Delray Beach, Florida, USA.

Craft C.B. and Richardson C.J. 1993. Peat accretion and N, P, and organic C accumulation in nutrient-enriched Everglades peatlands. Ecol. Appl. 3: 446–458.

Craft C.B., Vymazal J. and Richardson C.J. 1995. Response of Everglades plant communities to nitrogen and phosphorus additions. Wetlands 15: 258–271.

Cohen Y. and Rosenberg E. 1989. Microbial Mats: Physiological Ecology of Benthic Microbial Communities. American Society for Microbiology, Washington, DC, USA.

Davis S.M. and Ogden J.C. (eds) 1994. Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Delray Beach, Florida, USA.

DeBusk W.F., Reddy K.R., Koch M.S. and Wang Y. 1994. Spatial distribution of soil nutrients in a northern Everglades marsh: Water Conservation Area 2A. Soil Sci. Sot. Am. J. 58: 543–552.

Diaz M.R., Corredor J.E. and Morell J.M. 1990. Nitrogenase activity of *Microcoleus lyngbyaceus* mat communities in a eutrophic tropical marine environment. Limnol. Oceanogr. 35: 1788–1795.

Fay P. 1992. Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. 56: 340–373.

Flett R.J., Rudd J.W.M. and Hamilton R.D. 1975. Acetylene reduction for nitrogen fixation in freshwaters: a note of caution. Appl. Microbiol. 29: 580–583.

Flora M.D., Walker D.R., Scheidt D.J., Rice R.G. and Landers D.H. 1988. The response of Everglades marsh to increased nitrogen and phosphorus loading. Part I: Nutrient dosing, water chemistry, and periphyton productivity. Everglades National Park, Homestead, FL, USA.

France R., Holmquist J., Chandler M. and Cattaneo A. 1998. $\delta^{15}N$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system. Mar. Ecol. Progr. Ser. 167: 297–299.

Gallon J.R., Pedersen D.R. and Smith G.D. 1993. The effect of temperature on the sensitivity of nitrogenase to oxygen in the cyanobacteria Anabaena cylindrica (Lemmermann) and Gloeothece (Nageli). New Phytol. 124: 251–257.

Gleason P.J. and Spackman W. 1974. Calcareous periphyton and water chemistry in the Everglades. In: Gleason P.J. (ed.), Environments of South Florida: Past and Present, Memoir No. 2. Miami Geological Society, Coral Gables, FL, USA, pp. 225–248.

Grimshaw H.J., Wetzel R.G., Brandenburg M., Segerblom K., Wenkert L.J., Marsh G.A. et al. 1997. Shading of periphyton communities by wetland macrophytes: decoupling of algal photosynthesis from microbial nutrient retention. Arch. Hydrobiol. 139: 17–27.

Gu B. and Alexander V. 1993. Estimation of N_2 fixation based on differences in the natural abundance of ^{15}N among freshwater N_2-fixing and non-N_2-fixing algae. Oecologia 96: 43–48.

Hall G.B. and Rice R.G. 1990. Response of the Everglades marsh to increased nitrogen and phosphorus loading. Part III: Periphyton community dynamics. Everglades National Park, Homestead, FL, USA.

Howarth R.W., Marino R., Lane J. and Cole J.J. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. 33: 669–687.

Joye S.B. and Paerl H.W. 1994. Nitrogen cycling in microbial mats: rates and patterns of denitrification and nitrogen fixation. Mar. Biol. 119: 285–295.

Kendall C., Bemis B., Wankel S., Silva S., Chang C. and Campbell L. 2001. Lessons from the Everglades: atypical isotope patterns in a complex ecosystem. U.S. Geological Survey, Center for Coastal Geology, [Online] http://sofia.usgs.gov/publications/posters/lessons-evergl/print.sshtml.

Koch M.S. and Reddy K.R. 1992. Distribution of soil and plant nutrients along a trophic gradient in the Florida Everglades. Soil Sci. Soc. Am. J. 56: 1492–1499.

Koch M.S., Reddy K.R. and Chanton J.P. 1994. Factors controlling seasonal nutrient profiles in a subtropical peatlands of the Florida Everglades. J. Environ. Qual. 23: 526–533.

Kuo S. 1996. Phosphorus. In: Sparks D.L. (ed.), Methods of Soil Analysis, Part 3: Chemical Analysis. Book Series No. 5. Soil Science Society of America, Madison, WI, USA.

Loeppert R.H. and Suarez D.L. 1995. Carbonate and gypsum. In: Sparks D.L. (ed.), Methods of Soil Analysis, Part 3: Chemical Analysis. Book Series No. 5. Soil Science Society of America, Madison, WI, USA.

McCormick P.V., Chimney M.J. and Swift D.R. 1997. Diel oxygen profiles and water column community metabolism in the Florida Everglades, U.S.A. Arch. Hyrobiol. 140: 117–129.

McCormick P.V. and O'Dell M.B. 1996. Quantifying periphton responses to phosphorus in the Florida Everglades: a synoptic-experimental approach. J. N. Am. Bethol. Soc. 151: 450–468.

McCormick P.V., Rawlik P.S., Lurding K., Smith E.P. and Sklar F.H. 1996. Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. J. N. Am. Bethol. Soc. 15: 433–449.

McCormick P.V., Shuford R.B.E. III, Backus J.B. and Kennedy W.C. 1998. Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, USA. Hydrobiol. 362: 185–208.

Paerl H.W. 1990. Physiological ecology and regulation of N_2 fixation in natural waters. Adv. Microb. Ecol. 11: 305–344.

Paerl H.W., Bebout B.M. and Prufert L.E. 1989. Naturally occurring patterns of oxygenic photosynthesis and N_2 fixation in a marine microbial mat: physiological and ecological ramifications. In: Cohen Y. and Rosenberg E. (eds), Microbial Mats: Physiological Ecology of Benthic Microbial Communities. American Society for Microbiology, Washington, DC, USA.

Paerl H.W., Fitzpatrick M. and Bebout B.M. 1996. Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. Limnol. Oceanogr. 41: 419–427.

Paerl H.W., Joye S.B. and Fitzpatrick M. 1993. Evaluation of nutrient limitation of CO_2 and N_2 fixation in marine microbial mats. Mar. Ecol. Prog. Ser. 101: 297–306.

Paerl H.W., Prufert L.E. and Ambrose W.W. 1991. Contemporaneus N_2 fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium, *Lyngbya aestuarii*. Appl. Env. Micro. 57: 3086–3092.

Pinckney J., Paerl H.W., Reid R.P. and Bebout B. 1995. Ecophysiology of stromatolithic microbial mats, Stocking Island, Exuma Cays, Bahamas. Microb. Ecol. 29: 19–37.

Rader R.B. and Richardson C.J. 1992. The effects of nutrient enrichment on algae and macroinvertebrates in the Everglades: A review. Wetlands 12: 121–135.

Reddy K.R., DeLaune R.D., DeBusk W.F. and Koch M.S. 1993. Long term nutrient accumulation rates in the Everglades. Soil Sci. Soc. Am. J. 57: 1147–1155.

Reddy K.R., Wang Y., DeBusk W.F., Fisher M.M. and Newman S. 1998. Forms of soil phosphorus in selected hydrologic units of the Florida Everglades. Soil Sci. Soc. Am. J. 62: 1134–1147.

Reddy K.R., White J.R., Wright A. and Chua T. 1999. Influence of phosphorus loading on microbial processes in the soil and water column of wetlands. In: Reddy K.R., O'Connor G.A. and Schelske C.L. (eds), Phosphorus Biogeochemistry in Subtropical Ecosystems. CRC Press LLC, Boca Raton, FL, USA, pp. 249–273.

Rejmánková E. and Komárková J. 2000. A function of cyanobacterial mats in phosphorus-limited tropical wetlands. Hydrobiol. 431: 135–153.

Scinto L. 1997. Phosphorus cycling in a periphyton-dominated freshwater wetland. PhD Dissertation, University of Florida, Gainesville, USA.

Smith V. 1983. Low nitrogen-phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669–671.

Stal L.J. 1988. Nitrogen fixation in cyanobacterial mats. Methods of Enzymology 167: 474–484.

Stal L.J. 1995. Physiological ecology of cyanobacteria in microbial mats and other communities: Tansley Review No. 84. New Phytol 131: 1–32.

Steppe T.F., Olson J.B., Paerl H.W., Litaker R.W. and Belnap J. 1996. Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microb. Ecol 21: 149–156.

Swift D.R. 1984. Periphyton and water quality relationships in Everglades water conservation areas. Technical Publ. 87-2. South Florida Water Management District, West Palm Beach, Florida, USA.

Swift D.R. and Nicholas R.B. 1987. Periphyton and water quality relationships in the Everglades Water Conservation Areas, 1978–1982. Technical Publication 87-2. South Florida Water Management District, West Palm Beach, FL, USA.

Vymazal J. 1995. Algae and Element Cycling in Wetlands. CRC Press, Inc., Boca Raton, FL, USA.

Vymazal J. and Richardson C.J. 1995. Species composition, biomass, and nutrient content of periphyton in the Florida Everglades. J. Phycol 31: 343–354.

Vaithiyanathan P. and Richardson C.J. 1997. Nutrient profiles in the Everglades: examination along the eutrophication gradient. The Science of the Total Environment 205: 81–95.

Yamamuro M., Kayanne H. and Minagawa M. 1995. Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. Limnol. Oceanogr. 40: 617–621.

Zehr P.J., Mellon M., Braun S., Litaker W., Steppe T. and Paerl H.W. 1995.Appl. Environ. Microbiol. 61: 2527–2532.