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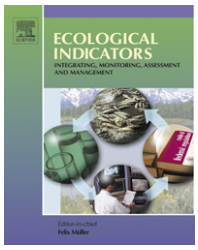

Isotopic indicators of environmental change in a subtropical wetland

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ABSTRACT

The $\delta^{15}N$ and $\delta^{13}C$ signatures of major organic matter (OM) pools were measured across chemical and hydrologic gradients in a large (58,800 ha) subtropical wetland to evaluate whether stable isotopes were useful indicators of environmental change. Once a rainfall-driven wetland, the Loxahatchee National Wildlife Refuge in the Florida Everglades now receives agricultural and urban drainage that has increased phosphorus (P) and mineral loads around the wetland perimeter. Additionally, water impoundment at the southern end has produced a latitudinal hydrologic gradient, with extended hydroperiods in the south and overdrained conditions in the north.

Detritus (−4.8‰ to 8.6‰), floc (−1.4‰ to 3.6‰), and metaphyton (−6.6‰ to +7.4‰) $\delta^{15}N$ declined southward with changes in hydrology as indicated by water depth. This pattern was attributed to higher mineralization rates under shorter hydroperiods. These signatures were also strongly correlated with increased nutrient and mineral loading. Rooted macrophyte $\delta^{15}N$, by contrast, appeared more responsive to soil nutrient pools. Cattail (−8.9‰ to +7.7‰) was restricted to the wetland perimeter and had the widest $\delta^{15}N$ range, which was positively correlated with soil P. Sawgrass (−5.3‰ to +7.7‰) occurred across most of the wetland, but its $\delta^{15}N$ was not strongly correlated to any gradient. Patterns for $\delta^{13}C$ were more strongly related to chemical gradients caused by canal intrusion than to latitude or hydrology. Again, metaphyton and detrital signatures were more sensitive to water chemistry changes than macrophytes. This pattern is consistent with their locations at the soil–water (detritus-floc), and air–water (metaphyton) interface. Metaphyton $\delta^{13}C$ (−36.1‰ to −21.5‰) which had the broadest range, was affected by DIC source and pool size. In contrast, cattail $\delta^{13}C$ (−28.7‰ to −26.4‰) was more closely related to soil P and sawgrass $\delta^{13}C$ (−30.1‰ to −24.5‰) was not related to any environmental gradient except latitude. There was no correlation between the two isotopes for any OM pool except cattail.

These results indicate that isotopic signatures of microbial (metaphyton and detrital) pools are more responsive to changes in wetland hydrology and water chemistry while those of rooted macrophytes respond only to the extent that soil chemistry is altered. Rooted macrophytes also differ in the sensitivity of their isotopic signatures to environmental change. The selection of OM pools for isotopic analysis will, therefore, affect the sensitivity of the analysis and the resulting patterns. Furthermore, $\delta^{15}N$ may be more robust and interpretable than $\delta^{13}C$ as an indicator of ecosystem change in wetlands exposed to multiple or complex anthropogenic gradients.

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1. Introduction

Stable isotopes are increasingly used to detect and understand causes of environmental change. They can integrate over long and short spatial and time scales, and have been used both to monitor ecosystem change and to make specific connections between ecology, land use, and geochemistry (Fry, 2006). In aquatic ecosystems, carbon (C) and nitrogen (N) isotopic signatures of organic matter (OM) have been used to detect changes in plant and microbial processes related to anthropogenic disturbance gradients. Heavier $\delta^{13}C$ values in plant tissue have been associated with increased nutrient availability and other conditions that stimulate photosynthetic rates (Grice et al., 1996; Fourquerean et al., 2005), stomatal conductance (reviewed by Dawson et al., 2002), internal CO_2 recycling (Grice et al., 1996), and elemental ratios (C:N, C:P, N:P) (Fourquerean et al., 2005). The $\delta^{15}N$ values vary among natural and anthropogenic (fertilizer, sewage, and manure) N sources (Kreitler, 1975, 1979; Kreitler and Jones, 1975; Shearer et al., 1978; Kohl et al., 1971; Heaton, 1986; Mariotti et al., 1982; Korom, 1992), and, consequently, plant and algal $\delta^{15}N$ signatures can reflect the relative contribution of these different sources when this nutrient is limiting (Grice et al., 1996; Elliott and Brush, 2006). The isotopic signature of detritus also is influenced by the rate of biogeochemical processes such as mineralization ($\delta^{15}N$ and $\delta^{13}C$) and denitrification ($\delta^{15}N$) (Hecky and Hesslein, 1995; Fogel and Turos, 1999; Fernandez et al., 2003; Fry, 2006).

Attempts to use stable isotopes as environmental markers have had mixed results, as the resulting signatures depend on the material being analyzed, the isotope, and the surrounding environmental conditions. For example, Jones et al. (2004) determined that the $\delta^{15}N$ of sediment and epilithon, but not macrophytes, was positively related to lake-water dissolved inorganic N (DIN) concentrations and was heaviest for N-limited lakes. Likewise, Cole et al. (2004), found macroalgae were better indicators of wastewater inputs than vascular plants and DIN concentrations. This was evident by higher $\delta^{15}N$ values as the percent of wastewater N increased from 4% to 8% in waters from eleven fresh and saline water bodies. Nitrogen and carbon stable isotopes often respond differently to the same environmental variable. Such differences were shown by McKee et al. (2002), who found that while the $\delta^{15}N$ of mangrove leaves increased along a gradient that shifted from P to N-limitation, $\delta^{13}C$ values were independent of nutrient limitation and were affected by stomatal conductance or carboxylation. Plants can discriminate against the heavy isotope when N availability is high (Dawson et al., 2002; Peterson and Fry, 1987; Pennock et al., 1996; Waser et al., 1998; Evans, 2001). For example, Evans et al. (1996) showed that the $\delta^{15}N$ of source and plants were similar only at low NH_4^+ concentrations, when the entire pool of N is assimilated, and Lake et al. (2001) reported that the $\delta^{15}N$ of plants was a useful indicator of eutrophication only when DIN concentrations were low.

Many stable isotope studies have focused on understanding the influence of specific anthropogenic and natural factors on various biotic components (e.g., Grice et al., 1996; Costanzo et al., 2003; Elliott and Brush, 2006). In contrast, our study was designed to evaluate the use of isotopes as an integrative measure of environmental change and to detect ecosystem responses to major anthropogenic environmental gradients in a large subtropical peatland, the Florida Everglades. We measured $\delta^{15}N$ and $\delta^{13}C$ in major OM pools across the northern part of this ecosystem and assessed their ability to detect changes across known environmental gradients. The sensitivity and consistency of isotopic patterns were evaluated in order to assess the utility of these metrics to detect different aspects of anthropogenic change.

2. Study location

The Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge), also known as WCA 1, occupies the northernmost 58,800 ha of the Everglades (Fig. 1). It is bounded by canals that convey discharges from Lake Okeechobee and the Everglades Agricultural Area (EAA) southward through the Everglades and to urban areas along the southeastern coast. Canal discharges into the rim canals occur at several locations (Fig. 1) and account for approximately 42% of the water entering the Refuge, with rainfall accounting for the remainder (Newman et al., 1997). The intrusion of canal waters into the Refuge has altered hydrology and nutrient and mineral concentrations around its perimeter. By contrast, environmental conditions remain strongly influenced by rainfall across the interior of the Refuge. A levee at the southern end of the Refuge allows this area to be managed as an impoundment. This has resulted in water ponding in areas of low elevation in the south and shortened hydroperiods in higher elevation areas to the north. These various hydrologic and water chemistry gradients are associated with changes in soils and vegetation. For instance, a gradient in soil P and minerals exists across the western side of the Refuge, where most canal water intrusion occurs (Doren et al., 1996; Newman et al., 1997; Corstanje et al., 2006). A more gradual decline in these soil parameters occurs from north to south (Corstanje et al., 2006). Sloughs, wet prairies, and tree islands comprise much of the habitat in the interior of the Refuge while sawgrass (*Cladium jamaicense*) is increasingly dominant in areas to the south and towards the perimeter of the Refuge. Dense stands of cattail (*Typha domingensis*) occupy a much narrower fringe adjacent to the canals, especially along the western edge.

3. Methods

3.1. Field sampling

Sampling followed a stratified-random sampling design, with strata defined based on proximity to the perimeter canals (Fig. 1). Eighty-five sites were selected randomly within a 4-km band around the perimeter of the Refuge where the steepest hydrologic and water quality gradients occur. An additional 22 sites were selected randomly in the interior of the Refuge (beyond the 4 km perimeter band) and with a minimum distance of 2 km between any pair of sites. Also included were 23 long-term monitoring sites for which extensive water chemistry information is available.

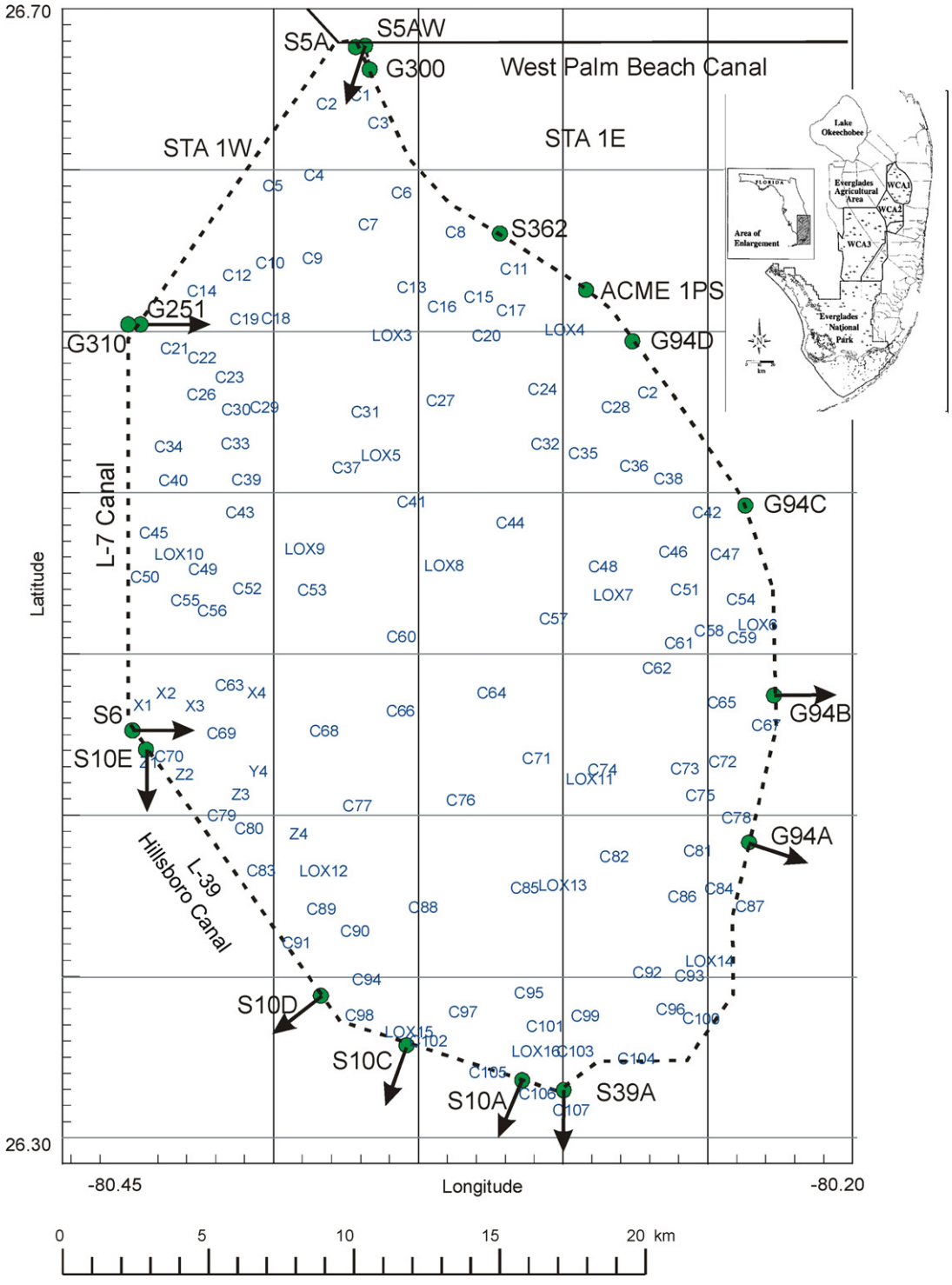

Fig. 1 – Location of the A.R.M. Loxahatchee National Wildlife Refuge within the Everglades of south Florida and the location of sampling locations within the Refuge. Circles around the perimeter of the Refuge show the water-control structures that regulate canal flows. Arrows indicate major inflow and discharge points.

In February 2004, plant, metaphyton (floating periphyton mat), detrital and water samples were collected across this sampling network. Grab samples of detritus, defined here as the surface soil layer containing highly decomposed plant material, were collected to a depth of ~5 cm just inside a stand of the dominant emergent macrophyte. Live leaves of each macrophyte were also collected. Sawgrass was the dominant species at 100 of the sites and throughout the Refuge interior. Cattail was dominant at 12 sites adjacent to the rim canals, and both sawgrass and cattail were common at 18 sites, where both species were sampled. Floc, an unconsolidated layer of plant and algal detritus and some live algae at the soil surface

in sloughs and wet prairies, was collected to a depth of ~5 cm using a fine-mesh dip net. Floc was collected at 60 sites where slough-wet prairie habitat was present and this layer was sufficiently deep to sample. Metaphyton was collected by dip net in slough-wet prairie habitat at 111 sites where it was present in sufficient quantity. All samples were temporarily stored at 4 °C and then frozen for later analysis.

Water depth was measured at each site using a meter stick and specific conductance and pH were measured using pre-calibrated Hydrolab and YSI data loggers and probes. Specific conductance was used to indicate the extent of intrusion of high conductivity (~1000 μS cm^{-1}) canal water into this low conductivity (~100–200 μS cm^{-1}) wetland at each sampling location.

3.2. Dissolved inorganic carbon (DIC) collection

Water for DIC analyses was collected in 40 mL clear EPA VOC vials with open-top septa-lined caps, triple rinsed in DI water and dried in a particle-free atmosphere. After drying, 5–10 mg of blue copper sulfate, which served as a bactericide, was added to each vial. A water sample was slowly drawn into a 60 mL syringe, taking care to exclude or remove bubbles and headspace. A 25-mm syringe filter (Whatman Polysulfone GD/X) containing a 10-μm prefilter and GF/F 0.7-μm filter) was then attached to the syringe, and 10 mL of water was pushed through the filter as a rinse. The remaining sample was slowly pushed into the vial until completely full and a positive (convex) meniscus formed over the vial opening. The cap was then screwed on until the septa popped slightly up, and the vials were chilled until analysis.

3.3. Laboratory analyses

A portion of each detritus sample was processed to determine soil concentrations of N, P, and calcium (Ca), which was used as an indicator of soil mineral concentration. All of these elements are present in higher concentrations in canal water than in surface waters in the Refuge. Nitrogen was measured by combustion analysis and P and Ca were measured using inductively coupled plasma spectroscopy.

Plant material was freeze-dried and ground to a fine powder with a ball mill. Approximately 18 mg of each sample was weighed into 11.5 mm × 7 mm silver capsules. Following the method of Yamamuro and Kayanne (1995), samples were acidified prior to analysis to eliminate fine-grained carbonate sediments and calcareous algae. This procedure showed no loss of organic C or N while quantitatively removing the carbonate. Carbon and N analysis runs were prepared with an EDTA working standard. Blanks consisting of empty silver capsules were run at the beginning and end of 50 sample runs. Samples were analyzed on a Carlo Erba 1500 elemental analyzer attached to a Micromass Optima Isotope Ratio Mass Spectrometer.

Nitrogen and C isotopic compositions were expressed in per mil relative to international standards of atmospheric air and Vienna PEE Dee Belemite (VPDB) respectively as described below:

$$\delta^{15}N_{Air} = \left\{ \left[\frac{(^{15}N/^{14}N)_X}{(^{15}N/^{14}N)_{Air}} \right] - 1 \right\} \times 1000$$

and

$$\delta^{13}C_{VPDB} = \left\{ \left[\frac{(^{13}C/^{12}C)_X}{(^{13}C/^{12}C)_{VPDB}} \right] - 1 \right\} \times 1000$$

In these equations, 'X' is the sample, 'Air' is the international N standard, and 'VPDB' is the international C standard. The analytical precision for our standards was approximately 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Concentrations and C isotopic composition of DIC were obtained using an OI Analytical 1010 TIC/TOC analyzer interfaced with a Micromass IsoPrime continuous flow mass spectrometer according to a method modified after St-Jean (2003). Sample vials sealed with open-top septa caps were loaded directly into the autosampler of the TIC/TOC analyzer. Inorganic and organic C concentrations from the TIC/TOC analyzer reagents ranged between 0.01 and 0.05 mg C L^{-1}, which is an order of magnitude lower than the precision of the C concentration measurements. Analysis of laboratory and field blanks of deionized water filtered through the syringe filters revealed no contribution of DOC from the syringe, filter cartridges, nor the combusted sample vials (0.04 ± 0.01 mg C L^{-1}, $n = 10$). Not all stations sampled for DIC were analyzed for $\delta^{13}C$ of DIC due to equipment malfunction. The analytical precision for DIC-$\delta^{13}C$ was ±0.5‰ determined from duplicate samples.

3.4. Statistical analysis

Data were analyzed using non-parametric statistics, which are insensitive to the distribution of the data (Hollander and Wolfe, 1999). The strength of relationships between environmental and isotopic parameters was determined using Spearman rank correlation coefficients. The distribution of isotopic data was evaluated using box plots, which showed the central tendency (median) and dispersion (quartiles) for isotope values for each OM pool.

4. Results

4.1. Environmental gradients

A major hydrologic gradient in the Refuge was a predictable increase in water depth and, by inference, hydroperiod, from north to south (Fig. 2). Water depths at the northern end of the Refuge at the time of sampling were generally <0.2 m while depths at the southern end often exceeded 1 m. This pattern was directly related to the north–south elevation gradient as indicated by a strong correlation between depth and latitude ($r = -0.791$, $p < 0.001$, $n = 130$). Water was deepest along the southwestern perimeter (0.59–1.50 m) and shallowest in the northwestern portion the Refuge (0.12–0.29 m). There was no consistent pattern for water depth with longitude ($r = 0.099$, $p > 0.050$, $n = 130$).

Specific conductance, an indicator of canal influences on water chemistry, also exhibited more than a 10-fold difference across the Refuge (Fig. 2), but was more strongly correlated with longitude ($r = -0.648$, $p < 0.001$, $n = 130$) than with latitude ($r = 0.233$, $p = 0.008$, $n = 130$) as a result of canal

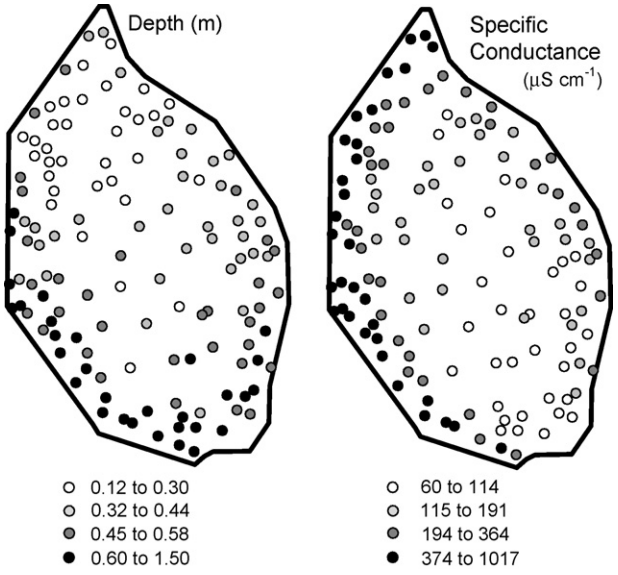

Fig. 2 – Spatial variation in water depth and specific conductance across the Refuge. Data for each parameter were divided into quartiles as shown in the legends below each image.

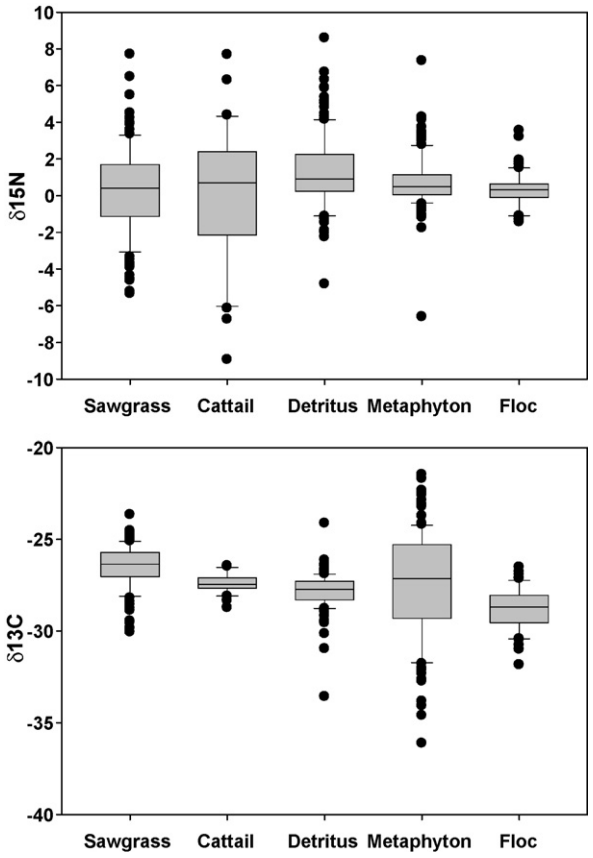

Fig. 3 – Boxplots showing variation in $\delta^{15}N$ and $\delta^{13}C$ for each OM pool. The top, mid-line, and bottom of each box represent the 75th, 50th (median), and 25th percentiles of data, respectively. The vertical lines represent the 90th and 10th percentiles. Closed circles are observations outside the 90th and 10th percentiles.

intrusion across the western perimeter. The influence of this intrusion on longitudinal trends in conductance was more clearly evidenced ($r = -0.765$, $p < 0.001$, $n = 66$) when sites along the eastern side of the Refuge (east of longitude −80.30), which also have some exposure to mineralized canal water, were removed from the analysis.

Chemical gradients in the surface soils (detritus samples) exhibited varying degrees of correlation with each other and with surface-water conductance (Table 1). Calcium and P concentrations ranged between 5.80 and 99.5 and 0.2 and 4.8 g kg^{-1}, respectively, and were strongly correlated with each other and with conductance. Nitrogen concentrations exhibited considerably less variation among sampling sites, with concentrations ranging between 16.90 and 50.50 g kg^{-1}, and were not correlated with any gradient.

4.2. *Patterns for $\delta^{15}N$*

All of the OM pools sampled showed a wide range of $\delta^{15}N$ across sampling sites, indicating the presence of strong environmental gradients (Figs. 3 and 4) The range of variation in $\delta^{15}N$ was similar for detritus (−4.8‰ to 8.6‰), sawgrass (−5.3‰ to +7.7‰), and metaphyton (−6.6‰ to +7.4‰), while floc samples showed a more limited range (−1.4‰ to +3.65‰). Detritus $\delta^{15}N$ was positively correlated with floc ($r = 0.651$, $p < 0.001$, $n = 60$) and metaphyton $\delta^{15}N$ ($r = 0.656$, $p < 0.001$, $n = 111$), and floc and metaphyton values were less strongly related ($r = 0.511$, $p = 0.002$, $n = 59$). Variation in sawgrass $\delta^{15}N$ was weakly correlated with detritus values ($r = 0.205$, $p = 0.032$, $n = 109$) but was not correlated with $\delta^{15}N$ from other OM pools, indicating a unique response to variation in environmental

Table 1 – Relationships (Spearman rank correlation coefficients) between environmental gradients measured in this study. Statistical significance is shown in parentheses next to each coefficient. Boldfaced values are significant at the $p < 0.05$ level.

	Depth	Sp Cond	Soil Ca	Soil P	Soil N
Latitude	**−0.791** (<**0.001**)	**0.233** (**0.008**)	0.150 (0.094)	0.072 (0.421)	0.159 (0.077)
Longitude	0.099 (0.261)	**−0.648** (<**0.001**)	**−0.542** (<**0.001**)	**−0.376** (<**0.001**)	−0.056 (0.537)
Depth		0.129 (0.144)	0.119 (0.186)	0.133 (0.140)	−0.084 (.352)
Sp Cond			**0.874** (<**0.001**)	**0.602** (<**0.001**)	0.086 (0.338)
Soil Ca				**0.559** (<**0.001**)	−0.013 (0.884)
Soil P					0.121 (0.180)

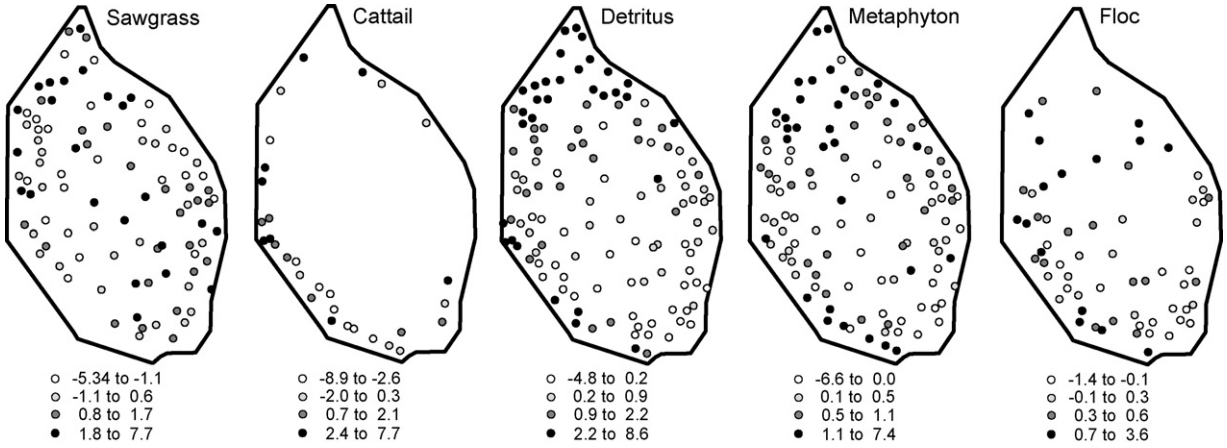

Fig. 4 – Spatial variation in $\delta^{15}N$ signatures for each OM pool across the Refuge. Data for each pool were divided into quartiles as shown in the legends below each image.

conditions across the Refuge. Despite its limited spatial distribution, $\delta^{15}N$ values for cattail varied more than for any other OM pool, ranging from −8.9‰ to +7.7‰. Cattail $\delta^{15}N$ was positively correlated with sawgrass $\delta^{15}N$ at sites where both species were sampled ($r = 0.630$, $p = 0.005$, $n = 18$), suggesting similar responses to environmental variation for these two macrophyte species. As for sawgrass, cattail values were not strongly correlated with the $\delta^{15}N$ from other pools. Furthermore, detritus and floc $\delta^{15}N$ were similar between stations containing sawgrass and cattail, indicating little influence of vegetation type on detrital signatures.

Detritus, floc, and metaphyton $\delta^{15}N$ showed a north–south decline across the Refuge that was associated with changes in hydrology as indicated by water depth (Table 2). This trend was strongest for the detritus layer and was more pronounced for all 3 pools when cattail-dominated sites near the edge of the Refuge were excluded from the analysis. Cattail $\delta^{15}N$ was positively correlated with latitude as well, but not with depth, and this relationship with latitude was largely determined by low values at 6 stations at the southern tip of the Refuge. Sawgrass $\delta^{15}N$ showed no relationship to this latitudinal gradient.

Relationships between $\delta^{15}N$ signatures and changes in water chemistry caused by canal-water intrusion also were evident although less pronounced than the latitudinal gradient. Detritus, floc, metaphyton, and cattail $\delta^{15}N$ values were significantly correlated with specific conductance (Table 2) while those for sawgrass were not. Independent responses of isotopic signatures in individual OM pools to latitude and conductance gradients were more clearly identified by a reanalysis of the data that excluded sites at the southern (south of latitude 26.405) and northern (north of latitude 26.599) perimeter of the Refuge, which were either extremely dry or extremely wet. There was no correlation between latitude and conductance for the remaining 90 stations ($r = 0.089$, $p > 0.05$) while latitude and depth were still strongly correlated ($r = -0.749$, $p < 0.001$). Across these stations, detritus, floc, and metaphyton $\delta^{15}N$ were most strongly correlated with latitude ($r = 0.416$–0.738, $p \leq 0.001$) and depth ($r = -0.330$ to -0.608, $p \leq 0.004$) while cattail $\delta^{15}N$ was significantly correlated with conductance ($r = 0.638$, $p = 0.019$).

Signatures for all OM pools except sawgrass also were significantly correlated with multiple soil chemical gradients caused by canal intrusion (Table 2). Correlations between detritus, floc, and metaphyton $\delta^{15}N$ and most or all measured soil gradients were significant, suggesting a general response to intrusion. Cattail $\delta^{15}N$ was correlated most strongly with soil P. Sawgrass $\delta^{15}N$ was weakly correlated with soil P.

Spatial variation in water depth and water and soil chemistry was greatest at the perimeter of the Refuge and lowest in the interior. Comparison of $\delta^{15}N$ values at the 33 stations closest to the perimeter with those at 28 interior stations showed 2–3-fold greater variation in detritus, floc, and metaphyton $\delta^{15}N$ at the perimeter, further illustrating the responsiveness of these signatures to environmental gradients. Variation in these pools around the perimeter was positively correlated ($p < 0.05$) with specific conductance and soil chemistry gradients but not latitude or water depth. By contrast, the range of sawgrass $\delta^{15}N$ values was identical between interior and perimeter sites, providing further evidence that sawgrass N signatures were insensitive to these major environmental gradients. However, sawgrass values exhibited a strong positive correlation with P at perimeter sites and were negatively correlated with conductance and Ca in the interior.

4.3. *Patterns for $\delta^{13}C$*

The range of $\delta^{13}C$ values varied among OM pools, with metaphyton having the greatest range (−36.1‰ to −21.5‰) and cattail having an extremely narrow range (−28.7‰ to −26.4‰) (Fig. 5). Metaphyton $\delta^{13}C$ was positively correlated with values for floc ($r = 0.417$, $p = 0.001$, $n = 59$), detritus ($r = 0.282$, $p = 0.003$, $n = 112$), and cattail ($r = 0.433$, $p = 0.034$, $n = 24$), and detritus and floc values also were correlated ($r = 0.348$, $p = 0.006$, $n = 60$). Cattail and sawgrass $\delta^{13}C$ were weakly correlated ($r = 0.416$, $p = 0.086$, $n = 18$). Detritus $\delta^{13}C$ values were lightest at stations dominated by cattails (−33.6‰ to −27.4‰) compared with those dominated by sawgrass (−29.5‰ to −24.1‰). The same

Table 2 – Relationships (Spearman rank correlation coefficients) between environmental gradients and isotope signatures of different OM pools. Statistical significance is shown in parentheses next to each coefficient. Boldfaced values are significant at the $p < 0.05$ level.

	15N					13C				
	Sawgrass	Cattail	Detritus	Metaphyton	Floc	Sawgrass	Cattail	Detritus	Metaphyton	Floc
Latitude	0.091 (0.335)	**0.451 (0.012)**	**0.633 (<0.001)**	**0.380 (<0.001)**	**0.402 (0.001)**	**−0.233 (0.012)**	−0.299 (0.109)	−0.080 (0.380)	0.126 (0.176)	0.168 (0.192)
Depth	−0.029 (0.758)	−0.023 (0.903)	**−0.388 (<0.001)**	**−0.241 (0.009)**	**−0.262 (0.040)**	0.147 (0.116)	0.153 (0.420)	−0.040 (0.664)	**−0.195 (0.035)**	**−0.310 (0.014)**
Sp Cond	−0.027 (0.777)	**0.449 (0.013)**	**0.573 (<0.001)**	**0.332 (<0.001)**	**0.512 (<0.001)**	−0.007 (0.940)	−0.123 (0.518)	**−0.347 (<0.001)**	**−0.475 (<0.001)**	**−0.516 (<0.001)**
Soil Ca	−0.056 (0.554)	0.175 (0.392)	**0.486 (<0.001)**	**0.298 (0.001)**	**0.389 (0.002)**	0.104 (0.913)	−0.020 (0.925)	**−0.347 (<0.001)**	**−0.483 (<0.001)**	**−0.482 (<0.001)**
Soil P	**0.192 (0.042)**	**0.631 (<0.001)**	**0.370 (<0.001)**	**0.231 (0.013)**	**0.345 (0.006)**	−0.062 (0.514)	**−0.456 (0.019)**	**−0.480 (<0.001)**	**−0.486 (<0.001)**	**−0.523 (<0.001)**
Soil N	0.063 (0.509)	0.234 (0.251)	**0.276 (0.002)**	0.116 (0.220)	**0.360 (0.004)**	0.027 (0.779)	−0.277 (0.170)	**−0.219 (0.016)**	0.107 (0.256)	0.036 (0.779)

pattern was observed for floc $\delta^{13}C$, but the differences were not as great.

The $\delta^{13}C$ signatures of OM pools were more strongly related to chemical gradients caused by canal intrusion than to the latitudinal gradient in hydrology (Table 2). Sawgrass $\delta^{13}C$ was weakly correlated with latitude, but was not correlated with water depth, while metaphyton and floc $\delta^{13}C$ were weakly correlated with depth. Cattail $\delta^{13}C$ was negatively correlated with soil P, while sawgrass $\delta^{13}C$ was not related to any chemical gradient. In contrast, detritus, floc, and metaphyton $\delta^{13}C$ were negatively correlated with specific conductance. The magnitude of change with increasing conductance across this gradient was greatest for metaphyton (~5‰), intermediate for floc (~2‰), and relatively small for detritus (1‰).

Variation in $\delta^{13}C$ values of detritus and metaphyton at the perimeter were 4- and 2-fold greater, respectively, than at the most interior sites while the range of values for floc and sawgrass was similar in the two areas. Detritus and metaphyton values were negatively correlated with conductance and/or soil chemistry gradients both at the perimeter and in the interior. Floc values also were negatively correlated with these gradients in the interior but not at the perimeter. Sawgrass $\delta^{13}C$ was significantly correlated with latitude (−), depth (+), and soil N (+) at perimeter sites and to conductance (+) and depth (−) in the interior.

4.4. *Relationship between $\delta^{15}N$ and $\delta^{13}C$*

Dual isotope plots of $\delta^{15}N$ and $\delta^{13}C$ (Fig. 6) were constructed for each OM pool by tracing a field that included at least 90% of the data for each variable (detritus 92%, sawgrass 95%, floc 97% and metaphyton 97%, cattail 100%). These plots showed a lack of strong correlation between $\delta^{15}N$ and $\delta^{13}C$ for most OM pools, indicating that these 2 signatures showed different response patterns across the Refuge. However, cattail $\delta^{15}N$ and $\delta^{13}C$ were significantly correlated ($r = -0.628$, $p < 0.001$, $n = 30$), while these isotopes in detritus showed a weaker correlation ($r = -0.337$, $p < 0.001$, $n = 122$).

4.5. *Relations between DIC and $\delta^{13}C$ of DIC*

Instrument problems prevented us from obtaining a complete set of DIC samples, thus limiting our ability to assess changes in this parameter across the full range of environmental conditions documented in the Refuge. In particular, we have no samples from the western perimeter of the Refuge where specific conductance was highest. Of the samples analyzed, DIC concentrations (1.07–27.63) were highest in the southwestern corner of the Refuge, which is heavily impacted by canal flows, and along the eastern perimeter where exposure is more modest (Fig. 7). Concentrations were strongly correlated with and proportional to specific conductance ($r = 0.837$, $p < 0.001$, $n = 54$) and pH ($r = 0.743$, $p < 0.001$, $n = 45$), indicating the importance of canal waters in supplying DIC to perimeter areas of the Refuge.

The $\delta^{13}C$ of DIC (−4.9‰ to −21.2‰) exhibited a spatial patterns similar to that for DIC (Fig. 7). The $\delta^{13}C$ values were positively correlated with DIC concentrations ($r = 0.705$, $p < 0.001$, $n = 53$), specific conductance ($r = 0.746$, $p < 0.001$, $n = 53$) and pH ($r = 0.714$, $p < 0.001$, $n = 44$). A sharp increase in

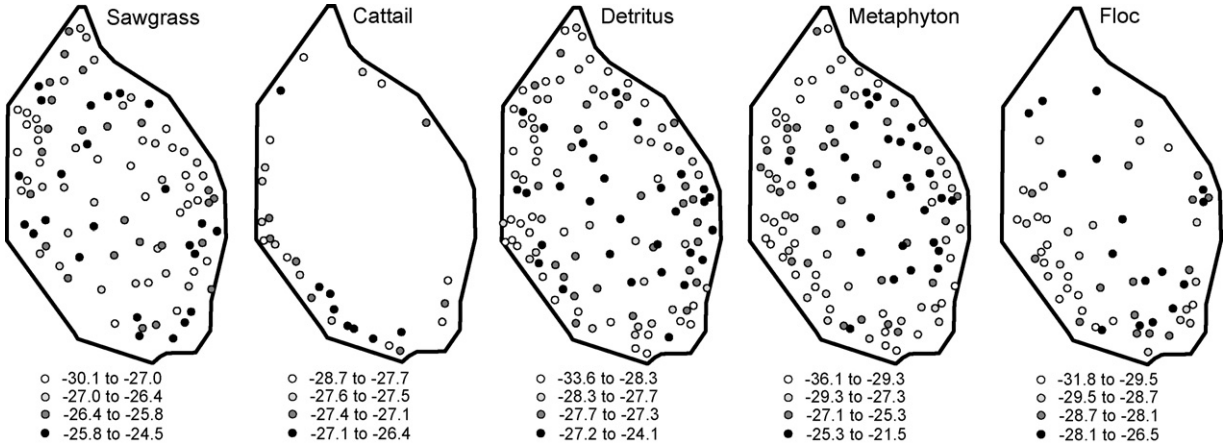

Fig. 5 – Spatial variation in $\delta^{13}C$ signatures for each OM pool across the Refuge. Data for each pool were divided into quartiles as shown in the legends below each image.

DIC $\delta^{13}C$ occurred between sites with background conductance (~100 μS cm^{-1}) and those with slightly elevated conductance (~200 μS cm^{-1}), while values were similar for sites with higher conductance levels.

The $\delta^{13}C$ of floc ($r = -0.670$, $p < 0.001$, $n = 24$), metaphyton ($r = -0.411$, $p = 0.002$, $n = 53$), and detritus ($r = -0.279$, $p = 0.043$, $n = 53$) were negatively correlated with DIC concentrations.

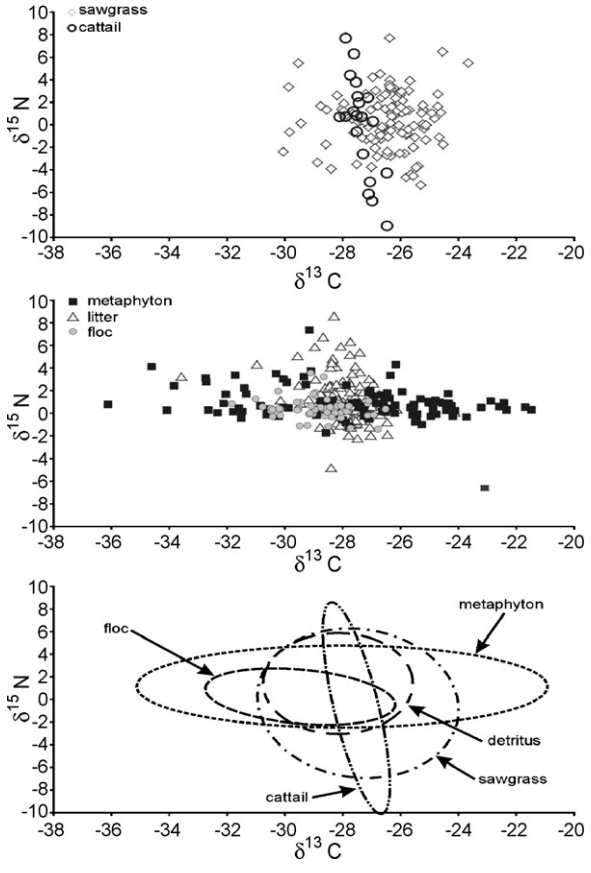

Fig. 6 – Relationships between spatial variation in $\delta^{13}C$ and $\delta^{15}N$ signatures for each OM pool. See Section 3 for details.

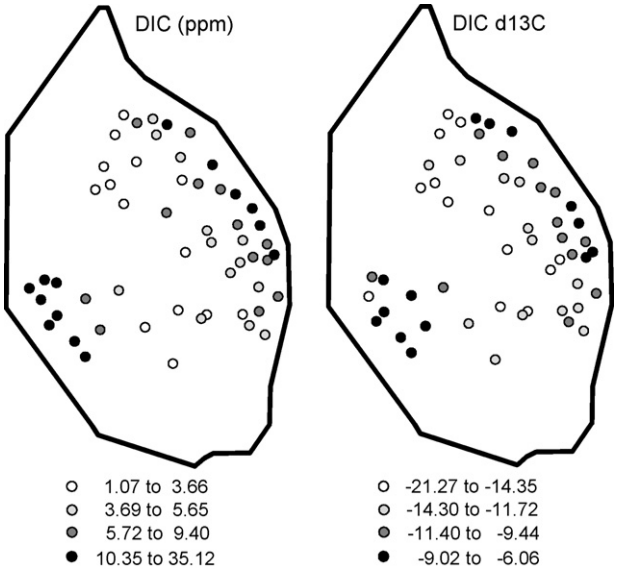

Fig. 7 – Spatial variation in water-column DIC concentrations and $\delta^{13}C$ signatures across the Refuge. Data for each measure were divided into quartiles as shown in the legends below each image.

However, only floc values also were correlated with the $\delta^{13}C$ of DIC ($r = -0.491$, $p = 0.017$, $n = 23$).

5. Discussion

5.1. *Comparisons of $\delta^{15}N$ and $\delta^{13}C$ patterns and responses*

The broad range of isotopic values we documented for most OM pools sampled appears typical for large, heterogeneous ecosystems (Fig. 3) (e.g., Cloern et al., 2002) and indicated high spatial variation in environmental conditions within the Refuge. Isotopic variation in OM across the Refuge was correlated with the considerable spatial variation in hydrology,

mineral concentrations, and P. This indicated that variation in these three environmental factors exerted a strong influence on isotopic signatures. Some of these influences may be direct, as for example with the effect of increasing concentrations of P – the primary limiting nutrient in the Everglades – on plant and periphyton growth rates and carbon acquisition (McCormick et al., 2002). Other influences may be indirect, such as the effect of hydrology on N cycling, with shorter-hydroperiod areas experiencing high rates of OM decomposition compared to longer-hydroperiod areas where decomposition is slow and reduced conditions predominate (White and Reddy, 2001).

In contrast to our findings, Inglett and Reddy (2006) documented relatively small shifts in sawgrass and cattail $\delta^{15}N$ and $\delta^{13}C$ along a 10-km gradient of canal influence in an Everglades wetland (WCA 2A) adjacent to the Refuge and exposed to the same canal sources. While steep P gradients occur near the perimeter of both wetlands, hydrology and mineral chemistry are much more similar across the WCA 2A gradient than across the much larger area of the Refuge.

While we detected many significant relationships between OM isotopic signatures and measured environmental gradients, unexplained variation was high for most signatures. Thus, we suspect that other environmental factors exerted an influence on these signatures. For example, local variation in isotopic signatures could confound responses across larger spatial scales such as those encompassed by the wetland hydrologic and water quality gradients we examined. Our data for sawgrass in particular suggested that there is considerable variability in environmental conditions other than those we measured even among sites in close proximity to each other. We did not measure within-site variation in isotopic signatures. However, significant variation in isotopic values among plants of the same species has been documented in response to local (e.g., at the scale of several meters) gradients and within individual stands (Guy et al., 1988; Leavitt and Long, 1988).

Despite this variability, the isotopic signatures of different OM pools showed distinct patterns of change across major environmental gradients in the Refuge. Two patterns in tissue $\delta^{15}N$ were detected, one for live tissues of rooted macrophytes and a second for metaphyton, detritus, and floc. Patterns for $\delta^{13}C$ were more complex. Sawgrass showed a spatial pattern similar to cattail and distinct from other pools, while patterns for cattail also were similar to those for metaphyton, and metaphyton and detrital pools again showed similarities.

Isotopic signatures for metaphyton and detrital (plant detritus and floc) pools were the most responsive to spatial variation in water chemistry and depth, a pattern that is consistent with their locations at the soil–water (detritus and floc) and air–water (metaphyton) interface. By contrast, isotopic variation in rooted macrophytes corresponded more closely to soil nutrient pools, and was thus affected only indirectly by surface–water chemistry and hydrology. Isotopic signatures for sawgrass, the dominant macrophyte at most locations in the Refuge, exhibited no strong relationships with any measured environmental gradient. These correlative results suggested that the metaphyton-detrital pools were exposed to different and more pronounced changes in N and C sources and availability across these gradients compared to rooted macrophytes, and that water-column and soil processes were at least partially isolated. We expect that these patterns will hold generally for wetlands and other shallow-water environments.

5.2. *Patterns for metaphyton and detritus*

Though metaphyton-detrital $\delta^{13}C$ signatures were most strongly correlated with gradients in surface-water specific conductance, soil P, DIC pool size (concentration), DIC-$\delta^{13}C$, and pH were also important. Elevated DIC concentrations are often associated with light $\delta^{13}C$ values for both algae and DIC in lacustrine systems when algal photosynthesis and, therefore, DIC uptake and fractionation, is low. However, this was not the case in the Refuge. Elevated DIC concentrations (large pool) coincided with heavy DIC-$\delta^{13}C$, indicating a heavy source of DIC. This is not the result of plant uptake, which would produce heavy DIC-$\delta^{13}C$ but also *decrease* pool size. Rather, the elevated DIC concentrations and heavy DIC-$\delta^{13}C$ along the periphery were likely the result of canal and or groundwater intrusion. Along the periphery, the inverse relation between DIC-$\delta^{13}C$ and metaphyton-$\delta^{13}C$ may be the due to the large pool size overwhelming the heavy source since a large pool size allows for greater algal fractionation against the heavy isotope.

In the interior, characterized by pH 6–7, low buffering capacity, and low DIC concentrations, it was likely that the dominant form of DIC was CO_2 which can be lost as a gas to the atmosphere. Here, the light DIC-$\delta^{13}C$ values may have been due to organic respiration. However, in the interior, where CO_2 is limited (small DIC pool), metaphyton may have to use bicarbonate, which is heavier than dissolved CO_2 (+1‰ vs. −7‰). If pool size is small and bicarbonate is unavailable, and metaphyton growth rate is high, metaphyton-$\delta^{13}C$ may also become heavy because there is no opportunity to fractionate.) In summary, the broader range of $\delta^{13}C$ values for metaphyton (−36.12‰ to −21.47‰) may be due to numerous factors: (1) differences in the amount of carbonate derived DIC between sites, (2) pool size effects, (3) variable contribution of respiration, which releases light $\delta^{13}C$ into the water where it is available for algal uptake, (4) differences in metaphyton community taxonomic composition, (5) possible C limitation of periphyton, resulting in a heavier $\delta^{13}C$ signature, (6) soil TP and hence growth rate, and (7) increased stagnant layer thickness related to mat size and hydrologic flow rate, resulting in less fractionation (Gu et al., 1995; Hecky and Hesslein, 1995). Both surface detritus and floc contain an algal component, albeit one with lower photosynthetic rates per unit biomass than metaphyton (McCormick et al., 1998; Grimshaw et al., 1997). Thus, all of these OM pools could show similar patterns for $\delta^{13}C$ across P gradients. However, detritus, which consists of dead material, may not be as affected by the DIC pool as metaphyton. Detritus and floc may be more affected by source material as evidenced by a $\delta^{13}C$ range within that for metaphyton and sawgrass $\delta^{13}C$.

Patterns of metaphyton-detrital $\delta^{15}N$ were also complex and correlated with both hydrologic and chemical gradients. Different processes likely affect OM $\delta^{15}N$ signatures across these two gradients. Changes in $\delta^{15}N$ of detrital material across hydrologic gradients may be related to higher decomposition and N oxidation rates under shorter hydroperiods

(DeBusk and Reddy, 1998). As previously mentioned, the drier, more oxidized conditions in the north are favorable for decomposition (White and Reddy, 2001). Decomposition proceeds with the preferential mineralization of lighter forms of N (Fogel and Turos, 1999) resulting in a heavier signature for the more refractory detritus that remains. Thus sites with shorter hydroperiods are predicted to have detrital material with heavier $\delta^{15}N$. In contrast, while $\delta^{13}C$ of bulk organic material becomes heavier in the early stages of decomposition (Fernandez et al., 2003), as decomposition continues, the bulk material becomes lighter because lipids and lignins, which are difficult to decompose, are light (Benner et al., 1987). The macrophyte detritus layer exhibited the strongest response while floc and metaphyton, which contain a greater photosynthetic component, showed a significant but weaker response across the hydrologic gradient. Increased loads of P and perhaps other elements caused by canal-water intrusion also may accelerate decomposition as demonstrated in the Refuge (Newman et al., 2001) and other Everglades wetlands (White and Reddy, 2000), and thus explain increases in detrital and metaphyton $\delta^{15}N$ near the Refuge perimeter.

Light $\delta^{15}N$ values can occur in an N-replete environment through preferential uptake of the depleted isotope (McKee et al., 2002; Clarkson et al., 2005; Inglett and Reddy, 2006), or discrimination against the heavy isotope during assimilation through the activity of the enzymes nitrate reductase and glutamate synthetase (Evans, 2001). Increased N demand in these P-enriched zones may result in less selectivity in biotic uptake of DIN and a consequent increase in the $\delta^{15}N$ of OM pools. This explanation is consistent with the finding that periphyton and bacterial communities are increasingly N limited in P-enriched portions of the Everglades closest to canal discharges (McCormick et al., 1996; White and Reddy, 1999).

5.3. *Patterns for rooted macrophytes*

Patterns for sawgrass and cattail isotopic signatures were broadly correlated, though each species showed distinct responses as well. Cattail, which occurred only along the perimeter, was exposed to the greatest canal effects (i.e., altered hydrology and water chemistry), and showed a narrower range of $\delta^{13}C$ values compared to sawgrass, which was found at most sites across the Refuge. In contrast, cattail had the broadest $\delta^{15}N$ range of any OM pools sampled. Inglett and Reddy (2006) also reported a wider range for cattail $\delta^{15}N$ relative to sawgrass across a gradient of canal influence in WCA 2A, and a broader range of $\delta^{13}C$ for sawgrass than for cattail. In contrast to our study, these investigators sampled both species at all of their sampling sites. Therefore, differences in location alone did not explain these differences between the two species. Cattail and sawgrass exhibit several differences in their physiological responses to environmental change with respect to growth rates, nutrient demand and partitioning, photosynthetic rates, and stomatal conductance (Miao and Debusk, 1999), and collectively, these factors may contribute to different ranges of isotopic values.

Of the environmental variables we measured, soil P concentration was most strongly correlated with tissue $\delta^{15}N$ values for both species and $\delta^{13}C$ values for cattail. Increases in $\delta^{15}N$ across the soil-P gradient in the Refuge were consistent with a shift towards increasing N limitation as the potential for plant uptake processes to discriminate against $\delta^{15}N$ decreases with increasing N demand. This effect was greatest for cattail, which has been shown to have higher nutrient requirements and a larger growth response to nutrient enrichment (Miao and Debusk, 1999). Decreases in cattail $\delta^{13}C$ with increasing soil P are inconsistent with increased photosynthetic rate in response to increased supply of this limiting nutrient (Dawson et al., 2002; Miao and Debusk, 1999) and with isotopic patterns observed across P-enrichment gradients elsewhere in the Everglades (Inglett and Reddy, 2006). Our data set does not provide us with a mechanistic explanation for this relationship.

6. Conclusions

The isotopic variation in major OM pools across this subtropical wetland suggested that $\delta^{15}N$ and $\delta^{13}C$ can be used to show complicated interactions between nutrient sources, nutrient pool size, biogeochemical processes, and anthropogenic manipulation. In addition, stable isotopes can be used to indicate hydrologic and chemical gradients. In this study, the isotopic signatures highlighted differences between the periphery and the interior, with periphery being more variable due to anthropogenic influences, and the drier north and inundated south.

Metaphyton and detrital pools were more responsive to hydrologic and water-chemistry changes while rooted macrophytes were more responsive to changes in soil chemistry. These patterns were predictable, and also suggested that wetland water-column and soil processes across these environmental gradients are at least partially isolated. The DIC and metaphyton data showed a complicated interaction between pool size effects, sources, and biological processes. These findings have implications both for the understanding of wetland biogeochemistry, wetland restoration efforts, and for tailoring the focus of stable isotope studies in these ecosystems.

Considerable variation in the overall sensitivity of different OM pools to environmental change also was detected. For example, sawgrass initially was considered an excellent candidate for detecting environmental gradients due to its widespread distribution across the Refuge, but its isotopic signatures exhibited no strong relationships with any measured environmental gradient in contrast to the other OM pools. The selection of OM pools for isotopic analysis will, therefore, affect both the ability to detect environmental gradients as well as the resulting patterns.

Finally, our results indicate that $\delta^{15}N$ may be the more robust isotopic indicator of ecosystem changes in wetlands exposed to multiple or complex anthropogenic gradients. Patterns for the C and N isotopes tended to be uncorrelated, suggesting that each provided unique information concerning environmental variation. However, isotopic signatures for N were more responsive to changes in hydrology and more easily interpreted across chemical gradients.

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REFERENCES

Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329, 708–710.

Clarkson, B.R., Schipper, L.A., Moyersoen, B., Silvester, W.B., 2005. Foliar ^{15}N natural abundance indicates phosphorus limitation of bog species. Oecologia 114, 550–557.

Cloern, J.E., Canuel, E.A., Harris, D., 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol. Oceanogr. 47, 713–729.

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand, C., McKinney, R.A., Grady, S.P., da Silva, M.H.C., 2004. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. J. Environ. Qual. 33, 124–132.

Corstanje, R., Grunwald, S., Reddy, K.R., Osborne, T.Z., Newman, S., 2006. Assessment of the spatial distribution of soil properties in a Northern Everglades Marsh. J. Environ. Qual. 34, 938–949.

Costanzo, S., Donohue, M.J., Dennison, W.C., 2003. Assessing the seasonal influence of sewage and agricultural nutrient inputs in a subtropical river estuary. Estuaries 26, 857–865.

Dawson, T.E., Mambelli, S., Plamboeck, H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. Ann. Rev. Ecol. Syst. 33, 507–559.

DeBusk, W.R., Reddy, K.R., 1998. Nutrient hydrology effects on soil respiration in a Northern Everglades Marsh. J. Environ. Quality 32, 702–710.

Doren, R.F., Armentano, T.V., Whitteaker, L.D., Jones, R.D., 1996. Marsh vegetation patterns and soil phosphorus gradients in the Everglades ecosystem. Aquat. Bot. 56, 145–163.

Elliott, E.M., Brush, G.S., 2006. Sedimented organic nitrogen isotopes in freshwater wetlands record long-term changes in watershed nitrogen source and land use. Environ. Sci. Technol. 40, 2910–2916.

Evans, R.D., Bloom, A.J., Sukrapanna, S.S., Ehleringer, J.R., 1996. Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill, CVT-5) grown under ammonium or nitrate nutrition. Plant Cell Environ. 19, 1317–1323.

Evans, R.D., 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci. 6, 121–126.

Fernandez, I., Mahieu, N., Cadisch, G., 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochem. Cycles 17 (3), 1075, doi:10.1029/2001GB001834.

Fogel, M.L., Turos, N., 1999. Transformation of plant biochemical to geological macromolecules during early diagenesis. Oecologia 120, 336–346.

Fourquerean, J.W., Escorcia, S.P., Anderson, W.T., Zieman, J.C., 2005. Spatial and seasonal variability in elemental content of $\delta^{13}C$ and $\delta^{15}N$ of *Thalassia testudiunum* from South Florida and its implication for ecosystem studies. Estuaries 28, 447–461.

Fry, B., 2006. Stable Isotope Ecology. Springer, New York, p. 390.

Grice, A.M., Loneragan, N.R., Dennison, W.C., 1996. Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. J. Exp. Mar. Biol. Ecol. 195, 91–110.

Grimshaw, H.J., Wetzel, R.G., Brandenburg, M., Sergerblom, M., Wenkert, L.J., Marash, G.A., Charnetzky, W.H., Haky, J.E., Carraher, G., 1997. Shading of periphyton communities by wetland emergent macrophytes: decoupling of algal photosynthesis from microbial nutrient retention. Arch. Hydrobiol. 139, 17–27.

Gu, B., Schleske, C.L., Brenner, M., 1995. Relationship between sediment and plankton isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and primary productivity in Florida lakes. Can. J. Fish. Aquat. Sci. 53, 875–883.

Guy, R.D., Warne, P.G., Reid, D.M., 1988. Stable carbon isotope ratio as an index of water-use efficiency in C3 halophytes – possible relationship to strategies for osmotic adjustment. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), Stable Isotopes in Ecological Research. Springer-Verlag, New York, pp. 55–75.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem. Geol. 59, 87–102.

Hecky, R.E., Hesslein, R.H., 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J. N. Am. Benth. Soc. 14, 631–653.

Hollander, M., Wolfe, D.A., 1999. Nonparametric Statistical Methods, 2nd ed. John Wiley & Sons, New York, p. 817.

Inglett, P.W., Reddy, K.R., 2006. Investigating the use of macrophytes stable C and N isotopic ratios as indicators of wetland eutrophication: patterns in the P-affected Everglades. Limnol. Oceanogr. 51, 2380–2387.

Jones, R.I., King, L., Dent, M.M., Maberly, C., Gibson, C.E., 2004. Nitrogen stable isotope ratios in surface sediments, epilithon and macrophytes from upland lakes with differing nutrient status. Freshwater Biol. 49, 382–391.

Kohl, D.H., Shearer, G.B., Commoner, B., 1971. Fertilizer nitrogen contribution to nitrate in surface water in a corn belt watershed. Science 174, 1331–1334.

Korom, S.F., 1992. Natural denitrification in the saturated zone: a review. Water Resour. Res. 28, 1657–1668.

Kreitler, C.W., 1975. Determining the source of nitrate in groundwater by nitrogen isotope studies: Rep. of Invest. #83, University of Texas at Austin, Austin, TX, Bureau of Econ. Geol., 57p.

Kreitler, C.W., 1979. Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. J. Hydrol. 42, 147–170.

Kreitler, C.W., Jones, D.C., 1975. Natural soil nitrate: the cause of the nitrate contamination of groundwater in Runnels County, Texas. Groundwater 13, 53–61.

Lake, J.L., McKinney, F.A., Osterman, R.J. Pruell, Kiddon, J., Ryba, S.A., Libby, A.D., 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Can. J. Fish. Aquat. Sci. 58, 870–878.

Leavitt, S.W., Long, A., 1988. Inter tree variability of $\delta^{13}C$ in tree rings. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), Stable Isotopes in Ecological Research. Springer-Verlag, New York, pp. 95–104.

Mariotti, A., Mariotti, F., Champigny, M.L., Amargar, N., Moyse, A., 1982. Nitrogen isotope fractionation associated with nitrate reductase and uptake of NO^{-}_{3} by pearl millet. Plant Physiol. 69, 880–884.

McCormick, P.V., Rawlik, P.S., Lurding, K., Smith, E.P., Sklar, F.H., 1996. Periphyton–water quality relationships along a

nutrient gradient in the northern Everglades. J. N. Am. Benthol. Soc. 15, 433–449.
McCormick, P.V., Shuford, R.B., Backus, J.K., Kennedy, W.C., 1998. Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, USA. Hydrobiologia 362, 185–208.
McCormick, P.V., Newman, S., Miao, S., Gawlik, D.E., Marley, D., Reddy, K.R., Fontaine, T.D., 2002. Effects of anthropogenic phosphorus inputs on the Everglades. In: Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, Boca Raton, FL, pp. 83–126.
McKee, K.L., Feller, I.C., Popp, M., Wanek, W., 2002. Mangrove isotopic ($\delta^{15}N$ and $\delta^{13}C$) fractionation across nitrogen vs. phosphorus limitation gradient. Ecology 83, 1065–1075.
Miao, S.L., Debusk, W.F., 1999. Effects of phosphorous enrichment on structure and function of sawgrass and cattail communities in the Everglades. In: Reddy, K.R., O'Connor, G.A., Schelske, C.L. (Eds.), Phosphorus Biogeochemistry in Subtropical Ecosystems. Lewis Publishers, Boca Raton, pp. 275–300.
Newman, S., Reddy, K.R., DeBusk, W.F., Wang, Y., 1997. Spatial distribution of soil nutrients in a northern Everglades marsh: water conservation area. 1. Soil Sci. Soc. Am. J. 61, 1275–1283.
Newman, S., Kumf, H., Laing, J.A., Kennedy, W.C., 2001. Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54, 229–250.
Pennock, J.R., Velinsky, D.J., Ludlan, J.M., Sharp, J.H., 1996. Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: implications for $\delta^{15}N$ dynamics under bloom conditions. Limnol. Oceanogr. 41, 451–459.
Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18, 293–320.
Shearer, G.B., Kohl, D.H., Chien, S.H., 1978. The nitrogen-15 abundance in a wide variety of soils. Soil Sci. Soc. Am. J. 42, 899–902.
St-Jean, G., 2003. Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyzer. Rapid Commun. Mass Spectrom. 17, 419–428.
Waser, N.A.D., Harrison, P.J., Nielson, B., Calvert, S.E., 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnol. Oceanogr. 43, 215–224.
White, J.R., Reddy, K.R., 1999. Influence of nitrate and phosphorus loading on denitrifying enzyme activity in Everglades wetland soils. Soil Sci. Soc. Am. J. 63, 1945–1954.
White, J.R., Reddy, K.R., 2000. Influence of phosphorus loading on organic nitrogen mineralization of Everglades soils. Soil Sci. Soc. Am. J. 64, 1525–1534.
White, J.R., Reddy, K.R., 2001. Influence of selected inorganic electron acceptors on organic nitrogen mineralization in Everglades soils. Soil Sci. Soc. Am. J. 65, 941–948.
Yamamuro, M., Kayanne, H., 1995. Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediments with a Yanaco MT-5 CHN analyzer. Limnol. Oceanogr. 40, 1001–1005.