

Potential N processing by southern Everglades freshwater marshes: Are Everglades marshes passive conduits for nitrogen?

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ABSTRACT

The degree of hydrological connectivity in wetlands plays a vital role in determining the flux of energy, material, and nutrients across these wet landscapes. During the last century, compartmentalization of hydrologic flows in the Florida Everglades by canals and levees has had a profound impact on the natural timing and supply of freshwater and nutrients across the southern Everglades. Nitrogen (N) is an understudied nutrient in the phosphorus-limited Everglades; it plays an important role in many Everglades processes. To gain a better understanding of the overall N-dynamics in southern Everglades marshes and the role that canals play in the distribution of N across this landscape, we analyzed $\delta^{15}\text{N}$ natural abundance data for the primary ecosystem components (the macrophyte *Cladium jamaicense*, marl soils, peat soils, and periphyton). Three sample transects were established in the three main basins of the southern Everglades: Shark River Slough, Taylor Slough, and the C-111 basin. Each transect included sample sites near canal inflows, in interior marshes, and at the estuarine ecotone. Natural abundance $\delta^{15}\text{N}$ signatures provided insights into processes that may be enriching the ^{15}N content of ecosystem components across the marsh landscape. We also conducted a combined analysis of $\delta^{15}\text{N}$ data, tissue N concentrations, and water column N data to provide a broad overview of N cycling in the freshwater marshes of the southern Everglades. The primary trend that emerged from each basin was a significant ^{15}N enrichment of all ecosystem components at near-canal sites, relative to more downstream sample sites. These data suggest that the phosphorus-limited marshes of the southern Everglades are not inactive conduits for N. Rather, these marshes appear to be actively cycling and processing N as it flows from the canal–marsh interface through downstream freshwater marshes. This finding has important implications to downstream coastal estuaries, including Florida Bay, and to nearshore coastal ocean ecosystems, such as coral reefs, where N is the limiting nutrient.

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1. Introduction

The degree of hydrological connectivity plays a vital role in determining the flux of energy, material, and nutrients across wetland landscapes (Odum et al., 1995). At the beginning of the 20th century, the freshwater marshes of the Florida Everglades possessed a high level of hydrological connectivity due to unimpeded sheetflow that followed a gravity driven gradient from north to south. Current freshwater flow in the Everglades is highly restricted by a water management network comprised of approximately 2500 km of

canals and levees. This compartmentalization of the Everglades has had a profound impact on the timing and supply of freshwater and nutrients across the landscape (Light and Dineen, 1994). However, over the last 20 years, several engineered restoration projects have attempted to restore the hydrologic regime of the southern Everglades, initially through enhanced water inflows from canals and more recently via water detention areas (along the eastern boundary of Everglades National Park (ENP)) where high stages are maintained in order to minimize seepage from ENP to adjacent canals (Rudnick et al., 1999; USACE, 2009). There remains considerable uncertainty as to how increased freshwater inflows (either directly canal-derived or via detention areas) will modify the supply of nutrients to the downstream freshwater oligotrophic marshes, to coastal estuaries

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such as Florida Bay, and to nearshore coastal ecosystems, including coral reefs.

Nitrogen (N) dynamics have received little attention in research being conducted in the phosphorus-limited Everglades landscape. Despite this disparity in research efforts, N plays important roles in many Everglades systems. Nitrogen is the limiting nutrient in many highly nutrient enriched areas of the northern Everglades (Inglett et al., 2011), has been shown to co-limit productivity with phosphorus (P) in some areas of Florida Bay (Glibert et al., 2004; Gardner and McCarthy, 2009; Inglett et al., 2011) and is the limiting nutrient in nearshore ecosystems (Lapointe and Clark, 1992; Davis et al., 2009). The point of freshwater inflow to Everglades' marshes (often the canal–marsh interface) typically has the highest rates of nutrient loading; N and P concentrations decrease rapidly with increased distance away from the canal (Craft and Richardson, 1993; McCormick and O'Dell, 1996; Childers et al., 2003, 2006a; Gaiser et al., 2006). While numerous studies have examined the role of P on marsh productivity and biogeochemistry (reviewed by Davis and Ogden, 1994; Noe et al., 2001), we currently lack similar information on N dynamics. Specifically, little is known about N distribution and processing, N transformation, and ultimately N transport to downstream coastal/estuarine ecosystems, where N is much more important and typically is the limiting nutrient.

In the southern Everglades, nutrient inputs have been shown to be largely determined by freshwater discharge volume at canal inflows (Rudnick et al., 1999). Along the canal–marsh interface, both total phosphorus (TP) and dissolved inorganic nitrogen (DIN) are quickly removed from the water column prior to downstream export (Rudnick et al., 1999; Parker, 2000; Childers et al., 2006a). In Taylor Slough, flow-weighted mean concentrations of DIN and TP decreased along a 3 km transect moving downstream from the canal–marsh interface (240–36 $\mu\text{g l}^{-1}$ and 11.6 to 6.1 $\mu\text{g l}^{-1}$, respectively; Rudnick et al., 1999). Following hydrologic restoration (i.e. levee removal) in the C-111 basin, Parker 2000 found the near-canal marsh sites had increased total nitrogen (TN) and TP concentrations, as compared to interior marsh sites located several kilometers downstream from the canal.

To date, the majority of research on canal-derived N in the southern Everglades has used water quality data to assess N flowing downstream through marshes from canals. It can be hypothesized that these P-limited marshes are not taking up or cycling N in appreciable quantities relative to water column concentrations. Stable isotope techniques offer an additional tool for clarifying potential N sources to the marsh, N transformations within the marsh, and the fate of N in downstream estuaries, as has been done in other aquatic ecosystems (McClelland and Valiela 1997; Cole et al., 2005; Dolenc et al., 2005; Wozniak et al., 2008). Sources of anthropogenic N-loading (e.g. sewage; often indicated by heavy/enriched $\delta^{15}\text{N}$ signatures) have been identified through the analysis of key ecosystem components (e.g. macroalgae and macrophytes) at sites directly impacted by anthropogenic influences (Heaton, 1986; Lapointe et al., 2004; Wigand et al., 2007). Conversely, when N originates from N-fixation (or fertilizer N), $\delta^{15}\text{N}$ signatures of the biota are lighter/less enriched (Wigand et al., 2007).

Several factors may lead to significant variation in $\delta^{15}\text{N}$ signatures, including $\delta^{15}\text{N}$ ratio of N sources, the rate of isotope fractionation, and the type N transformation(s) (Hogberg, 1997). While the relative contributions of “light and heavy” N sources are important, understanding the type and duration of N processing within a system (via biochemical transformations and physical transport) is critical. Each N transformation (e.g. denitrification, N fixation and volatilization) has a unique fractionation factor (α) that describes the enrichment or depletion of the heavier ^{15}N isotope (nitrification $\alpha = \sim 1.000$ – 1.035 ; denitrification $\alpha = 1.028$; and nitrogen fixation $\alpha = 1.000$; Fig. 1; Lathja and Michener, 1994;

Hogberg, 1997). Fractionation that occurs during N processing usually results in products which are depleted in ^{15}N relative to the source material. For example, NO_3^- produced during nitrification of ammonium will be depleted in ^{15}N , while residual ammonium is enriched in ^{15}N (Nadelhoffer and Fry, 1994) and the prolonged processing of DIN (nitrification, denitrification, and volatilization) can lead to the overall enrichment of the resulting DIN, through the loss of the lighter ^{14}N (Macko and Ostrum, 1994; Cifuentes et al., 1989). Other processes such as biotic assimilation and remineralization can result in varied α values, depending on the organism and environmental conditions, leading to further enrichment of the N pool (Wada and Hattori, 1978; Lettole, 1980; Mariotti et al., 1981).

To gain a better understanding of the overall N-dynamics in the region and the role that canals are playing in the distribution of N across the Everglades' landscape, we analyzed the $\delta^{15}\text{N}$ stable isotope natural abundance of the primary ecosystem components in the three main basins of the southern Everglades (Shark River Slough, Taylor Slough, and C-111 basin). A sample transect of three sites (near-canal, interior marsh, and estuarine ecotone) was established in each basin. The specific goals of this study were:

- 1) Determine if the three main drainage basins of the southern Everglades (Shark River Slough, Taylor Slough and C-111 basin) possess enriched $\delta^{15}\text{N}$ signatures at the canal–marsh interface, as compared to locations further downstream from canals.
- 2) Compare the degree of variability in $\delta^{15}\text{N}$ signatures of the dominant ecosystem components found within each of these basins.
- 3) Assess the potential importance of N cycling in these P-limited marshes and the relevance of this to downstream N-limited coastal ecosystems.

2. Methods

2.1. Study area

The southern Everglades is part of a larger wetland landscape that extends across 28,000 km^2 of south-central Florida (Fig. 1; Childers et al., 2003). The Everglades' landscape is comprised of sawgrass marshes, deep water sloughs, and forested tree island ecosystems which are situated on a topographic gradient (1 m per 56 km) extending from Lake Okeechobee to Florida Bay (Fig. 2; Light and Dineen, 1994). This sub-tropical system has a distinct wet season which historically occurs from May through November (Hela, 1952). The seasonal wetting of the Everglades' marshes is an important natural driver of several ecosystem processes, including macrophyte and periphyton productivity (Ewe et al., 2006; Iwaniec et al., 2006; Childers et al., 2006b). However, the creation of the water management network has resulted in a highly modified hydrological regime including altered patterns of flow, hydroperiod, and nutrient delivery to Everglades' marshes (Childers et al., 2003).

Each of the three main drainage basins of the southern Everglades has freshwater inflows originating from a canal or water control structure (owned by the South Florida Water Management District). Freshwater inflows from Water Conservation Area 3A (WCA-3A) are distributed into Shark River Slough via four S-12 gate structures, while freshwater inflows to Taylor Slough originate from the S-332D pump station and the L-31N canal, with water pumped into a managed detention area to the east of ENP to minimize eastward seepage from ENP wetlands. However, some water from this detention area enters L-31W, and then can flow directly into ENP (Fig. 1). Following the removal of the southern levee of the C-111 canal in the late 1990's, the C-111 basin receives over-bank flow directly from the C-111 canal. Prior to levee

removal, the C-111 canal acted to severely limit the historical north-to-south flow of freshwater through the C-111 Basin and essentially cut off the flow of freshwater flow to northeastern Florida Bay. For each of our study basins the predominant sources of nutrients are atmospheric deposition, surface water inflow and groundwater inflow. The relative importance of these nutrient sources varies greatly between the wet and dry seasons and is primarily driven by the hydrology of the basin (See Sutula et al., 2001).

2.2. Experimental design

A sample transect was established in each of the three main drainage basins of the Everglades (Shark River Slough, Taylor

Slough, and C-111 Basin; Fig. 1); here we considered the basins, and therefore the transects within the basins, as landscape-scale replicates, though not replicates in a strict, traditional statistical sense. Each individual transect included 3 sample sites (near-canal, NC; interior marsh, IM; and estuarine ecotone, ECO), which occurred at increasing distance away from canal inflows of freshwater (Fig. 1). These 9 sample sites (3 NC, 3 IM, and 3 ECO) were sampled two times during the 2004 wet season (August and November). At each site, triplicate samples of the predominant ecosystem components were collected and processed for $\delta^{15}\text{N}$ isotope analysis including aboveground macrophyte, belowground macrophyte (sawgrass; *Cladium jamaicense*), marl and peat soils at several depths, periphyton, and flocculent material.

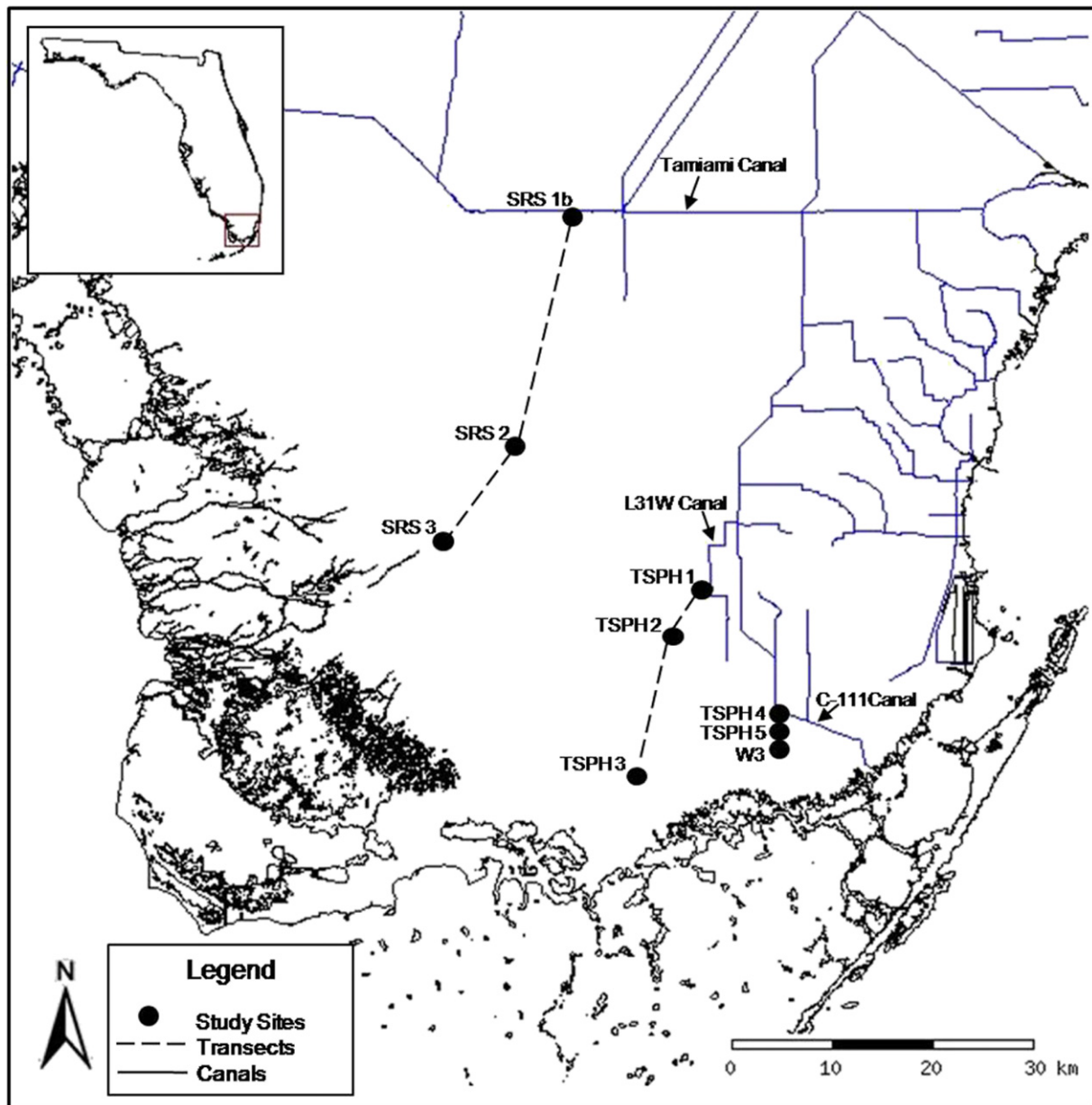


Fig. 1. Map of the Everglades showing the three major basins in which sample transects were established: Shark River Slough (SRS), Taylor Slough (TS), C-111 Basin (C-111). Sample sites (near-canal: SRS 1, TSPH 1, and TSPH 4; interior marsh: SRS 2, TSPH 2, and TSPH 5; ecotone: SRS 3, TSPH 3, and W3) are also shown along each sample transect. In addition, the freshwater inflows to each basin are also depicted: SRS = Tamiami canal; TS = L-31W canal; and C-111 = C-111 canal. *Everglades Map*. 1:503,112; J.R. Wozniak; using "Florida Coastal Everglades LTER Mapserver project". <http://fcelter.fiu.edu/gis/everglades-map/> (22 March 2010).

2.3. Basin descriptions

2.3.1. Shark River Slough

Shark River Slough (SRS), located approximately 50 km west of Miami (Fig. 1) is the main drainage basin in Everglades National Park (ENP). The soils of SRS consist of a mosaic pattern

of peat soils mixed with marl soils and the dominant macrophyte, sawgrass (*Cladium jamaicense*), is often interspersed with spike rush (*Eleocharis cellulosa*) in deeper water slough habitats. Sample locations along the SRS transect are shown in Fig. 1 and include a near-canal site (30 m from canal; FCE LTER site SRS-1b), interior marsh site (24 km from canal; FCE LTER site SRS-

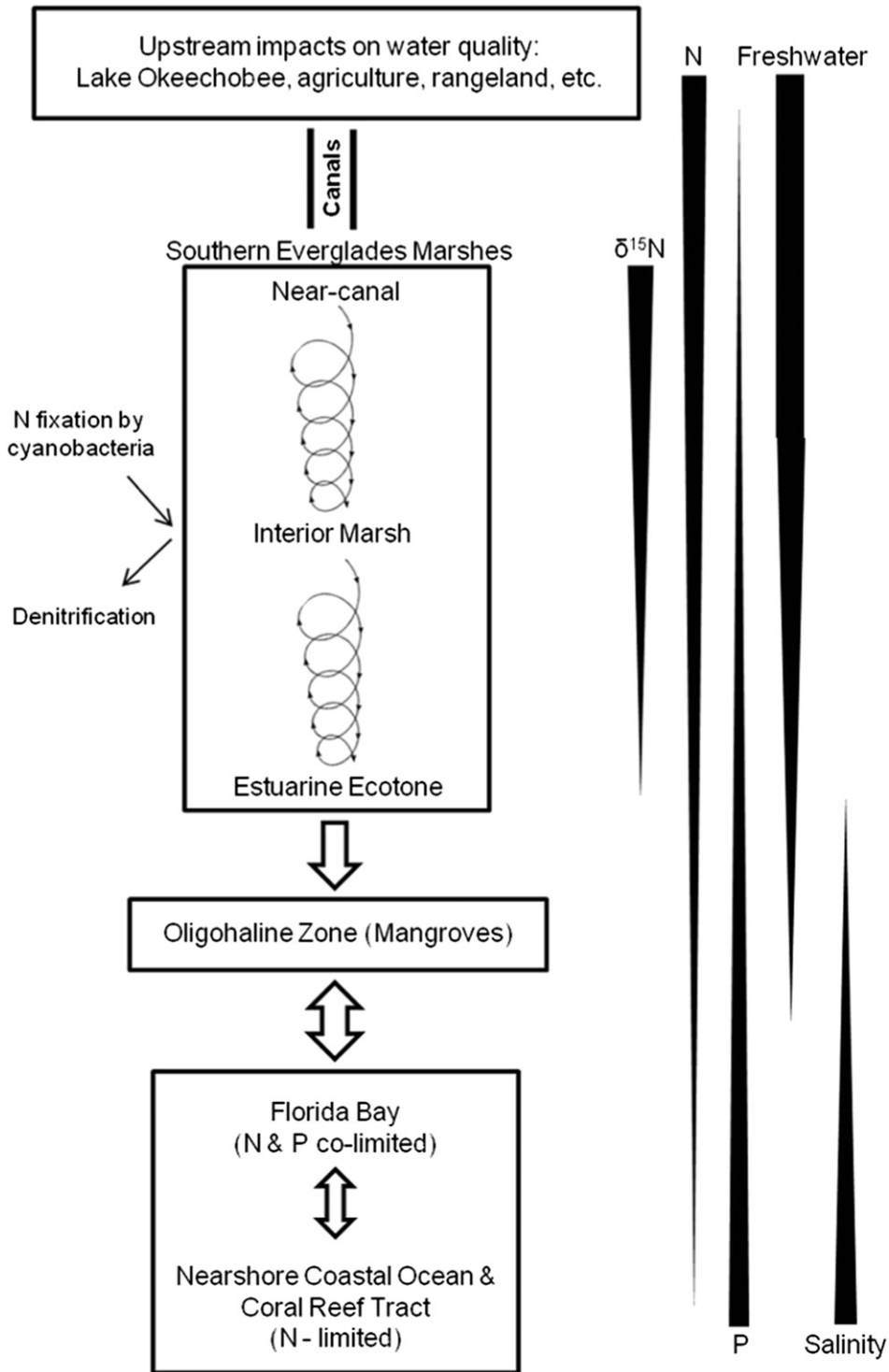


Fig. 2. Conceptual diagram illustrating the hydrologic connectivity, potential sources of nitrogen (N), the canal distribution of N to the southern Everglades' marshes, and the flux of N between Everglades' ecosystems. Boxes represent the main systems of the Everglades discussed in this manuscript and arrows represent points of connection for surface water flows and or the flux of nitrogen between systems. Spiraling arrows represent processing of N as it cycles through the marsh ecosystem. Black wedges to the right depict the "relative" changes in $\delta^{15}\text{N}$, nitrogen (N), phosphorus (P), freshwater and salinity abundance the system.

2), and estuarine ecotone site (35 km from canal; FCE LTER site SRS-3).

2.3.2. C-111

The C-111 basin (C-111) is a freshwater marl prairie dominated by calcareous periphyton and sawgrass (*Cladium jamaicense*). Located to the north of the ENP panhandle, this basin is bordered by the C-111 canal to the north and Florida Bay to the south (Fig. 1; Parker, 2000), and is a major source of freshwater flows to northeastern Florida Bay (Schaffranek, 1996; Rudnick et al., 1999; Sutula et al., 2003). In 1997, in an effort to increase freshwater flows to the C-111 basin and to improve the distribution of freshwater, the southern levee (spoil mounds) was removed. This allowed freshwater to flow from the canal, via over-bank flow, into the marl prairies and to northeastern Florida Bay (Parker, 2000; Troxler-Gann and Childers, 2006). The C-111 basin transect included a near-canal site (20 m from canal; FCE LTER site TS/Ph-4), interior marsh site (2 km from canal; FCE LTER site TS/Ph-5), and an estuarine ecotone site (4 km from canal; Fig. 1).

2.3.3. Taylor Slough

Prior to the hydrological modifications that have occurred over the last 50 years, Taylor Slough (TS) was the major overland source of freshwater to central Florida Bay (Light and Dineen, 1994; Sutula et al., 2003). Taylor Slough is structurally similar to the C-111 basin, with the main habitat type being freshwater marl prairie dominated by *Cladium jamaicense*. The TS transect included a near-canal site (10 m from the L-31W canal; FCE LTER site TS/Ph-1), interior marsh site (4 km from canal; FCE LTER site TS/Ph-2) and an estuarine ecotone site (22 km from canal; FCE LTER site TS/Ph-3; Fig. 1).

2.4. Field sampling techniques

Sampling took place during the 2004 wet season in August and November. Individual macrophyte culms (sawgrass; *Cladium jamaicense*) were harvested and the aboveground and belowground tissues were separated, rinsed to remove attached soil or epiphytic algae and placed in separate sample bags. Soil cores (10 cm length and 3.81 cm diameter) were collected in marl (TS and C-111) and peat (SRS) soils. Cores were sectioned in the field into core sections (0–1, 1–5, 5–10 cm) and placed in separate sample bags. Approximately 25 g dry weight of calcareous periphyton was collected at each site in TS and C-111. Because calcareous periphyton was not abundant at the SRS sites, periphyton samples were composed of both periphyton and detrital material (colloquially known as “floc”) along the SRS transect. “Floc” is low density detrital organic material found at the soil–water interface, and is comprised of sloughed periphyton material, detritus from vegetation, and microorganisms (Wood, 2005; Leonard et al., 2006). Floc samples were collected with a modified core and plunger device, following the methods of Wood (2005). All samples were immediately placed on ice for transport to the laboratory. In the laboratory, samples were dried (70 °C) for 48 h to a constant weight and homogenized using a mortar and pestle or Wiley mill mechanical grinder (Thomas Scientific, USA).

2.5. ¹⁵N laboratory analysis

All isotopic analyses were conducted at the Southeast Environmental Research Center Stable Isotope Lab at Florida International University using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures. The EA was used to combust organic material, producing N₂ and CO₂, which were then

measured on a Thermo Electron Delta C IRMS in a continuous flow mode. We report isotopic ratios (*R*) in the standard delta (δ) notation (‰) using the international standard of atmospheric nitrogen (AIR, N₂):

$$\delta(\text{‰}) = \left[\left(R_{\text{Sample}} / R_{\text{Standard}} \right) - 1 \right] \times 1000.$$

Analytical reproducibility based on replicates of internal standards is better than $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$. Marl soils and calcareous periphyton samples were decarbonated with 1 M HCl prior to isotopic measurement.

2.6. Water column and tissue nitrogen data

By design, our nine sample sites coincided with sites of the Florida Coastal Everglades Long-Term Ecological Research Program (FCE LTER; <http://fcelter.fiu.edu>). This allowed us to use additional data on water column N (DIN and DON) and tissue N concentrations for periphyton, aboveground macrophyte, belowground macrophyte (sawgrass; *Cladium jamaicense*), and soils. Water quality samples were collected as “grab” samples during monthly FCE LTER site visits between 2003 and 2006. Samples were collected from 10cm below the water surface using sample-rinsed bottles and placed on ice in the dark for transport (Childers et al., 2006a). See Childers et al. (2006a) for additional details on water quality sampling protocols and specific methods of nutrient analysis.

2.7. Statistical analyses

We combined our August and November samples into a single dataset for all statistical analyses. A single-factor analysis of variance (ANOVA) confirmed no significant difference between these sample events ($df = 1, f = 0.1504, p = 0.6989$). We first examined the data to see if there was significant variation in $\delta^{15}\text{N}$ signatures between the three basins (SRS, TS and C-111). To do this we pooled all ecosystem component data (periphyton, aboveground macrophytes, etc.) within each basin. Data from the three basins was then compared by using a one-way ANOVA analysis, with basin as the factor of interest. Secondly, we sought to determine the degree of variability in $\delta^{15}\text{N}$ signatures among sites (NC, IM and ECO) within each individual basin. To do this we used a one-way ANOVA to compare the three sample sites (NC, IM and ECO) in a basin. An ANOVA analysis was repeated for each of the three basins (SRS, TS and C-111). Post hoc Tukey’s HSD test was used to ascertain specific site differences when ANOVA tests generated significant ($p < 0.05$) results. Finally we attempted to determine how each ecosystem components $\delta^{15}\text{N}$ signature varied by site within a given basin. Here we used individual one-way ANOVAs for each ecosystem component (periphyton, soil, aboveground macrophytes, etc.) to determine variation in $\delta^{15}\text{N}$ signatures between the three sample sites (NC, IM and ECO) within each basin (SRS, TS and C-111). Post hoc Tukey’s HSD test was used to ascertain specific differences when ANOVA tests generated significant ($p < 0.05$) results. Nitrogen tissue concentrations and water column DIN and DON concentrations were also tested for sample site effects (NC, IM, and ECO) within a given basin. Post hoc Tukey’s HSD test was used to ascertain specific differences when ANOVA tests generated significant ($p < 0.05$) results. All data were first checked to confirm normality and homogeneity of variance to justify the use of parametric tests. Statistical procedures were performed using JMP Start Statistics and SAS (SAS Institute Inc. 2005).

3. Results

3.1. $\delta^{15}\text{N}$ isotope variations by basin and sample site

We found no significant variation in $\delta^{15}\text{N}$ signatures between the three basins (ANOVA, $df = 2$, $F = 0.91$, $p = 0.4533$). We then sought to determine how isotope signatures differed at sample sites within individual basin. In the C-111 basin, $\delta^{15}\text{N}$ signatures varied significantly by sample site (ANOVA, $p < 0.0001$; Table 1). Nitrogen isotope signatures were the heaviest at the near-canal site, which was significantly more enriched than both interior marsh and estuarine ecotone sites (Tukey's HSD; Table 1). In the Taylor Slough basin, $\delta^{15}\text{N}$ signatures also showed significant variation by sample site (ANOVA, $p < 0.0001$; Table 1). $\delta^{15}\text{N}$ signatures at the NC site were more enriched relative to both interior marsh and estuarine ecotone sites (Tukey's HSD; Table 1). In Shark River Slough basin, $\delta^{15}\text{N}$ signatures again showed a significant difference among sample sites (ANOVA, $p < 0.0001$; Table 1). The NC site values were more enriched than at IM, while the ECO and IM sites were not significantly different (Tukey's HSD; Table 1).

3.2. $\delta^{15}\text{N}$ isotope variations by ecosystem component

All ecosystem components in the C-111 basin showed a similar trend of more enriched/heavy $\delta^{15}\text{N}$ signatures at the near-canal site compared to the interior marsh and estuarine ecotone sites (Tukey's HSD; Table 1). At the near-canal site, soil 0–1 cm, soil 1–5 cm and periphyton were the most enriched ecosystem components (Table 1). In the TS basin, periphyton was the only ecosystem component in the Taylor Slough transect that did not have more enriched isotope signatures at the NC site as compared

to IM and ECO sites (Tukey's HSD, Table 1). All individual ecosystem components in the SRS basin were more enriched at NC relative to the IM site. Periphyton and surface soil (0–1 cm) were not different at NC and ECO while the 1–5 cm and 5–10 cm soil depths were actually more enriched at ECO relative to NC (Tukey's HSD; Table 1).

3.3. Nitrogen tissue concentrations and water column DIN:DON

Aboveground macrophyte tissue N content showed no inter-site variability in any of the three basins. Belowground macrophyte tissue N did vary throughout the Taylor Slough basin (ANOVA, $df = 2$, $F = 6.4474$, $p = 0.0033$; Table 2). Values at ECO were highest while the NC site showed lowest belowground N content (Tukey's HSD, Table 2). Periphyton N content varied significantly along the C-111 (ANOVA, $df = 2$, $F = 5.2715$, $p = 0.0076$) and Taylor Slough (ANOVA, $df = 2$, $F = 5.5790$, $p = 0.0091$) basins, but not in the SRS basin (ANOVA, $df = 2$, $F = 1.4316$, $p = 0.2484$; Table 2). The C-111 NC site had higher periphyton N than the IM site, but was not different from periphyton N at ECO (Table 2). Soil nitrogen concentrations did not vary significantly along the SRS basin (ANOVA, $df = 2$, $F = 2.0068$, $p = 0.1689$), but we did find significant variability along both the C-111 (ANOVA, $df = 2$, $F = 5.6309$, $p = 0.0305$) and Taylor Slough (ANOVA, $df = 2$, $F = 7.3113$, $p = 0.0033$; Table 2) basin. In the C-111 basin, soil N content at IM site was higher than at NC (Tukey's HSD; Table 2). We found the same pattern along the Taylor Slough basin (Tukey's HSD; Table 2).

The DIN:DON ratio in the C-111 basin at the near-canal site (0.35 was higher than the value at the interior marsh site (0.21)) although this difference was not significant (ANOVA, $df = 1$, $F = 2.9959$, $p = 0.0891$; Table 2). The trend of increased DIN:DON at the NC site was again displayed in Taylor Slough and Shark River Slough,

Table 1

Mean (basin Averages) $\delta^{15}\text{N}$ ‰ isotope signatures and standard deviations (\pm) are first shown for the near-canal, interior marsh and estuarine ecotone sample sites in Shark River Slough, Taylor Slough, and C-111 basin. Mean (Ecosystem Component Averages) $\delta^{15}\text{N}$ ‰ isotope signatures and standard deviations (\pm) are the listed for each individual ecosystem component (periphyton, aboveground macrophyte, belowground macrophyte, and soil 0–1 cm, soil 1–5 cm, and 5–10 cm) at each sample location (near-canal, interior marsh, and ecotone) in each basin. The results from one-way ANOVAs and post hoc Tukey's HSD are also shown. Statistically significant results are designated by an asterisk "*" and different letters.

	$\delta^{15}\text{N}$ ‰			ANOVA			Tukey's HSD			
	Near-canal	Interior marsh	Estuarine ecotone	df	F ratio	P	NC	IM	ECO	q*
Basin averages										
Basin										
C-111 Basin	5.95 (1.98)	2.07 (1.43)	2.31 (1.26)	2	79.0385	<0.0001*	A	B	B	2.37199
Taylor Slough	3.28 (0.91)	1.72 (1.56)	2.02 (2.02)	2	11.4416	<0.0001*	A	B	B	2.37245
Shark River Slough	5.29 (0.55)	2.62 (2.10)	4.46 (1.40)	2	31.1379	<0.0001*	A	B	A	2.37502
Ecosystem component averages										
C-111 Basin										
Periphyton	6.58 (1.38)	2.77 (0.69)	3.30 (0.36)	2	39.249	<0.0001*	A	B	B	2.52998
Soil 0-1	8.03 (0.38)	3.08 (0.35)	2.70 (0.16)	2	672.062	<0.0001*	A	B	B	2.55216
Soil 1-5	7.88 (0.42)	2.81 (0.19)	2.54 (0.31)	2	608.9976	<0.0001*	A	B	B	2.55216
Soil 5-10	4.90 (0.30)	3.29 (0.52)	3.71 (0.40)	2	25.0912	<0.0001*	A	B	B	2.55216
Aboveground macrophyte	3.48 (0.49)	0.09 (1.13)	0.77 (0.35)	2	32.8866	<0.0001*	A	B	B	2.56536
Belowground macrophyte	3.75 (0.68)	0.78 (0.76)	0.73 (1.03)	2	26.7154	<0.0001*	A	B	B	2.54545
Taylor Slough										
Periphyton	3.27 (0.99)	1.75 (0.73)	4.42 (1.63)	2	7.8775	0.0042*	AB	B	A	2.58033
Soil 0-1	3.53 (0.15)	2.51 (0.31)	2.68 (0.25)	2	38.6332	<0.0001*	A	B	B	2.55216
Soil 1-5	3.74 (0.23)	2.75 (0.59)	2.85 (0.20)	2	16.4365	<0.0001*	A	B	B	2.55216
Soil 5-10	4.20 (0.17)	3.56 (0.28)	3.57 (0.28)	2	13.2735	<0.0001*	A	B	B	2.55216
Aboveground macrophyte	1.97 (0.44)	-0.27 (0.51)	-0.53 (0.43)	2	57.7817	<0.0001*	A	B	B	2.54045
Belowground macrophyte	2.84 (1.01)	0.09 (0.79)	-0.17 (0.49)	2	34.0772	<0.0001*	A	B	B	2.54045
Shark River Slough										
Periphyton	5.15 (0.09)	3.98 (0.89)	5.07 (0.12)	2	6.8753	0.00102*	A	B	A	2.66776
Soil 0-1 cm	5.12 (0.26)	3.69 (0.24)	5.17 (0.25)	2	80.7306	<0.0001*	A	B	A	2.52998
Soil 1-5 cm	4.94 (0.16)	4.28 (0.13)	5.55 (0.29)	2	59.0894	<0.0001*	B	C	A	2.10982
Soil 5-10 cm	5.01 (0.29)	4.17 (0.32)	5.76 (0.25)	2	52.4847	<0.0001*	B	C	A	2.56536
Aboveground macrophyte	6.26 (0.73)	-0.45 (1.26)	2.67 (0.98)	2	62.8644	<0.0001*	A	C	B	2.56536
Belowground macrophyte	5.61 (0.28)	0.67 (1.13)	2.94 (0.91)	2	36.6701	<0.0001*	A	C	B	2.59747

Table 2

Average tissue nitrogen concentrations (mgN/g dry weight) for periphyton, above-ground macrophyte, belowground macrophyte (sawgrass; *Cladium jamaicense*) and soil from near-canal, interior marsh, and ecotone sites in Shark River Slough, Taylor Slough, and C-111 basin. The ratio of dissolved inorganic nitrogen (DIN) to dissolved organic nitrogen (DON) from each site along the sample transects are also shown. Statistically different values for each ecosystem component from near-canal interior marsh and estuarine ecotone sites (within each individual basin) are designated by different subscripted letters (a, b, etc.; Tukey's HSD).

	Nitrogen Concentration (mgN/g dry weight)				DIN:DON
	Periphyton	Aboveground macrophyte	Belowground macrophyte	Soil	
C-111 Basin					
Near-canal	8.26 ^a	8.24 ^a	5.76 ^a	3.77 ^b	0.35 ^a
Interior marsh	7.18 ^b	7.61 ^a	6.08 ^a	5.45 ^a	0.21 ^a
Estuarine ecotone	7.68 ^{a,b}	—	—	—	—
Taylor Slough					
Near-canal	8.77 ^{a,b}	8.53 ^a	6.36 ^b	4.28 ^b	0.35 ^a
Interior marsh	8.38 ^b	7.88 ^a	7.44 ^{a,b}	6.29 ^a	0.27 ^a
Estuarine ecotone	10.00 ^a	8.27 ^a	8.12 ^a	6.47 ^a	0.32 ^a
Shark River Slough					
Near-canal	21.31 ^a	7.87 ^a	6.47 ^a	33.51 ^a	0.21 ^a
Interior marsh	18.63 ^a	7.23 ^a	7.99 ^a	34.99 ^a	0.12 ^a
Estuarine ecotone	19.01 ^a	8.04 ^a	8.14 ^a	36.94 ^a	0.15 ^a

C-111 and Taylor Slough periphyton data from Iwaniec et al. (2006).

Shark River Slough periphyton data aboveground macrophyte, belowground macrophyte, soil and DIN:DON data from Florida Coastal Everglades.

Long-Term Ecological Research Program (www.fcelter.fu.edu).

however statistical analysis showed no significant difference between sample sites in both basins (ANVOA, $df = 2$, $F = 0.2348$, $p = 0.7913$ and ANVOA $df = 2$, $F = 2.3627$, $p = 0.0989$, respectively; Table 2).

4. Discussion

4.1. Importance and rationale for understanding N in a P-limited system

Nitrogen is an understudied nutrient that influences both macrophyte and periphyton production in Everglades wetlands. In areas of documented P loading impacts, N may at times become the limiting nutrient (Inglett et al., 2011). Much of the N provided to the southern Everglades originates from the north (e.g. Lake Okeechobee and the Everglades Agricultural Area; Capone et al., 1995 and Inglett et al., 2011). This N is delivered by the water management network (i.e. canals) and via the southern Everglades' marshes to downstream coastal ecosystems (Fig. 2). Where Everglades' restoration (www.evergladesplan.org) increases freshwater flows, there may be a corresponding increase in N loading to the oligotrophic southern Everglades marshes and downstream N-limited coastal ecosystems (Glibert et al., 2004). The negative effects of increased N-loading have been well documented in nearshore coastal and coral reef ecosystems and include harmful algal blooms, sea-grass die offs and loss of coral communities (Lapointe et al., 2005).

4.2. The role of isotope fractionation in the enrichment of canal N

The predominant trend from each of our study basins was that the $\delta^{15}\text{N}$ of all ecosystem components was enriched at the canal-marsh interface (NC) sites (Table 1). These results suggest that the $\delta^{15}\text{N}$ signature of N in canal water may also be enriched in ^{15}N , or "heavy." Because canals are the main N source to marshes located at or near canal inflows, mechanisms by which canal-borne N may become more enriched in ^{15}N are an important part of our story.

During the dry season in the Everglades, water in canals is impounded behind water control structures (gates and pump stations) and has considerably longer residence times relative to the wet season. Biological activity continues in canal water during the dry season, though, including the uptake, assimilation, and subsequent fractionation of N. The repeated cycling of N in water with long residence times leads to the isotopic enrichment of the DIN pool (Lamb and Swart, 2008). Assimilation of DIN progressively enriches the residual water column DIN pool with ^{15}N (Altabet and Deuser, 1985; Goering et al., 1990; Altabet and Francois, 1994; Benner et al., 1997). When water control structures are opened at the onset of the wet season, the impounded water enters the marsh landscape and organisms taking up this enriched DIN pool will acquire an enriched $\delta^{15}\text{N}$ signature. Notably, this enriched DIN pool will first come in contact with marshes at the canal-marsh interface and [we posit that] this explains the enriched $\delta^{15}\text{N}$ signatures we found in all ecosystem components at near-canal sites.

4.3. The role of isotope fractionation in the marsh cycling of N

In general across the three basins of the southern Everglades, the more downstream sample sites (interior marsh and estuarine ecotone) were isotopically depleted in relation to the near-canal locations. One explanation is the addition of new N at the ECO and IM sites and N_2 -fixation by cyanobacterial periphyton mats is likely a significant source of this less enriched $\delta^{15}\text{N}$ signatures. Periphyton mats are highly productive at these downstream sample sites (Iwaniec et al., 2006) and also play a vital role in N_2 -fixation in oligotrophic ecosystems (Doyle and Fisher, 1994; Currin and Paerl, 1998; Vargas and Novelo, 2006). The $\delta^{15}\text{N}$ signatures of cyanobacterial mats in tropical marshes have been shown to have a strong negative correlation with N_2 -fixation, suggesting the link between more depleted isotopic signatures at downstream sites and an increased reliance on atmospheric N sources (Rejmankova et al., 2004). This negative correlation is a result of atmospherically fixed N possessing a $\delta^{15}\text{N}$ signature of 0.00‰ (N-fixation $\alpha = 1.000$; Lathja and Michener, 1994; Hogberg, 1997).

It appears that there are several possible N sources and processes at work at NC, IM and ECO sites across the freshwater marshes of the southern Everglades. This fact highlights the need for additional research in the region to quantify N processing on a site by site basis. Future assessments should specifically include the determination of N-assimilation and N-mineralization rates for individual ecosystem components (e.g., periphyton, sawgrass, soil and fish) both along the canal-marsh interface and at more downstream sites. In addition, little is known about denitrification rates throughout the region and their importance on the N-cycle and N pools. Further information on N-fixation rates by cyanobacterial periphyton mats at all study sites would be helpful to determine the magnitude and importance of atmospheric N sources to the freshwater marshes of the southern Everglades.

4.4. Supplementing $\delta^{15}\text{N}$ data with DIN:DON and Tissue N Concentrations

While natural abundance $\delta^{15}\text{N}$ signatures provide insight into the location of $\delta^{15}\text{N}$ enriched ecosystem components across the marsh landscape, the combined analysis of $\delta^{15}\text{N}$ data, tissue N concentrations and water column N data together provide a broad overview of N-cycling in the freshwater marshes of the southern Everglades. Previous work by Rudnick et al. (1999) showed that the mean concentration of DIN flowing into the Taylor Slough and C-111 basins was two times higher than in water flowing into the Shark River Slough basin (Rudnick et al., 1999). Our DIN:DON data agree with this finding as the near-canal ratios in TS (0.35) and C-

111(0.35) were both higher than in the SRS basin (0.21; Table 2). In the Taylor Slough basin Rudnick et al. (1999), determined that water column TN concentrations remained constant along a 3 km transect moving away from the canal–marsh interface; however, there was a decrease in DIN concentration from 240 $\mu\text{g l}^{-1}$ to 36 $\mu\text{g l}^{-1}$ along the transect. This downstream decrease in DIN concentration of 204 $\mu\text{g l}^{-1}$ is noteworthy, as it indicates that DIN likely was removed from the water column, presumably through assimilation into organically-bound N. We found the highest DIN:DON ratios at the NC sites and subsequently lower DIN:DON ratios at the downstream IM sites in each basin (Table 2); however, these differences proved not to be significant.

In the C-111 basin, periphyton tissue N concentrations were significantly higher at the NC site compared to the IM site (Table 2). The combination of increased tissue N concentration (periphyton), increased water column DIN concentration, and elevated water column DIN:DON at the NC site compared to the IM site suggests that N is being actively processed between the NC and IM sites in the C-111 basin. This result is supported by the significantly enriched $\delta^{15}\text{N}$ signatures noted for each ecosystem component sampled at the canal–marsh interface. If we assume that canal-derived DIN possesses an enriched $\delta^{15}\text{N}$ signature, as described above, then the biological assimilation of this enriched DIN, which is apparent through the decrease in DIN concentrations between NC and IM sites, would impart an enriched isotope signature on any organism that utilizes the enriched DIN source.

4.5. Addressing variability in $\delta^{15}\text{N}$ signatures

When addressing the variability in $\delta^{15}\text{N}$ signatures between basins, it is important to note that the C-111 and TS basins are both marl (calcium carbonate) soil systems, while the SRS basin is peat (organic) based. Other potential causes of this variation may be the presence of deeper water slough habits found in SRS (as compared to the shallower freshwater marl prairie habitats of TS and C-111) and the decreased abundance of calcium carbonate/cyanobacterial periphyton mats associated with these deep water slough habitats. Specifically, the difference in soil type may be one of the primary reasons SRS displayed less variability in average ecosystem component $\delta^{15}\text{N}$ values between NC, IM and ECO sites (Ecosystem Component Averages; Table 1). The Tukey's comparison of individual ecosystem components in SRS showed that soil (1–5 and 5–10 cm) was most enriched in ^{15}N at the ECO site and above-ground and belowground macrophytes were both less enriched in ^{15}N at the ECO site than the IM location (Table 1). In SRS, only periphyton and soil (0–1 cm) followed the pattern of the highest amount of ^{15}N enrichment at the NC site, as was also displayed in the TS and C-111 basins. The relatively high N concentration found in SRS soils (~ 35 mgN/g dry weight; Table 2) may act to nullify the effects of isotopically enriched N which may enter the SRS basin from canal inflows. Also, the peat soils of SRS may be an additional source of nitrogen (via the oxidation of peat) at the IM and ECO locations, which is not available in the calcium carbonate rich, marl-based systems of TS and C-111 (Table 2; Inglett et al., 2011).

When comparing the two marl-based systems (C-111 and TS), every ecosystem component sampled was more enriched in ^{15}N at the NC site in the C-111 basin relative to the same site in Taylor Slough (Table 1). Despite the fact that tissue N concentrations and DIN:DON are comparable between C-111 and TS (Table 2), it is unclear why the C-111 basin possesses enriched $\delta^{15}\text{N}$ signatures. One plausible explanation is that the water in the L31W canal, that flows into TS, comes from the same South Dade Conveyance canal system as the C-111 basin water, except that it takes that water considerably longer to flow south, down to the C-111 basin (via the C-111 canal; Fig. 1). So the water reaching C-111 marshes has been

in canals longer, has been processed/fractionated more and thus should contain a heavier DIN $\delta^{15}\text{N}$ signature than the water entering TS. Another explanation is that the NC site in the C-111 basin receives a much larger proportion of water input directly from canal over-bank flow than does the NC site in TS. Since 1999, canal water inputs to Taylor Slough were decreased by pumping canal water into a 23 km² ha detention area east of the slough to decrease seepage from the slough (Munoz-Carpena and Li, 2003). This decreased canal water inputs to TS and presumably increased surface water inputs from adjacent Everglades' marshes that were derived more from local rainfall than canals.

5. Conclusions

It is clear that there are multiple factors at play in driving the near-canal ^{15}N enrichment of ecosystem components in the C-111, TS and SRS basins. While this data set illustrates significant trends in $\delta^{15}\text{N}$ isotope enrichment at multiple sample sites across the southern Everglades landscape, additional process-based studies are required that address N-cycling rates (assimilation, mineralization, N-fixation, denitrification, etc.) to confirm the degree of isotope fractionation occurring and the potential N sources (e.g. canals, atmosphere and soil) that provide N to each sample site. In sum, the $\delta^{15}\text{N}$ data set indicates that the marshes of the southern Everglades are not passive conduits for N, directly transporting N from more northern sources to the downstream coastal ecosystems. Rather these marshes are actively processing N as it “spirals” through the ecosystem with downstream freshwater flows (Fig. 2). The processing of N in the P-limited marshes of the southern Everglades if of critical important to “downstream” coastal ecosystems including Florida Bay (which is co-limited in some regions by N and P) and the nearshore coral reef tract (N-limited) where the implication for increased N-loading is much more important.

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References

- Altabet, M.A., Deuser, W.G., 1985. Seasonal-variations in natural abundance of ^{15}N in particles sinking to the deep Sargasso Sea. *Nature* 315, 218–219.
- Altabet, M.A., Francois, R., 1994. Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. *Global Biogeochemical Cycles* 8, 103–116.
- Benner, R., Biddanda, B., Black, B., McCarthy, M., 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Marine Chemistry* 57, 243–263.
- Capone, L.T., Izuno, F.T., Botcher, A.B., Sanchez, C.A., Coale, F.J., Jones, D.B., 1995. Nitrogen concentrations in agricultural drainage water in south Florida. *Agricultural Engineering* 38, 1089–1098.
- Childers, D.L., Doren, R.F., Jones, R., Noe, G.B., Ruggie, M., Scinto, L.J., 2003. Decadal change in vegetation and soil phosphorus pattern across the Everglades landscape. *Journal of Environmental Quality* 32, 344–362.
- Childers, D.L., Boyer, J.N., Davis, S.E., Madden, C.J., Rudnick, D.T., Sklar, F.H., 2006a. Relating precipitation and water management to nutrient concentrations in the

- oligotrophic "upside down" estuaries of the Florida Everglades. *Limnology & Oceanography* 50, 602–616.
- Childers, D.L., Iwaniec, D., Rondeau, D., Rubio, G., Verdon, E., Madden, C.J., 2006b. Primary productivity in Everglades marshes demonstrates the sensitivity of oligotrophic ecosystems to environmental drivers. *Hydrobiologia* 569, 273–292.
- Cifuentes, L.A., Fogel, M.L., Pennock, J.R., Sharp, J.H., 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. *Geochimica et Cosmochimica Acta* 53, 2713–2721.
- Cole, M.L., Kroeger, K.D., McClelland, J.W., Valiela, I., 2005. Macrophytes as indicators of land-derived wastewater: application of a delta N-15 method in aquatic systems. *Water Resources Research* 41, 1–12.
- Craft, C.B., Richardson, C.J., 1993. Peat accretion and N, P and organic C accumulation in nutrient-enriched and unenriched Everglades peatlands. *Ecological Applications* 3, 446–458.
- Currin, C.A., Paerl, H.W., 1998. Environmental and physiological controls on diel patterns of N₂ fixation in epiphytic cyanobacterial communities. *Microbial Ecology* 35, 34–45.
- Davis, S.M., Ogden, J.C., 1994. *Everglades: The Ecosystem and Its Restoration*. St Lucie Press, USA.
- Davis, S.E., Lirman, D., Wozniak, J.R., 2009. Nitrogen and phosphorus exchange among tropical coastal ecosystems. In: Nagelkerken, I. (Ed.), *Ecological Connectivity Among Tropical Coastal Ecosystems*. Springer-Verlag, NY, pp. 9–44.
- Dolenec, T., Vokal, B., Dolenc, M., 2005. Nitrogen-15 signals of anthropogenic nutrient loading in *Anemonia sulcata* as a possible indicator of human sewage impacts on marine coastal ecosystems: a case study of Pirovac Bay and the Murter Sea (Central Adriatic). *Croatica Chemica Acta* 78, 593–600.
- Doyle, R.D., Fisher, T.R., 1994. Nitrogen-fixation by periphyton and plankton on the Amazon floodplain at Lake Calado. *Biogeochemistry* 26, 41–66.
- Ewe, M.L., Gaiser, E.E., Childers, D.L., Iwaniec, D., Rivera-Monroy, V.H., Twilley, R.R., 2006. Spatial and temporal patterns of aboveground net primary productivity (ANPP) along two freshwater-estuarine transects in the Florida Coastal Everglades. *Hydrobiologia* 569, 459–474.
- Gaiser, E.E., Childers, D.L., Jones, R., Richards, J.H., Scinto, L.J., Trexler, J.C., 2006. Periphyton response to eutrophication in the Florida Everglades: cross-system patterns of structural and compositional change. *Limnology Oceanography* 51, 617–630.
- Gardner, W.S., McCarthy, M.J., 2009. Nitrogen dynamics at the sediment-water interface in shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with increased eutrophication. *Biogeochemistry* 95, 185–198.
- Glibert, P.M., Heil, C.A., Hollander, D., Revilla, M., Hoare, A., Alexander, J., Murasko, S., 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. *Marine Ecology-Progress Series* 280, 73–83.
- Goering, J., Alexander, V., Haubensack, N., 1990. Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific Bay. *Estuarine Coastal Shelf Science* 30, 239–260.
- Heaton, T.H., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a Review. *Chemical Geology* 59, 57–102.
- Hela, J., 1952. Remarks on the climate of southern Florida. *Bulletin of Marine Science* 2, 438–447.
- Hogberg, P., 1997. Tansley review no 95: ¹⁵N natural abundance in soil-plant systems. *New Phytologist* 137, 179–203.
- Inglett, P.W., Rivera-Monroy, V., Wozniak, J.R., 2011. Biogeochemistry of nitrogen across the everglades landscape. *Critical Reviews in Environmental Science and Technology* 41, 187–216.
- Iwaniec, D.M., Childers, D.L., Rondeau, D., Madden, C.J., Saunders, C., 2006. Effects of Hydrologic and water quality drivers on periphyton dynamics in the southern Everglades. *Hydrobiologia* 569, 223–235.
- Lamb, K., Swart, P.K., 2008. The carbon and nitrogen isotopic values of particulate organic material from the Florida Keys: a temporal and spatial study. *Coral Reefs* 27, 351–362.
- Lapointe, B.E., Clark, M.W., 1992. Nutrient inputs from the watershed and coastal eutrophication in the Florida Keys. *Estuaries* 15, 465–476.
- Lapointe, B.E., Barile, P.J., Matzie, W.R., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the lower Florida Keys: discrimination of local versus regional nitrogen sources. *Journal of Experimental Marine Biology and Ecology* 308, 23–58.
- Lapointe, B.E., Barile, P.J., Littler, M.M., Littler, D.S., 2005. Macroalgal blooms on southeast Florida coral reefs. II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. *Harmful Algae* 4, 1106–1122.
- Lathja, K., Michener, R.H., 1994. *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, London, p. 316.
- Leonard, L., Croft, A., Childers, D.L., Mitchell-Bruker, S., Solo-Gabriele, H., Ross, M., 2006. Characteristics of surface-water flows in the ridge and slough landscape of Everglades National Park: implications for particulate transport. *Hydrobiologia* 569, 5–22.
- Lettolle, R., 1980. Nitrogen-15 in the natural environment. In: Fritz, P., Fontes, J. (Eds.), *Handbook of Environmental Isotope Geochemistry*. Elsevier, Amsterdam, pp. 407–433.
- Light, S.S., Dineen, J.W., 1994. Water control in the Everglades: a historical perspective. In: Davis, S.M., Ogden, J.C. (Eds.), *Everglades: The Ecosystem and Its Restoration*. St Lucie Press, USA, pp. 47–84.
- Macko, S.A., Ostrom, N.E., 1994. Pollution studies using stable isotopes. In: Lajtha, K., Michener, R.H. (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell, Oxford, pp. 45–62.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles, illustrations for the denitrification and nitrification processes. *Plant Soil Science* 62, 413–430.
- McClelland, J.W., Valiela, I., 1997. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. *Limnology Oceanography* 42, 930–937.
- McCormick, P.V., O'Dell, M.B., 1996. Quantifying periphyton responses to phosphorus in the Florida Everglades: a synoptic-experimental approach. *Journal of the North American Benthological Society* 15, 450–468.
- Munoz-Carpena, R., Li, Y., 2003. Study of the Frog Pond Area Hydrology and Water Quality Modifications Introduced by the C-111 Project Detention Pond Implementation. IFAS-University of Florida, Homestead, FL. Project Report No. TREC-RMC-2003-01.
- Nadelhoffer, K.J., Fry, B., 1994. Nitrogen isotope studies in forested ecosystems. In: Lajtha, K., Michener, R.H. (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell, Oxford, pp. 22–44.
- Noe, G.B., Childers, D.L., Jones, R.D., 2001. Phosphorus biogeochemistry and the impact of phosphorus enrichments: why is the Everglades so unique? *Ecosystems* 4, 603–624.
- Odum, W.E., Odum, E.P., Odum, H.T., 1995. Nature's pulsing paradigm. *Estuaries and Coasts* 18, 547–555.
- Parker III, F.E., 2000. Quantifying spatial and temporal variability in marsh-water column interactions in a southern Everglades marsh. Master's thesis, Florida International University, Miami, FL, USA.
- Rudnick, D.T., Chen, Z., Childers, D.L., Boyer, J.N., Fontaine III, T.D., 1999. Phosphorus and nitrogen inputs to Florida Bay: the importance of the Everglades watershed. *Estuaries* 22, 398–416.
- Rejmankova, E., Komarkova, J., Rejmanek, M., 2004. $\delta^{15}\text{N}$ as an indicator of N₂ fixation by cyanobacterial mats in tropical marshes. *Biogeochemistry* 67, 353–368.
- Schaffranek, R.W., 1996. Coupling models for canal and wetland interactions in the south Florida ecosystem. US Geological Survey Fact Sheet FS-139096. 4.
- Sutula, M., Day, J.W., Cable, J., Rudnick, D., 2001. Hydrological and nutrient budgets of freshwater and estuarine wetlands of Taylor Slough in southern Everglades, Florida (U.S.A.). *Biogeochemistry* 56, 287–310.
- Sutula, M.A., Perez, B.C., Reyes, E., Childers, D.L., Davis, S., Day Jr., J.W., Rudnick, D., Sklar, F., 2003. Factors affecting spatial and temporal variability in material exchange between the Southern Everglades wetlands and Florida Bay (USA). *Estuarine, Coastal Shelf Science* 57, 757–781.
- Troxler-Gann, T.G., Childers, D.L., 2006. Relationships between hydrology and soils describe vegetation patterns in seasonally flooded tree islands of the southern Everglades, Florida. *Plant and Soil* 279, 271–286.
- US Army Corps of Engineers and South Florida Water Management District. 2009. Central and southern Florida project Comprehensive Everglades Restoration Plan, C-111 spreader canal western project. Draft Project Implementation report and Environmental Impact Statement.
- Vargas, R., Novelo, E., 2006. Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. *Biology and Fertility of Soils* 43, 367–372.
- Wada, E., Hattori, A., 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. *Geomicrobiology* 1, 85–101.
- Wigand, C., McKinney, R.A., Cole, M.L., Thursby, G.B., Cummings, J., 2007. Varying stable nitrogen isotope ratios of different coastal marsh plants and their relationships with wastewater nitrogen and land use in New England, USA. *Environ Monit Assess* 131, 71–81.
- Wood, A.D., 2005. Dynamics of detrital particulate organic material: in the ridge and slough landscape of the Everglades. Master's thesis, Florida International University, Miami, Florida, USA.
- Wozniak, J.R., Childers, D.L., Anderson, W.T., Rudnick, D.T., Madden, C.J., 2008. An in situ mesocosm methods for quantifying nitrogen cycling rates in oligotrophic wetlands using ¹⁵N tracer techniques. *Wetlands* 28, 502–512.