

Investigating the use of macrophyte stable C and N isotopic ratios as indicators of wetland eutrophication: Patterns in the P-affected Everglades

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Abstract

We investigated the use of stable C and N isotopic ratios as indicators of shifts in nutrient limitation of aquatic macrophytes in native *Typha* (*Typha domingensis* Pers.) and *Cladium* (*Cladium jamaicense* Crantz) communities growing along the well-established phosphorus enrichment gradient of Water Conservation Area 2A of the Florida Everglades. Both *Typha* and *Cladium* had significantly different $\delta^{15}\text{N}$ ($\sim 4\%$ and 6% , respectively) in affected areas, with live leaves of *Typha* showing elevated $\delta^{15}\text{N}$ up to 7 km from nutrient inflows. In contrast, changes in $\delta^{13}\text{C}$ were inconsistent, with an $\sim 2\%$ increase in *Typha* and a corresponding 2% decrease in *Cladium* of nutrient-affected areas. The isotopic patterns of live leaves were well represented in standing dead leaves of *Cladium*, but not for *Typha*, indicating a significant alteration of isotopic signature during senescence for this emergent species. Correlations of isotopic values with tissue nutrients (total C, N, and P) indicated a greater effect of P on the $\delta^{13}\text{C}$ of both plants and the $\delta^{15}\text{N}$ of *Typha*, and a greater importance of N content in determining $\delta^{15}\text{N}$ of *Cladium*. These results support the use of macrophyte biomass $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as an indicator of eutrophication and shifts between N and P limitation. However, the results also highlight potential pitfalls arising from differences in species-specific response to nutrient enrichment.

The process of wetland eutrophication, like that of any other aquatic system, often involves a transition in the limiting nutrient. Commonly, subtropical and tropical freshwater systems are limited by phosphorus (P) (Reddy et al. 1999). When P is added to these systems, productivity increases, and if continued, other nutrients may become limiting. Most frequently, nitrogen (N) is the element limiting freshwater wetland systems apart from P; therefore P inputs to a P-limited system are likely to result in a shift to increasing N limitation (Verhoeven et al. 1996). Such shifts in nutrient limitation can result in drastic changes in the functioning of a wetland, and thus, there has long been interest in identifying indicators of this transition.

Macrophytes are generally the dominant producers in wetlands, and for this reason many approaches have used their biomass to assess N and P limitation. These methods have included direct measurements of biomass N and P concentrations (Gerloff and Kromholz 1966), stoichiometric ratios between carbon (C) and N (C:N) and N and P (N:P) (e.g., Gusewell et al. 2003), and time-consuming factorial fertilization experiments (Chapin et al. 1986). Natural abundance ratios of stable C and N isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) have also been proposed as indicators of nutrient

limitation in aquatic macrophytes (McKee et al. 2002; Jones et al. 2004).

Isotopic ratios of plant biomass provide insight into plant C and N cycling processes (reviewed by Dawson et al. 2002). Plant tissue $\delta^{13}\text{C}$ has long been used to discern primary photosynthetic pathways as well as relative CO_2 limitation. Decreased stomatal conductance (or closed stomates) results in less enzymatic discrimination against $^{13}\text{CO}_2$, and therefore, higher tissue $\delta^{13}\text{C}$. Thus, $\delta^{13}\text{C}$ of plants can provide information regarding physiological controls and stresses (e.g., water and nutrient availability) for plant growth.

Plant $\delta^{15}\text{N}$ has also been used to study plant processes such as N uptake and translocation (Evans 2001; Dawson et al. 2002). Early work attempted to utilize plant $\delta^{15}\text{N}$ to elucidate the source of N plant communities. Since then, other factors have been reported to affect plant $\delta^{15}\text{N}$ including external N form and concentration, plant type, mycorrhizal status, and environmental conditions (Handley et al. 1998). In general, plant assimilation is believed to discriminate against ^{15}N , leaving plant biomass with a $\delta^{15}\text{N}$ somewhat more depleted than its source N. Under conditions of N scarcity, or at high growth rates, however, available N pools can be more completely utilized, thereby reducing the potential for uptake-associated fractionation.

The link between isotopic fractionation and environmental conditions indicates that plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may be used to assess the shifts in nutrient limitation observed during eutrophication. In this study, we wished to examine the isotopic changes resulting from a shift from P to N limitation, where $\delta^{13}\text{C}$ may be useful to assess the photosynthetic changes resulting from increased growth rates, and $\delta^{15}\text{N}$ could indicate increased N demand or decreased N availability under conditions of N limitation. We tested these hypotheses by investigating the patterns of

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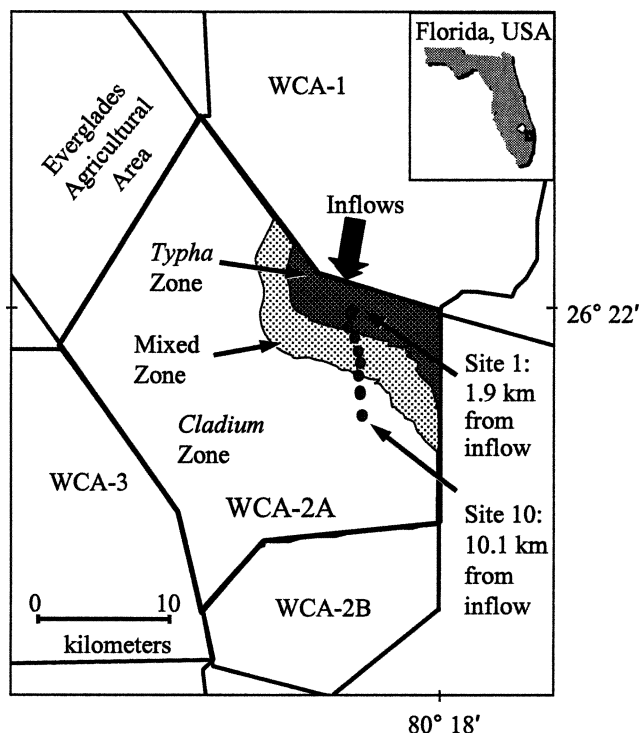


Fig. 1. Location of Water Conservation Area 2A (WCA-2A) and the transect of sampling sites used in this study.

macrophyte $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a well-documented gradient of P pollution in a historically P-limited Florida Everglades ecosystem.

Materials and methods

Site description—The Florida Everglades is a large, subtropical wetland in the southern tip of the Florida peninsula (Fig. 1). During the last century, the Everglades has been fragmented through the construction of a system of dikes, levees, and canals into distinct hydrologic units known as Water Conservation Areas (WCAs). Of these units, WCA-2A is a diked, 547-km² portion of the northern Everglades (Fig. 1). WCA-2A is characterized as a peat-based, freshwater wetland underlain by limestone. In its natural state, WCA-2A is a highly P-limited ecosystem; however, inputs of P-rich agricultural drainage water along the northeastern perimeter have lessened P limitation in affected areas. The result of the discharges has been the creation of a P enrichment gradient in the surface water and soil with high nutrient levels nearer the spillways and unenriched, background levels in the interior of WCA-2A (Table 1) (Reddy et al. 1993).

The addition of P to the WCA-2A marsh has resulted in alterations to the microbial and algal communities, and in more affected areas, the macrophyte community has also undergone a transition favoring the replacement of native Everglades marshes (dominated by sawgrass, *Cladium jamaicense* Crantz) and openwater sloughs (dominated by periphyton mats, *Utricularia purpurea* Walt., and *Nymphaea odorata* Ait.) with extensive stands of eutrophically

Table 1. Nutrient concentrations at the enriched (Near inflow) and oligotrophic (Marsh interior) WCA-2A transect extremes.

Parameter	Units	WCA-2A location	
		Near inflow	Marsh interior
Porewater*			
NH ₄ ⁺	mg N L ⁻¹	3.6	2.3
PO ₄ ³⁻	mg P L ⁻¹	0.6	0.1
Floodwater†			
Total N	mg N L ⁻¹	2.0	1.5
NH ₄ ⁺	μg N L ⁻¹	35.0	30.0
NO ₃ ⁻	μg N L ⁻¹	7.0	7.0
Total P	μg P L ⁻¹	70.0	7.0
PO ₄ ³⁻	μg P L ⁻¹	17.0	5.0

* DeBusk et al. 1994 (0–10 cm soil depth).

† Vaithyanathan and Richardson (1997).

adapted cattail, *Typha domingensis* Pers. (Reddy et al. 1999). Reduced P limitation and subsequent increased demand for other essential nutrients (e.g., N) is believed to be one of the principal causes of the WCA-2A plant, algal, and microbial changes. Thus, in P-affected areas near the inflows, there is a higher demand for N and an increased N limitation of the WCA-2A ecosystem (reviewed by Noe et al. 2001). This transition from P to N limitation near the WCA-2A inflows has been observed through decreasing total N to total P ratios (TN:TP) in the soil and macrophytes near the WCA-2A inflows, as well as increased microbial N assimilation, and higher biological nitrogen fixation rates in affected areas (Inglett et al. 2004).

Transect sampling—Spatial patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were assessed using samples collected in September 2000 from a transect of 10 sites spanning the WCA-2A nutrient gradient (Fig. 1) (Inglett 2005). Particular site locations were chosen on the basis of the dominance of emergent macrophytes (open-water slough areas were avoided), and the presence of sufficient plants of both *Typha* and *Cladium*. Water depth varies little between these sites and is generally shallow (~0.25 m). At each of these sites, four replicate samples of soil (0–10 cm) and aboveground plant materials were collected at random within an area of 100 m². Live and senescent (standing dead) plant leaves of *Typha* and *Cladium* were collected at each site by clipping four healthy, intact, mature plants at the soil surface. Soil samples were taken using a sharpened, thin-wall aluminum tube (10-cm diameter). Samples were placed on ice and returned to the laboratory where plant leaves were sorted into live and senescent (brown) portions. Recently senesced (i.e., yellow) portions were not included in the analysis. Live and dead leaves were gently wiped with a clean, damp cloth to remove surface debris, and cut into approximately 10-cm sections for drying. Soil samples were screened to remove larger organic components (e.g., large roots). All samples were dried at 55°C and ground to pass a 2-mm mesh using a Wiley mill. Subsamples of soil and live and

senescent plant materials were then ball milled for chemical and isotopic analyses.

Chemical and isotopic analyses—Total P was measured colorimetrically using a Technicon Autoanalyzer (method 365.1, U.S. EPA 1993) following Kjeldahl digestion (method 351.2, U.S. EPA 1993). Total N and C and bulk C and N isotopic ratios were determined simultaneously using a Costech model 4010 elemental analyzer (Costech Analytical Industries) coupled to a Finnigan MAT DeltaPlusXL mass spectrometer (CF-IRMS, Thermo Finnigan) via a Finnigan ConFlo III interface. Elemental calibration was accomplished using acetanilide (10.4% N, 71.1% C), and measurements were verified using a standard wheat flour (1.85% N, 40.2% C Iso-Analytical).

Ratios of C- and N-stable isotopes (R_{sample}) are expressed as per mille (‰) differences from the ratio of a standard (R_{std} , atmospheric N_2 and Pee Dee Belemnite, for N and C, respectively) using delta notation (δ) as: $\delta_{\text{sample}} = [(R_{\text{sample}}/R_{\text{std}}) - 1] \times 1000$. Low N-containing samples were corrected for mass dependency of isotopic measurement using an equation derived from the isotopic values of the acetanilide standards (6) with each analysis. Such mass-dependent corrections were generally small (<1‰), and necessary only for samples with mass 28 peak intensities less than 0.5 V. Following mass dependency correction, all $\delta^{15}N$ values were adjusted for accuracy using known isotopic standards (wheat flour, $\delta^{15}N = 2.55\text{‰}$, $\delta^{13}C = -26.43$; IAEA-N1, $\delta^{15}N = 0.4\text{‰}$; ANU-Sucrose, $\delta^{13}C = -10.5\text{‰}$). Analytical precision for isotopic standards was less than $\pm 0.1\text{‰}$ and $\pm 0.3\text{‰}$ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Statistical analysis—Correlations of $\delta^{13}C$ and $\delta^{15}N$ with plant tissue nutrient concentrations and ratios (TC, TN, TP, TC:TN, TN:TP) were assessed through Pearson product-moment correlation with casewise deletion of missing data using Statistica[™] (StatSoft). In select cases, log transformations were applied to improve normality.

Results

Patterns of soil nutrients along the transect reveal the clear P enrichment of the soil and plant components near the WCA-2A inflows (Fig. 2). Soil TP increased threefold (from 0.05% to 0.16% P). Over this same gradient, the P content of live *Cladium* increased almost threefold (from 0.02% to 0.06% P), and that of *Typha* increased approximately twofold (from 0.05% to 0.11% P). In contrast, soil TN remained essentially constant along the transect at 3%; however, there were small increases in the TN of live *Cladium* (0.75% to 0.90%) and *Typha* (0.75% to 1.0%). Because of the small change in TN relative to the increase in TP, molar TN:TP ratios of soil and plants decreased near the inflows. For soil, TN:TP ranged from 150 at the interior sites to approximately 40 at site 1, whereas a narrower range was encountered for both *Cladium* (66 to 33) and *Typha* (35 to 18).

Isotopic compositions of both *Typha* and *Cladium* changed measurably along the WCA-2A transect (Fig. 3).

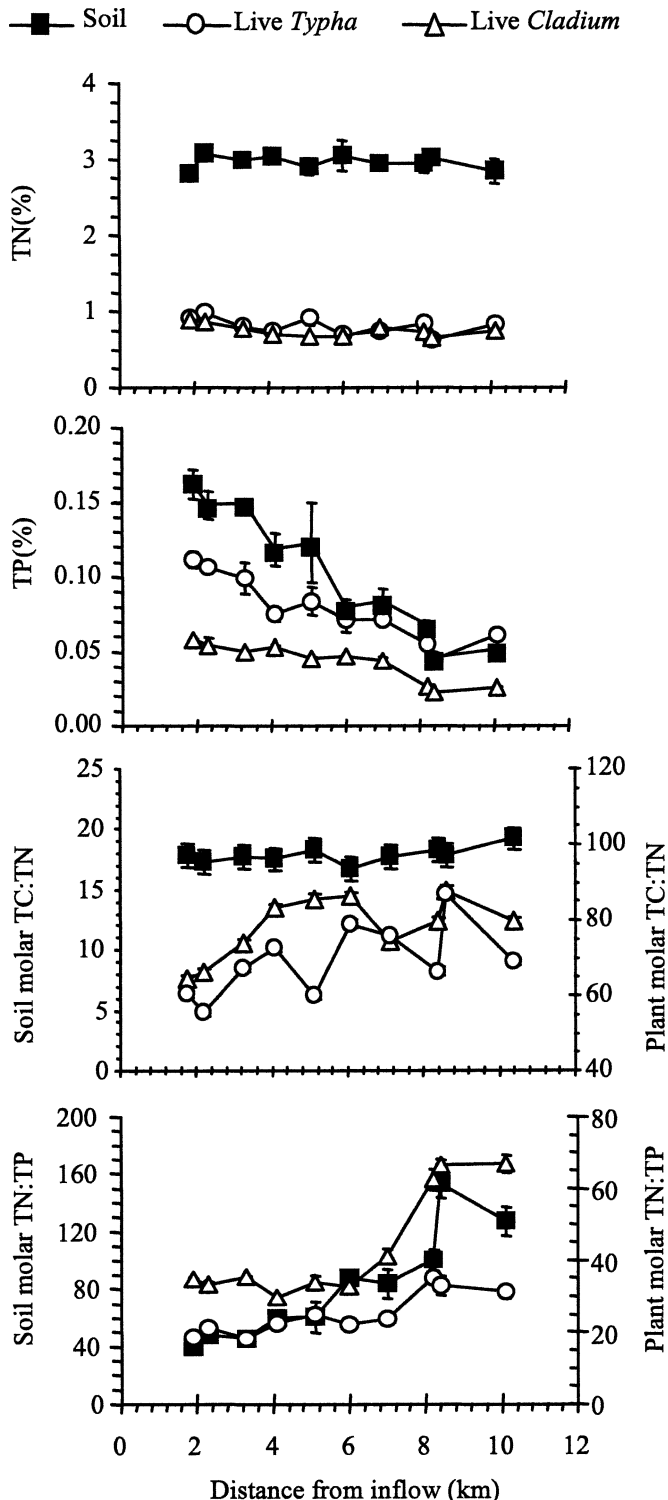


Fig. 2. Patterns of nutrients (TC, TN, TP) in soils and live plant leaves (*Typha* and *Cladium*) across the WCA-2A transect of the Florida Everglades. Points represent the mean (\pm SE) of four replicate samples.

Live leaf $\delta^{13}C$ ranged from -26.3‰ to -27.8‰ for *Typha*, and -25.0‰ to -27.0‰ for *Cladium*. In general, *Typha* biomass was more ^{13}C -depleted than that of *Cladium* at a given site, but the two were approximately equal near the

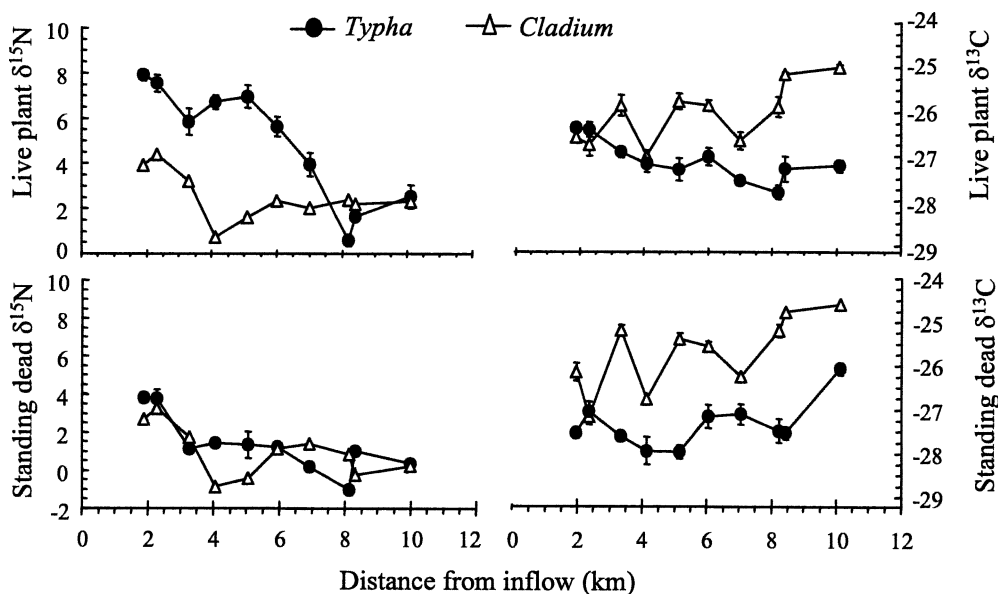


Fig. 3. Patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of live and standing dead leaves of *Cladium* and *Typha* along the WCA-2A nutrient gradient. Points represent the mean (\pm SE) of four replicate plant samples.

inflows. The patterns of $\delta^{13}\text{C}$ in live plants were not consistent between *Typha* (becoming $\sim 1.5\text{‰}$ more positive) and *Cladium* (becoming $\sim 2\text{‰}$ more negative) near the inflows (Fig. 2). This between-species difference in $\delta^{13}\text{C}$ was not seen in the standing dead leaf materials, which showed decreases ($\sim 1.5\text{‰}$ to 2‰) for both species near the inflows.

In both species, there was an increase in live leaf $\delta^{15}\text{N}$ of plants nearer to the inflows, with enrichments of 4% and 6% in *Cladium* and *Typha*, respectively. The pattern of increased $\delta^{15}\text{N}$ near the WCA-2A inflows was also seen in the standing dead leaf materials of both species (Fig. 3). The $\delta^{15}\text{N}$ of standing dead materials was generally lower relative to corresponding live biomass; however, in *Cladium*, the difference was consistently $\sim 2\text{‰}$ throughout the transect, while the live–dead difference in *Typha* varied from $\sim 1\text{‰}$ in the interior locations to $\sim 4\text{‰}$ near the inflow (Fig. 3).

Pearson product-moment correlation demonstrated that $\delta^{15}\text{N}$ in live *Cladium* was largely related to plant tissue N (TN and TC:TN) while that of live *Typha* correlated better with plant P (TP and TN:TP) (Table 2). In standing dead materials, $\delta^{15}\text{N}$ was again better correlated with TN and TC:TN in *Cladium* and TP and TN:TP in *Typha*. Phosphorus levels explained more variability in $\delta^{13}\text{C}$ of live *Typha* and *Cladium*, and again in the standing dead material of *Cladium*. In contrast, TC offered the best correlation with $\delta^{13}\text{C}$ in the standing dead *Typha*.

Discussion

$\delta^{13}\text{C}$ patterns—Carbon-stable isotopic composition of plant tissues is often used to indicate photosynthesis because unlike instantaneous gas exchange measurements, $\delta^{13}\text{C}$ can integrate the net effects of a variety of processes regulating photosynthetic rates (e.g., humidity, irradiance,

Table 2. Pearson product-moment correlation coefficients (r) between leaf isotopic composition and leaf nutrient concentrations and ratios for plants collected along the WCA-2A nutrient enrichment gradient.

Plant type	n	TN	TC	TP	TC:TN	TN:TP
$\delta^{13}\text{C}$						
<i>Cladium</i> live	39	-0.46**	0.46**	-0.76***	0.47**	0.65***
<i>Cladium</i> dead	37	-0.70***	-0.38*	-0.79***	0.69***	0.76***
<i>Typha</i> live	40	0.20	-0.28	0.64***	-0.23	-0.68***
<i>Typha</i> dead	38	-0.13	-0.41*	0.14	0.14	-0.20
$\delta^{15}\text{N}$						
<i>Cladium</i> live	39	0.44**	-0.38*	0.25	-0.42**	-0.09
<i>Cladium</i> dead	37	0.65***	-0.24	0.47**	-0.66***	-0.32
<i>Typha</i> live	40	0.43**	-0.59***	0.85***	-0.49*	-0.83***
<i>Typha</i> dead	38	0.56***	0.45**	0.67***	-0.52**	-0.53**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

and CO₂ availability) (reviewed by Dawson et al. 2002). In general, variation in the $\delta^{13}\text{C}$ of fixed C within a given species is based on physiological changes affecting stomatal conductance in relation to the rate of CO₂ fixation. When stomates are more open or rates of photosynthesis are low, internal leaf CO₂ levels nearly equal atmospheric, and enzymatic discrimination against ¹³C becomes near maximal, leaving fixed C more isotopically depleted. Conversely, plants use internal CO₂ more completely when stomates are closed or photosynthesis is high, resulting in less discrimination and more positive $\delta^{13}\text{C}$ values closer to atmospheric CO₂ ($\sim -8\%$).

In WCA-2A, differences were seen in the C isotopic composition of both *Typha* and *Cladium* along the transect, where $\delta^{13}\text{C}$ of *Typha* showed a gradual increase ($\sim 2\%$), and *Cladium* an overall decrease ($\sim 2\%$) (Fig. 3). These plants are both C₃ species, and the overall ranges in $\delta^{13}\text{C}$ ($<3\%$) are similar to the $\sim 2\%$ shifts in mangrove $\delta^{13}\text{C}$ along a gradient from P to N limitation reported by McKee et al. (2002).

Unpolluted Everglades systems are known to be severely P-limited; therefore, patterns of increasing $\delta^{13}\text{C}$ could represent the effects of P addition on increasing rates of C fixation. Several studies have measured rates of photosynthesis and gas exchange in Everglades *Typha* and *Cladium* (e.g., Koch and Rawlik 1993; Miao et al. 1997) with the overall conclusions that nutrient additions do enhance rates of photosynthesis, and that stomatal conductance of *Typha* and *Cladium* remains essentially constant along the WCA-2A nutrient gradient. The lack of significant changes in stomatal conductance suggests that variation in live plant $\delta^{13}\text{C}$ of WCA-2A may be the result of added nutrients stimulating photosynthesis. This mechanism may explain the $\sim 2\%$ increase in $\delta^{13}\text{C}$ of live *Typha* near the inflows (Fig. 3); however, the $\delta^{13}\text{C}$ of *Cladium* decreased toward the inflows. Decreased photosynthesis in *Cladium* near the inflows is puzzling, given the demonstrated ability of nutrient additions to enhance *Cladium* photosynthesis (Miao et al. 1997).

Several processes can be invoked to explain the inconsistencies in $\delta^{13}\text{C}$ patterns of *Typha* and *Cladium* in WCA-2A. First, low $\delta^{13}\text{C}$ of *Cladium* near the inflows could represent photosynthetic inhibition, which is likely the result of the highly reducing soil conditions in these eutrophic areas. Reducing conditions have been shown to reduce photosynthetic activity of *Cladium* relative to *Typha* (Pezeshki et al. 1996). Alternatively, the different responses of $\delta^{13}\text{C}$ could be due to other differences between *Typha* and *Cladium* such as changes in leaf morphology with increasing nutrient availability, altered biomass distribution (root vs. shoot), or even anatomical differences promoting the internal cycling of soil-derived CO₂. The different isotopic responses suggest a more species-specific physiological difference; however, other potential biogeochemical explanations make it difficult to attribute the observed patterns in *Typha* and *Cladium* to differences in photosynthesis alone.

$\delta^{15}\text{N}$ patterns—It was hypothesized that increased P availability and consequent N demand would lead to

altered $\delta^{15}\text{N}$ of the plant biomass along the WCA-2A transect. Significant changes were observed in the live tissues of both *Typha* and *Cladium* near the inflows, with the $\delta^{15}\text{N}$ of each species increasing by $\sim 8\%$ and 4% , respectively (Fig. 3). The ranges observed in this study are comparable to those of other similar studies. For example, McKee et al. (2002) observed a $\sim 5\%$ increase in $\delta^{15}\text{N}$ of N-limited red mangrove trees relative to corresponding P-limited ones, whereas Jones et al. (2004) observed a $\sim 4\%$ range in $\delta^{15}\text{N}$ of macrophytes of lakes of varying N status.

Increased $\delta^{15}\text{N}$ in plants is often interpreted as an indicator of sewage or pollution N, and therefore one explanation for the higher N isotopic ratios of macrophytes near the inflows is the incorporation of isotopically “heavy” N from the drainage water discharges of the Everglades Agricultural Area (e.g., Cole et al. 2004). Isotopic measurements of inflow total N indicate that the $\delta^{15}\text{N}$ of N discharged into WCA-2A is $\sim 4\%$ (Inglett 2005); therefore this could only partially explain the observed isotopic enrichment of *Cladium*, which shows increased $\delta^{15}\text{N}$ only in areas near the inflows. Unlike *Cladium*, however, the increase in *Typha* $\delta^{15}\text{N}$ occurs up to 7 km removed from the inflows in areas still apparently limited by P. Other measurements also indicate that the $\delta^{15}\text{N}$ of porewater NH₄⁺ (the dominant N species in reduced soils) is similar at both the eutrophic and nonaffected WCA-2A sites ($\sim 7.5\%$; Inglett 2005). Given these results, it appears that the WCA-2A inflows are affecting plant $\delta^{15}\text{N}$ independently of a direct influence on N sources.

Organic matter diagenesis has also been proposed to increase $\delta^{15}\text{N}$ of organic matter (e.g., Novak et al. 1999). By this mechanism, isotopically light forms of organic N are preferentially mineralized, leaving the residual organic N in the soil system isotopically heavier (Fogel and Tuross 1999). If P loading of WCA-2A stimulated organic N mineralization and associated loss processes, this theory would predict that the $\delta^{15}\text{N}$ of soil organic matter would gradually increase near inflows, leading to a similar, gradual $\delta^{15}\text{N}$ increase in the macrophyte communities utilizing mineralized soil N. This may be the case in the Everglades where additions of P to the highly P-limited soil do stimulate microbial activities with a concomitant increase in both N mineralization and NH₄⁺ flux (White and Reddy 2000). Despite the plausibility of this argument, however, such a mechanism fails to explain the different responses of *Typha* and *Cladium* growing along an identical eutrophication gradient.

One difference between *Cladium* and *Typha* that could affect their $\delta^{15}\text{N}$ is the plants' differing demand for N after P addition. *Typha* and *Cladium* maintain different life strategies, where *Cladium* is adapted to survive under conditions of drought, fire, and extremely low levels of available P, and *Typha* is adapted to rapidly take up available P (lowering TN:TP) and colonize areas of relatively high nutrient availability (Newman et al. 1996). Differing N demand after P addition has been demonstrated for *Cladium* and *Typha* grown in solution culture, with *Typha* significantly increasing its N assimilation capacity from 2.9 to 54 mg g⁻¹ root dry wt d⁻¹, when grown at P supply rates of 10 and 500 $\mu\text{g P L}^{-1}$ (Lorenzen et al. 2001).

At the same P levels, *Cladium* showed no such increase in N assimilation; therefore, the increased $\delta^{15}\text{N}$ in *Typha* of WCA-2A may represent a higher N demand and N assimilation rate by *Typha* relative to *Cladium*.

Evans (2001) summarized the factors affecting isotopic fractionation of plant N relative to source N. In this model, no fractionation is observed during the process of N uptake into the cell (either at high or low external N concentrations), but rather occurs during N assimilation inside the cell through enzymatic action of nitrate reductase (15%) and glutamine synthetase (17%). Discrimination (favoring incorporation of ^{14}N) can only occur when there is a net efflux of N (^{15}N -enriched) from the cell, as would be the case under non-N-limiting conditions. In this case, the assimilated N would be isotopically lighter than the source N. When N becomes limiting, or increased assimilation limits N efflux, the discrimination against ^{15}N is reduced, resulting in a plant tissue $\delta^{15}\text{N}$ more representative of the external N supply. This could be the case along the WCA-2A transect, where *Typha* decreases its discrimination against ^{15}N with increased demand for N, while *Cladium* fails to exhibit increased N assimilation rates (Lorenzen et al. 2001), and thus, equally discriminates against ^{15}N at all P levels along the transect.

Live–dead comparison—Many studies use stable isotopic ratios of organic matter as a record of paleoproductivity or nutrient conditions in lakes (e.g., Brenner et al. 1999) and wetlands (Novak et al. 1999; Smallwood et al. 2003). In systems driven by macrophyte production, the use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to indicate past nutrient conditions is based on the assumption that isotopic signatures of soil or sediment organic matter ultimately reflect those of the living source plant (Wooller et al. 2003b). In the current study, there were contrasting patterns of $\delta^{13}\text{C}$ and a significant increase in the $\delta^{15}\text{N}$ of live leaves of *Cladium* and *Typha* in areas nearing the WCA-2A inflows (Fig. 3). It is thus important to compare the isotopic ratios of the live and standing dead biomass to assess any effect of plant senescence.

For *Cladium*, there was a near-perfect agreement between $\delta^{13}\text{C}$ of the live and standing dead leaves along the WCA-2A transect (Fig. 4). The isotopic agreement between live and dead leaves in this species is similar to that observed for mangroves (Wooller et al. 2003a,b) and suggests a potential use of *Cladium* $\delta^{13}\text{C}$ to indicate long-term nutrient effects. The same relation was not observed for *Typha*, where there was no discernable relation in the $\delta^{13}\text{C}$ in live versus standing dead (Fig. 4). Thus, the $\delta^{13}\text{C}$ of *Typha* biomass appears to be less useful as a palaeoindicator and may limit the use of *Typha* detritus to directly infer nutrient conditions present at the time of leaf deposition.

Unlike the findings of Wooller et al. (2003a,b), there was a general decrease in $\delta^{15}\text{N}$ as leaf materials of WCA-2A *Typha* and *Cladium* senesced (Figs. 3, 4). In *Cladium*, this decrease in $\delta^{15}\text{N}$ during senescence was almost constantly 2‰, whereas in *Typha*, there was a greater and more variable decrease in $\delta^{15}\text{N}$ of senescent relative to the live tissues (1–5.5‰) (Fig. 4). Decreasing $\delta^{15}\text{N}$ during senescence (including both translocation and early aerial decay processes) could indicate the removal of high $\delta^{15}\text{N}$

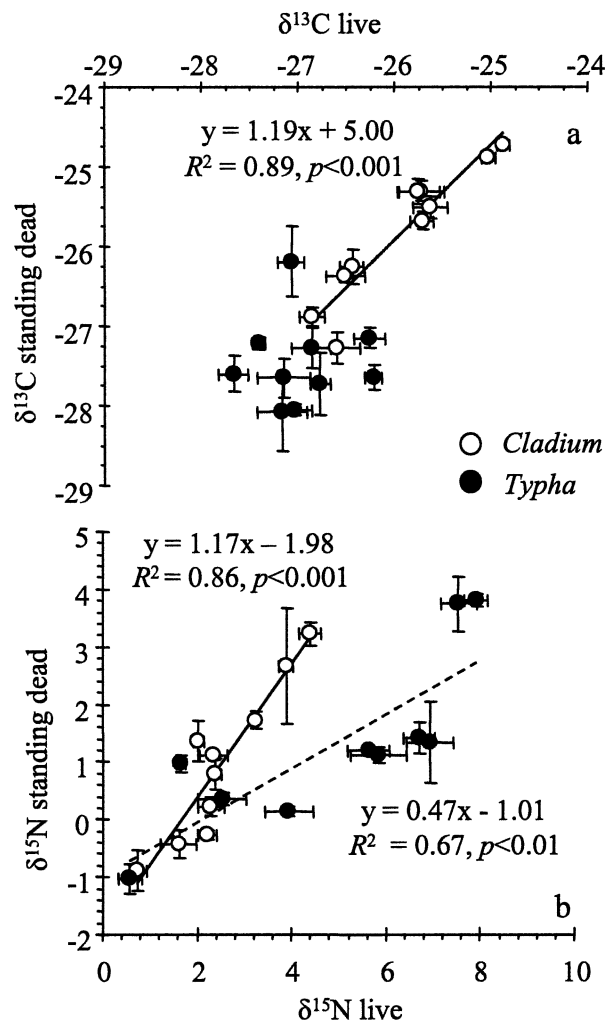


Fig. 4. Correlations between (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ in live and standing dead leaf biomass of *Typha* and *Cladium* along the WCA-2A nutrient gradient. Points represent the means (\pm SE) of four samples collected at each of the 10 transect stations.

compounds (e.g., amino acids) from the leaf (Handley et al. 1998). In this manner, the variability in the isotopic change upon leaf senescence between *Cladium* and *Typha* may further illustrate the difference between these two species, both in terms of the composition of N forms in senescent material, and in their efficiency of nutrient resorption.

Correlation with tissue nutrients—An additional explanation of the differences between *Cladium* and *Typha* involves correlations of macrophyte $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with tissue nutrient concentrations (Table 2). The correlation between live leaf $\delta^{13}\text{C}$ and tissue P content (measured as TP or TN:TP) in both species is interesting considering the opposite relations for *Typha* (positive) and for *Cladium* (negative). As earlier discussed, enhanced P levels could result in increased rates of photosynthesis (yielding increased $\delta^{13}\text{C}$) in *Typha*. In *Cladium*, however, there is no physiological reason to expect decreased photosynthesis as a result of increasing levels of the limiting nutrient. The

negative correlation of $\delta^{13}\text{C}$ with P in *Cladium* may be a consequence of the correlation between P and its effects on soil redox conditions on the WCA-2A gradient. TP content also explained most of the variability in $\delta^{13}\text{C}$ of standing dead *Cladium*, indicating, like the strong correlation between $\delta^{13}\text{C}$ of the live and standing dead forms, that there is a consistent effect of senescence on *Cladium*. In contrast, the relation between $\delta^{13}\text{C}$ and P content seen in live *Typha* was not observed in the standing dead form. In this case, tissue TC showed the best correlation with $\delta^{13}\text{C}$ in the standing dead, suggesting that much of the isotopic change with senescence in *Typha* came through a loss of labile C compounds.

The $\delta^{15}\text{N}$ of *Cladium* correlated best with N content and C:N ratio (indicating control by N availability), whereas that of *Typha* best correlated with tissue P and N:P ratios (indicating control by P availability). TN:TP ratios are often viewed as an indication of nutrient limitation by N and P (Gusewell et al. 2003). In this regard, the high values of TN:TP of the interior WCA-2A marsh are characteristic of the P-limited status of the Everglades system as a whole. With P loading in the inflows, both *Cladium* and *Typha* respond with slightly increasing leaf N content and large decreases in TN:TP (Fig. 2). Despite the similar responses, however, there is a profound difference in TN:TP between *Cladium* and *Typha*, with the values of *Typha* in the P-limited interior sites being approximately equal to those of *Cladium* in the highly affected inflow areas.

In their study of Everglades plant communities, Daoust and Childers (1999) observed the TN:TP of *Cladium* to average 53 and 70 during the dry and wet seasons, respectively. They concluded that TN:TP ratios above 35 indicated *Cladium* was continually P-limited while other macrophytes (*Peltandra virginica* and *Pontederia cordata*) exhibited N limitation when their TN:TP ratios fell below 30. In this current study, TN:TP ratios of *Cladium* were 66 in the interior WCA-2A sites, but decreased to approximately 33 near the inflows (Fig. 2). On the basis of the arguments of Daoust and Childers (1999), the *Cladium* of this study at most became colimited by N and P, but never became solely N-limited. In contrast, *Typha* during this study displayed TN:TP ratios of 35 in the interior marsh, indicating either colimitation by N and P or that of P alone. Thus, the lack of change in *Cladium* $\delta^{15}\text{N}$ along the transect may have resulted from lack of strong N limitation in the *Cladium* sampled in this study.

Isotopes as indicators of eutrophication—Natural abundance studies of C and N isotopes are often used to assess environmental processes and trace C and N flow through ecosystems. In this study, analysis of plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ demonstrated an additional use of the natural abundance stable isotopic approach by indicating the effects of P-induced eutrophication within a wetland ecosystem. The magnitudes of changes in $\delta^{13}\text{C}$ (~2‰) and $\delta^{15}\text{N}$ (up to 6‰) across the P gradient of WCA-2A are similar to those reported in other studies. Patterns of $\delta^{13}\text{C}$ most likely represented the effects of nutrient additions on photosynthetic and physiological processes that differed between *Typha* and *Cladium*, and thus, possibly represent physio-

logical differences between these two species in response to varying nutrient regimes. Similarly, $\delta^{15}\text{N}$ demonstrated a potential to identify changing plant N demand with increasing P availability and also indicated a difference between *Typha* and *Cladium* in response to added P.

Correlations of isotopic ratios with tissue nutrient content indicate a dominance of P control of $\delta^{13}\text{C}$ in live *Typha* and *Cladium* and of $\delta^{15}\text{N}$ in live *Typha*, whereas tissue N content explained most of the variability of $\delta^{15}\text{N}$ in live *Cladium* leaves. Though plausible explanations were given for the species-dependent variation in the response of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, several questions remain. For example, it is uncertain why *Cladium* and *Typha* exhibited opposite patterns of $\delta^{13}\text{C}$ in live biomass along the same nutrient enrichment gradient. Also, it is unclear why *Cladium* failed to exhibit increased $\delta^{15}\text{N}$ in the same manner as *Typha*. More detailed studies addressing plant age and seasonal patterns, perhaps including hydroponic P addition experiments, are required before satisfactory explanations can be found.

Higher-resolution sampling at lower ambient P levels could also help determine the response of *Typha* $\delta^{15}\text{N}$ during the initial stages of P enrichment. It is possible that $\delta^{15}\text{N}$ of *Typha* leaf tissue may indicate the increased N demand before TN:TP ratios have been altered. Thus, *Typha*, which responds more rapidly to P than *Cladium*, could provide a sensitive early warning indicator of P enrichment. Similarly, other Everglades plant species (e.g., *Eleocharis* spp. and *Nymphaea odorata*) could also be tested for this response to P enrichment. The isotopic similarity between the live and senescent leaf materials also illustrates a potential use of *Cladium* detritus to infer past nutrient conditions (e.g., in a peat profile). Such live–dead similarity was not observed, however, for *Typha* in this study, indicating that isotopic patterns in macrophyte-derived detritus are potentially species specific. For this reason, knowledge of the effects of nutrient availability on isotopic composition of individual macrophyte species must be known to make an accurate assessment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ patterns in a given system.

Nutrient availability is a primary factor controlling wetland productivity and species composition, and is also a direct determinant of wetland function. As a result, much effort has been focused on identifying and predicting how nutrient loading alters biogeochemical cycles surrounding the shift between N and P limitation. Natural abundance levels of C and N isotopes are believed to act as an integrator of ecosystem processes, and thus, analysis of these ratios may offer a promising technique to elucidate the nature and extent of nutrient limitation. If properly calibrated with other indicators (e.g., TN:TP) and experimental manipulation of nutrient limitation (i.e., fertilization experiments), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may provide an easily measured indicator of the transition between N and P limitation in wetlands.

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