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Estimating Sources of Soil Organic Matter in Natural and Transplanted Estuarine Marshes using Stable Isotopes of Carbon and Nitrogen

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Stable isotopes of carbon (δ^{13}C)[a] and nitrogen (δ^{15}N)[a] were used to determine the origin of soil organic matter in irregularly flooded natural and transplanted estuarine marshes.

$$a_X = [R(\text{sample}) - R(\text{standard})]/R(\text{standard}) \times 1000$$

where X is δ^{13}C or δ^{15}N and R is ^{13}C/^{12}C or ^{15}N/^{14}N of the sample and the international standards Pee Dee Belemnite (CO_2) and atmospheric nitrogen (N_2).}

Emergent and aquatic plants, soils, detritus and adjacent forest vegetation were collected from one natural and two transplanted marshes and the δ^{13}C and δ^{15}N values were measured.

The isotopic composition of natural and transplanted marsh soils was similar to emergent vegetation. The δ^{15}N of marsh soils fell within the range of emergent macrophytes (+1 to +4‰), while soil δ^{13}C ranged from −18 to −26‰. The δ^{13}C of natural marsh soils (−20 to −24‰) reflected mixing between C_3 (*Juncus*) and C_4 (*Spartina, Distichlis*) marsh plants while the soil δ^{13}C (−24 to −26‰) of one transplanted marsh was attributed to top soil applied prior to establishment of the marsh. The δ^{13}C (−25‰) and δ^{15}N (+5‰) of estuarine detritus suggested mixing between terrestrial material and phytoplankton.

Marsh emergent vegetation appears to be the principal source of organic matter in soils of both natural and transplanted marshes. However, the young transplanted marsh soils also reflect external inputs of C (terrestrial material) contributed during marsh establishment.

Introduction

Stable isotope measurements (δ^{13}C, δ^{15}N) have been used to determine sources of detritus in estuaries and to delineate estuarine food webs (Haines, 1976*a, b*; 1977; Haines & Montague, 1979; Hackney & Haines, 1980; Hughes & Sherr, 1983; Mariotti *et al.*, 1983; Peterson *et al.*, 1985, 1986). The δ^{13}C value of plants is determined primarily by the mode of photosynthesis. Plants utilizing the C_3 pathway, which include terrestrial forest

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vegetation, exhibit $\delta^{13}C$ values ranging from -20 to -34‰, while C_4 plants, such as the marsh emergent *Spartina,* have $\delta^{13}C$ values of -6 to -19‰ (Smith & Epstein, 1971; Sternberg & DeNiro, 1983). The $\delta^{13}C$ values of phytoplankton in temperate waters is intermediate between C_3 and C_4 plants, ranging from -20 to -23‰ (Sackett *et al.*, 1965; Gearing *et al.*, 1984). The $\delta^{15}N$ in vegetation also varies for terrestrial forest vegetation ($+3$ to -5‰), phytoplankton ($+6$ to $+11$‰) and marsh emergent vegetation ($+3$ to $+6$‰) (Wlotzka, 1972; Wada *et al.*, 1975; Peters *et al.*, 1978; Sweeney & Kaplan, 1980; Macko, 1983; Mariotti *et al.*, 1983; Peterson *et al.*, 1985).

Stable carbon isotope analysis of estuarine sediments and detritus suggests that the contribution of marsh emergent vegetation to estuarine organic matter pools may not be as important as previously believed. Haines (1976*b*) and Sherr (1982) found the $\delta^{13}C$ of detritus collected from Georgia tidal creeks and shelf waters (-18 to -25‰) were similar to phytoplankton. Hackney & Haines (1980) measured $\delta^{13}C$ values of -25 to -27‰ for detritus in a Mississippi estuary and suggested that terrestrial material was the principal source of carbon in this estuary. The $\delta^{13}C$ of estuarine marsh soils and sediments ranges from -16 to -25‰ (Johnson & Calder, 1973; Haines, 1976*a*, *b*; Haines, 1980; Sherr, 1982; Hughes & Sherr, 1983; Ember & Williams, 1985). Haines (1976*b*) suggested that the $\delta^{13}C$ of marsh soils may result from mixing of C_3 and C_4 carbon sources, inputs from sources with intermediate $\delta^{13}C$ values (e.g. benthic algae, phytoplankton) or isotopic fractionation during decomposition. Although not as widely used as $\delta^{13}C$, stable isotopes of N can be applied in conjunction with $\delta^{13}C$ measurements to provide better resolution in tracing the sources of estuarine organic matter (Peterson *et al.*, 1985).

In recent years, emergent vegetation has been established to stabilize dredge material and eroding shorelines and to mitigate damage to estuarine marshes from development and surface mining (Woodhouse *et al.*, 1974; Broome *et al.*, 1982, 1983). Although these young transplanted marsh soils lack the large reservoirs of organic materials characteristic of many natural marshes, rates of net primary production (NPP) of emergent vegetation and soil organic matter accumulation are similar in the two marsh types (Broome *et al.*, 1986; Craft *et al.*, 1986). However, it is unknown whether this accumulating soil organic matter is derived from marsh emergent vegetation or from other sources.

The objectives of this study were to estimate the sources of soil organic matter in transplanted estuarine marshes by measuring the natural abundances of the stable isotopes of C and N. $\delta^{13}C$ and $\delta^{15}N$ were measured in above-ground emergent vegetation and soils of natural and transplanted marshes. Other sources of marsh organic matter (terrestrial forest vegetation, benthic algae) also were measured.

Methods

Sample collection and preparation

In 1985, emergent vegetation and soils were collected from a natural and two transplanted marshes on the Pamlico River estuary, NC (Figure 1). The three marshes were similar with respect to hydrology and salinity but differed in age (Craft *et al.*, 1986). The transplanted marshes, North Carolina phosphate (NCPC) and Texasgulf were approximately two and five years old at the time of sampling. Soil organic matter content (top 10 cm) averaged 1% in the NCPC transplanted marsh and 44% in the natural marsh (Craft *et al.*, 1986). The addition of top soil during establishment of the Texasgulf transplanted marsh resulted in soil organic matter levels (5%) that were higher than those in the NCPC transplanted marsh.

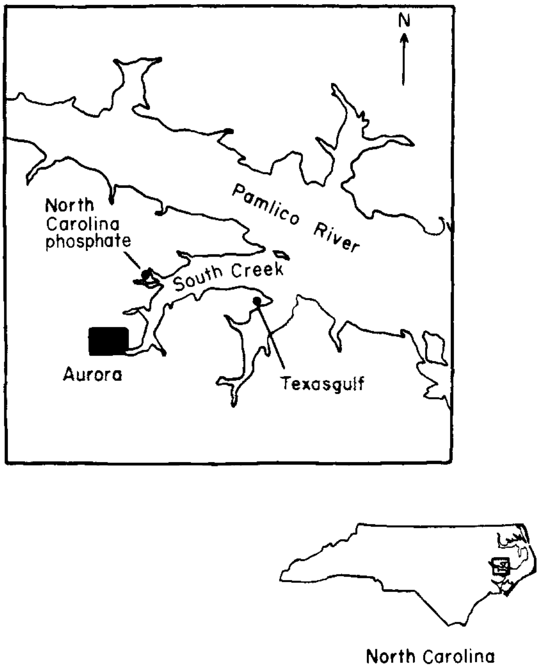

Figure 1. Location of sampling sites.

Three samples of above-ground leaf material were taken from each of the dominant macrophytes [*Spartina alterniflora* Loisel., *S. cynosuroides* (L.), *S. patens* (Ait.) Muhl., *Juncus roemerianus* Scheele, *Distichlis spicata* (L.)] in the natural and Texasgulf transplanted marsh. Other sources of estuarine organic matter [e.g. leaves from adjacent forest vegetation (*Acer rubrum* L., *Liquidambar styraciflua* L., *Pinus taeda* L.), *Cladium jamaicense* Crantz, *Eleocharis* sp., *Myriophyllum spicatum* L., *Ruppia maritima* L., benthic algae] were also collected from natural and transplanted marshes. Soils were sampled by taking five cores (8·5 cm diameter, 10 cm deep) beneath each of the dominant marsh macrophytes in the three marshes. Vegetation and soils were prepared for combustion according to Hayes (1983).

Detritus in estuarine water was sampled during July 1986 in South Creek, a tributary of the Pamlico River (Figure 1). Five 750-ml water samples were collected along a transect of South Creek, from the North Carolina phosphate marsh to its confluence with the Pamlico River. Samples were filtered through precombusted (450 °C for 4 h) Gelman-type AE glass fibre filters and the material trapped by the filters was dried at 105 °C.

Combustion and mass spectrometry

Samples of vegetation (10 mg) and soil (10–100 mg) were placed in 9-mm diameter quartz combustion ampoules with 25 mg of silver foil (1 cm^2, 0·025 mm thick), 300 mg CuO and 1 m of 0·5 mm diameter copper wire (twisted into 5-cm long braids). The ampoules were evacuated to 1×10^{-3} torr and combusted for 4 h at 850 °C, cooled to 550 °C and maintained at that temperature for 10 h. Analysis of the NBS-22 carbon isotope standard using this method yielded $\delta^{13}C$ values of $-29{\cdot}48 \pm 0{\cdot}07‰$ ($N=4$) compared to the value of $-29{\cdot}81 \pm 0{\cdot}06‰$ measured by Schoell *et al.* (1983). Filters containing detritus were placed in 9-mm diameter quartz ampoules containing CuO, and copper and silver wire. The

TABLE 1. Mean coefficients of variation (CV) for randomly selected replicate samples of $\delta^{13}C$ and $\delta^{15}N$

	N	CV	Range of CV
Vegetation			
Carbon	8	1·0	(0·1–3·6)
Nitrogen	11	16·4	(2·2–60·1)
Soil			
Carbon	6	1·2	(0·5–2·8)
Nitrogen	7	8·5	(0·4–26·5)

ampoules were then inserted into larger (12 mm) ampoules, evacuated and combusted as described previously.

After combustion, the samples were purified using a double cryogenic trap system. Water and CO_2 were frozen with liquid nitrogen and the remaining N_2 was adsorbed on a molecular sieve (5A, Absorbents and Dessicants of America, Gardena, CA). Carbon dioxide was separated from water by freezing the water with liquid nitrogen/methanol slush and trapping the CO_2 with liquid nitrogen. The isotopic composition of the purified CO_2 and N_2 was determined on a Finigan-MAT 251 ratio mass spectrometer at the Department of Marine, Earth and Atmospheric Sciences at North Carolina State University.

Working standards for $\delta^{13}C$ [Coleman instrument grade, (99·99%) tank CO_2 containing 99·96% $^{12}CO_2$ and 0·04% $^{13}CO_2$, Matheson Gas Products Inc., East Rutherford, NJ] and $\delta^{15}N$ [ultra-high purity grade (99.998%) tank N_2, Air Products and Chemicals Inc., Allentown, PA] yielded values of −10·73‰ and −3·06‰ relative to the international standards for C and N, respectively. The $\delta^{15}N$ values were corrected for the percentage contribution of atmospheric argon (Q factor; Mariotti, 1984). Blank samples were run regularly and, with the exception of the filters, were insignificant because of the small volume (<1% of the sample) of CO_2 and N_2 produced. Blank corrections for the filters were made according to Hayes (1983).

Coefficients of variation for randomly chosen replicate samples indicated greater variability in the $\delta^{15}N$ values than in the $\delta^{13}C$ measurements (Table 1).

Results and discussion

Vegetation

The $\delta^{13}C$ values of C_4 plants in natural and transplanted marshes were similar, ranging from −12 to −14‰ (Table 2). *J. roemerianus*, the dominant C_3 emergent in the natural marsh, had a $\delta^{13}C$ of −26‰. Terrestrial forest vegetation also had $\delta^{13}C$ values (−27‰) characteristic of the C_3 pathway. The $\delta^{13}C$ of benthic and floating algae collected from a transplanted marsh ranged from −14 to −19‰ and were similar to values reported by Haines (1976*b*) in Georgia salt marshes. Aquatic vascular plants (*Eleocharis*, *Myriophyllum*, *Ruppia*) growing in transplanted marshes had $\delta^{13}C$ values intermediate between C_3 and C_4 vegetation (Table 2).

Stable N isotope analysis also revealed distinctly different values for terrestrial material (−3 to −4‰) and marsh emergent and aquatic vegetation (+1 to +4‰) (Table 2). Peterson *et al.* (1985) measured similar $\delta^{15}N$ values for *S. alterniflora* (+3·8‰) and forest

TABLE 2. Mean $\delta^{13}C$ and $\delta^{15}N$ of terrestrial and marsh vegetation

Source	Vegetation		C_3/C_4[a]	N	$\delta^{13}C$		$\delta^{15}N$	
Terrestrial		*Acer*	C_3	2	−27·3	<0·1[b]	−4·1	±0·9
		Liquidambar	C_3	2	−26·7	±0·9	−2·9	±1·1
		Pinus	C_3	2	−26·9	±0·5	−2·9	±1·9
Marsh (natural)	Emergent vascular	*Distichlis*	C_4	3	−13·7	<0·1	+2·6	±0·2
		S. cynosuroides	C_4	3	−12·2	±0·1	+2·3	±0·1
		S. patens	C_4	3	−12·8	±0·1	+1·4	±0·4
		Juncus	C_3	3	−26·0	±0·3	+1·0	±0·3
		Cladium	C_3	1	−26·2	—	+2·4	—
Marsh (transplanted)	Emergent vascular	*S. alterniflora* (TG)[3]	C_4	3	−12·0	±0·2	+1·6	±0·4
		S. cynosuroides (TG)	C_4	3	−12·4	<0·1	+4·3	±0·3
		S. patens (TG)	C_4	3	−13·3	<0·1	+2·4	±0·2
	Aquatic vascular	*Myriophyllum* (TG)	?	1	−16·3	—	+3·0	—
		Eleocharis (NC)	?	1	−19·7	—	+1·9	—
		Ruppia (NC)	?	1	−14·7	—	+1·2	—
		Benthic algae (NC)	?	2	−16·7	±2·3	+0·8	±0·4
		Floating algae (NC)	?	1	−16·8	—	+0·7	—

[a]Photosynthetic pathway based on $\delta^{13}C$.
[b]Standard error of the mean.
[c]TG, collected from Texasgulf; NC, collected from North Carolina Phosphate.

TABLE 3. Mean $\delta^{13}C$ and $\delta^{15}N$ of soils collected under various marsh macrophytes in natural and transplanted marshes

Marsh	Vegetation	N	$\delta^{13}C$	$\delta^{15}N$
Natural	*S. cynosuroides*	5	−21·2±0·4[a]	+3·5±0·2
	Distichlis/S. patens	5	−19·7±0·6	+2·3±0·2
	Juncus	5	−23·8±0·5	+0·9±0·1
Transplanted (North Carolina phosphate)	*S. cynosuroides*	5	−18·1±1·1	+4·2±0·4
	S. patens	5	−21·8±0·2	+3·4±0·2
	S. alterniflora	5	−18·4±1·9	+3·5±0·4
Transplanted (Texasgulf)	*S. cynosuroides*	5	−26·1±0·2	+3·3±0·2
	S. patens	5	−24·3±0·7	+3·2±0·2
	S. alterniflora	5	−25·8±0·5	+2·8±0·2

[a]Standard error of the mean.

vegetation (−0·6‰) in a Massachusetts salt marsh, while Mariotti *et al.* (1983) found higher values for *S. alterniflora* (+6·2‰), *J. roemerianus* (+2·8‰) and benthic algae (+3·9‰) in a Georgia salt marsh.

Soil

The mean $\delta^{15}N$ of natural and transplanted marsh soils (top 10 cm) were similar and ranged from +2·2 to +3·7‰ (Table 3). Soil $\delta^{13}C$ values also were similar in the natural (−20 to −24‰) and the NCPC transplanted marsh (−18 to −22‰) (Table 3). However, application of top soil during establishment of the Texasgulf transplanted marsh resulted in soil $\delta^{13}C$ values (−24 to −26‰) reflective of terrestrial material.

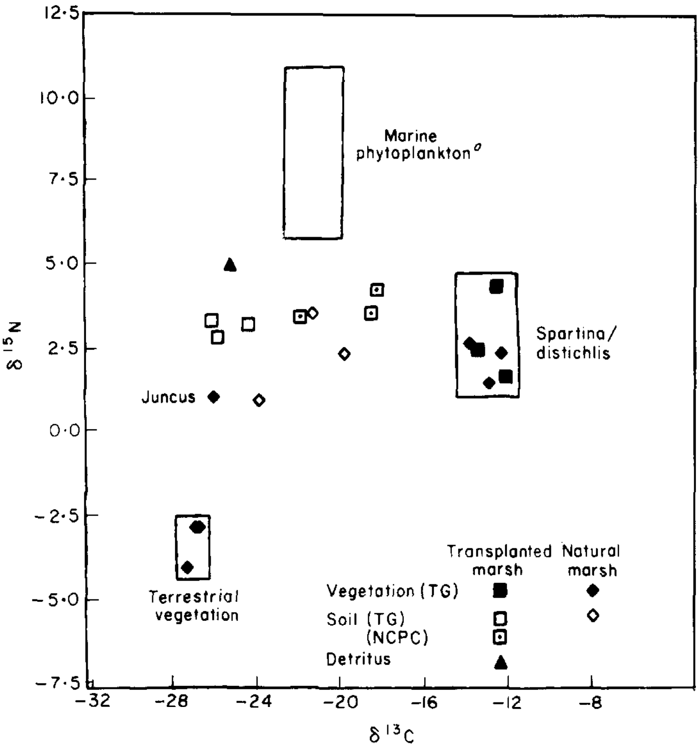

Figure 2. The relationship between $\delta^{13}C$ and $\delta^{15}N$ of vegetation, marsh soils and estuarine detritus. [a]From: Sackett *et al.* (1965), Peters *et al.* (1978), Sweeney and Kaplan (1980), Macko (1983), Gearing *et al.* (1984), Peterson *et al.* (1985).

Sources of estuarine organic matter

Soil organic matter in natural and transplanted marshes generally reflected the contribution of emergent vegetation (Figure 2). The $\delta^{15}N$ fell within the range of marsh vegetation (+1 to 4‰), while $\delta^{13}C$ varied from −18 to −26‰. In the natural marsh, the $\delta^{13}C$ of soils collected under *Juncus* (−23·8‰) were very similar to above ground *Juncus* material (−26‰), while soils taken under *S. cynosuroides* and *Distichlis/S. patens* stands suggested mixing between C_3 and C_4 marsh vegetation (Figure 2).

The soil $\delta^{13}C$ of the Texasgulf transplanted marsh was attributed to top soil derived from C_3 terrestrial vegetation ($\delta^{13}C = -25$ to -30‰; Deines, 1980), while the soil $\delta^{13}C$ values of the C_4 dominated NCPC marsh could have resulted from aquatic vascular plants, phytoplankton and/or benthic algae, mixing of *Spartina* and terrestrial material or, perhaps, isotopic fractionation during decomposition of marsh vegetation.

Several researchers have suggested that the discrepancy in the $\delta^{13}C$ of *Spartina* dominated marsh soils and *Spartina* tissue may result from accumulation of lignin in the substrate (Spiker *et al.*, 1985; Benner *et al.*, 1986*a*, *b*). Marsh emergent vegetation such as *Spartina* and *Juncus* contains large amounts (75%) of lignocellulose (Hodson *et al.*, 1983). Chemically isolated lignin is depleted in $\delta^{13}C$ compared to cellulose and readily decomposable compounds such as proteins and carbohydrates (Benner *et al.*, 1986*a*). During decomposition, these labile compounds are degraded much faster than lignin, resulting in accumulation of highly refractory lignin-derived carbon in the soil (Benner *et al.*, 1986*b*). Thus, the depletion in $\delta^{13}C$ (compared to *Spartina* tissue) in the NCPC marsh could result from accumulation of isotopically light lignin compounds. However, accumulation of lignins (which contain little or no nitrogen; Brauns, 1952; Schubert, 1965) in marsh soils does not explain the low soil C : N ratios (11–37) observed in these and other estuarine marshes of the Atlantic and Gulf coasts (Haines *et al.*, 1977; DeLaune *et al.*, 1979; Coultas, 1980; Bowden, 1984; Craft *et al.*, 1986). Furthermore, if lignin accumulation is responsible for the isotopically light soil $\delta^{13}C$ values in *Spartina alterniflora* marshes, why is no depletion observed in soils beneath other lignocellulose-rich marsh emergents such as *Juncus roemerianus*? (Johnson & Calder, 1973; Hackney & Haines, 1980; this study). Perhaps both mixing of organic matter sources (e.g. *Spartina, Juncus*, aquatic vascular plants, benthic algae, terrestrial material) and isotopic fractionation during decomposition (lignification, formation of recalcitrant organic N compounds) should be considered when assessing the origins of estuarine organic matter.

The isotopic composition of estuarine detritus in a tributary of the Pamlico River suggested that phytoplankton and, to a lesser extent, terrestrial material were the major contributors to the detrital pool (Figure 2). Other investigators noted similar $\delta^{13}C$ (-20 to -26‰) and $\delta^{15}N$ values ($+2$ to $+5$‰) in detritus collected from estuarine waters (Stephenson & Lyon, 1982; Mariotti *et al.*, 1983; Matson *et al.*, 1983; Owens, 1985; Conkright & Sackett, 1986).

In conclusion, the stable isotope composition of natural and transplanted marsh soils suggests that emergent vegetation is the major contributor to marsh soil organic matter pools. The $\delta^{15}N$ values of marsh soils were similar to emergent vegetation, while the soil $\delta^{13}C$ values indicated mixing of organic matter sources and/or isotopic fractionation during decomposition and soil development.

The similarity in the $\delta^{13}C$ and $\delta^{15}N$ of soils and vegetation collected from natural and transplanted marshes suggests that the processes (i.e. hydrology, NPP of emergent vegetation) responsible for accumulation of organic matter in natural marshes (Craft *et al.*, 1986) also function in the young transplanted marshes. It is likely that, as transplanted marshes evolve, soil organic matter pools will develop and become comparable to natural marshes. Over time, transplanted marshes will contribute to the biogeochemical cycling of C, N and P by serving as reservoirs of organic materials for estuarine ecosystems.

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