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δ^{15}N Studies of Nitrogen Use by the Red Mangrove, *Rhizophora mangle* L. in South Florida

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To help define nitrogen (N) sources and patterns of N processing in mangrove ecosystems, mangrove leaf nitrogen contents and δ^{15}N values were assayed in three marshes along the south Florida coast. In each marsh, leaf samples were collected from dwarf mangroves at interior locations and taller mangroves at the ocean fringe. Leaf % N and δ^{15}N values did not differ consistently between dwarf and tall mangroves, even though there were large variations in δ^{15}N (18‰ range, −5 to +13‰) and % N (1·2% range, 0·9–2·1%). Highest % N and δ^{15}N values occurred along the western margin of Biscayne Bay where canals draining agricultural lands deliver high-nitrate waters to fringing mangrove marshes. High mangrove δ^{15}N values may be good biomonitors of anthropogenic N loading to south Florida estuaries. Lower values likely reflect less anthropogenic N entering the mangrove marshes, as well as differences in plant physiology that occur along the fringe-dwarf gradient.

Keywords: mangroves; *Rhizophora mangle*; δ^{15}N; nitrogen isotopes; Florida coast

Introduction

Many coastal mangrove ecosystems exhibit a gradation between large fringing trees along the shoreline and smaller dwarf trees at interior marsh locations. Several kinds of stresses increase with distance from the shoreline, and their single or cumulative effects may result in poorer conditions for plant growth and eventual dwarfism. These stresses are not restricted to mangrove marshes, but can also have pronounced effects in intertidal marshes outside of the tropical mangrove zone. For example, tall creekside *vs* short inland forms of cord grass, *Spartina alterniflora* and *Spartina patens*, are a common feature of intertidal marshes in the temperate zone (Howes *et al.*, 1981; Burdick *et al.*, 1989). One hypothesis explaining the small stature of plants in interior marshes is that root metabolism is inhibited in sulphide-rich waterlogged sediments, thereby depressing energy-demanding processes such as N uptake required for normal tree development (Koch *et al.*, 1990). Consistent with this hypothesis are observations that increased plant growth in shoreline plants correlates positively with better drainage and increased root zone oxidation (Howes *et al.*, 1981). Also, several long-term nutrient additions have shown that dwarfism can be partially reversed by fertilization (Howes *et al.*, 1981; Feller, 1995), and N and P analysis of dwarf plants shows evidence of nutrient deficiency (Koch, 1997; Koch & Snedaker, 1997). Nutrient limitation and poor drainage thus may be proximate and ultimate causes, respectively, of marsh plant dwarfism.

To help examine nutrient cycling in Florida mangrove marshes, a field survey of red mangrove, *Rhizophora mangle* L. was conducted. Three coastal marshes of south Florida were studied to test for possible higher δ^{15}N values near sources of anthropogenic N loading, a phenomenon that has been recently observed in several coast locations (Hansson *et al.*, 1997; McClelland *et al.*, 1997; Voss & Struck, 1997). Mangrove leaves were collected at each site for % N and δ^{15}N analysis to also investigate possible N-related physiological differences between dwarf, inland mangroves *vs* tall, fringing mangroves along the shoreline.

Study sites

Three mangrove marshes were studied along the south Florida coast (Figure 1). The relatively pristine Sugarloaf Key site in the lower Florida Keys (25°15′N, 80°27′W) has been described by Sternberg *et al.* (1991) and Lin and Sternberg (1992). A second site in the Upper Keys (25°17′N, 80°18′W) lies in the Crocodile Lakes National Wildlife Refuge in Key Largo. This site borders Manatee Bay, and may

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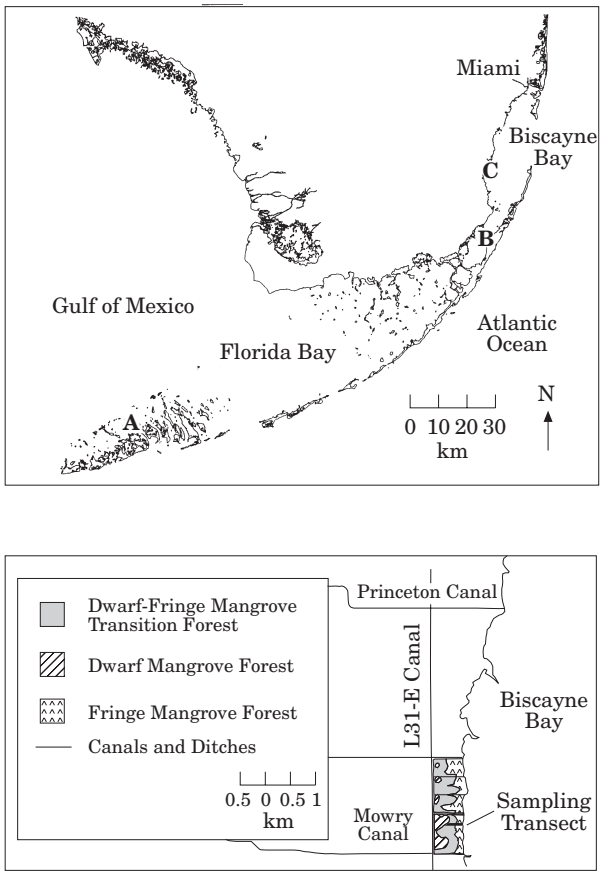

FIGURE 1. Top panel: location of mangrove marsh study sites A–C along the south Florida coast (A=Sugarloaf Key, B=Crocodile Lakes National Wildlife Refuge, C=Biscayne Bay). Bottom panel: expanded view of marsh site C along Biscayne Bay.

receive some anthropogenic N loading from canals draining the Everglades that empty at the opposite shore of this semi-enclosed bay. The third site is located on the western shore of Biscayne Bay (25°28′N, 80°21′W), just north (<0·5 km) of Mowry Canal in Biscayne Bay National Park. This canal and several others along a 5 km stretch of the western shoreline are major conduits for nitrate-rich surface waters exiting into Biscayne Bay, with highest canal flows during the summer–autumn wet season. Local agricultural croplands are the likely source of nitrate-N that can reach 60 μM in near-shore waters along the mangrove marsh (unpublished data from water quality surveys of the Southeast Environmental Research Program). A north–south canal constructed in the 1960s forms the western boundary of this marsh (Figure 1). Construction of this L31E canal and associated levees led to impoundment and salinization of the coastal wetlands seawards of the canal, with replacement of the freshwater wetlands by mangrove marsh. Levees along a second canal, the Mowry Canal, form the southern boundary of the Biscayne mangrove marsh sampled in this study (Figure 1).

Methods

Leaf samples were collected over a 2·5 year period from autumn 1994 to April 1997. Leaves were generally collected at 0·5–2 m height from 5–10 individual trees at each location, then composited. Leaf midribs were removed before overnight drying at 60 °C. Dried samples were ground to a fine powder with an automated mortar and pestle (Wig-L-Bug™). Samples were combusted to nitrogen gas with a Carlo-Erba elemental analyser and analysed for δ^{15}N values *vs* nitrogen in air using continuous-flow stable isotope mass spectrometry (Barrie & Prosser, 1996). Replicate analyses of individual samples usually showed agreement of better than 0·2‰ δ^{15}N and 0·05% N.

Results

At the relatively pristine Sugarloaf site, dwarf trees at the ocean edge had relatively δ^{15}N low values (near −5‰), while taller fringe trees and inland dwarf trees had higher values near +2‰ (Figure 2, left panels). There was not a strong contrast in % N for dwarf *vs* taller trees at this site (Figure 2).

At the North Key Largo site, contrasts in % N and δ^{15}N for dwarf *vs* tall trees were more pronounced (Figure 2, middle panels). Dwarf trees located at the ocean edge again had lowest −3‰ δ^{15}N values, with values rapidly climbing to 7‰ in the centre of the fringe where tall trees were growing on peat soils in a natural berm elevated 50–80 cm above mean sea level. These taller trees also showed a maximum % N for this site of about 1·55% N. Dwarf plants at the ocean edge of the marsh had lower δ^{15}N values than inland dwarf plants. The dwarf trees at the ocean edge were also relatively poor in nitrogen content compared to fringe trees and inland dwarf trees (Figure 2, middle panels).

At the third site along Biscayne Bay, there were no oceanside dwarf trees. Seaside trees were rooted in the mid-intertidal, with branches extending out over the water. Highest δ^{15}N values occurred in the middle of the fringe (Figure 2, right panels), and particularly high N contents (2·1% N) were found in young trees that were rapidly recolonizing this zone after Hurricane Andrew that struck this coast in August 1992. Both % N and δ^{15}N declined inland along the transect as tall fringe trees were replaced by dwarf trees (Figure 2, right panels). Mangroves were also sampled just beyond the western end of the marsh,

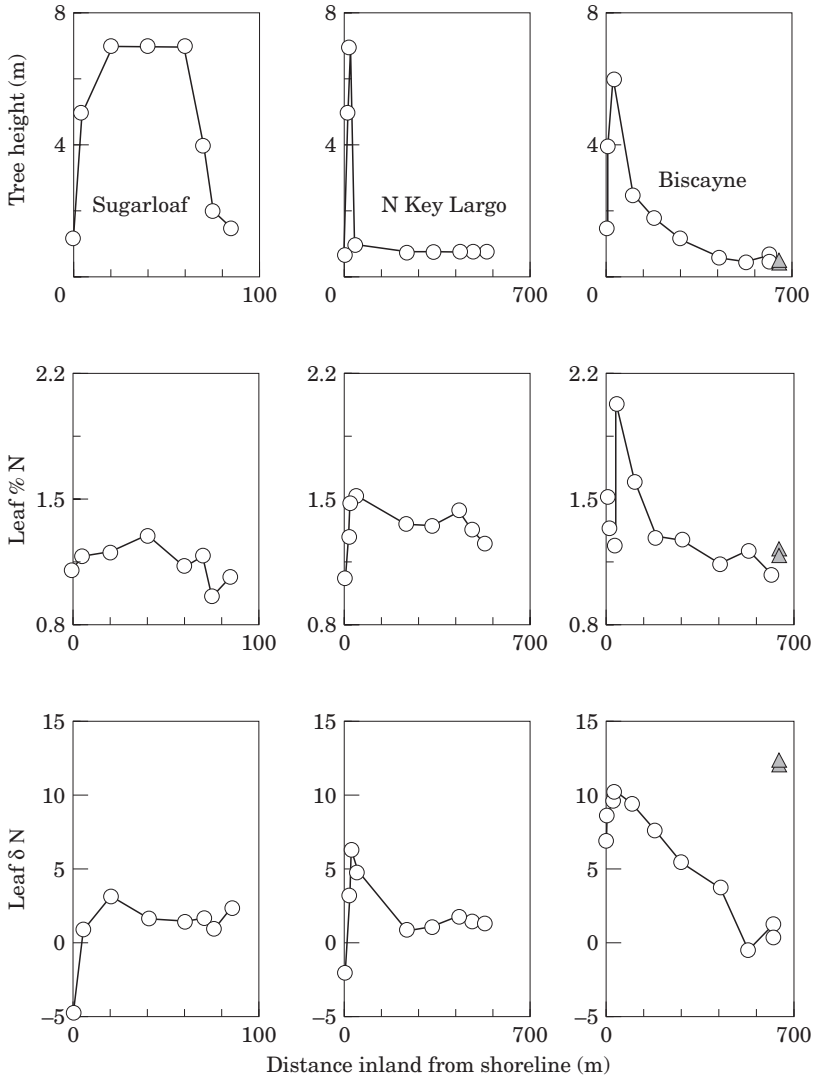

FIGURE 2. Tree height, leaf % N and δ^{15}N in south Florida mangrove forests. Shaded triangles at the right in graphs for the Biscayne Bay site show results for small, immature mangroves sampled across a levee and in the L31E canal that forms the back end of the Biscayne mangrove marsh.

over a levee in the north–south L31E canal that forms the back boundary of this marsh (see Figure 1). Young mangroves (<1 m) growing in the canal had very elevated leaf δ^{15}N values near 12‰ (Figure 2, triangles in right panels). A larger red mangrove tree growing on carbonate bedrock from Mowry Canal outlet at the southern side of the marsh also had a high 11·3‰ leaf δ^{15}N values, as did several grass and shrub samples from Princeton and Military Canals located 1–3 km from Mowry Canal (δ^{15}N plant range of 11–16‰).

Discussion

Two indicators of mangrove ecosystem N cycling were measured during this study—mangrove leaf % N and mangrove leaf δ^{15}N. There are many factors that could potentially affect these indicators, including the long-term nitrogen supply to the sites, soil development and fertility, and competition among plants for N. In spite of these possible complexities and the potential for micro-site variation in soil processes, rather regular patterns in plant δ^{15}N across the three transects were observed (i.e. little change for most of the Sugarloaf transect, increased δ^{15}N in the tall fringe at N Key Largo and Biscayne, Figure 2). Here some first perspectives on factors likely to control leaf δ^{15}N in mangrove marshes are discussed.

In general, nitrogen nutrient inputs should set an ecosystem baseline for δ^{15}N values. For example, nitrogen fixation leads to values near − 1‰ in planktonic marine systems, while higher values occur in

systems where deep-water nitrate (usually 4–7‰) is important (Macko & Ostrom, 1994; Carpenter *et al.*, 1997). The 11–16‰ values observed for rooted plants in the canals feeding into western Biscayne Bay are much higher than the −10 to +2‰ values normally found in freshwater and terrestrial plants (Fry, 1991; Nadelhoffer *et al.*, 1996), and these unusual values likely indicate that nutrient δ^{15}N is elevated in canals draining local cropland areas. Groundwater N entering coastal marshes from agricultural areas has been shown to lead to high plant δ^{15}N values of emergent coastal vegetation (Page, 1995).

If external nutrient sources set an average δ^{15}N baseline for mangrove systems, within-system processes that fractionate ^{15}N can lead to plants that have either elevated or depressed δ^{15}N values *vs* this baseline. For example, denitrification occurring in nitrate-rich systems can remove ^{15}N-depleted nitrogen, leaving residual nitrate enriched in ^{15}N (Nadelhoffer & Fry, 1994). Plants using ^{15}N-enriched nitrate, or ^{15}N-enriched ammonium left behind in the below-ground rhizosphere by nitrification reactions that occur in oxygenated microzones (Reddy *et al.*, 1989), could become enriched in ^{15}N (Jordan *et al.*, 1997). Alternatively, plants could have ^{15}N-depleted values if they fractionate against ^{15}N during uptake of nitrate or ammonium (Yoneyama & Kaneko, 1989; Yoneyama *et al.*, 1991; Yoneyama, 1995). Isotopic changes during nutrient cycling can thus be complex (Shearer *et al.*, 1974; Mariotti *et al.*, 1981, 1984), but plants should integrate many of these variations because δ^{15}N of tree leaves represents the long-term N acquired over growing seasons (Garten, 1993). Below two models are discussed of how external source inputs and within-system N processing might interact to produce the observed leaf δ^{15}N values.

Model 1. High source δ^{15}N, lower plant δ^{15}N due to plant isotopic fractionation during N uptake

In this model, between-site differences in plant isotopic compositions would be explained by changes in source δ^{15}N values across the three sites, with sites having higher anthropogenic N loading having higher plant δ^{15}N values. Within sites, the model focuses on plant N uptake as the important point of isotopic fractionation.

Laboratory studies show that isotope fractionation can range widely during plant N uptake. Conditions favour little fractionation when nitrate is the dominant N source for terrestrial plants (Mariotti *et al.*, 1982) or when N supplies are limited and all N is used regardless of isotopic composition (Montoya & McCarthy, 1995). Larger fractionations often occur for plants that use ammonium rather than nitrate (Yoneyama *et al.*, 1991; Pennock *et al.*, 1996). Ammonium is the dominant plant N nutrient in mangrove marsh porewater, and according to current models of plant isotopic fractionation (Goericke *et al.*, 1994), the combination of slow plant growth and high ammonium concentrations should produce the largest fractionations (lowest plant δ^{15}N). Model 1 predicts that δ^{15}N changes observed within a site are due to changes at the plant level, such as a switch from nitrate to ammonium nutrition, or alterations in the rate of ammonium supply *vs* plant N demand.

This model correctly predicts important aspects of the δ^{15}N transects, i.e. that proximity to anthropogenic N sources influences plant δ^{15}N across the three sites, and that tall, fast-growing fringe trees have higher δ^{15}N values within each site than slower-growing dwarf plants.

Model 2. Low source δ^{15}N, higher plant δ^{15}N due to microbial ^{15}N fractionation during nitrification and denitrification

In this model, δ^{15}N values of external nutrients could be low and similar across the sites, with between-site differences explained by overall site fertility. Hoegeberg (1997) showed that fertilized upland forest systems are enriched in ^{15}N *vs* source inputs because mechanisms of N loss from these systems usually involve faster loss of ^{14}N than^{15}N, and because these systems usually lose a greater fraction of their total N than non-fertilized systems. Similar factors could potentially apply across the three mangrove sites which receive more (Biscayne Bay) and less (North Key Largo, Sugarloaf) anthropogenic N loading. Extending this reasoning, areas of highest fertility within each study site would also have highest plant δ^{15}N values.

Strict application of this model fails in three different tests. First, the lowest plant δ^{15}N would indicate an upper limit for source δ^{15}N, so that for example, the −5‰ value for oceanside dwarf plants at the Sugarloaf site would represent the source value. This value is possible, but unlikely in marine systems where inputs from nitrogen fixation and nitrogenous runoff are −1‰ or higher (Carpenter *et al.*, 1997). A second prediction of this model is that some plants should have considerably higher δ^{15}N values than source inputs. However, in the Biscayne site, fringe mangrove δ^{15}N values were lower than those measured in plants from nearby nutrient-rich canals, so that mangrove leaf values were lower than expected for this nutrient-rich area. This result is contrary to the model 2 prediction. Lastly, this model predicts that within

sites, highest δ^{15}N values should be found in areas of highest fertility, i.e. in taller, faster-growing fringe trees *vs* dwarf trees. This prediction was consistent with results at Biscayne and North Key Largo, but not at Sugarloaf (Figure 2).

In summary, model 1 fits the current field observations better than model 2. Future long-term field experiments are needed to more rigorously test these models, e.g. whether waterlogged soils lead to plants with low δ^{15}N (model 1), while better growth with soil aeration (Wilsey *et al.*, 1992) leads to plants whose δ^{15}N values are higher than those of soil nutrients (model 2). These kinds of experiments have been performed to test controls of carbon isotopic distributions in mangroves (Lin & Sternberg, 1992), and need to be repeated to test controls of nitrogen isotopic distributions in mangroves.

These considerations also point to the importance of determining δ^{15}N values of source ammonium and nitrate. Nutrient δ^{15}N measurements have been a great technical challenge in the past, and it has not been feasible to really develop a field programme that matches continuous monitoring of soil nutrient δ^{15}N values with the more easily measured plant δ^{15}N values. Recent technical advances in measurement of δ^{15}N values of ammonium and nitrate (Sigman *et al.*, 1997; Holmes *et al.*, 1998) will help remedy this situation. In the current study, canal plants were used at the Biscayne Bay site as biomonitors and accumulators of pollutant N, rather than embarking on a long-term and technologically challenging set of nutrient δ^{15}N measurements. These bioassay plants indicated that, as has been found in several other recent coastal studies (Hansson *et al.*, 1997; McClellan *et al.*, 1997; Voss & Struck, 1997), high δ^{15}N values accompany nutrient enrichment. Continued measurement of δ^{15}N bioindicator plants along with nutrient δ^{15}N assays may well prove generally useful in following N-related ecosystem degradation and recovery in mangrove marshes (Cifuentes *et al.*, 1996) and other coastal zone areas.

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References

Barrie, A. & Prosser, S. J. 1996 Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In *Mass Spectrometry of Soils* (Boutton, T. W. & Yamasaki, S., eds). Marcel Dekker, New York, pp. 1–46.

Burdick, D. M., Mendelssohn, I. A. & McKee, K. L. 1989 Live standing crop and metabolism of the marsh-grass *Spartina patens* as related to edaphic factors in a brackish, mixed marsh community in Louisiana. *Estuaries* **12,** 195–204.

Carpenter, E. J., Rahvey, R. R., Fry, B. & Capone, D. G. 1997 Biogeochemical tracers of the marine cyanobacterium *Trichodesmium*. *Deep-Sea Research* **44,** 27–38.

Cifuentes, L. A., Coffin, R. B., Solorzano, L., Cardenas, W., Espinoza, J. & Twilley, R. R. 1996 Isotopic and elemental variations of carbon and nitrogen in a mangrove estuary. *Estuarine, Coastal and Shelf Science* **43,** 781–800.

Feller, I. C. 1995 Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle* L.). *Ecological Monographs* **65,** 477–505.

Fry, B. 1991 Stable isotope diagrams of freshwater food webs. *Ecology* **72,** 2293–2297.

Garten, C. T. 1993 Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker branch watershed. *Ecology* **74,** 2098–2113.

Goericke, R., Montoya, J. P. & Fry, B. 1994 Physiology of isotopic fractionation in algae and cyanobacteria. In *Stable Isotopes in Ecology and Environmental Science* (Lajtha, K. & Michener, R., eds). Blackwell Scientific Publications, Oxford, pp. 187–221.

Hansson, S., Hobbie, J. E., Elmgren, R., Larsson, U., Fry, B. & Johansson, S. 1997 The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* **78,** 2249–2257.

Hoegberg, P. 1997 Tansley Review no. 95: ^{15}N natural abundance in soil-plant systems. *New Physiologist* **137,** 179–203.

Holmes, R. M., McClelland, J. W., Sigman, D. M., Fry, B. & Peterson, B. J. 1998 Measuring ^{15}N-NH_4^+ in marine, estuarine, and fresh waters: an adaptation of the ammonium diffusion method for samples with low ammonium concentrations. *Marine Chemistry* **60,** 235–243.

Howes, B. L., Howarth, R. W., Valiela, I. & Teal, J. M. 1981 Oxidation-reduction potential in a salt marsh: Spatial patterns and interactions with primary production. *Limnology and Oceanography* **26,** 350–360.

Jordan, M. J., Nadelhoffer, K. J. & Fry, B. 1997 Nitrogen cycling in forest and grass ecosystems irrigated with treated wastewater enriched in ^{15}N. *Ecological Applications* **7,** 864–881.

Koch, M. S. 1997 *Rhizophora mangle* (red mangrove) seedling development into the sapling stage across resource and stress gradients in subtropical Florida. *Biotropica* **29,** 427–439.

Koch, M. S., Mendelssohn, I. A. & McKee, K. L. 1990 Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. *Limnology and Oceanography* **35,** 399–408.

Koch, M. S. & Snedaker, S. C. 1997 Factors influencing *Rhizophora mangle* (red mangrove) seedling development in Everglades carbonate soils. *Aquatic Botany* **59,** 87–98.

Lin, G. & Sternberg, L. da S. L. 1992 Effect of growth form, salinity, nutrient and sulfide on photosynthesis, carbon isotope discrimination and growth of red mangrove (*Rhizophora mangle* L.). *Australian Journal of Plant Physiology* **19,** 509–517.

Macko, S. A. & Ostrom, N. E. 1994 Pollution studies using stable isotopes. In *Stable Isotopes in Ecology and Environmental Science* (Lajtha, K. & Michener, R., eds). Blackwell Scientific Publications, 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; illustration for the denitrification and nitrification processes. *Plant and Soil* **62,** 413–430.

Mariotti, A., Mariotti, F., Champigny, M. L., Amaeger, N. & Moyse, A. 1982 Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO_3^- by pearl millet. *Plant Physiology* **69,** 880–884.

Mariotti, A., Lancelot, C. & Billen, G. 1984 Natural isotopic composition of nitrogen as a tracer of origin for suspended

organic matter in the Scheldt estuary. *Geochimica et Cosmochimica Acta* **48,** 549–555.

McClelland, J. W., Valiela, I. & Michener, R. H. 1997 Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography* **42,** 930–937.

Montoya, J. P. & McCarthy, J. J. 1995 Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. *Journal of Plankton Research* **17,** 439–464.

Nadelhoffer, K. J. & Fry, B. 1994 Nitrogen isotope studies in forest ecosystems. In *Stable Isotopes in Ecology and Environmental Science* (Lajtha, K. & Michener, R., eds). Blackwell Scientific Publications, Oxford, pp. 22–44.

Nadelhoffer, K., Shaver, G., Fry, B., Giblin, A., Johnson, L. & McKane, R. 1996 ^{15}N natural abundances and N use by tundra plants. *Oecologia* **107,** 386–394.

Page, H. M. 1995 Variation in the natural abundance of ^{15}N in the halophyte, *Salicornia virginica*, associated with groundwater subsidies of nitrogen in a southern California salt-marsh. *Oecologia* **104,** 181–188.

Pennock, J. R., Velinsky, D. J., Ludlam, J. M., Sharp, J. H. & Fogel, M. L. 1996 Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: Implications for δ^{15}N dynamics under bloom conditions. *Limnology and Oceanography* **41,** 451–459.

Reddy, K. R., Patrick, W. H. Jr. & Lindau, C. W. 1989 Nitrification–denitrification at the plant root-sediment interface in wetlands. *Limnology and Oceanography* **34,** 1004–1013.

Shearer, G., Duffy, J., Kohl, D. H. & Commoner, B. 1974 A steady-state model of isotopic fractionation accompanying nitrogen transformations in soil. *Soil Science Society of America, Proceedings* **38,** 315–322.

Sigman, D., Altabet, M., Holmes, R. M. & Fry, B. 1997 Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry* **57,** 227–242.

Sternberg, L. da S. L., Ish-Shalom-Gordon, N., Ross, M. & O'Brien, J. 1991 Water relations of coastal plant communities near the ocean/freshwater boundary. *Oecologia* **88,** 305–310.

Voss, M. & Struck, U. 1997 Stable nitrogen and carbon isotopes as indicator of eutrophication of the Oder river (Baltic sea). *Marine Chemistry* **59,** 35–49.

Wilsey, B. J., McKee, K. L. & Mendelssohn, I. A. 1992 Effects of increased elevation and macro- and micronutrient additions of *Spartina alterniflora* transplant success in salt-marsh dieback areas in Louisiana. *Environmental Management* **16,** 505–511.

Yoneyama, T. 1995 Nitrogen metabolism and fractionation of nitrogen isotopes in plants. In *Stable Isotopes in the Biosphere* (Wada, E., Yoneyama, T., Minagawa, M., Ando, T. & Fry, B., eds). Kyoto University Press, Kyoto, Japan, pp. 92–102.

Yoneyama, T. & Kaneko, A. 1989 Variations in the natural abundance of ^{15}N in nitrogenous fractions of komatsuna plants supplied with nitrate. *Plant and Cell Physiology* **30,** 957–962.

Yoneyama, T., Omata, T., Nakata, S. & Yazaki, J. 1991 Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. *Plant and Cell Physiology* **32,** 1211–1217.